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PRIMARY RESEARCH PAPER

Impact of freshwater inputs on the spatial structure of benthic macroinvertebrate communities in two landlocked coastal lagoons

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Abstract Landlocked lagoons are naturally stressed environments. They are strongly influenced by freshwater input which not only varies naturally, but which is also impacted by anthropogenic activities. This study investigated the direct influence of freshwater discharges on the distribution patterns and abundance of benthic communities in two neighbouring landlocked coastal lagoons, assessing the whole system and the confluence area of each tributary. Sampling occurred in the wet and dry seasons of 2011 at two distances from freshwater discharge locations. Both lagoons were colonized by species from tw
o different pools, freshwater and marine. Freshwater flow rates had a direct influence on the spatial structure of the benthic communities of brackish-water/freshwater interface areas, where also specific taxa can act as early indicators of freshwater input variations. The intensity of this influence is highly dependent on lagoon size, creating spatial heterogeneity or affecting the entire system. The benthic fauna at the confluence of the tributary that depends almost exclusively on groundwater showed the lowest variability, suggesting

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that the biogeochemical nature of the groundwater may be a central cause for setting specific ecotones. The results suggest that benthic communities of landlocked coastal lagoons can be highly impacted by flow reduction from freshwater aquifers under drought conditions or water abstraction activities.

Keywords Macroinvertebrates · Spatial distribution · Groundwater · Brackish systems · Freshwater influence · Interface areas

Introduction

Coastal lagoons are highly productive brackish-water systems that provide a wide range of natural services, granting them a high level of importance, both economical and ecological (Kjerfve, 1994). Despite their significance to human societies, these are highly stressed systems that constrain the establishment of biological communities, not only due to their natural variability, but also due to the anthropogenic pressures they are exposed to. These particular characteristics require the management of these systems, which poses several difficulties (Viaroli et al., 2010) and needs scientific support. Scientific knowledge on coastal lagoons has been increasing, but mostly focused on open lagoons, leaving a gap as to the functioning of lagoons that are closed, *i.e.* that have short period or no connection to the adjoining sea, termed as landlocked or enclosed lagoons (Bamber et al., 2001; Beer & Joyce, 2013). This knowledge gap is particularly significant with regard to the factors that modulate the dynamics of the system and how they affect the biological communities and, more importantly, if those factors are subject to human exploitation, like water abstraction activities (Correia et al., 2012; Félix et al., 2013a).

Landlocked coastal lagoons are typically shallow and confined brackish-water systems at the interface between the continent and sea (Healy, 2003), highly dependent on freshwater discharges, either from run-off or groundwater. Freshwater inputs, which vary naturally in their flow rates and biogeochemical characteristics, are important drivers for the settlement and structure of biological communities of these enclosed systems (Cancela da Fonseca et al., 2001a; Basset et al., 2006; Cañedo-Argüelles & Rieradevall, 2010). However, the level of their impact is also highly dependent on the morphological characteristics of each lagoon, such as the surface area or depth (Basset et al., 2006; Cañedo-Argüelles & Rieradevall, 2010). Thus, each lagoon can have its particular traits and biological role. Most studies on enclosed lagoons have focused mainly on the nutrient enrichment provided by freshwater sources and very little on the direct impact on the ecosystems and biological communities. Although it has been suggested that freshwater inputs can have a high impact in the settlement and composition of biological communities (e.g. Cañedo-Argüelles & Rieradevall, 2010), no information on the direct influence and impact on the stream/lagoon interface areas is available. Hence, the biodiversity in different confluence areas (different streams can discharge into a single lagoon) might be influenced by the biogeochemical processes due to freshwater discharge. This influence is expected to decrease gradually with increasing distance from the confluence, due to the dilution effect of brackish water. The hydraulic management of these basins and groundwater exploitation policies are particularly difficult to manage due to the lack of knowledge on the influence of freshwater inputs into the lagoonal system and its ecological susceptibility (cf. Smith, 1994). This may be particularly relevant in years of drought or heavy rainfall and in medium- and long-term scenarios of reduced rates of aquifer recharge, caused by increasing effects of climate change.

Enclosed lagoons are also the final reservoir of continental watershed material, which is transported by freshwater and accumulate. This adds to the lagoons own decay products, due to active beach ridges, resulting in high primary production conditions and consequent eutrophication (see Beer & Joyce, 2013). These phenomena lead to a natural ageing process in most landlocked coastal lagoons, i.e. towards eutrophicated freshwater ponds (Heydorn & Tinley, 1980). In some Portuguese SW coastal lagoons this process is prevented by man-made openings of the sand barrier, typically conducted once a year, according to management decisions. The opening connects the lagoons to the sea promoting the export of nutrients and organic matter and the modification of physical, chemical and biological conditions (Cancela da Fonseca et al., 2001a; Costa et al., 2003). On the other hand, freshwater discharges are the main water input during the isolation period from the sea. This freshwater input is important for setting ecotones in enclosed lagoons, by influencing parameters like salinity, sediment type or organic matter. Additionally, it determines the colonization of the lagoon by freshwater species, particularly benthic invertebrates (Cancela da Fonseca et al., 2001a; Basset et al., 2006; Oyedele & Momoh, 2009; Cañedo-Argüelles & Rieradevall, 2010).

Benthic macroinvertebrates play an important role in the metabolism of aquatic ecosystems and are a key biological group in the energy flow of these brackish-water systems (Casagranda et al., 2006). The marine colonization of landlocked coastal lagoons with periodic openings to the sea occurs essentially during these openings, after which diversity is mainly determined by the plasticity of the species, based on their tolerance to variations of environmental factors. The colonization by freshwater macroinvertebrate species, mainly aquatic insects, also occurs in these lagoons and depends mostly on less constant abiotic factors, like salinity, which varies considerably on both temporal and spatial scales (Cancela da Fonseca et al., 1989; Correia et al., 2012; Beer & Joyce, 2013; Félix et al., 2013a).

This study addressed the influence of freshwater inputs and associated environment on the distribution and abundance of macroinvertebrates in two neighbouring enclosed coastal lagoons, located in the southwest region of Portugal. The hypotheses tested are the following: (1) there were differences in the structure of benthic communities between freshwater confluence areas and the lagoonal environment; (2) there was temporal variation (between wet and dry seasons) of benthic communities within confluence areas—both testing the spatial distribution patterns of the benthos, as a consequence of freshwater inputs—and (3) there were differences in the abundance of individual species/taxa between freshwater confluence areas and the lagoonal environment—the latter hypothesis will identify which specific species or taxa can be early indicators of freshwater input variations within these interface areas.

Differences in the benthic communities and individual taxa between the confluence areas and lagoonal environments are expected to occur in both lagoons, particularly marked after the wet season, due to increased freshwater inputs (groundwater and run-off). The reduced freshwater flow during the dry season should allow a brackish-water intrusion into the confluence areas, increasing the similarities between both areas. Understanding if freshwater has an influence on the biological communities is of particular importance in groundwater-dependent systems, tributaries that commonly sustain human exploitation through water abstraction activities.

Materials and methods

Study area

Melides (38°08′N, 8°47′W) and Santo André (38°6′N, 8°48′W) are small coastal lagoons—with an average area of 0.4 and 2.5 km^2, respectively—located in the southwest of continental Portugal (Fig. 1), 2.5 km apart, with catchment areas of 63 and 96 km^2, respectively. Melides Lagoon has one major tributary, the Melides stream, and an adjacent groundwater outflow, Unnamed outflow, while three major tributaries, namely Badoca/Ponte, Serradinha and Cascalheira streams, and an adjacent groundwater outflow, Caniços outflow, discharge into Santo André Lagoon. Most tributaries depend on base flow and on the top detritic layer of the Sines aquifer system. In addition, the Melides stream is highly dependent on the deep carbonate aquifer (Monteiro et al., 2008). Both lagoons are isolated from the sea (Atlantic Ocean) by sand barriers, except for a short period, during early spring (coinciding with the end of the rainy season), when the connection to the sea is artificially made by the opening of an inlet in the sand barrier. Brief periods of sea-lagoon connection can also occur during occasional sand-barrier overwash episodes, which may not occur every year, but are typical of the late summer/early autumn spring tides (after the dry season). The inlet, which persists for a variable period (a few days to a few weeks) until its sealing off by waves and currents, provides the main exchange pathway between the lagoons and the sea allowing discharge of retained materials and recruitment of marine species (Cancela da Fonseca et al., 1989; Costa et al., 2003). In 2011, during which the present study was made, Melides Lagoon remained open for 3 days in April and Santo André Lagoon remained open for 56 days, between March and May. At least one overwash episode was reported for each lagoon, during a spring tide in late August 2011.

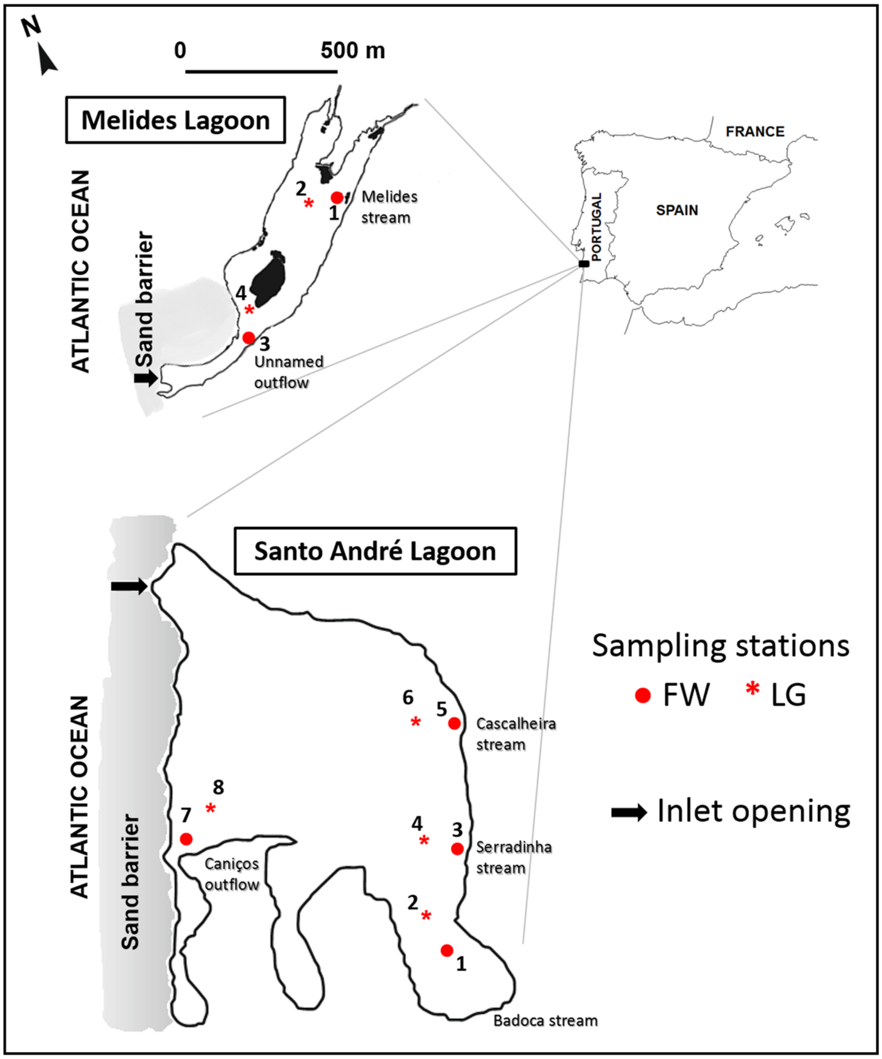

Fig. 1 Study area and location of the sampling stations, with indication of freshwater tributaries and groundwater outflows. *FW* direct freshwater influence zone (50 m of the discharge); *LG* away from the direct freshwater influence (200 m of the discharge). Each odd number is a FW zone and its subsequent even number its respective LG

Sampling

Sampling was carried out twice during the closed period of each lagoon, in May (end of wet season) and September (end of dry season) 2011, corresponding to two periods of the same hydrological year, with different environmental conditions. The location of known freshwater discharges was termed as Confluence. In the Melides Lagoon, there are two known Confluences: Melides stream and an Unnamed outflow, and in Santo André Lagoon, there are four: Badoca, Serradinha and Cascalheira streams, and Caniços outflow (Fig. 1). Sampling was conducted at two distances from the freshwater discharge point, specifically 50 m from freshwater discharge (FW) and 200 m from the freshwater discharge (LG) (depicted in Fig. 1). This experimental design assumed that benthic communities were directly influenced by freshwater at FW sites and not directly influenced by the freshwater inputs at LG sites.

Three random replicates were collected at each sampling station (site) using a van Veen grab (0.05 m^2). The samples were sieved in situ with a 500-μm mesh sieve, and subsequently fixed in a 4% formalin solution buffered with borax and stained with Rose Bengal. In the laboratory, samples were rinsed to remove excess formalin and sediment, the organisms were hand sorted into major taxonomic groups and preserved in 70° ethanol. All specimens were identified to the lowest possible taxonomic level (frequently species level) and counted. Several environmental parameters were measured in the water, namely bottom temperature, dissolved oxygen (DO), total dissolved solids (TDS), pH and salinity, during each sampling event and at each sampling station, using a multi-parametric sonde. Bottom water samples were also collected at each site using an adapted horizontal van Dorn water sampler for the determination of nutrient (NO_3, NO_2, NH_4, P, PO_4) and chlorophyll *a* (Chl*a*) concentrations. The Chl*a* was determined by Spectrophotometry, using the method by Lorenzen (1967) and nitrates, nitrites and ammonium by Segmented Flow Analyser using the Skalar continuous flow system (autoanalyzer) (Grasshoff et al., 1983). The orthophosphate concentration was measured through colorimetry using the ascorbic acid method (Grasshoff et al., 1983). All analyses were run by the accredited laboratory of Administração da Região Hidrográfica do Alentejo.

Additional grab samples were collected at each site for determination of total organic matter (TOM), which was obtained by loss on ignition (480°C), and sediment grain size. The latter was determined using 63 μm to 2 mm sieves to separate, respectively, the silt–clay, sand and gravel fractions. Each fraction was dried and weighed, and sediments assorted according to their percentages and classified with Shepard diagrams. Sediments were then grouped as follows: Mud < 63 μm; 0.063 mm $\leq$ Muddy sand/Sandy mud < 0.2 mm; 0.2 mm $\leq$ Sand < 2 mm; Gravel > 2 mm, following the classification system adopted in the sediment charts of the Portuguese Navy Hydrographic Institute (Moita, 1985).

Data analysis

The spatial and temporal differences in benthic macroinvertebrate communities were assessed using a Permutational Analysis of Variance—PERMANOVA (Anderson, 2001), with four factors: Lagoon (fixed with two levels, Melides and Santo André), Period (fixed with two levels, May and September), Distance (fixed with two levels, FW and LG) and Confluence (each FW/LG pair) nested in Lagoon (random with two levels in Melides Lagoon: Melides stream and Unnamed outflow; and with four levels in Santo André Lagoon: Badoca, Serradinha and Cascalheira streams and Caniços outflow) (see Fig. 1). The abundance data was log $(X + 1)$ transformed and Bray-Curtis similarity coefficient was used as a resemblance measure. The Monte Carlo permutations (P(MC)) were used whenever there were not enough possible permutations (number of possible permutations <100) to get a reasonable test in PERMANOVA. The PERMDISP routine tested the dispersion of the data and the Pair-Wise tests were run as a posteriori comparisons of levels for single factors, in the case of significant interactions or significant main effects. The SIMPER routine was used to identify which taxa and environmental variables (normalized) provided discrimination between any differing factors, with a cut-off level selected wherever the individual contribution of variables was higher than 10% or at a major leap between high and low percentage of individual contribution. A multi-dimensional scaling (MDS) ordination was performed using benthic abundance data, and environmental variables most likely to influence the benthic structure were superimposed on the MDS (bubble plots). All statistical analyses were run in Primer 6 + PERMANOVA (Clarke & Gorley, 2006; Anderson et al., 2008).

A non-parametric Wilcoxon test was used to analyse differences in each species' abundance between sampling sites located closer to the freshwater discharges (FW) and those located farther apart (LG), using Statistica v11 (Statsoft®). These tests were run independently for each lagoon and each sampling period.

Results

A total of 78799 macroinvertebrate specimens of 80 different taxa was collected, 32 in Melides Lagoon and 68 in Santo André Lagoon, with 20 taxa common to both systems (Table 1). Most consisted of typical lagoonal taxa and Chironomidae, Ostracoda, *Peringia ulvae*, Oligochaeta, *Lekanesphaera hookeri* and *Abra segmentum*, accounted for about 90% of the total average abundance observed in both lagoons.

In Melides Lagoon, 24 taxa were recorded in May and 18 in September, with 10 common taxa between periods and with most Insecta families occurring only in September. Thus, there was a species reduction and also a shift in community composition (described below) between sampling at the end of the wet and dry seasons, and hence between periods, differing according to freshwater input. A similar trend was observed

Table 1 Presence of macroinvertebrate taxa during both sampling periods in Melides and Santo André lagoons

Taxa	Melides		Santo André	
	May	September	May	September
Oligochaeta ni.	x	x	x	x
Polychaeta				
Protodorvillea keferstein (McIntosh, 1869)			x	
*Nephtys*sp.			x	
Alitta succinea (Leuckart, 1847)				x
Hediste diversicolor (O.F. Müller, 1776)	x		x	x
Perinereis cultrifera (Grube, 1840)			x	x
Exogone sp.		x		
Syllis gracilis (Grube, 1840)			x	
Syllis hyalina (Grube, 1863)		x		
Owenia fusiformis (DelleChiaje, 1844)	x		x	
Desdemona ornata (Banse, 1957)			x	x
Aonides oxycephala (Sars, 1862)	x	x	x	x
Dipolydora coeca (Örsted, 1843)				x
Dipolydora flava (Claparède, 1870)			x	
Polydora ciliata (Johnston, 1838)	x		x	x
Polydora cornuta Bosc, 1802	x		x	x
Polydora sp.I			x	x
Polydora sp.II				x
Prionospio cirrifera (Wirén, 1883)				x
Scolelepis bonnieri (Mesnil, 1896)			x	
Streblospio shrubsolii (Buchanan, 1890)	x		x	x
Caulleriella bioculata (Keferstein, 1862)			x	
Tharyx sp.	x			
Capitellidae ni				x
Capitella sp.				x
Capitella capitata (Fabricius, 1780)				x
Mediomastus fragilis (Rasmussen, 1973)		x	x	x
Saccocirrus sp.				x
Arachnida				
Acarina ni	x			
Branchiopoda				
Dunhevedia crassa King, 1853				x
Simocephalus vetulus (O.F. Müller, 1776)		x		x
Insecta				
Dytiscidae ni				x
Hydrophilidae ni	x	x	x	x
Ceratopogonidae ni		x		x
Chironomidae ni	x	x	x	x
Ephydridae ni			x	x
Limonidae ni		x	x	
Baetidae ni		x		
Heptageniidae ni				x
Corixidae ni				x

Table 1 continued

Taxa	Melides		Santo André	
	May	September	May	September
Mesoveliidae ni			x	x
Coenagrionidae ni		x		
Libellulidae ni		x		
Malacostraca				
Gammarus chevreuxi (Sexton, 1913)			x	
Microprotopus maculatus (Norman, 1867)			x	
Liocarcinus marmoreus (Leach, 1814)			x	
Carcinus maenas (Linnaeus, 1758)			x	x
Lekanesphaera hookeri(Leach, 1814)	x	x	x	x
Maxillopoda				
Harpacticoida ni	x	x	x	x
Balanus crenatus (Bruguière, 1789)				x
Ostracoda ni	x	x	x	x
Bryozoa				
Hyalinella sp.	x			
Hydrozoa				
Anthomedusae ni	x			
Cordylophora caspia (Pallas, 1771)	x		x	x
Entoprocta				
cf. *Barentsia benedeni* (Foettinger, 1887)			x	
Foraminifera ni			x	
Bivalvia				
Corbula gibba (Olivi, 1792)	x			
Modiolus modiolus(Linnaeus, 1758)				x
Cerastoderma edule (Linnaeus, 1758)			x	x
Cerastoderma glaucum (Bruguière, 1789)			x	x
Cerastoderma sp.				x
Laevicardium crassum (Gmelin, 1791)			x	
Kellia suborbicularis (Montagu, 1803)			x	
Spisula elliptica (Brown, 1827)			x	
Spisula subtruncata (da Costa, 1778)			x	
Ervilia castanea (Montagu, 1803)			x	
Abra alba (W. Wood, 1802)			x	
Abra segmentum (Récluz, 1843)			x	x
Abra sp.			x	
Angulus tenuis (da Costa, 1778)			x	
Polititapes virgineus (Linnaeus, 1767)			x	
Venerupis corrugata (Gmelin, 1791)			x	
Bivalvia ni			x	
Gastropoda				
Retusa obtusa (Montagu, 1803)				x
Peringia ulvae (Pennant, 1777)	x		x	
Ecrobia ventrosa (Montagu, 1803)	x	x		x
Obtusella intersecta (S. Wood, 1857)	x	x		

Table 1 continued

Taxa	Melides		Santo André	
	May	September	May	September
Nematoda ni	x		x	x
Nemertea				
Amphiporidae ni			x	
Platyhelminthes				
Turbellaria ni	x			

in Santo André Lagoon, with 49 and 42 taxa registered, respectively in May and September and with 23 common taxa between periods. Several marine Bivalvia species were registered exclusively in this lagoon and only in May, just after the closure of the inlet (see Table 1), all of which were only present in the downstream area of the lagoon.

The MDS ordination of the biological assemblages indicates a clear separation between the benthic communities of both lagoons (Fig. 2a), corroborated by the PERMANOVA results (Table 2). Up to 74% of the dissimilarity between lagoons was explained by (i) the exclusive occurrence of *A. segmentum* and *Desdemona ornata* in Santo André; (ii) higher abundance of Oligochaeta and *Peringia ulvae* in Santo André and; (iii) higher abundance of Ostracoda, Chironomidae, *Lekanasphera hookeri* and *Hediste diversicolor* in Melides(SIMPER analysis). Environmental variables that most discriminated the two lagoons (up to 80%, SIMPER results) were salinity, temperature and percentage of gravel, all higher in Santo André Lagoon; and depth, percentage of mud (negatively correlated to % of gravel) and total orthophosphate (PO_4) concentration, higher in Melides Lagoon (Fig. 2).

The PERMANOVA results also showed a significant interaction between the factors Period, Distance and Confluence (Table 2). The PERMDISP routine indicated that the significance found in the interaction of factors is not the result of heterocedasticity (P(perm) = 0.352). The Pair-wise tests performed within this interaction to factors Period and Distance have shown that the benthic communities of Melides Lagoon did not vary significantly between periods in any of the sampling sites (either FW or LG). However, in Santo André Lagoon, significant variations between wet and dry seasons were found in all FW and LG sites, except in Badoca LG. In this lagoon, there were several taxa that explained this dissimilarity (up to 74%, SIMPER results) and that were more abundant in September, namely *A. segmentum*, Oligochaeta, Ostracoda, *D. ornata*, Chironomidae, *P. ulvae*, *Polydora cornuta* and *Cerastoderma glaucum*. The environmental conditions showing a variation between wet and dry seasons at Santo André Lagoon were Chl*a*, TOM and Gravel showing higher values in May (wet season), while DO was higher in September (dry season) (up to 73%, SIMPER results).

In Melides Lagoon, no effect of freshwater was found, as there were no differences in the benthic communities between FW and LG sites. In Santo André Lagoon, there were significant differences between FW and LG in two confluence areas, the Caniços outflow ($p < 0.04$) and the Serradinha stream. However, in the latter these differences were only evident in May ($P = 0.009$). Although in the Caniços outflow there were consistent differences between FW and LG, the species that explained those dissimilarities (75%, SIMPER results) fluctuated. In May, the Caniços LG site showed higher abundance of *D. ornata*, *P. ulvae*, Foraminifera, Harpacticoida, *Cerastoderma edule*, *A. segmentum* and Chironomidae, while FW showed higher abundance of *C. glaucum*. On the other hand, in September, LG showed higher abundances of Oligochaeta, *P.cornuta*, *Capitella capitata*, *P. ulvae*, Ostracoda, Harpacticoida and *C. glaucum* and FW of Chironomidae. The differences between FW and LG concerning the Serradinha stream (in May) are explained by the higher abundance of Chironomidae, *P. ulvae*, Ostracoda, *Gammarus chevreuxi* in FW and *A. segmentum* in LG. In the Caniços outflow, that showed consistent biological differences, the most important abiotic parameters discriminating sites were depth, DO and temperature, all higher in FW (up to 94% of SIMPER results).

The lagoonal bivalve *A. segmentum* was the taxon with a more consistent presence and higher abundance at LG sites. Most taxa that gave higher contributions to

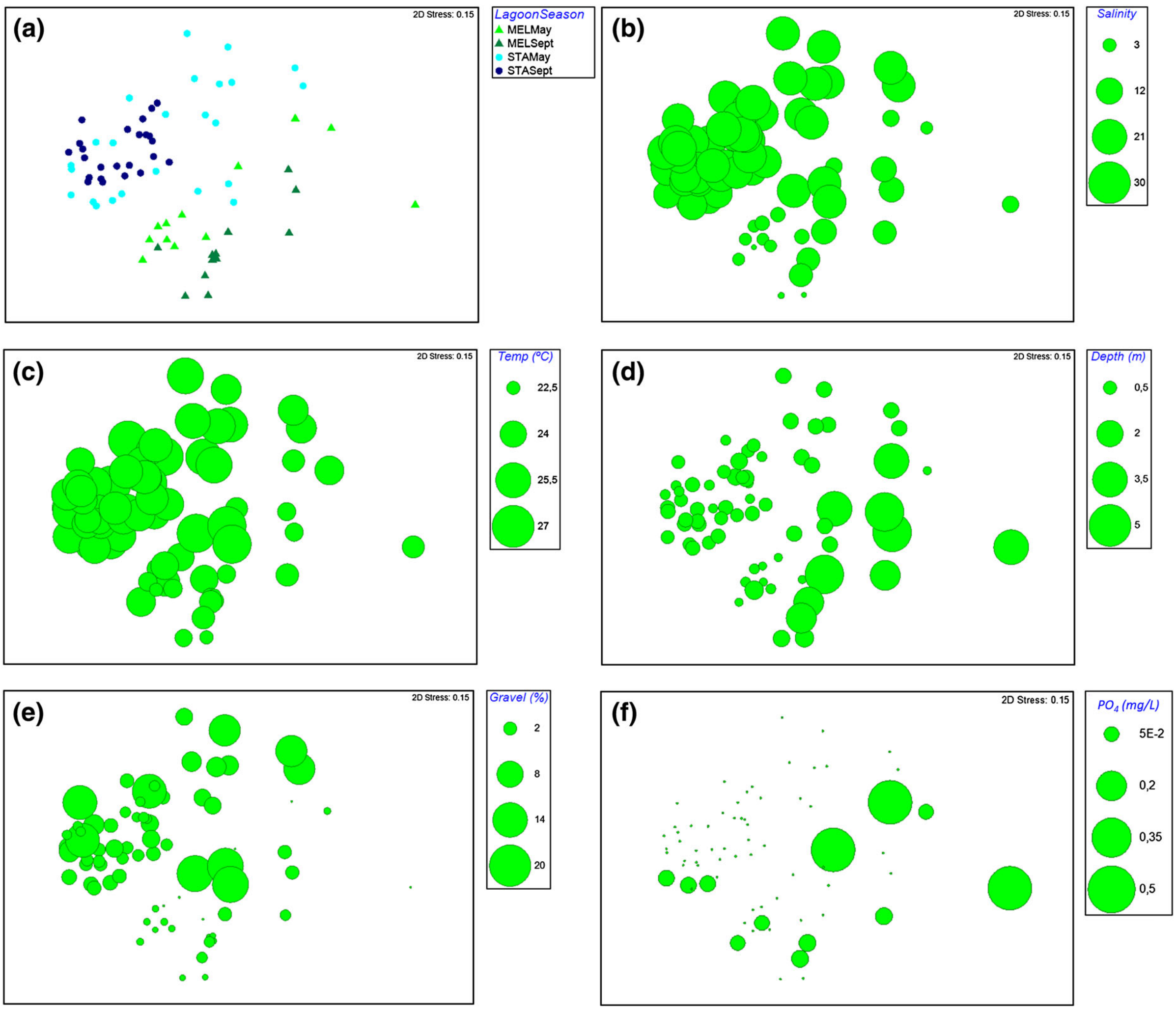

Fig. 2 Multi-Dimensional Scaling plots depicting the sampling stations, **a** based on species densities (*MEL* Melides Lagoon; *STA* Santo André Lagoon) and MDS-Bubble plots, overlaying environmental variables that most discriminated the two lagoons, explaining up to 80% of the taxa's distribution (SIMPER analysis), **b** salinity, **c** temperature, **d** depth, **e** gravel percentage (negatively correlated to the Mud%), and **f** total orthophosphate concentration

dissimilarities found between different periods (from the SIMPER results), varied considerably on a spatial and temporal scale. The taxa Ostracoda, *P. ulvae* and Chironomidae had their highest abundance either in LG or FW sites, depending on the season.

Wilcoxon tests showed that there were significant differences in the abundance of individual taxa between FW and LG sites, as listed in Table 3. In Melides Lagoon, differences were only found in May for *H. diversicolor* and Ostracoda, which were more abundant in LG locations while in Santo André Lagoon there were significant differences for Chironomidae and *P. cornuta* in September, which were more abundant in FW and LG locations, respectively.

Discussion

The present study indicates that freshwater inputs influence the structure of benthic communities and spatial distribution of specific taxa, but that influence seems to depend on the characteristics of the lagoonal system, i.e. significant differences between FW and LG occurred, but only in Santo André lagoon.

Table 2 Results of the four-way PERMANOVA analysis on the variation of the benthic community structure to the following tested design

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Lagoon	1	24,206	24,206	4.9795	0.0646	15	***0.007***
Period	1	5593.5	5593.5	2.9733	0.0688	9929	0.0339
Distance	1	1721.8	1721.8	0.89357	0.4820	9911	0.5151
Confluence (Lagoon)	4	19,445	4861.2	9.0396	*0.0001*	9918	0.0001
Lagoon × Period	1	4591.8	4591.8	2.4408	0.0986	9930	0.0641
Lagoon × Distance	1	3886	3886	2.0167	0.1566	9912	0.1117
Period × Distance	1	719.96	719.96	0.50096	0.7269	9950	0.7917
Period × Confluence (Lagoon)	4	7525	1881.3	3.4983	*0.0001*	9907	0.0001
Distance × Confluence (Lagoon)	4	7707.7	1926.9	3.5832	*0.0001*	9908	0.0001
Lagoon × Period × Distance	1	1779.3	1779.3	1.2381	0.3234	9954	0.3356
Period × Distance × Confluence (Lagoon)	4	5748.6	1437.1	2.6725	***0.0001***	9907	0.0006
Residuals	48	25,812	537.76				
Total	71	109,480					

Three fixed-factors with two levels each: Lagoon (Santo André and Melides); Period (May and September); and Distance [FW—direct freshwater influence zone (50 m of the discharge) and LG—away from the direct freshwater influence (200 m of the discharge)]; and the random factor Confluence nested in the factor Lagoon, with two levels for Melides Lagoon and four levels for Santo André Lagoon, corresponding to each tributary of each lagoon (stream or outflow). Italic and bold numbers highlight are all significant values to consider and those of interest (interaction and significant factor without interactions), respectively

Table 3 Differences in the abundances of individual taxa between FW and LG sites

	Ind/m^2 ± SE		Wilcoxon paired test
	FW	LG	*P* value
Melides (May)			
Hediste diversicolor	83 ± 53	237 ± 109	0.043
Ostracoda	3870 ± 2635	30870 ± 8450	0.046
Santo André (September)			
Chironomidae	6473 ± 828	2940 ± 524	0.010
Polydora cornuta	53 ± 40	312 ± 94	0.012

Only taxa and periods that showed significant differences between the two Distance levels are presented. Average density *per* site (individuals/m^2 ± SE) and respective *P* value are also indicated

Although both studied lagoons are neighbouring systems, their benthic communities are dissimilar, suggesting that specific morphological and dynamic characteristics of each lagoon might determine the settlement of different species. Corroborating previous studies, benthic communities, as well as other biological groups, seem to be particularly dependent on the lagoon size (Félix et al., 2013a, b), where smaller lagoons show less evident macrobenthic zonation, as found in Melides Lagoon (see also Cancela da Fonseca et al., 2001b; Félix et al., 2013a). In these smaller enclosed systems occasional phenomena like heavy or abrupt freshwater inputs can have a widespread effect across the entire system. Santo André lagoon, on the other hand, appears to buffer freshwater input and displays a certain degree of zonation (e.g. Elliott & Whitfield, 2011; Correia et al., 2012; Félix et al., 2013a, b).

Seawater intrusion into landlocked lagoons allows the colonization of marine species and, hence, potentially modifies the influence of freshwater input. After the wet season, seawater entered the lagoon through the artificial opening and in late summer through an overwash episode. During the former there is persistent influence and a high input of seawater whilst, in the latter, seawater intrusion is occasional and has a lower input. Thus, one might expect a higher pressure of marine tidal forces on the confluences in May. However, this was not the case. After the rainy season (in May) freshwater taxa (e.g. Chironimidae, *G. chevreuxi*) expanded further into the Santo André lagoonal environment (LG) despite the opening of the sand barrier. In contrast, a large portion of marine/brackish taxa was found in September, (from the overwash) and the presence of freshwater taxa was more confined to FW areas. This indicates that freshwater flow rate is the main factor determining the extent to which seawater incursion affects community structure. In summary, the temporal shift in the community composition suggests that stream/lagoon interface areas are dynamic and dependent on freshwater flow rates. This may allow freshwater species to colonize areas further into the lagoon or be more confined to confluence areas, depending on the higher or lower freshwater flow rate, respectively.

Like the freshwater flow rate, the geochemical nature of freshwater input can also affect several other key parameters such as nutrients, Chl*a* and silicates (Duarte et al., 2006; Medina-Gómez & Herrera-Silveira, 2006; Young et al., 2008). The structure of the benthic community in the Caniços outflow, opposing other tributaries, showed no variation between periods. This singularity could likely be related to the biogeochemical nature of the freshwater, as it is the only tributary whose flow depends almost exclusively on groundwater, potentially contributing for the establishment of specific ecotones.

The analysis of individual taxa, support the hypothesis that some taxa may be good early indicators of flow rate changes. Some of the species with marine affinities, like *H. diversicolor* and *P. cornuta*, were generally more abundant in LG sites while Chironomids, typical of freshwater habitats, were more abundant in FW areas. Although also significant in this analysis, considerations about the Ostracoda distribution would require identifications to the species level, to determine their optimal habitat type, as this group can cover a relatively wide range of ecotones (*e.g.* Loureiro et al., 2009; Martins et al., 2010). The fact that these differences for individual taxa were found in May for Melides and September for Santo André Lagoon is consistent with the results obtained for the spatial distribution of the benthic community in both lagoons, suggesting that these were the most heterogeneous periods in each lagoon. This pattern enhances the higher heterogeneity of a small lagoon (Melides) after inlet opening and higher patchiness of a wider lagoon (Santo André) after the dry season. Again, a reduction in the flow rate of tributaries seems to reduce the area under the effect of freshwater modifying the distribution boundaries for several species.

The present results showed that, like in open brackish systems, such as estuaries (Silva et al., 2012), the structure of benthic communities is strongly associated and dependent of freshwater inputs. Thus, and although additional information is required to quantify this relationship, it is reasonable to consider that unsustainable groundwater abstraction can have a strong impact on the biological communities of enclosed lagoons. Considering the fact that groundwater is subject to human exploitation, future research should aim to understand the real ecological importance of these systems, as it may require specific exploitation policies. Additionally, severe reductions in freshwater flow rate may cause sea water intrusion, with strong effects on the interface areas (Oyedele & Momoh, 2009) and, like any other abrupt environmental shift in a system, may impair the ecosystem dynamics.

It is therefore recommended that the exploitation of the aquifers ensure a sustainable management, particularly in years of drought that can be aggravated in the medium- and long-term by the effects of climate change (Klein Tank & Können, 2003). In conclusion, a science-based groundwater management of groundwater-dependent ecosystems, such as enclosed lagoons, is particularly relevant for the sustainability of these sensitive habitats.

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References

Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson, M. J., R. N. Gorley & K. R. Clarke, 2008. PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth: 213.

Bamber, R. N., P. M. Gilliland & E. A. Shardlow, 2001. Saline Lagoons: A Guide to Their Management and Creation. English Nature, Peterborough.

Beer, N. A. & C. Joyce, 2013. North Atlantic coastal lagoons: conservation, management and research challenges in the 21st century. Hydrobiologia 701: 1–11.

Basset, A., N. Galuppo & L. Sabetta, 2006. Environmental heterogeneity and benthic macroinvertebrate guilds in italian lagoons. Transitional Waters Bulletin 1: 48–63.

Cancela da Fonseca, L., A. M. Costa & J. M. Bernardo, 1989. Seasonal variation of benthic and fish communities in a shallow land-locked coastal lagoon (St. André, SW Portugal). Scientia Marina 53: 663–669.

Cancela da Fonseca, L., J. M. Bernardo, A. M. Costa, M. Falcão & C. Vale, 2001a. Seasonal Chemical changes and eutrophication of a land-locked coastal lagoon (St. André, SW Portugal). Boletim do Museu Municipal Funchal 6: 167–183.

Cancela da Fonseca, L., P. Duarte & F. Magalhães, 2001b. Trophic group patterns of Macrobenthos in brackish coastal systems. Boletim do Museu Municipal Funchal 6: 139–165.

Clarke, K.R. & R.N. Gorley, 2006. PRIMER v6: User Manual/ Tutorial. PRIMER-E, Plymouth.

Cañedo-Argüelles, M. & M. Rieradevall, 2010. Disturbance caused by freshwater releases of different magnitude on the aquatic macroinvertebrate communities of two coastal lagoons. Estuarine, Coastal and Shelf Science 88: 190–198.

Casagranda, C., C.F. Boudouresque & P. Francour, 2006. Trophic flows in the macroinvertebrate community of a Mediterranean brackish lagoon, Lake Ichkeul (Tunisia) using a functional model. Proceedings of the international workshop on "The Protection of Coastal and Marine Environment": 153–162.

Correia, M. J., J. L. Costa, P. Chainho, P. M. Félix, M. L. Chaves, J. P. Medeiros, G. Silva, C. Azeda, P. Tavares, A. Costa, A. M. Costa, J. Bernardo, H. N. Cabral, M. J. Costa & L. Cancela da Fonseca, 2012. Inter-annual variations of macrobenthic communities over three decades in a land-locked coastal lagoon (Santo André, SW Portugal). Estuarine Coastal and Shelf Science 110: 168–175.

Costa, A. M., L. Cancela da Fonseca & M. Cristo, 2003. Annual cycle of the benthic community of a coastal lagoon: Lagoa de Melides (Grândola, SW Portugal). Revista de Biologia 21: 71–89.

Duarte, P., M. F. Macedo & L. Cancela da Fonseca, 2006. The relationship between phytoplankton diversity and community function in a coastal lagoon. Hydrobiologia 555: 3–18.

Elliott, M. & A. K. Whitfield, 2011. Challenging paradigms in estuarine ecology and management. Estuarine Coastal and Shelf Science 94: 306–314.

Félix, P. M., P. Chainho, J. L. Costa, M. J. Correia, M. L. Chaves, J. P. Medeiros, H. N. Cabral, N. Wouters, J. Bernardo, A. M. Costa, M. Cristo, G. Silva, C. Azeda, P. Tavares, M. J. Costa & L. Cancela da Fonseca, 2013a. Short-term versus long-term changes in the benthic communities of a small coastal lagoon: implications for ecological status assessment. Vie Milieu 63: 11–22.

Félix, P. M., M. J. Correia, P. Chainho, M. J. Costa, H. N. Cabral, I. Domingos, J. L. Costa & L. Cancela da Fonseca, 2013b. Influence of streams discharges on the structure of fish communities of Portuguese land-locked coastal lagoons. Cahiers de Biologie Marine 54: 427–435.

Grasshoff, K., M. Ehrhardt, K. Kremling & T. Almgren, 1983. Methods of Seawater Analysis. Verlag Chemie, Weinheim.

Healy, B., 2003. Coastal lagoons. In Otte, M. L. (ed.), Wetlands of Ireland. Distribution, Ecology, Uses and Economic Value. University College Dublin Press, Dublin: 51–78.

Heydorn, A. E. F. & K. L. Tinley, 1980. Estuaries of the Cape, Part I. Synopsis of the Cape Coast. Natural features, dynamics and utilization. CSIR Research Report 380: 1–97.

Kjerfve, B., 1994. Coastal lagoon processes. Elsevier Oceanography Series. Elsevier, Amsterdam.

Klein Tank, A. M. G. & G. P. Können, 2003. Trends in indices of daily temperature and precipitation extremes in Europe, 1946–1999. Journal of Climate 16: 3665–3680.

Lorenzen, C., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnology and Oceanography 12: 343–346.

Loureiro, I. M., M. C. Cabral & F. Fatela, 2009. Marine influence in ostracod assemblages of the Mira river estuary: comparison between lower and mid estuary tidal marsh transects. Journal of Coastal Research SI56: 1365–1369.

Martins, M. J. F., T. Namiotko, M. C. Cabral, F. Fatela & M. J. Boavida, 2010. Contribution to the knowledge of the freshwater Ostracoda fauna in continental Portugal, with an updated checklist of Recent and Quaternary species. Journal of Limnology 69: 160–173.

Medina-Gómez, I. & J. A. Herrera-Silveira, 2006. Primary production dynamics in a pristine groundwater influenced coastal lagoon of the Yucatan Peninsula. Continental Shelf Research 26: 971–986.

Moita, I., 1985. Carta dos sedimentos superficiais da Plataforma Continental: Cabo S. Vicente ao Rio Guadiana (SED 7 e 8), 1st ed. Instituto Hidrográfico, Lisbon.

Monteiro, J.P., A. Chambel &J. Martins, 2008. Conceptual and Numerical Flow Model of the Sines Aquifer System (Alentejo, South Portugal). Proceedings of the International Groundwater Symposium. International Association of Hydraulic Engineering and Research (IAHR): 76–84.

Oyedele, K. F. & E. I. Momoh, 2009. Evaluation of Sea water intrusion in freshwater aquifers in a Lagoon Coast: a case study of the University of Lagos Lagoon, Akoka, Nigeria. New York Science Journal 2: 32–42.

Silva, A. C. F., P. Tavares, M. Shapouri, T. Y. Stigter, J. P. Monteiro, M. Machado, L. Cancela da Fonseca & L. Ribeiro, 2012. Estuarine biodiversity as an indicator of groundwater discharge. Estuarine, Coastal and Shelf Science 97: 38–43.

Smith, N.P. 1994. Water, salt, and heat balances of coastal lagoons. In Kjerfve, B. (ed), Coastal lagoon processes. Elsevier Oceanography Series 60, Amsterdam: 69–101.

Viaroli, P., P. Lasserre & P. Campostrini, 2010. Lagoons and coastal wetlands in the global change context: impact and management issues. Developments in Hydrobiology 192: 1–170.

Young, M. B., M. E. Gonneea, D. A. Fong, W. S. Moore, J. Herrera-Silveira & A. Paytan, 2008. Characterizing sources of groundwater to a tropical coastal lagoon in a karstic area using radium isotopes and water chemistry. Marine Chemistry 109: 377–394.