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SHORT-TERM VERSUS LONG-TERM CHANGES IN THE BENTHIC COMMUNITIES OF A SMALL COASTAL LAGOON: IMPLICATIONS FOR ECOLOGICAL STATUS ASSESSMENT

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MACROINVERTEBRATES
SPATIAL-TEMPORAL VARIATIONS
BIOTIC INDICES
LAND-LOCKED LAGOON
TRANSITIONAL WATERS
MELIDES

ABSTRACT. – The characteristic high variability and low predictability of coastal lagoons, due to strong changes in marine and freshwater inputs, make these ecosystems an interesting case-study. The small Melides landlocked coastal lagoon in SW Portugal is a paradigmatic example, with a biological community highly stressed by these phenomena. Benthic macroinvertebrate samples were collected in 1998/99 and 2009 and each year, in different seasons and addressing different environmental conditions influenced by the connection to the sea and rainfall regime. Major spatial and temporal patterns in benthic communities were investigated using some invertebrate attributes (e.g. community composition, density, species richness and diversity). A very low taxonomic species richness and diversity was found in the Melides lagoon and only a much reduced number of species occurred along all sampling periods and in both sampling campaigns. Although the colonization events play a crucial role, the persistence of the observed species was mainly associated to abiotic factors, such as salinity, temperature and dissolved oxygen. Despite the potential reduction in anthropogenic pressure, by the construction of a sewage treatment plant and a reduction of urban occupation, the ecological status did not improve and the high level of natural environmental variably in the lagoon seems to be the dominant stressor influencing benthic invertebrate communities.

INTRODUCTION

Land-locked coastal lagoons are a particular type of transitional waters, with high variability and low predictability events. Enclosed lagoon systems are the final reservoir of organic and inorganic continental material proceeding from their watersheds. This occurs through freshwater inputs carrying these materials, which accumulate due to active beach ridges, resulting in high primary production conditions and consequent eutrophication (Colombo 1977; Cauwet 1988). The natural ageing of coastal lagoons (Heydorn & Tinley 1980) is, in some Portuguese SW coast lagoonal systems, prevented by an annual man-made opening of the sand barrier connecting the lagoon to the sea, allowing the nutrient export and the renewal of the system and biologic communities (Cancela da Fonseca *et al*. 2001, Costa *et al*. 2003). The enclosed lagoons occurring in the southwest Portuguese coast are brackish lagoonal environments where freshwater input depends on streams that convey water from groundwater systems and run-off from the hydrographical basins. Apart from the periodic man-made inlet openings, the marine water input depends also on spontaneous communication with the sea, either by the high water levels due to heavy rainfall periods pushing and breaking the sand barrier or spring tides and storms that may also result on overwash episodes (Cancela da Fonseca *et al*. 1989, Costa *et al*. 2003, Whitfield *et al*. 2008). These unpredictable events create abrupt variations on abiotic conditions, especially on salinity levels, that have a significant impact on the benthic communities, not only limiting species richness and diversity and allowing tolerant species to thrive, but also allowing new colonization events (Cognetti 1992, Munari & Mistri 2008, Correia *et al*. 2012).The knowledge on this impact is of the uttermost importance for a better quality assessment and an improved management of transitional water systems.

Benthic invertebrates are commonly used as bioindicators for the assessment of environmental water quality due to its rapid response to anthropogenic and environmental stress (Pearson & Rosenberg 1978, Grall & Glemarec 1997, Eaton 2000, Simboura & Zenetos 2002). This approach is now a baseline for the determination of Ecological Quality Status (EQS) required by the European Water Framework Directive (WFD). Several indices using macroinvertebrates were, thus, developed and adapted to be used as assessment tools for EQS (e.g. Borja *et al*. 2000, Simboura & Zenetos 2002, Diaz *et al*. 2004, Perus *et al*. 2004, Rosenberg *et al*. 2004, Dauvin & Ruellet 2007, Cañedo-Argüelles *et al*. 2012). However, inconsistencies in the use of different indices promote a persistent debate on their limitations, regarding its application (e.g. Chainho *et al*. 2008). Particularly, in transitional (brackish) waters, this evaluation is hampered by the natural variability associated with these ecosystems (Munari & Mistri 2008). Species in these highly variable environments are tolerant, adapted to this variability (Cognetti 1992; Simonini *et al*. 2004; Munari & Mistri 2007), and they tend to be opportunists (Healy 2003). Also the distinction between anthropogenic disturbances and environmental natural stressors may not be easily identified (ICES 2004, Chainho *et al*. 2007, Zettler *et al*. 2007, Munari & Mistri 2008, Cardoso *et al*. 2011). The high variability and low predictability events, characteristic of coastal lagoons, pose increased difficulties in the EQS assessment, when compared to other transitional waters (e.g. Whitfield *et al*. 2008, Cañedo-Argüelles & Rieradevall 2010, Correia *et al*. 2012).

The aims of the present work were (i) to assess spatial and short-term versus long-term variations in the benthic invertebrate communities and understand which are more significant, (ii) to investigate which are the major environmental factors responsible for the dissimilarities in these communities and (iii) to determine if these variations constrain the assessment of ecological status.

METHODOLOGY

Study area: The Melides coastal lagoon is a small system (0.4 km^2) located in the south of Portugal (38°7'55 N; 8°47'11 W), included in the Natura 2000 network (PTCON0034). It is isolated from the sea by a sand barrier which is artificially opened and naturally sealed off by tidal phenomena and sea currents (Costa *et al*. 2003, Freitas *et al*. 2008). These openings determine the input and colonization of the marine faunal component in the lagoon and are one of the causes of the strong seasonal changes in salinity and other abiotic factors. The freshwater input depends mainly on groundwater inflow and run-off of Ribeira de Melides, a 14 km stream with a 65 km^2 catchment area. There are, however, other factors responsible for salinity variations: water discharges from the rice fields located upstream create a rapid decrease in salinity and heavy rainfall periods can also lower salinity values and, additionally, cause the rupture of the sand barrier allowing a marine water input, which may increase it abruptly. There are also sand barrier overwash episodes during storms and particular strong tides, causing an input of sea water.

Major human pressures are sewage discharge, livestock and rice production, but some changes occurred during the 20 years' study period: half of the rice producers changed to more sustainable practices by implementing integrated production rules; and Melides and Vale Figueira sewage treatment plants started

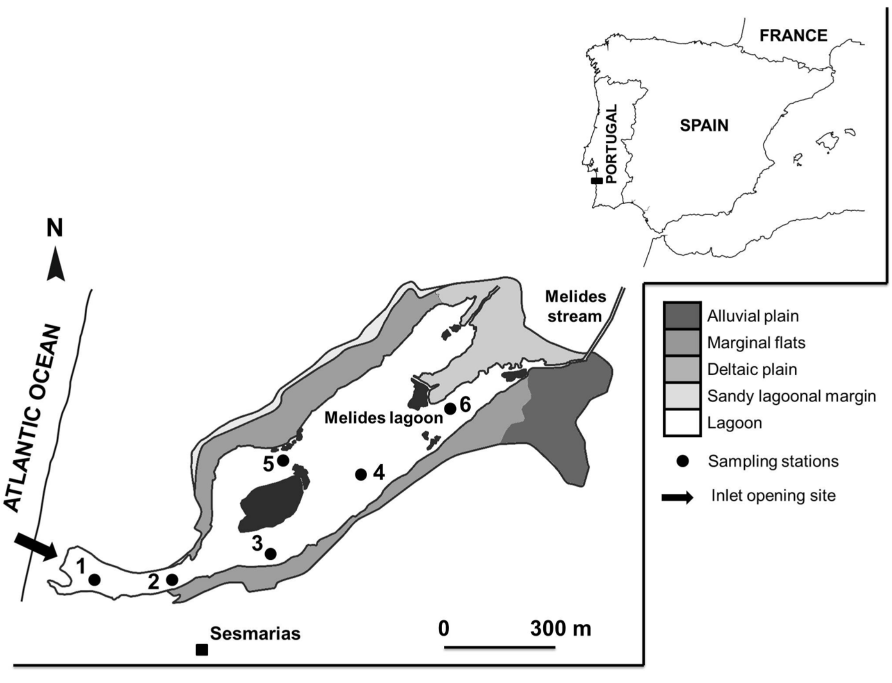

Fig. 1. – Study area with the location of sampling stations.

operating in 1983 and 2007, respectively, and both discharge to Ribeira de Melides. The livestock effluent discharge occurs in the soil, received by the lagoon through run-off (Freitas *et al.* 2008).

Sampling and analysis: Six stations were sampled (Fig. 1) on two main periods, 1988/89 and 2009, on sequential months throughout the hydrological year, from after the artificial inlet opening of the lagoon until just before the next year's re-opening. Station 3 was not sampled in 2009. Only months common to both campaigns were included in this study (March, April, May, June, September 1988 and February, March 1989; February, March, April, May, June, September 2009). Dates of the occurrence of natural and artificial openings of the sand barrier were provided by Administração da Região Hidrográfica Alentejo, the competent authority for the management of the Alentejo hydrographic region. Rainfall data was acquired from SNIRH (Sistema Nacional de Informação de Recursos Hídricos).

Macroinvertebrates were collected using a van Veen grab sampler (0.05 m^2 of sampled area), 3 replicates for each station (giving a total area of 0.15 m^2 per sampling site), sieved *in situ* with a 500 μm square mesh sieve, and after fixed in a 10 % formalin solution buffered with borax and stained with Rose Bengal until further analysis. In the laboratory samples were then rinsed to remove excess of formalin and sediment, and the organisms were hand sorted into major taxonomic groups, followed by preservation in 70º ethanol. All specimens were then identified to the lowest practical taxonomic level (usually species level) and counted. Several environmental variables, namely temperature, dissolved oxygen (DO), pH and salinity were measured at the bottom during each sampling event using a multi-parametric sonde.

Additional grab samples were collected for sediment grain size analysis, which was obtained using 63 µm and 2 mm sieves to separate the silt-clay, sand and gravel fractions, respectively. Each fraction was dried and weighed and sediments assorted according to their percentages (Buller & McManus 1979) and classified with Shepard diagrams (Buchanan & Kain 1971). Sediments were then grouped into sand, muddy sand/sandy mud and mud (Mud < 63 μm; 0.063 mm ≤ Muddy sand/Sandy mud < 0.2 mm; 0.2 mm ≤ Sand < 2 mm), following the classification system adapted from that of sediment charts of the Portuguese Navy Hydrographic Institute (Moita 1985).

Data analysis: Spatial and temporal differences on benthic macroinvertebrate communities were assessed using a Permutational Analysis of Variance - PERMANOVA (Anderson 2005) performed on PRIMER® v6, with a three-way fixed-effect crossed design (Factors: Year; Month; and Station) (for sampling sites see Fig. 1). For this analysis replicate samples were log transformed (log (X+1)) and Bray-Curtis zero-adjust similarity coefficient was used as resemblance measure, since there were replicates with zero counts (Clarke *et al.* 2006). Correspondence Analysis (CA) was the multivariate ordination technique used to identify patterns within sampling stations, based on the taxa density (ter Braak 1986). Environmental variables were used to interpret the patterns extracted from all variation by an indirect gradient analysis and plotted as supplementary variables. All taxa were considered in the analysis, but only taxa with a weight of more than 6 % were represented in the CA plot. Data was log transformed (log (X+1)) and the sediment related variables (Sand, Muddy Sand/Sandy Mud and Mud) were classified and included in the environmental data matrix as dummy variables. The CA was performed in Canoco v4.5 (Biometris®).

To identify and categorise the permanency of the benthic community within each year, a constancy index was adapted from Bachelet *et al.* (1996) and calculated as follows:

$$C_{ij} = (n_{ij} / n_j) \times 100$$

where n_{ij} is the number of occurrences of taxa i in month group j, and n_j is the number of months in month group j. Taxa were termed Permanent (C = 100 %), Constant (100 % > C ≥ 50 %), Frequent (50 % > C ≥ 25 %) or Temporary (C < 25 %).

The Benthic Assessment Tool (BAT) is a multimetric benthic invertebrate index proposed to assess the ecological status in Portuguese transitional waters, which combines Margalef, Shannon-Wiener and AMBI indices (Teixeira *et al.* 2009). Since type specific reference conditions are still under discussion, only single metrics (i.e. Shannon-Wiener and AMBI) were used in the present study. Margalef index was not calculated due to an identified redundancy with the Shannon-Wiener results (Chainho *et al.* 2008) and also because available reference values for this index do not specify if they were based on a density data matrix or absolute values, which might underestimate the results, according to Gamito (2010). The metrics used to assess spatial and temporal variations in the structure of the benthic community and ecological status were density (N) expressed in ind.m^{-2}, taxonomic richness (S) (Legendre & Legendre 1998), the Shannon-Wiener diversity index (H') (Shannon & Weaver 1963) and the AZTI Marine Biotic Index, AMBI (Borja *et al.* 2000). The latter was calculated using the software AMBI v4.1 with the February 2010 update of species list. Taxa not included in the AMBI classification list, particularly species with freshwater affinities, were assigned to an equivalent score of the freshwater biotic index IBMWP (Iberian Bio-monitoring Working Party), whenever possible (Alba-Tercedor *et al.* 2002, Medeiros *et al.* 2011). Temporal comparison between these parameters was assessed with a two-way crossed factor ANOVA (Factors: Year and Month), using Statistica v10 (Statsoft®). All statistical tests were considered significant at $p < 0.05$.

RESULTS

Environmental conditions

Environmental variables measured in the lagoon showed a high variability along the year. Salinity always increased to euhaline values across all sampling stations after the opening of the sand barrier and decreased after the rainfall period and the drainage of rice fields (Fig. 2). Nevertheless, in 1988/89 a saline spatial gradient between freshwater conditions (i.e. confluence area

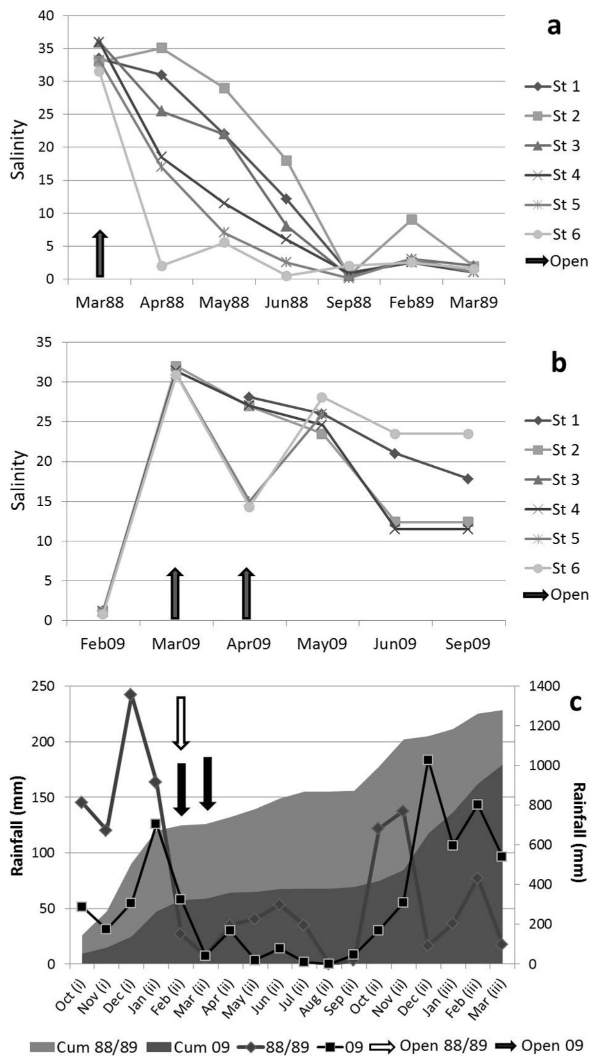

Fig. 2. – Salinity variations along the hydrological years of (a) 1988/89 and (b) 2009, for each station (series); and monthly and cumulative rainfall data (mm) for both hydrological years (1988/89 and 2009) (c). Arrows indicate the opening period of the lagoon to the sea. Each set of months from the three years represented in the plot are symbolised as (i), (ii) and (iii). Each i corresponds to a civil year, and the data set comprises the whole influencing period of the hydrological year in the study. For the 88/89 period, the labels start at October 1987 – Oct (i) – to March 1989 – Mar (iii). For the 2009 period, labels start at October 2008 – Oct (i) – to March 2010 – Mar (iii).

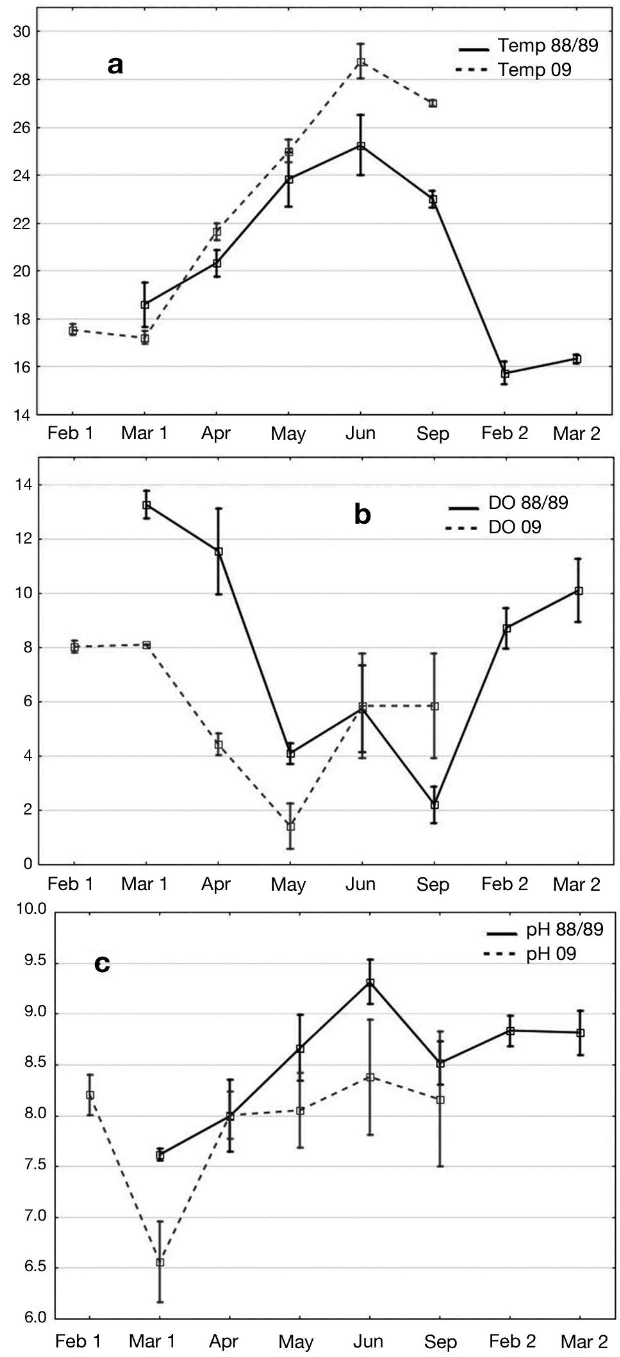

Fig. 3. – Mean monthly ± SE (a) temperature (ºC), (b) dissolved oxygen (DO in mg/L) and (c) pH for each year (1988/89 and 2009).

with the Melides stream) and euhaline conditions (i.e. close to the sand barrier) was established, while in 2009 salinities varied less, only between mesohaline and polyhaline conditions, except for February, when the whole lagoon was oligohaline (Fig. 2a, b). Monthly rainfall and cumulative rainfall along the studied periods indicated that highest precipitations occurred between December and January in both years, but 1988/89 registered higher rainfall values (Fig. 2c). The highest freshwater input in 1988/89 is clearly stressed by the mean salinity decrease along the year, trend that is less marked in 2009 (Fig. 2).

Temperature varied between 14 ºC and 30 ºC, with higher values during summer months and lower during the winter months (Fig. 3a). In general, lower temperature values were registered during 1988/89. DO and pH varied between 0.1 mg/L and 14.8 mg/L and 5.5 and 10, respectively (Fig. 3b, c). Overall, 2009 was the year registering lower DO and pH values.

Benthic community

A total of 67 taxa were identified in both sampling periods, mainly Bivalvia (18) Insecta (16), Polychaeta (15) and Gastropoda (7) species, as can be inferred from the abundance values of Table I. Chironomidae was the most representative taxon, accounting for 54 % of total abundance, followed by *Potamopyrgus antipodarum* (Gray, 1843) and Oligochaeta, both with 13 %, and Ostracoda with 11 %. Despite the clear predominance of Chironomidae (29 % in 1988/89 and 73 % in 2009), important differences regarding other taxa should be stressed: (i) Oligochaeta was scarcely represented in 2009 with less than 0.1 %, but abundant in 1988/89 (29 %), (ii) *P. antipodarum* occurred only in 1988/89 (30 %), while *Hydrobia ulvae* (Pennant, 1777), another Hydrobiidae gastropod, occurred in both periods but with higher abundances in 2009, (iii) Ostracoda was abundantly represented in 2009 (20 %) but absent in 1988/89 and (iv) although 16 different insect taxa were identified in 1988/89, only Chironomidae occurred in 2009. Overall, the number of invertebrate taxa was drastically reduced between sampling periods ($p < 0.0001$): 66 taxa were identified in 1988/89 against only 7 in 2009. Major differences in the number of taxa between periods were found after inlet opening (Fig. 5a). The taxon identified as Bivalvia in 2009 was a single species but individuals were too small to allow identification to a lower taxonomical level. These differences in taxonomic richness were also emphasized by the results of the constancy index, which identified three (i.e. Chironomidae, *Hediste diversicolor* (O.F. Müller, 1776), *P. antipodarum*) and five (i.e. Chironomidae, *H. diversicolor*, *H. ulvae*, *Lekanesphaera hookeri* (Leach, 1814), Ostracoda permanent taxa in 1988/89 and 2009, respectively (Fig. 4). Those permanent taxa represented only 5 % of the total fauna in 1988/89 and most taxa (64 %) were considered temporary, since they occurred in less

Table I. – Abundance of macroinvertabrate species for the Melides lagoon (average ind.m^{-2} ±SE), for the 1988/89 and 2009 periods.

	1988/89		2009	
	Mean	± SE	Mean	± SE
Hydrozoa				
Cordylophora caspia (Pallas, 1771)	1.9	0.5	0	0
Gastropoda				
Haminoea hydatis (Linnaeus, 1758)	0.2	0.2	0	0
Haminoea sp.	0.2	0.2	0	0
Hydrobia ulvae (Pennant, 1777)	0.5	0.4	64	44
Lymnaea peregra (Müller, 1774)	0.2	0.2	0	0
Physa fontinalis (Linnaeus, 1758)	5.8	3.0	0	0
Potamopyrgus antipodarum (Gray, 1843)	582.4	131.5	0	0
Pusillina inconspicua (Alder, 1844)	0.2	0.2	0	0
Rissoa parva (da Costa, 1778)	0.3	0.3	0	0
Bivalvia				
Abra alba (Wood, 1802)	0.2	0.2	0	0
Abra segmentum (Récluz, 1843)	0.5	0.4	0	0
Atrina fragilis (Pennant, 1777)	0.2	0.2	0	0
Barnea candida (Linnaeus, 1758)	0.6	0.5	0	0
Bivalvia	0.2	0.2	16	6
Cerastoderma edule (Linnaeus, 1758)	1.1	0.6	0	0
Cerastoderma glaucum (Bruguière, 1789)	0.5	0.4	0	0
Cerastoderma sp.	0.2	0.2	0	0
Ensis ensis (Linnaeus, 1758)	0.2	0.2	0	0
Ensis siliqua (Linnaeus, 1758)	0.3	0.2	0	0
Ervilia castanea (Montagu, 1803)	0.5	0.4	0	0
Lutraria angustior Philippi, 1844	0.2	0.2	0	0
Mactra glauca Born, 1778	0.6	0.5	0	0
Modiolus barbatus (Linnaeus, 1758)	0.8	0.8	0	0
Musculus subpictus (Cantraine, 1835)	0.3	0.3	0	0
Scrobicularia plana (da Costa, 1778)	0.5	0.5	0	0
Spisula solida (Linnaeus, 1758)	0.2	0.2	0	0
Spisula subtruncata (da Costa, 1778)	0.2	0.2	0	0
Polychaeta				
Capitella capitata (Fabricius, 1780)	3.8	1.4	0	0
Hediste diversicolor (Müller, 1776)	136.8	36.8	80	26
Nephtys caeca (Fabricius, 1780)	0.2	0.2	0	0
Nephtys cirrosa (Ehlers, 1868)	1.4	1.3	0	0
Nephtys hombergii Savigny in Lamarck, 1818	1.4	0.9	0	0
Scolelepis squamata (Müller, 1806)	0.2	0.2	0	0
Ophelia bicornis Savigny in Lamarck, 1818	0.2	0.2	0	0
Raphidrilus nemasoma Monticelli, 1910	0.2	0.2	0	0
Sabella pavonina Savigny, 1822	0.3	0.2	0	0

Table I. - Continued.

	1988/89		2009	
	Mean	± SE	Mean	± SE
Saccocirrus papillocercus Bobretzky, 1872	1.6	1.2	0	0
Malacoceros tetracerus (Schmarda, 1861)	0.8	0.6	0	0
Malacoceros fuliginosus (Claparède, 1870)	0.2	0.2	0	0
Scoloplos armiger (Müller, 1776)	1.6	1.1	0	0
Spio filicornis (Müller, 1776)	0.2	0.2	0	0
Streblospio shrubsoli (Buchanan, 1890)	0.2	0.2	0	0
Oligochaeta	587.8	238.8	1	1
Crustacea				
Gammarus chevreuxi Sexton, 1913	17.0	11.0	0	0
Lekanesphaera hookeri (Leach, 1814)	18.9	7.0	32	10
Ostracoda	0.0	0.0	592	335
Simocephalus vetulus (Müller, 1776)	2.2	1.8	0	0
Tanais dulongii (Audouin, 1826)	0.2	0.2	0	0
Tylos latreillei Audouin, 1826	0.2	0.2	0	0
Insecta				
Aepophilus bonnairei Signoret, 1879	0.2	0.2	0	0
Baetis sp.	0.2	0.2	0	0
Berosus sp.	0.3	0.3	0	0
Ceratopogonidae	0.5	0.3	0	0
Chironomidae	579.2	95.0	1861	851
Cloeon sp.	3.7	1.9	0	0
Crocothemis erythraea (Brullé, 1832)	0.3	0.2	0	0
Diptera n.d.	1.4	0.6	0	0
Haliplus sp.	0.2	0.2	0	0
Ichneumonidae n.d.	0.2	0.2	0	0
Ischnura elegans (Vander Linden, 1820)	0.2	0.2	0	0
Ischnura graellsi (Rambur, 1842)	1.0	0.4	0	0
Ischnura pumilio (Charpentier, 1825)	2.2	0.9	0	0
Naucoris maculatus Fabricius, 1798	11.0	7.3	0	0
Philydrus sp.	0.2	0.2	0	0
Plea leachi MacGregor & Kirkaldy, 1899	0.6	0.4	0	0
Bryozoa				
Conopeum seurati (Canu, 1928)	0.5	0.3	0	0
Ophiurida				
Amphipholis squamata (Delle Chiaje, 1828)	0.2	0.2	0	0

than 25 % of the sampling occasions, whilst in 2009 only 14 % were temporary and the majority were permanent (71 %).

Concerning benthic invertebrate variations in density, these ranged from 20 ind.m^{-2} to 64840 ind.m^{-2}, with some differences between months and sampling stations, but an exceptional increase on densities was registered in September 2009 (Fig. 5b), mainly due to high abundance of Chironomidae. The PERMANOVA results showed high spatial and temporal variability in the benthic macroinvertebrate communities (significant differences within each factor and significant interactions between all factors) (Table II). Pairwise comparisons rendered significant differences for all groups within factors (Year, Month and Station), except between stations 3-4 and 4-5, but even so, showing a small degree of similarity.

Two major groups of samples were identified in the CA ordination diagram, corresponding to different sampling periods, with a more uniform distribution of the community composition in 2009 than in 1988/89 (Fig. 6). Stations 1 and 2 were clearly different from other 1988/89 stations, which had a more similar and homogeneous benthic community. These differences between sampling stations seem to be related to higher salinity and coarser sediments (stations closer to the sand barrier). A more homogeneous and stable biological community occurred throughout the year 2009 and among sampling stations, which seems to be influenced by lower salinity variations along time. Differences between periods are mainly associated with higher temperature and lower DO values in 2009. Among the most representative taxa, Ostracoda, *H. ulvae* and *L. hookeri* were markedly more present in the 2009 samples. Higher abundances of Oligochaeta and *H. diversicolor* were found on 1988/89, but more related to the downstream stations (1 and 2). For the same 1988/89 period, but in an opposite trend, *P. antipodarum* was more abundant in sandy mud stations, occurring when the salinity was low.

Ecological status

Shannon-Wiener diversity values were always low (Fig. 7a), with higher values registered in 1988/89 than in 2009 ($p < 0.001$). Highest diversity was obtained in March 1988/89, after the inlet opening, although in 2009 the highest values were obtained just before the opening

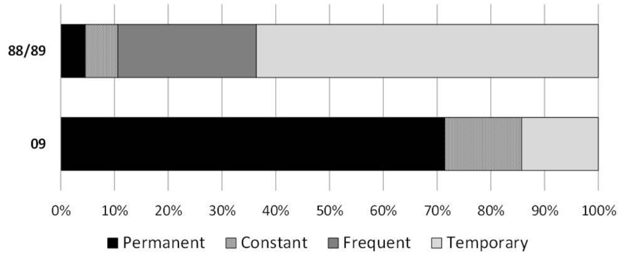

Fig. 4. – Percentage of species classified according to the adapted Constancy Index, as Permanent (C = 100 %), Constant (100 % > C ≥ 50 %), Frequent (50 % > C ≥ 25 %) or Temporary (C < 25 %). The total number of taxa was 66 and 7 for 1988/89 and 2009, respectively.

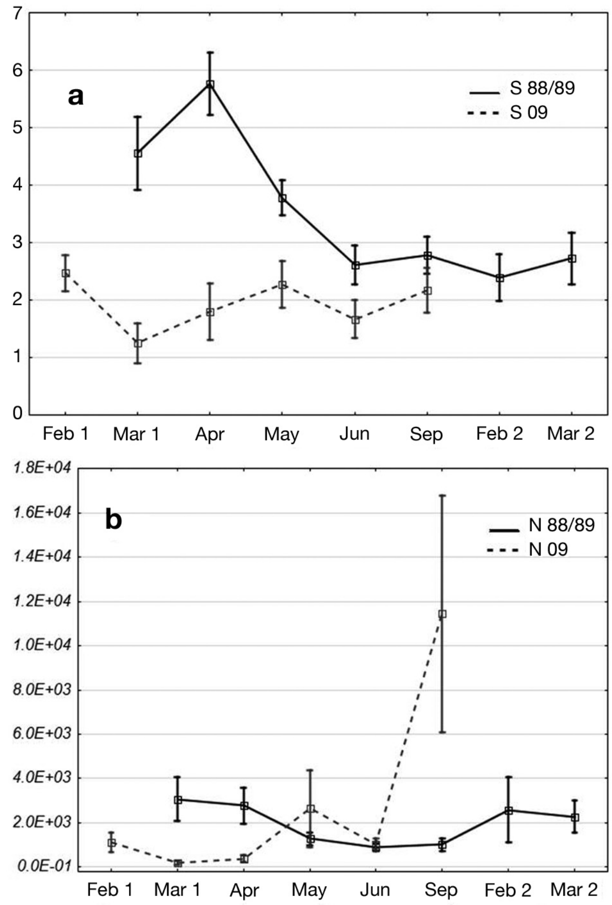

Fig. 5. – Mean monthly ±SE values of (a) number of taxa (S) and (b) density (N, ind.m^{-2}), for each year (1988/89 and 2009).

(February). All stations were classified as in Poor-Bad status in 1988/89 and in a Bad status in 2009, following thresholds proposed by Bettencourt *et al.* (2004) for Portuguese transitional and coastal waters. According to AMBI values, 1988/89 samples were classified as in a Good to Moderate status and significantly lower in the 2009 samples ($p < 0.0001$) as in a Moderate status (Fig. 7b), with the lowest quality status obtained in March. Overall, results obtained for both periods, with both indices, point towards an ecological quality decrease in 2009.

Table II. – Results from a three-fixed factor PERMANOVA (Year – Ye; Month – Mo; Sampling Station – St), assessing spatial and temporal differences on benthic macroinvertebrate communities.

Source	df	SS	MS	Pseudo-F	P(perm)	Perms
Ye	1	70760	70760.0	45.958	0.001	998
Mo	5	42999	8599.8	55.855	0.001	999
St	5	54748	10950	71.117	0.001	998
Ye x Mo	5	43535	8707.0	56.551	0.001	998
Ye x St	4	26272	6568.0	42.658	0.001	999
Mo x St	25	75888	3035.5	19.715	0.001	997
Ye x Mo x St	17	68749	4044.0	26.266	0.001	994

DISCUSSION

Short-term versus long-term variations

The benthic community of the Melides lagoon showed high spatial and temporal variability, mainly due to annual variations, both natural and artificial, on system's environmental conditions, related to: marine water inputs caused by an annual man-made inlet opening, and spring tides and storms that overwash and/or cause occasional breaking of the sand barrier, but also due to freshwater inputs associated to groundwater inflow and Melides stream discharges from run-off and drainage of the rice fields. On a short-term analysis, these abrupt and periodical changes of lagoonal environment cause the almost exclusive occurrence of stress tolerant species. A higher number of species was registered immediately after the opening of the lagoon to the sea, but it was significantly reduced in the following months. In fact, differences in the number of taxa during the annual cycle may be a consequence of the different taxa tolerance limits according to the Remane's statement (Muus 1967, Nybakken 1993). As pointed out by Wolf (1971), in brackish water systems a salinity increase promotes a consequent escalation of species richness. Thus, the drastic decrease of salinity values after the inlet closing of Melides lagoon may act as a major determinant factor in the reduction of taxa to the few brackish species that are able to endure the prevalent environmental conditions (e.g. Bamber *et al.* 1992, Healy 2003).

However, long-term changes are distinctly more relevant than short-term, suggesting that there are other factors weighing more than annual environmental variations. Further evidences point towards the predominant influence of salinity as the major factor for the significant differences between the benthic community of 1988/89 and 2009. The variety of salinities during 1988/89 would be expected to provide sustainability to a larger and more diversified group of taxa (e.g. Odum 1969, Pianka 1969, 1973, Schoener 1974). Studies conducted on estuarine systems indicated a salinity value of 5 (limit of oligohaline zone) as a threshold for the occurrence of brack-

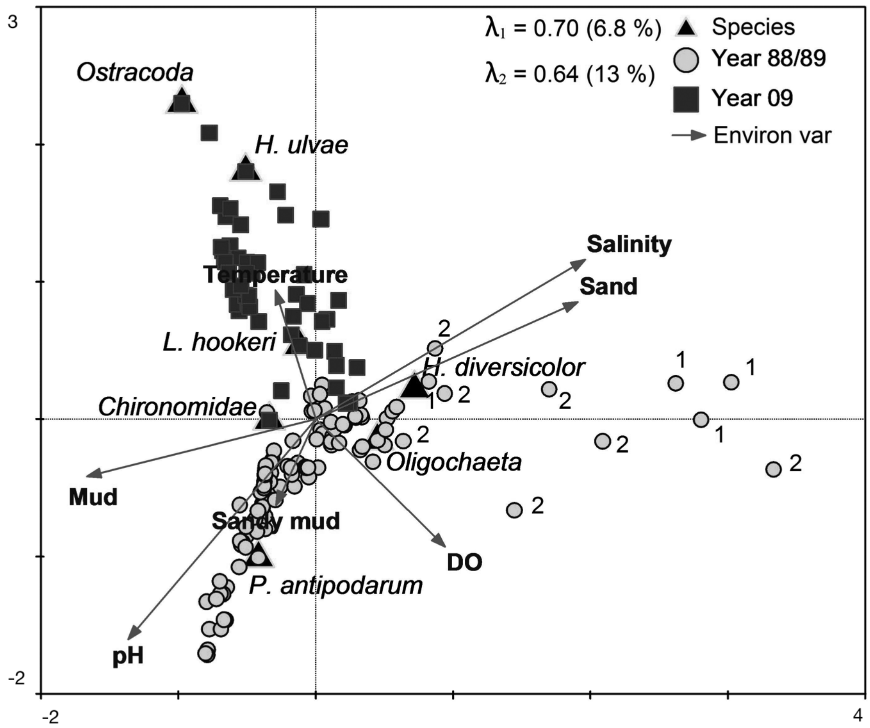

Fig. 6. – Correspondence Analysis of benthic invertebrate abundance (Log+1 transformed) sampled monthly in 1988/89 and 2009 in the Melides lagoon, comprising 67 taxa. Only taxa with a weight of more than 6 % are represented (Chironomidae; *Hediste diversicolor*; *Hydrobia ulvae*; *Lekanesphaera hookeri*; Oligochaeta; Ostracoda; *Potamopyrgus antipodarum*). Environmental variables were plotted on the ordination as supplementary variables. Eigenvalues (λ_i) and cumulative percentage explained (between brackets) for the first two axes are also provided. Stations 1 and 2 are indicated since they were emphasized in the discussion.

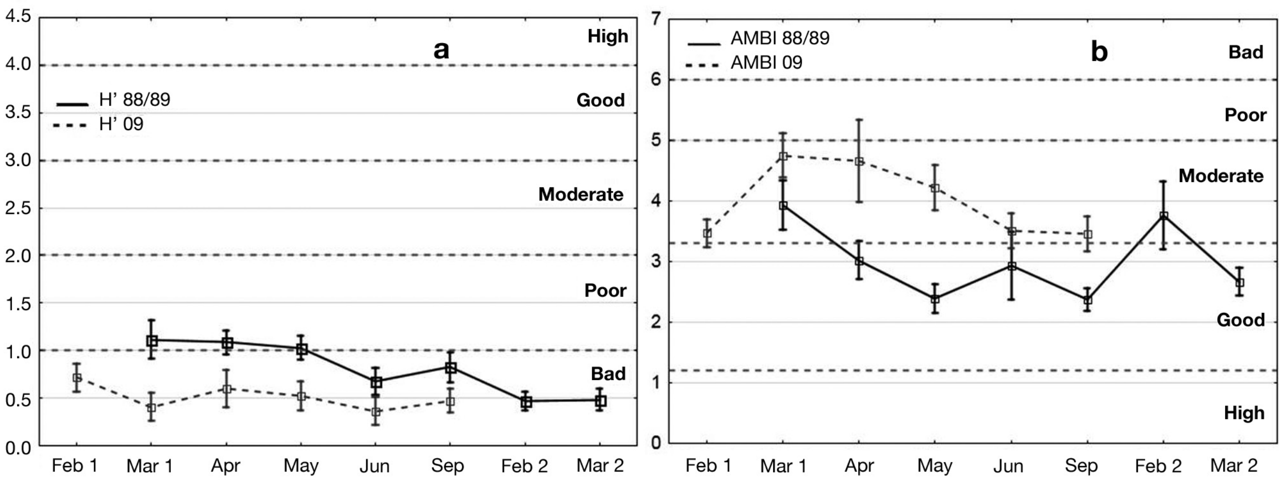

Fig. 7. – Mean monthly ±SE values for (a) Shannon-Wiener index (H') and (b) AMBI index, for each year (1988/89 and 2009).

ish water fauna (Remane & Schlieper 1971, Cognetti & Maltagliati 2000) and while most brackish species cannot tolerate lower salinities, several insect taxa have this threshold as the upper limit of the salinity optimal range (Williams & Williams 1998, Cañedo-Argüelles & Rieradevall 2010). Accordingly, while in 2009 salinities vary only between mesohaline and polyhaline, except for February (prior to opening of the inlet), in 1988/89 a wide range of salinities occurred across the lagoon covering the entire scale between fresh and salt water. This provided more diverse habitat conditions and allowed species with marine affinities to colonise the area closer to the sand barrier (e.g. Polychaeta) and freshwater species to colonise the upstream area (e.g. Insecta). Thus, while in 1988/89 mainly freshwater and marine taxa were found, in 2009 only brackish taxa occurred. Moreover, the replacement of the gastropod *P. antipodarum* by *H. ulvae* between periods provides an additional evidence in favour of this hypothesis, substantiating the importance of salinity on the benthic community structure, since the first is a freshwater to brackish water species (Siegismund & Hylleberg 1987, Hughes 1996) and the second occurs predominantly in salinities higher than 20 (Komendantov & Smurov 2009).

Although salinity may not be the sole factor influencing the structure of benthic communities, there are some lagoonal characteristics that precludes the use of the confinement theory, proposed for lagoonal environments

(Guélorget & Perthuisot 1983, Pérez-Ruzafa & Marcos 1993). The size of the lagoon and its closed nature is a key factor originating a lack of zonation gradients. Here there are many potential abiotic factors overlapping (oxygen concentration, depth, temperature, substrate or even ionic composition), that neutralizes the effect of confinement (Barnes 1994, Pérez-Ruzafa *et al.* 2011). A given event easily affects the whole lagoon, instead of creating a zonation effect, probably justifying the high variability found for the Melides lagoon, on both spatial and temporal levels.

Nevertheless, besides salinity variations, mainly due to higher precipitation and the establishment of a temporal salinity gradient across the lagoon, some other factors, such as temperature and DO, needed to be examined. Their synergetic influence, together with salinity, accounts for a major factor, constraining benthic communities (Hartog 1971, Pearson & Rosenberg 1978, Barnes 1987, Bamber *et al.* 1992, Healy 2003). Although human pressure on this lagoonal system appears to have been reduced along this 20 year period (Freitas *et al.* 2008), species richness and diversity decreased dramatically. The change observed in these environmental variables suggests a direct effect of abiotic factors on the benthic community, namely, higher salinity and temperature values and lower DO, from 1988/89 to 2009 (Duarte *et al.* 2002, Rosenberg *et al.* 2002). Considering human pressures related to rice fields and sewage discharge in the Melides river basin were reduced between both studied periods, a reduction of nutrients concentrations and Chl*a* would be expected in the lagoon. Although Chl*a* and nitrate measurements were conducted simultaneously with benthic invertebrates collections in the 1988/89 period (unpublished data), in the most recent surveys Chl*a* and nitrates were measured in 2007 and 2008 (Freitas *et al.* 2008), while benthic invertebrates were collected in 2009. Nevertheless, those measurements do not provide indication of degradation or improvement of the ecological status of the lagoon. Although no reference values are available for Portuguese coastal lagoons, Brito *et al.* (2012) proposed 8 mg/L as boundary between Good and High status for the Ria Formosa coastal lagoon and all measurements available for the Melides lagoon are lower than 0.05 mg/L (unpublished data; Freitas *et al.* 2008). Moreover, although a concentration of 25 mg/L $NO_3$was established as the boundary between Bad and Good status for Portuguese southern rivers (INAG 2009) (no references are available for coastal lagoons), all measurements available for the Melides lagoon are lower than 2 mg/L.

Benthic communities can be strongly conditioned by these environmental unfavourable conditions, which can justify the fact that most taxa in 1988/89 are temporary and occurring only after the inlet opening (when the renewal of the lagoon promotes a mitigation of extreme abiotic conditions), and all permanent taxa are tolerant brackish species. These environmental conditions could play an important and limiting role, *per se*, on the species richness and diversity, creating environmental settings that fall outside the tolerance range of several species. However, the differences of the benthic community between both periods may be explained by an additional factor. The oligohaline conditions of Feb09, prior to the opening of the lagoon to the sea, do not provide significant differences in diversity when compared to the following months, after the opening, suggesting that salinity or temperature cannot entirely explain the substantial change in the macroinvertebrate community. These shifts in the ecosystem's regime are not systematic and depend greatly on climatic, stochastic events that result in inputs of sea water, freshwater and diverse hydrodynamic conditions due to wind driven mixing (Cancela da Fonseca *et al.* 1989; Costa *et al.* 2003; Vignes *et al.* 2009; Barbone *et al.* 2012). Thus, the timing of the disturbance may influence significantly the recruitment of new species either through larvae entrance (availability) at the opening time of the sand barrier or the suitable conditions for egg deposition of insect species (Zajac & Whitlatch 1982, Cañedo-Argüelles & Rieradevall 2010). The absence of good conditions for recruitment constitutes a possible cause for the low diversity in 2009 and Melides Lagoon may be considered this year a "shock lagoon" (Healy 2003) which is usually poor in species. Most species collected in that period are tolerant species already present previously to and during the disturbance occurrences – over 70 % classified as permanent taxa (Fig. 4). Thus, the randomness of colonization may have been a factor establishing the differences found between the two periods (Pérez-Ruzafa *et al.* 2011).

In addition, the Ecological island concept (Pimm 1984) fits this type of coastal lagoons, since a huge number of accidental taxa were found during its annual cycle. Thus, their biological diversity is a function of species immigration and extinction rates, as it was defined for island systems (MacArthur 1972, Diamond & May 1976).

Ecological status assessment in coastal lagoons

Similarly to what was found for other brackish systems (e.g. Zettler *et al.* 2007, Chainho *et al.* 2008, Puente & Diaz 2008), in the present study, classifications obtained using diversity (i.e. Poor to Bad status) were always lower than those indicated by the AMBI index (i.e. Good to Moderate status). This is mainly related to the dominance of a reduced number of tolerant taxa due to constraints imposed by the extremely stressful environmental conditions of coastal lagoons. AMBI overestimates the ecological status classification when tolerant species included in ecological group III are dominant, as indicated previously by other authors (e.g. Quintino *et al.* 2006, Puente & Diaz 2008). On the other hand, benthic diversity can underestimate ecological status since it is highly influenced by natural stressors, such as cold winters (Kröncke & Reiss 2010), floods (Chainho *et al.* 2007) and frequent salinity fluctuations (Cañedo-Argüelles & Rieradevall 2010, Puente & Diaz 2008). Consequently, thresholds used for both indices would need to be modified in order to be adapted to coastal lagoons' characteristics, as well as different habitat types (Gamito *et al.* 2012). However, a modification of reference conditions and thresholds require some assumptions, namely that (i) indices respond predictably to changes on human pressures and (ii) response to human pressures can be separated from variations due to cyclical oscillations. Assuming that there was a decrease on the levels of human pressure in the Melides lagoon between 1988/89 and 2009 (Freitas *et al.* 2008), an improvement on the results of biotic indices should have been registered, but both indices indicated a worse ecological status in 2009. When reference conditions for this type of land-locked lagoons are established, ecological status will have to be assessed in periods with similar characteristics for, at least, the following factors: (i) annual precipitation regime, (ii) influence of sea water in the lagoon water mass balance, (iii) temperature annual cycle.

The use of indices developed for estuarine and coastal areas, such as AMBI, in lower salinity areas raises additional problems concerning the assignment of taxa to ecological groups according to their sensitivity/tolerance to human pressures. Some taxa classified by both AMBI and IBMWP do not have equivalent scores, namely, Chironomidae, *Potamopyrgus antipodarum* and *Gammarus chevreuxi* Sexton, 1913. Criteria for determining sensitivity might be different because of different taxonomic resolution, since AMBI assigns most taxa to the species level, while IBMWP used the family level. Specific indices for coastal lagoons have also been proposed, such as Chironomid Exuviae in Transitional Water index (EQAT), based on the identification of one dominant taxonomic group in these aquatic systems to the species level (e.g. Cañedo-Argüelles *et al.* 2012). Although being a promising tool, it requires high taxonomic skills for the identification of Chironomid species.

These results corroborated several previous studies that raise problems concerning the implementation of the WFD assessment tools in transitional waters, showing that land-locked coastal lagoons have specific constraints that are not realistic in a monitoring scenario with assessments conducted every three years.

CONCLUSIONS

In spite of the strong intra-annual variations observed in the Melides lagoon, reflecting a marked spatial and temporal variability, long-term are clearly more significant than short-term changes. Salinity, temperature and dissolved oxygen seem to be the major environmental factors driving the variations observed on the benthic communities between 1988/89 and 2009. Although an improvement on water quality would be expected along these 20 years due to a reduction on human pressures, environmental and benthic data demonstrate the opposite and the diversity and AMBI indices indicate a decrease on the ecological status. The results indicate that assessment tools proposed for coastal lagoons are influenced by the high natural variations observed, presenting important limitations to the assessment of ecological quality status, due to the dominance of a reduced number of tolerant taxa, given the constraints imposed by the extremely stressful environmental conditions.

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