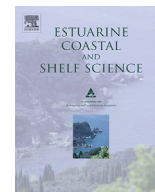


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The influence of submarine groundwater discharges on subtidal meiofauna assemblages in south Portugal (Algarve)

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ABSTRACT

Submarine groundwater discharges (SGD) have been documented as contributing to the biological productivity of coastal areas, through a bottom-up support to higher trophic levels. Nevertheless, the effects on the bottom levels of the coastal food web, namely the meiofauna, are still very poorly known. The “Olhos de Água” beach is the only area on the South coast of Portugal where submarine freshwater seepages have been identified. In this study, meiofauna assemblages in the area impacted by SGD were compared with the meiofauna from a similar area, but without SGD. Samples were taken in Spring and Summer 2011, under different hydrological regimes, aquifer recharge (after Winter) and dryness (after Spring), respectively. The major changes in the community were recorded at a seasonal level, with higher abundances and number of taxa in Spring, when compared to Summer. This may be explained by better sediment aeration during spring along with higher food availability from the sedimentation of spring phytoplankton blooms. Although no significant differences were detected by multivariate analysis on the meiofauna abundances between Control and Impact areas, pair-wise tests on the interactions between factors in number of taxa (*S*) and species richness (Margalef's *d*) suggested that the discharge of groundwater stimulated an increase in meiofauna diversity. Such effect can be observed between the meiofauna assemblages from impacted and control areas and also between periods with different discharge regimes (Spring and Summer) in the impacted area. These findings highlight the role that freshwater discharges from coastal aquifers have on meiofauna assemblages and suggest that SGD contribute to enhance the transfer of energy from the lower levels of the trophic web to upper levels.

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1. Introduction

Submarine Groundwater Discharge (SGD) can be, volumetrically and chemically, significant for coastal waters worldwide, influencing productivity, species biomass, composition and zonation (Johannes, 1980). Estimates of $2\text{--}4 \times 10^{13} \text{ m}^3 \text{ yr}^{-1}$ have been made for the Atlantic Ocean, which is around the same amount of freshwater that enters this ocean from rivers (Moore et al., 2008). This water might be pure freshwater from a coastal aquifer, recirculated seawater, or a mixture of fresh and seawater. Recirculation may further enhance biogeochemical reactions in the aquifer and increase the nutrient supply to coastal areas (Moore, 2006). This flow of fluids through shelf sediments transport nutrients like silica, nitrogen and phosphorous (e.g. Valiela et al., 1990; Leote et al.,

2008; Waska and Kim, 2011) to the coastal zone, which in turn have the ability to affect the biological zonation (Johannes, 1980; Miller and Ullman, 2004). For these conceptual similarities, SGD have been termed ‘submarine estuaries’ (Moore, 1999). As in “conventional” estuaries, where the freshwater inflow is a major structuring factor of biological communities (Montagna and Kalke, 1992), changes in salinity due to freshwater flow from SGD also has the ability to influence the distribution of organisms (Miller and Ullman, 2004; Dale and Miller, 2007; Cave and Henry, 2011). SGD have also been shown to affect local sediments characteristics. Fine sediments and higher amounts of clay particles have been found in association with freshwater seepages (Zipperle and Reise, 2005). Other studies have identified the ability of aquifers to transport fine sediments (Mahler and Lynch, 1999; Herman et al., 2007; Goldscheider et al., 2010).

Benthic infauna can be particularly useful to study the long-term effects of environmental factors on biota, since these organisms are limited in mobility and long-lived, when compared to planktonic

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organisms of equivalent size (Montagna and Kalke, 1992). For its life history characteristics, namely ubiquitous distribution, rapid generation times, direct benthic development and sessile life-style (Kennedy and Jacoby, 1999), meiofauna holds a high potential for environmental monitoring programs (e.g. Hicks, 1991; Mirto and Danovaro, 2004; Balsamo et al., 2012).

The effects of SGD on coastal marine infaunal assemblages have been usually focused on macrofauna. Increased abundances of polychaetes have been found to be associated with zones with lower salinity caused by SGD (e.g. Bussmann et al., 1999; Miller and Ullman, 2004; Dale and Miller, 2008; Silva et al., 2012). Effects on meiofaunal assemblages are, to date, restricted to one study in Roscoff Aber Bay, France, where no differences were registered in meiofauna abundances in seepage zone, when compared to a control zone (Ouisse et al., 2011), while on biomass, a significant decrease was identified at the freshwater seepages site during spring months (Migné et al., 2011).

Distribution and abundance of meiofauna change according to a whole range of factors, from abiotic (e.g. grain size, sediment water content, hydrodynamics factors like wave exposure and tidal currents, temperature, salinity and sediment oxygen content) to biotic factors (e.g. food supply, organic matter content, interactions with macrofauna). Interactions with tubes and burrows of macrofauna, around which better sediment chemistry conditions exist, namely higher oxygen levels, can also create microhabitats at certain sediment depths where meiofauna usually do not occur due to adverse sediment chemistry conditions (Meyers et al., 1987; Ólafsson, 2003). The grain size of sediments can also play an important role on meiofaunal abundances, directly through the availability of interstitial habitats, or indirectly through changes in

the availability of food and oxygen (McIntyre, 1969; Martens and Schockaert, 1986; Giere, 2009).

In the present study, community composition and ecological indexes of benthic meiofauna assemblages were studied aiming to assess if: 1) assemblages differed between areas under the influence of SGD and control areas without SGD; 2) if the observed patterns were consistently maintained in distinct seasons of the year (Spring vs. Summer).

2. Materials and methods

2.1. Study area

Two study areas located in the south coast of Portugal were selected: a non-impacted (Control) area and an Impact area (Fig. 1), in front of the beach of Olhos de Água (where SGD occurs). These two locations are separated by 7 km and both are belong to the same water mass according to the Water Framework Directive (2000/60/EC) classification: a mesotidal (3.4 m), euhaline (35) sheltered coast (Bettencourt et al., 2003). This south-facing coast receives waves mainly from W (31%) with an average height of 1 m, while significant heights greater than 3 m usually arrive from SW in Winter months (Costa et al., 2001).

The existence of SGD in Olhos de Água beach is known for a long time and was already described in 1841 by João Baptista Lopes on a document concerning the Algarve “kingdom” (Lopes, 1841). Arrifes beach was chosen as a Control location, as it has no direct influence of aquifers (Almeida et al., 2000). The Albufeira-Ribeira de Quarteira aquifer, which supports various points of discharge in the Olhos de Água area, is mainly formed by limestone and detrital-carbonate

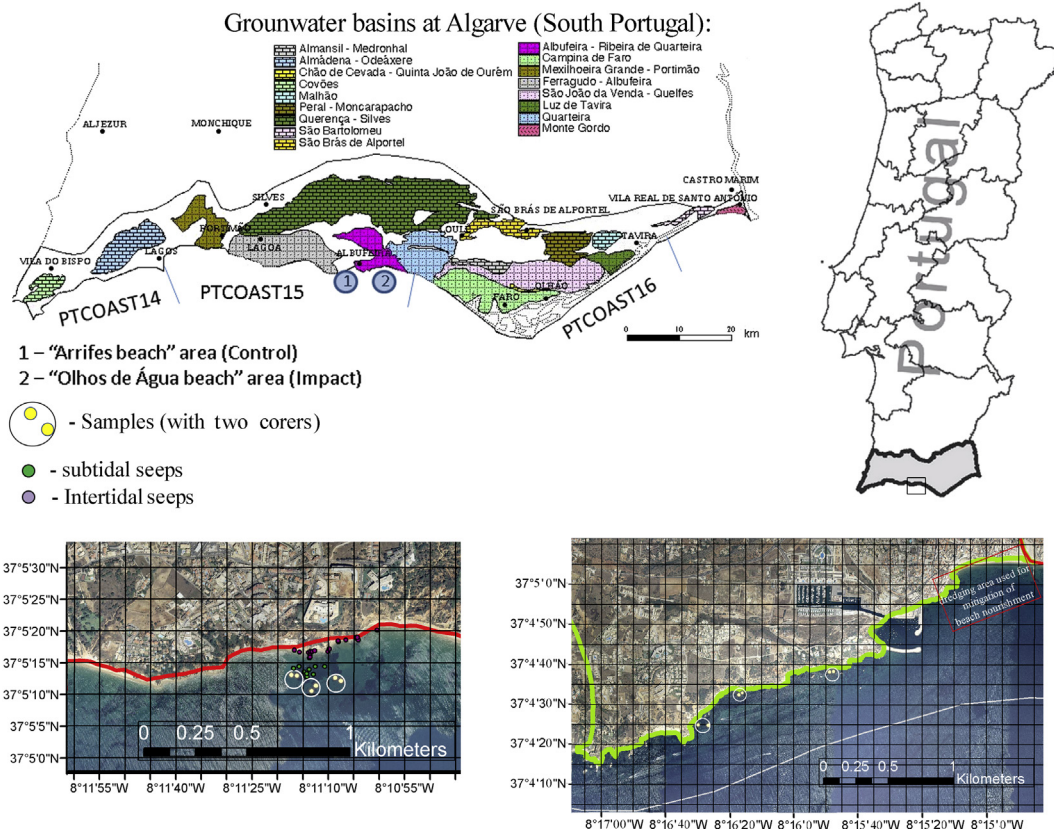


Fig. 1. Location of the major aquifer systems in Algarve, the study area and respective sampling sites (Control and Impact), locations of the samples and the positions of the intertidal and subtidal seeps (adapted from Almeida et al., 2000).

Miocene and Jurassic lithologies (Almeida and Silva, 1990). The volume of water discharged fluctuates according to the aquifer water table. However, freshwater discharges are observed throughout the year, with several discharge points in intertidal and subtidal areas (Almeida et al., 2000). Despite the difficulties associated with measuring the volumes of discharged groundwater to Olhos de Água beach, estimations of 100 litres *per second* have been proposed (Almeida and Silva, 1990).

Until recently, groundwater was the major source of water for human consumption in the central Algarve region (Monteiro et al., 2007). Socio-economical changes in the region, mainly a higher tourism demand and changes in the type of agriculture practiced, led to a change in water politics and to an increasing use of dam waters for public consumption, rather than groundwater (Monteiro et al., 2003). This change in water supply in the beginning of the 21st century allowed the restoration of some coastal aquifers in the Algarve to their natural hydrodynamic conditions (Monteiro et al., 2007).

2.2. Experimental design and sampling

Sampling took place according to a Control–Impact design, with seasonal replication. In order to account for the natural changes in the hydrological regime of these coastal aquifers, sampling was done in Spring and Summer and to have replication within seasons there were two sampling periods *per season*. Spring was considered since it is when the aquifers have been recharged by winter precipitation, as is characteristic of the Mediterranean climate (Korkmaz, 1990) and so it is expected that a higher volume of water is discharged. On the other hand, Summer is the season of the year when the volume of discharged water is expected to reach its minimum due to a lack of refilling of the aquifers and a higher human demand for water (Monteiro et al., 2007). The Control area was specifically chosen, as it is the only area in Algarve that belongs to the same water mass as the Impact area but has no influence of any coastal aquifer system (Fig. 1). The Impact area of Olhos de Água is also a unique area within the south coast of Portugal, as to our knowledge, no other SGD occur in the coastal area of Algarve. In order to keep the design as conservative as possible, there could only be one Control area and one Impact area.

Samples were taken by SCUBA diving in subtidal soft-bottom sediments, at depths between 4 and 7 m. At each sampling period, 3 replicate samples were randomly taken in each location (Control and Impact), each sample consisting of 2 corers with 3.5 cm of diameter and 15 cm deep, which were subsequently pooled. In the Impact location, samples were randomly collected within the area delimited by seepages, but not directly on the seepages.

In parallel with each sample, two identical corers (3.5 × 15 cm) were collected: one for organic matter and one for grain size analysis. Meiofauna samples were subsequently preserved in 4% buffered formalin and stained with Rose Bengal until further laboratory processing.

2.3. Laboratory procedures

2.3.1. Meiofauna separation protocol

The technique used to separate the meiofauna from the sediment was based in the protocol by Burgess (2001) which relies on density differences. Samples were initially washed with a 500 µm sieve to remove formalin, Rose Bengal excess and macrofauna organisms, while meiofauna and sediment were retained in a 55 µm sieve. The remaining sample was placed in 500 ml PE tubes with Ludox[®] (DuPont) HS 40 solution in a 3:2 Ludox/sediment proportion. Samples were homogenized manually for 30 s and subsequently with an automatic vortex mixer (Cassel) for 4 min. Organisms were separated from sediment on a centrifuge

(Beckman Coulter, Avanti™ J-25) at 900 × g for 5 min. The supernatant was removed from the sample by decanting and passed through a 55 µm sieve, where meiofauna was retained. Meiofauna samples were then washed with freshwater, stored in tubes and preserved in 96% ethanol. Meiofauna organisms were then identified and counted according to major taxonomic groups.

2.3.2. Sediment analysis

The method of “loss on ignition” was used for organic matter quantification. Dry weight was determined after drying the sediment samples in an oven (WTC Blender) for 12 h at 30 °C. The samples were subsequently ashed in a muffle (DINKO) at 450 °C for 4 h and the remaining sediment was weighted. The organic matter (OM) percentage was then estimated by dividing ash-free weight (the difference between ash weight and dry weight) by dry weight.

The analysis of grain fractions started by placing the sediment in hydrogen peroxide until all organic matter was oxidized and then dried at 60 °C (WTC Blender oven). Sediment samples were then passed through a stack of sieves that comprised six fractions: silt and clay (<0.063 mm), very fine sand (0.063–0.125 mm), fine sand (0.125–0.250 mm), medium sand (0.250–0.500 mm), coarse sand (0.500–1.000 mm) and very coarse sand (>1.000 mm). The sediment retained on each fraction was then weighted and the percentage of each fraction, in relation to the total weight of each sample, calculated.

2.4. Data analysis

Variations in organic matter and grain fractions were evaluated through a two-way analysis of variance (ANOVA) in order to test for significance between seasons, locations and interactions between the two factors (significance level of $\alpha = 0.05$). Prior to ANOVA, data were analysed to test normality (Kolmogorov–Smirnov’s test) and homogeneity of variance (Levene’s test) among treatments. Average and standard deviation of sediment parameters were calculated for Control and Impact, in Spring and Summer 2011. In order to cope with seasonal variability, data was previously tested for differences between sampling dates within each season. No significant differences within seasonal replicate data were detected, so this factor was not considered in subsequent analyses. Overall, there were 4 lost samples, so the degrees of freedom were reduced accordingly (from 23 to 19 df), to compensate for these “missing” replicates.

Differences in abundance of each meiofauna taxonomic group between locations (Control and Impact) and between seasons (Spring and Summer) were tested with two-way ANOVA (significance level of $\alpha = 0.05$). Whenever significant interactions between the two factors (season × location) were identified, pair-wise comparisons were used (Holm–Sidak method). The average and standard deviation of abundance of meiofauna taxonomic groups was calculated.

For each meiofauna sample, total taxa (S), abundance (N), species richness (Margalef’s d) and Shannon–Wiener’s diversity (H') were estimated. Differences in ecological indices between seasons, locations and interactions between the two factors were tested for significance with two-way ANOVA (significance level of $\alpha = 0.05$). Whenever significant interactions between the two factors were identified, pair-wise comparisons were also used. Average and standard deviation of these ecological indices was calculated for Control and Impact, in Spring and Summer 2011.

Multivariate statistical methods were used to assess the community structure across seasons (Spring and Summer) and locations (Control and Impact). The non-metric multidimensional scaling (Kruskal and Wish, 1978) ordination technique was performed, as it

is considered as one of the most robust ordination techniques for the analysis of biotic communities (Clarke and Warwick, 2001).

Permutational multivariate analysis of variance (PERMANOVA) was used to test for statistical differences in meiofauna composition among seasons, sampling locations and interactions between the two variables (Anderson, 2001). As the design was unbalanced at the lowest level (i.e. unequal numbers of replicate samples within each factor level of the design) it was decided to use Type I sums of squares in the analyses (Anderson et al., 2008). A two-factor PERMANOVA was performed, using 999 permutations and was based in Bray–Curtis measure of similarity of $\log_{(x+1)}$ transformed data.

A Canonical Analysis of Principal coordinates (CAP) (Anderson and Willis, 2003) was performed to better understand and visualize the relationships between meiofauna, organic matter and sediment grain size fractions. According to its authors (Anderson and Willis, 2003), CAP was designed to identify the strongest relationship between environmental data and the biological data cloud and also considering the correlation structure among variables.

MDS, PERMANOVA and CAP were all based on the Bray–Curtis matrix of similarity of $\log_{(x+1)}$ transformed meiofauna abundance data. The above statistical analyses were conducted using PRIMER 6 and SigmaStat 3.5 software.

3. Results

3.1. Sediment characteristics

Organic matter percentages remained close to 1%, with an average value of $1.0 \pm 0.4\%$ and a maximum average value of $1.2 \pm 0.6\%$ in the Impact location, during summer. ANOVA showed no significant differences between seasons or between locations among organic matter percentages (Table 1).

Fine sand was the dominant grain fraction, always comprising values around 50% of the sediment in both locations (Table 1). Significant differences were only detected by ANOVA between locations in clay and silt, very fine sand and medium sand, while no significant differences were found between seasons or interactions between the two factors. Silt and clay and very fine sand showed

the same pattern, increasing from Control to Impact, while medium sand had a higher percentage in Control than in Impact (Table 1).

3.2. Meiofauna assemblages

In the present study area, the average meiofauna abundance was $14,691.8 \pm 15,642.3 \text{ ind m}^{-2}$. A total of 14 taxonomic groups were identified throughout this study: Amphipoda, Bivalvia, Cladocera, Copepoda + nauplii, Crustacea larvae, Cumacea, Egg, Foraminifera, Gastropoda, Nematoda, Nemertea, Ostracoda, Polychaeta + larvae and Turbellaria. Nematoda dominated the meiofauna assemblages, with an average percentage of abundance of $53 \pm 22\%$ (present in all Control and Impact samples), followed by Turbellaria (average of $16 \pm 17\%$ and present in all Impact samples) and Copepoda + nauplii (average of $13 \pm 14\%$ and present in all Control samples). Significant differences in the abundance of taxonomic groups were only found between seasons for Nematoda and Polychaeta + larvae, both decreasing from Spring to Summer (Table 1). No significant differences were found between locations or interactions between the two factors.

Significant differences between seasons and locations were detected by ANOVA for some of the ecological indices. The number of taxa and abundance showed significant changes among seasons, but not among locations, while in species richness significant differences were recorded between Control and Impact (Table 1). Interactions between factors (seasons \times locations) were also identified for the number of taxa and species richness, which required a more detailed examination of the results. Pair-wise comparisons revealed similar patterns for the number of taxa and species richness. Both indices increased significantly from Control to Impact in Spring, but not in Summer. In the Impact location, significantly higher values were found in Spring than in Summer, which did not occurred in Control.

The MDS ordination showed a separation between Spring and Summer meiofauna samples, but not among Control and Impact (Fig. 2). The PERMANOVA results also showed a significant ($p = 0.006$) difference between Spring and Summer, but not among locations (Table 2).

The main results from CAP were that silt and clay were correlated with meiofauna abundances from Impact, while coarse sand was

Table 1

Average and standard deviation of sediment variables, meiofauna taxa abundances and ecological indices (S – number of taxa; N – abundance; d – species richness; H' – Shannon's diversity) in Control and Impact of Spring and Summer 2011. Two-way ANOVA (d.f._{Season} = 1; d.f._{Local} = 1; d.f._{SexLo} = 1; d.f._{Residual} = 16; d.f._{Total} = 19) results of variables between seasons, locations and interactions between the two factors. * $p < 0.05$; ** $p < 0.001$.

		Spring		Summer		p value		
		Control	Impact	Control	Impact	Season	Local	SexLo
Sediment variables	Organic matter	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.2 ± 0.6	0.21	0.65	0.79
	Silt and clay	0.2 ± 0.0	1.4 ± 0.5	0.2 ± 0.1	0.9 ± 0.3	0.31	**	0.22
	Very fine sand	14.7 ± 0.5	39.7 ± 2.1	18.5 ± 2.6	36.4 ± 3.0	0.95	**	0.53
	Fine sand	49.3 ± 21.9	54.1 ± 2.8	57.3 ± 5.1	54.2 ± 4.0	0.80	0.42	0.86
	Medium sand	10.7 ± 0.2	2.4 ± 0.2	17.1 ± 5.5	4.5 ± 0.9	0.05	**	0.31
	Coarse sand	23.5 ± 20.7	1.6 ± 0.5	6.3 ± 1.8	2.8 ± 0.8	0.42	0.06	0.28
	Very coarse sand	1.6 ± 1.4	0.7 ± 0.1	0.6 ± 0.3	1.3 ± 0.5	0.83	0.66	0.46
Meiofauna taxa	Copepoda + nauplii	1846.2 ± 1909.9	153.3 ± 115.4	831.8 ± 752.9	1061.0 ± 1208.2	0.94	0.05	0.07
	Egg	971.4 ± 475.4	721.5 ± 1238.5	360.8 ± 195.2	523.4 ± 841.4	0.19	0.81	0.69
	Foraminifera	462.1 ± 727.1	377.3 ± 603.6	35.4 ± 14.1	268.8 ± 340.1	0.10	0.96	0.73
	Gastropoda	0	905.4 ± 40.0	0	438.6 ± 580.2	0.61	0.16	0.61
	Nematoda	15,722.2 ± 11,751.7	15,189.3 ± 15,142.2	3367.0 ± 1104.4	2405.0 ± 4369.6	*	0.87	0.96
	Nemertea	70.7 ± 20.0	938.4 ± 888.2	773.4 ± 962.6	325.4 ± 214.9	0.63	0.30	0.08
	Ostracoda	1471.3 ± 0	492.3 ± 431.7	0	396.1 ± 520.2	0.07	0.90	0.60
	Others	160.3 ± 58.9	82.3 ± 63.8	84.9 ± 80.0	207.5 ± 278.8	0.77	0.81	0.22
	Polychaeta + larvae	914.8 ± 722.7	999.7 ± 1366.7	90.5 ± 46.5	277.3 ± 224.9	*	0.51	0.95
	Turbellaria	7413.1 ± 9803.4	4682.7 ± 6269.7	871.5 ± 539.6	466.9 ± 352.8	0.07	0.88	0.97
Ecological indices	S	7.67 ± 1.15	11.50 ± 1.38	7.60 ± 1.14	7.33 ± 2.80	*	0.06	*
	N	26,134.4 ± 24,225.6	24,578.2 ± 17,952.8	6807.6 ± 1626.4	5654.1 ± 7970.7	*	0.82	0.98
	d	0.68 ± 0.07	1.10 ± 0.12	0.75 ± 0.12	0.76 ± 0.25	0.11	*	*
	H'	1.05 ± 0.33	1.19 ± 0.38	1.34 ± 0.11	1.38 ± 0.35	0.12	0.55	0.74

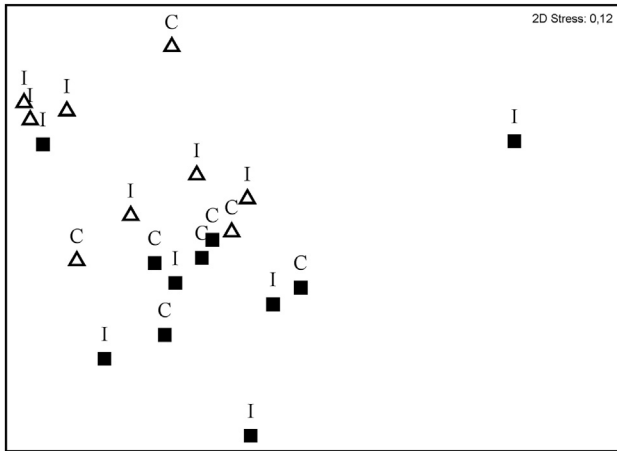


Fig. 2. Non-metric multidimensional scaling ordination (MDS) plot for meiofauna assemblages. (Δ – Spring; \blacksquare – Summer; C – Control; I – Impact).

Table 2

Results from the two-factor PERMANOVA on the meiofauna abundances (Se – season; Lo – location). The number of permutations used was 999.

Source	df	SS	MS	Pseudo-F	P (perm)	U. perms
Se	1	1808.5	1808.5	3.925	0.006	999
Lo	1	833.8	833.8	1.810	0.142	999
SexLo	1	41.3	41.3	0.090	0.951	999
Res	16	7371.7	460.7			
Total	19	10,055.0				

more correlated with some Control samples (Fig. 3A). Very fine sand also showed a positive correlation with the Impact samples. Three main groups of meiofauna taxa, presenting correlations higher than 0.6 with sediment variables, could be identified: Ostracoda, Crustacea larvae, Gastropoda and Cladocera seemed to be more correlated with silt and clay, while Nematoda, Polychaeta + larvae and Foraminifera, although also correlated with silt and clay, seemed to have a higher influence from organic matter (Fig. 3B). Egg appeared more spaced from other groups but with a positive correlation with coarse sand.

4. Discussion

Meiofauna can be found at very high abundances in shallow and subtidal marine sediments, with average values usually ranging from 10^5 and 10^6 ind m^{-2} (e.g. Albertelli et al., 1999; Rodríguez

et al., 2001; Gheskiere et al., 2005; Kotwicki et al., 2005a, 2005b; Leonardis et al., 2008; Gomes and Filho, 2009; Sandulli et al., 2010). Our average meiofauna abundances (1.5×10^4 ind m^{-2}) were below the values usually found in similar marine systems. Even looking at the Spring average abundances (2.5×10^4 ind m^{-2}), that were significantly higher than the Summer abundances, the values can still be considered low. Meiofauna abundances are highly influenced by food availability, namely microphytobenthos, organic matter, bacteria or microalgae (Blanchard, 1990; Ansari et al., 1993; Pinckney et al., 2003) and sediment chemistry, namely oxygen and sulphide levels which may be of utmost importance (e.g. Meyers et al., 1987; Steyaert et al., 2003). In the present study, 53% of the sediment was constituted by fine sand, possibly with a thin oxygenated layer and low food supply, here represented by low organic matter percentages (average of 1%). The combination of these two factors, may explain the relatively low abundances of meiofauna found in the present study.

The influence of wave action and grain size on meiofauna assemblages is documented. For example, in northern Spain beaches an exponential increase in meiofauna abundance, biomass and species richness has been related to beach exposure and average grain size (Rodríguez et al., 2003). In our study, the fractions of silt and clay and very fine sand were the only sediment parameters that showed a significant increase from Control to Impact. CAP also showed that these two fractions were indeed more correlated with the Impact samples, with silt and clay showing a high correlation with some taxa. Although these differences in sediment composition may have some influence in the distribution of meiofauna, SGD may also have a role in the higher amounts of finer sediments found in the Impact site. In fact, the finer sediments may be supplied by the aquifers, as has been reported in other studies (e.g. Mahler and Lynch, 1999; Zipperle and Reise, 2005; Herman et al., 2007; Goldscheider et al., 2010). During field sampling a re-suspension effect caused by the seepages was also recorded *in-situ*, which seems to support this hypothesis.

The more evident patterns were detected on a seasonal basis, with significant differences in some ecological indices, namely number of taxa (S) and abundance (N). PERMANOVA results also showed significant changes in meiofauna assemblages between Spring and Summer. During spring months in the south coast of Portugal, the average wave height remains above 1 m, but periods of 1–3 m waves occur, while during summer months, around 80% of the time the average wave height remains lower than 1 m (Costa et al., 2001). Accordingly, a higher aeration of the sediment occurs during spring months, when compared to summer months.

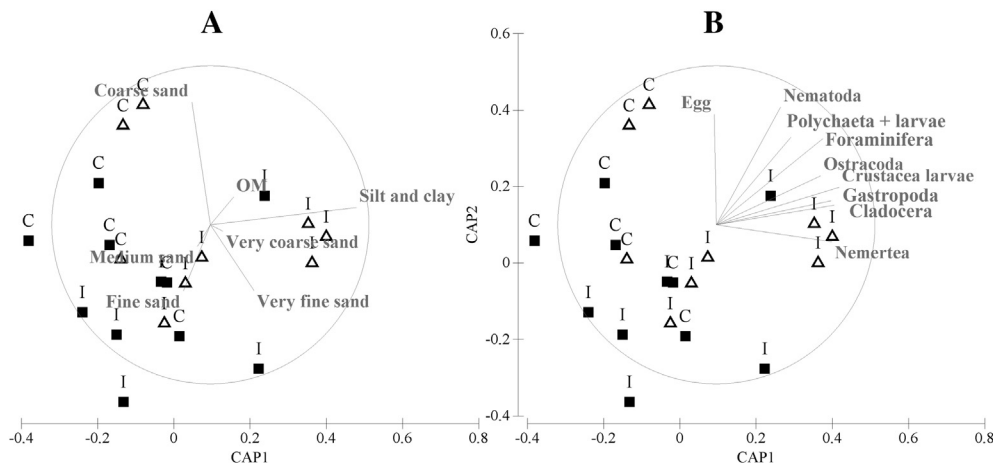


Fig. 3. Canonical analysis of principal coordinates (CAP) with vectors of sediment parameters (A) and taxa with correlations higher than 0.6 (B). The circle represents a correlation of 1, while the vector length represents the correlation strength. (Δ – Spring; \blacksquare – Summer; C – Control; I – Impact).

These higher oxygen levels enhance the abundance and diversity of meiofauna, as previously mentioned for other shallow water and beach environments (e.g. Meyers et al., 1987; Rodríguez et al., 2003; Giere, 2009). In addition, at our latitudes, the peak of primary production occurs during spring months (Longhurst, 1995). Spring phytoplankton blooms and their sedimentation, have already been coupled with meiofauna growth (Rudnick et al., 1985; Ólafsson and Elmgren, 1997; Nascimento, 2010). Indeed, Rudnick et al. (1985), in Narragansett Bay (USA), also found seasonal cycles very similar to the present results, with higher abundances and biomasses in May and June, which the author related to the sedimentation of phytoplankton from early spring, while the lowest values were found in late summer months, when these phytoplankton blooms have already been depleted.

The significant decrease in the number of meiofauna taxa and abundance from Spring to Summer may, therefore, be part of a seasonal cycle, driven by physical and biological factors: higher sediment dynamism during spring and consequent higher interstitial oxygen levels (McLachlan, 1989) and higher food availability due to spring phytoplankton blooms (Longhurst, 1995). However, this high natural environment variability, together with the natural variability characteristic of meiofaunal assemblages, namely rapid generation times, creates difficulties for the assessment of fine distribution patterns.

SGD have been documented as a nutrient source to coastal areas that enhance biological productivity (e.g. Johannes, 1980; Moore, 1999, 2006; Rocha et al., 2009; Waska and Kim, 2011), which then would be expected to influence the lower levels of the trophic chain, namely meiofauna, due to its rapid generation times and direct benthic development (Kennedy and Jacoby, 1999). In other trophic levels, increases in abundance and diversity of organisms have been found for similar coastal soft-bottom environments under effect of SGD. However, in the case of meiofauna, the identification of such effects may become challenging due to the natural variability of these assemblages. In fact, although multivariate statistics did not reveal significant differences between Control and Impact (e.g. MDS, PERMANOVA), significant interactions were found in number of taxa and species richness between factors (seasons and locations). This indicates that differences between Control and Impact locations existed, with seasonality also playing a role in establishing those distribution patterns. The pair-wise tests applied on the interactions of these two parameters (*S* and *d*) showed that an increase in diversity occurred in the area under effect of SGD during the Spring period. The volume of discharged freshwater does fluctuate on a seasonal basis, caused by variations in the water level in the aquifer due to seasonal changes in precipitation (Valiela et al., 1990). In spring the aquifers are usually well recharged, which ensures a substantial flow of water to the coastal zone. Thus, the higher diversity of meiofauna during Spring in the area under effect of SGD can be due to a higher volume of discharged water and nutrients to the coastal area.

Meiofauna is a major component of marine ecosystem and play a significant role in energy transfer, acting as a link between primary producers and higher trophic levels (Moens and Vincx, 1996; Li et al., 1997; Schmid-Araya et al., 2002; Giere, 2009). Thus, the nutrient supplied by SGD enables a higher primary productivity, namely of microphytobenthos and phytoplankton, which may be nutrient limited (Webster et al., 2002). Bacteria, microphytobenthos and phytoplankton are all food sources for soft-bottom meiofauna (Rudnick et al., 1985; Sundbäck et al., 1996; Ólafsson and Elmgren, 1997; Coull, 1999) and thus higher food availability due to higher nutrient supply, especially during spring, would explain the higher diversity of meiofauna observed.

In subtidal soft-bottom substrates, such as the present study area, a high percentage of meiofauna is consumed by organisms

from upper trophic levels, with Polychaetes and Nematodes being the main contributors for this energy transfer (Danovaro et al., 2007). The higher diversity of organisms in the lower levels of the trophic chain, in the present case meiofauna, increases the number of links in the food web. This enables the transfer of energy through a higher number of trophic pathways that has the ability to support a more diverse range of organisms on the higher trophic levels (Sokołowski et al., 2012). This means that the observed increase in diversity of meiofauna assemblages in the present area under effect of SGD may also trigger trophic cascade responses.

Studies regarding the effect of SGD on meiofauna assemblages are almost inexistent worldwide, which strengthens the need of more studies in this area, in order to understand how these phenomena affect the bases of the food web of coastal marine ecosystems. Although the natural variability of meiofauna assemblages may difficult the identification of clear effects from SGD, this study shows that these natural freshwater inputs play a role in the dynamics of these soft-bottom communities, namely in increasing the diversity of meiofaunal assemblages.

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References

- Albertelli, G., Covazzi-Harriague, A., Danovaro, R., Fabiano, M., Fraschetti, S., Pusceddu, A., 1999. Differential responses of bacteria, meiofauna and macrofauna in a shelf area (Ligurian Sea, NW Mediterranean): role of food availability. *Journal of Sea Research* 42, 11–26.
- Almeida, C., Mendonça, J.J.L., Jesus, M.R., Gomes, A.J., 2000. Sistemas Aquíferos de Portugal Continental – Sistema Aquífero: Albufeira-Ribeira de Quarteira (M6). Instituto da Água.
- Almeida, C., Silva, M.L., 1990. Hidrogeologia do miocénico entre Albufeira e Ribeira de Quarteira. *Geolis* 4 (1 and 2), 199–216.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.
- Anderson, M.J., Gorley, R.N., Clarke, K.B., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.
- Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84, 511–525.
- Ansari, Z.A., Sreepada, R.A., Matondkar, S.G.P., Parulekar, A.H., 1993. Meiofaunal stratification in relation to microbial food in a tropical mangrove mud flat. *Tropical Ecology* 34 (2), 204–216.
- Balsamo, M., Semprucci, F., Frontalini, F., Coccioni, R., 2012. Meiofauna as a tool for marine ecosystem biomonitoring. In: Cruzado, A.E. (Ed.), *Marine Ecosystems*, pp. 77–104. Available from: <http://www.intechopen.com/books/marine-ecosystems/meiofauna-as-a-tool-for-marine-ecosystem-monitoring>.
- Bettencourt, A.M., Bricker, S.B., Ferreira, J.G., Franco, A., Marques, J.C., Melo, J.J., Nobre, A., Ramos, L., Reis, C.S., Salas, F., Silva, M.C., Simas, T., Wolff, W., 2003. Typology and Reference Conditions for Portuguese Transitional and Coastal Waters. Development of Guidelines for the Application of the European Union Water Framework Directive, TICOR, IMAR/INAG.
- Blanchard, G.F., 1990. Overlapping microscale dispersion patterns of meiofauna and microphytobenthos. *Marine Ecology Progress Series* 68, 101–111.
- Burgess, R., 2001. An improved protocol for separating meiofauna from sediments using colloidal silica sols. *Marine Ecology Progress Series* 214, 161–165.
- Busmann, I., Dando, P.R., Niven, S.J., Suess, E., 1999. Groundwater seepage in the marine environment: role for mass flux and bacterial activity. *Marine Ecology Progress Series* 178, 169–177.
- Cave, R.R., Henry, T., 2011. Intertidal and submarine groundwater discharge on the west coast of Ireland. *Estuarine, Coastal and Shelf Science* 92, 415–423.
- Clarke, K.B., Warwick, R.M., 2001. Change in Marine Communities: an Approach to Statistical Analysis and Interpretation, second ed. PRIMER-E, Plymouth.

- Costa, M., Silva, R., Vitorino, J., 2001. Contribuições para o estudo do clima de água marítima na costa portuguesa. In: 2^o JECF, Sines, Portugal, p. 20.
- Coull, B.C., 1999. Role of meiofauna in estuarine soft-bottom habitats. *Australian Journal of Ecology* 24, 327–343.
- Dale, R.K., Miller, D.C., 2007. Spatial and temporal patterns of salinity and temperature at an intertidal groundwater seep. *Estuarine, Coastal and Shelf Science* 72, 283–298.
- Dale, R.K., Miller, D.C., 2008. Hydrologic interactions of infaunal polychaetes and intertidal groundwater discharge. *Marine Ecology Progress Series* 363, 205–215.
- Danovaro, R., Scopa, M., Gambi, C., Fraschetti, S., 2007. Trophic importance of subtidal metazoan meiofauna: evidence from in situ exclusion experiments on soft and rocky substrates. *Marine Biology* 152, 339–350.
- Gheskiere, T., Vincx, M., Weslawski, J.M., Scapini, F., Degraer, S., 2005. Meiofauna as descriptor of tourism-induced changes at sandy beaches. *Marine Environmental Research* 60, 245–265.
- Giere, O., 2009. *Meiobenthology: the Microscopic Motile Fauna of Aquatic Sediments*. Springer-Verlag, Berlin.
- Goldscheider, N., Pronk, M., Zopfi, J., 2010. New insights into the transport of sediments and microorganisms in karst groundwater by continuous monitoring of particle-size distribution. *Geologia Croatica* 63 (2), 137–142.
- Gomes, A.J., Filho, J.S.R., 2009. Composição e variabilidade espaço-temporal da meiofauna de uma praia arenosa na região amazônica (Ajuruteua, Pará). *Iheringia, Série Zoologia* 99 (2), 210–216.
- Herman, E.K., Tancredi, J.H., Toran, L., White, W.B., 2007. Mineralogy of suspended sediment in three karst springs. *Hydrogeology Journal* 15 (2), 255–266.
- Hicks, G.R.F., 1991. Monitoring with meiofauna: a compelling option for evaluating environmental stress in tidal inlets. In: 10th Australasian Conference on Coastal and Ocean Engineering, Auckland, New Zealand, pp. 387–391.
- Johannes, R.E., 1980. The ecological significance of the submarine discharge of groundwater. *Marine Ecology Progress Series* 3, 365–373.
- Kennedy, A.D., Jacoby, C.A., 1999. Biological indicators of marine environmental health: meiofauna – a neglected benthic component? *Environmental Monitoring and Assessment* 54, 47–68.
- Korkmaz, N., 1990. The estimation of groundwater recharge from spring hydrographs. *Hydrological Sciences Journal* 35 (2), 209–217.
- Kotwicki, L., De Troch, M., Urban-Malinga, B., Gheskiere, T., Weslawski, J.M., 2005a. Horizontal and vertical distribution of meiofauna on sandy beaches of the North Sea (The Netherlands, Belgium, France). *Helgoland Marine Research* 59, 255–264.
- Kotwicki, L., Szymelfenig, M., De Troch, M., Urban-Malinga, B., Weslawski, J.M., 2005b. Latitudinal biodiversity patterns of meiofauna from sandy littoral beaches. *Biodiversity and Conservation* 14, 461–474.
- Kruskal, J.B., Wish, M., 1978. *Multidimensional Scaling*. Sage Publications, Beverly Hills.
- Leonardis, C.D., Sandulli, R., Vanaverbeke, J., Vincx, M., Zio, S.D., 2008. Meiofauna and nematode diversity in some Mediterranean subtidal areas of the Adriatic and Ionian Sea. *Scientia Marina* 72 (1), 5–13.
- Leote, C., Ibáñez, J.S., Rocha, C., 2008. Submarine groundwater discharge as a nitrogen source to the Ria Formosa studied with seepage meters. *Biogeochemistry* 88, 185–194.
- Li, J., Vincx, M., Herman, P.M.J., 1997. Carbon flows through meiobenthic nematodes in the Westerschelde Estuary. *Fundamental & Applied Nematology* 20 (5), 487–494.
- Longhurst, A., 1995. Seasonal cycles of pelagic production and consumption. *Progress in Oceanography* 36, 77–167.
- Lopes, J.B.S.L., 1841. *Corografia ou Memoria Economica, Estadistica, e Topografica do Reino do Algarve*. Academia Real das Ciencias de Lisboa, Lisboa.
- Mahler, B.J., Lynch, F.L., 1999. Muddy waters: temporal variation in sediment discharging from a karst spring. *Journal of Hydrology* 214, 165–178.
- Martens, P.M., Schockaert, E.R., 1986. The importance of turbellarians in the marine meiobenthos: a review. *Hydrobiologia* 132, 295–303.
- McIntyre, A.D., 1969. Ecology of marine meiobenthos. *Biological Reviews* 44 (2), 245–288.
- McLachlan, A., 1989. Water filtration by dissipative beaches. *Limnology and Oceanography* 34 (4), 774–780.
- Meyers, M.B., Fossing, H., Powell, E.N., 1987. Microdistribution of interstitial meiofauna, oxygen and sulfide gradients, and the tubes of macro-infauna. *Marine Ecology Progress Series* 35, 223–241.
- Migné, A., Ouisse, V., Hubas, C., Davoult, D., 2011. Freshwater seepages and ephemeral macroalgae proliferation in an intertidal bay: II. Effect on benthic biomass and metabolism. *Estuarine, Coastal and Shelf Science* 92, 161–168.
- Miller, D.C., Ullman, W.J., 2004. Ecological consequences of ground water discharge to Delaware Bay, United States. *Ground Water – Oceans Issue* 42 (7), 959–970.
- Mirto, S., Danovaro, R., 2004. Meiofaunal colonisation on artificial substrates: a tool for biomonitoring the environmental quality on coastal marine systems. *Marine Pollution Bulletin* 48, 919–926.
- Moens, T., Vincx, M., 1996. Do meiofauna consume primary production? About many questions and how to answer them. In: Baeyens, J., et al. (Eds.), *Integrated Marine System Analysis*. European Network for Integrated Marine System Analysis, FWO Vlaanderen: Minutes of the First Network Meeting, pp. 188–202.
- Montagna, P.A., Kalke, R.D., 1992. The effect of freshwater inflow on meiofaunal and macrofaunal populations in the Guadalupe and Nueces estuaries, Texas. *Estuaries* 15 (3), 307–326.
- Monteiro, J.P., Martins, R.R., Nunes, P., Diogo, A., 2003. Evolução do uso de águas subterrâneas nas redes urbanas de abastecimento público entre Albufeira e Quarteira (Algarve central) (Evolution of groundwater use in urban supply between Albufeira and Quarteira – central Algarve). In: Ribeiro, L., Peixinho de Cristo, F. (Eds.), *As Águas Subterrâneas no Sul da Península Ibérica*. AIH, APRH, p. 10.
- Monteiro, J.P., Oliveira, M.M., Costa, J.P., 2007. Impact of the replacement of groundwater by dam waters in the Albufeira-Ribeira de Quarteira and Quarteira coastal aquifers. In: XXXV AIH Congress. Groundwater and Ecosystems. Lisbon, Portugal, p. 10.
- Moore, W.S., 1999. The subterranean estuary: a reaction zone of ground water and sea water. *Marine Chemistry* 65, 111–125.
- Moore, W.S., 2006. The role of submarine groundwater discharge in coastal biogeochemistry. *Journal of Geochemical Exploration* 88, 389–393.
- Moore, W.S., Sarmiento, J.L., Key, R.M., 2008. Submarine groundwater discharge revealed by ²²⁸Ra distribution in the upper Atlantic Ocean. *Nature Geoscience* 1, 309–311.
- Nascimento, F.J.A., 2010. *Trophic Ecology of Meiofauna: Response to Sedimentation of Phytoplankton Blooms in the Baltic Sea*. Ph.D. thesis. Stockholm University, Sweden.
- Ólafsson, E., 2003. Do macrofauna structure meiofauna assemblages in marine soft-bottoms? A review of experimental studies. *Vie Milieu* 53 (4), 249–265.
- Ólafsson, E., Elmgren, R., 1997. Seasonal dynamics of sublittoral meiobenthos in relation to phytoplankton sedimentation in the Baltic Sea. *Estuarine, Coastal and Shelf Science* 45, 149–164.
- Ouisse, V., Riera, P., Migné, A., Leroux, C., Davoult, D., 2011. Freshwater seepages and ephemeral macroalgae proliferation in an intertidal bay: I. Effect on benthic community structure and food web. *Estuarine, Coastal and Shelf Science* 91, 272–281.
- Pinckney, J.L., Carman, K.R., Lumsden, S.E., Hymel, S.N., 2003. Microalgal-meiofaunal trophic relationships in muddy intertidal estuarine sediments. *Aquatic Microbial Ecology* 31, 99–108.
- Rocha, C., Ibáñez, J., Leote, C., 2009. Benthic nitrate biogeochemistry affected by tidal modulation of Submarine Groundwater Discharge (SGD) through a sandy beach face, Ria Formosa, Southwestern Iberia. *Marine Chemistry* 115, 43–58.
- Rodríguez, J.G., Lastra, M., López, J., 2003. Meiofauna distribution along a gradient of sandy beaches in northern Spain. *Estuarine, Coastal and Shelf Science* 58, 63–69.
- Rodríguez, J.G., López, J., Jaramillo, E., 2001. Community structure of the intertidal meiofauna along a gradient of morphodynamic sandy beach types in southern Chile. *Revista Chilena de Historia Natural* 74, 885–897.
- Rudnick, D.T., Elmgren, R., Frithsen, J.B., 1985. Meiofaunal prominence and benthic seasonality in a coastal marine ecosystem. *Oecologia* 67, 157–168.
- Sandulli, R., Leonardis, C.D., Vanaverbeke, J., 2010. Meiobenthic communities in the shallow subtidal of three Italian marine protected areas. *Italian Journal of Zoology*, 1–11. iFirst.
- Schmid-Araya, J.M., Hildrew, A.G., Robertson, A., Schmid, P.E., Winterbottom, J., 2002. The importance of meiofauna in food webs: evidence from an acid stream. *Ecology* 83 (5), 1271–1285.
- Silva, A.C.F., Tavares, P., Shapouri, M., Stigter, T.Y., Monteiro, J.P., Machado, M., Cancela da Fonseca, L., Ribeiro, L., 2012. Estuarine biodiversity as an indicator of groundwater discharge. *Estuarine, Coastal and Shelf Science* 97, 38–43.
- Sokolowski, A., Wolowicz, M., Asmus, H., Asmus, R., Carlier, A., Gasiunaitė, Z., Grémare, A., Hummelf, H., Lesutienė, J., Razinkovas, A., Renaud, P.E., Richard, P., Kędra, M., 2012. Is benthic food web structure related to diversity of marine macrobenthic communities? *Estuarine, Coastal and Shelf Science* 108, 76–86.
- Steyaert, M., Vanaverbeke, J., Vanreusel, A., Barranguet, C., Lucas, C., Vincx, M., 2003. The importance of fine-scale, vertical profiles in characterising nematode community structure. *Estuarine, Coastal and Shelf Science* 58, 353–366.
- Sundbäck, K., Nilsson, P., Nilsson, C., Jönsson, B., 1996. Balance between autotrophic and heterotrophic components and processes in microbenthic communities of sandy sediments: a field study. *Estuarine, Coastal and Shelf Science* 43, 689–706.
- Valiela, I., Costa, J., Foreman, K., Teal, J.M., Howes, B., Aubrey, D., 1990. Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. *Biogeochemistry* 10, 177–197.
- Waska, H., Kim, G., 2011. Submarine groundwater discharge (SGD) as a main nutrient source for benthic and water-column primary production in a large intertidal environment of the Yellow Sea. *Journal of Sea Research* 65, 103–113.
- Webster, I.T., Ford, P.W., Hodgson, B., 2002. Microphytobenthos contribution to nutrient-phytoplankton dynamics in a shallow coastal lagoon. *Estuaries* 25 (4A), 540–551.
- Zipperle, A., Reise, K., 2005. Freshwater springs on intertidal sand flats cause a switch in dominance among polychaete worms. *Journal of Sea Research* 54, 143–150.