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ORIGINAL ARTICLE

# Spatial and temporal variation of the benthic macrofauna in a grossly polluted estuary from southwestern Spain

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**Abstract** The spatial–temporal variation of subtidal macrofauna communities of the Odiel–Tinto estuary, one of the most polluted areas in the world, was studied along a sampling period of 4 years (and 3 sampling events). This system has shown typical water and sediment characteristics of estuarine areas although the inner stations showed high concentrations of heavy metals. The structure of the macrofauna community was associated with granulometry, the percentage of organic matter and the heavy metals. Like in other estuaries, the community was dominated by polychaetes (especially by small size opportunistic taxa), meanwhile the crustaceans were the least abundant. Some changes during the sampling period were slight increment in richness and diversity; greater presence of molluscs and crustaceans in the inner zones; a more homogeneous spatial distribution of opportunistic taxa and a higher number of taxa involved in the differences among the estuary areas. The period of study does not allow assuring that these changes have been due to a true improvement or to natural cycles of the communities in naturally stressed systems. So that it would be necessary the establishment of a long-term monitoring programme to study the evolution of the macrofauna communities to state whether the corrective measures could achieve an improvement of this environment. This programme should focus on the study of macrobenthic community's structure and on those selected parameters, which have been the major structuring factors for these communities.

**Keywords** Heavy metal · Odiel estuary · Tinto River · Macrofauna · Recovery · Southwestern Spain

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## Introduction

The Tinto–Odiel estuary (SW Spain) is one of the most polluted areas in the world, with extremely high concentrations of heavy metals in the sediments (Nelson and Lamothe 1993; Ruiz 2001; Sainz and Ruiz 2006) and very acidic waters (pH 2–4; Elbaz-Poulichet et al. 2001). Both rivers flow through the Iberian Pyrite Belt, one of the most important mining areas in western Europe, which have been worked since the Phoenician and Roman times. The estuarine zone includes an area of salt marsh and, since the 1960 s, a heavily industrialised urban area. This industrial activity includes phosphate fertilizer plants, oil refinery, power plants and other chemical industries. Furthermore, in this area exists an important port activity that has supposed the construction of a long breakwater, causing an interruption of the littoral sedimentary fluxes, and the periodic dredging of the bottom. However, it is one of the more important wetlands for migrating birds from southern Europe together to the nearby Doñana National Park. This zone was declared as a biosphere reserve by UNESCO's MAB Programme in 1983, Natural Protected Area in 1989 by Andalusia Government, and is a RAMSAR site and a Special Zone for Birds Protection in the European Union.

To minimize the industrial impact on this system, between 1986 and 1998, the Environmental Agency of the Government of Andalusia established the Odiel and Tinto River Correction Plan (Usero et al. 2000). Since then, the

evolution of the estuary has been studied from the chemical point of view, in relation to the origin, variation and nature of the heavy metals (Elbaz-Poulichet et al. 2001; Ruiz 2001; Bermejo et al. 2003; Sainz and Ruiz 2006; among others). There are some works related to distribution and migrations of birds (e.g., Sanchez et al. 2006) and ecophysiology of salt-marsh macrophytes (e.g., Nieva et al. 2001), but there are few studies related to the contamination effects on organisms, except some on Ostracoda and Foraminifera (Ruiz et al. 2004; Ruiz et al. 2008), and ecotoxicology (Luque et al. 1999; Morillo et al. 2005). On subtidal soft-bottom macrofauna, there is only one investigation previous to the implementation of the Correction Plan (Cano and García 1987) and a sampling survey enclosed in a general study of the Gulf of Cadiz communities (Drake et al. 1999).

The soft-bottom macrofauna is one of the key components of the food web of estuaries and is considered a key element of many marine and estuarine monitoring programmes (Ysebaert and Herman 2002). An extensive literature has described the relationships between the benthic estuarine community and the effect of contaminants (e.g., Pearson and Rosenberg 1978; Warwick and Clarke 1993; Dauvin 2008). However, estuaries are stressful environments due to the interaction of local physical, geological, chemical and biological factors (Saiz-Salinas and González-Oreja 2000; Dauvin et al. 2006) and, as a consequence, the estuarine macrofauna communities exhibit high resistance to pollution (Boesch and Rosenberg 1981). This often makes difficult in the interpretation of the effects of disturbance on the animal communities and confounds the impacts of anthropogenic activity on estuarine biotic integrity (Rakocinski et al. 1997; Dauvin et al. 2006; Dauvin 2008).

The temporal scale of sampling is a key issue for monitoring programmes in the coastal zone (Comín et al. 2004). Many authors argue that the temporal variability of environmental parameters remains high, but the changes in the community can be undetected, so that the structure of the communities remains stable for long periods of time (Govaere et al. 1980; Livingston 1987; Turner et al. 1995). Some aspects on environmental disturbance can be identified only if there are sufficient data to show long-term trends, which are not usually comparables with short-time scale fluctuations. Without a long-term perspective, natural variations in community structure could be mistakenly attributed to anthropogenic disturbance (Thrush et al. 1994; de Paz et al. 2008).

The main aim of the present study was to contribute to the evaluation of the environmental quality of the Odiel–Tinto system in function of the spatial–temporal variation of subtidal macrofauna communities. This work spanned a sampling period of 4 years (and 3 sampling events) although our future research objective will be to establish a long-term monitoring programme of the soft-bottom macrofauna that allows us to see if the corrective measures established since 1986 have resulted in remarkable improvement of this environment.

## Materials and methods

The Odiel and Tinto Rivers have 128 and 92 km in length, a drainage basin area around 2,300 and 1,680 $km^2$ and an average annual water discharge of 405 and 160 $Hm^3$, respectively. To the west of the Huelva city, the Odiel River is a well mixed estuary and is divided in numerous channels and islands. The Tinto River also makes up a less extensive mixed estuary to the east of Huelva before the rivers confluence in the Padre Santo Channel. This channel is directed toward south-east along 13 km until the mouth, in the Atlantic Ocean.

Sampling was undertaken during ebb tide in the summer of 1998, 2000 and 2002 at 8 subtidal stations: 3 in Odiel River, 1 in Tinto River and 4 in Padre Santo Channel (Fig. 1). The criterion of this spatial design was covering all the subtidal environments of the study area. At each station, six replicates samples (five for biological analysis and one for sediment analysis) were taken with a 0.05 $m^2$ van Veen grab. Each replicate was sieved in seawater through a mesh of 0.5 mm, fixed with 4% formalin and stained with Bengal rose. Macrofauna was sorted and, whenever possible, identified to family level. Identification of animals to taxonomic levels equal to or even higher than family has been used in many benthic studies (Herman and Heip 1988; Warwick and Clarke 1991; Vanderklift et al. 1996; Pagola-Carte et al. 2001) and has been found to be sufficient to determine changes in the composition of the soft-bottom benthic macrofauna (Sánchez-Moyano et al. 2006).

For sediment analysis, granulometry was assessed following the Boyoucos method (Boyoucos 1934), and organic matter percentage was obtained as weight loss by ignition at 450°C for 24 h (mean value of 3 replicates per station). The other sediment parameters were measured by laboratories of the Environmental Agency of the government of Andalusia (South Spain): total organic carbon (TOC) was determined by EPA 415.1; fats and hydrocarbons were measured by extraction and FT-IR spectrophotometry; total nitrogen in the sediment was assessed via Kjedahl digestion; phosphate was measured using UV visible spectrophotometry; and the metal contents were measured by SM 3111 A and B for Cd, Zn, Cu and Cr, and EPA 245.1 for Hg. The index of geoaccumulation ($I_{geo}$) has been used as a relative measure of metal pollution in the sediments for Cr, Cu and Zn according to the regional background established by Ruiz (2001) for unpolluted sandy and silty–clayey sediments.

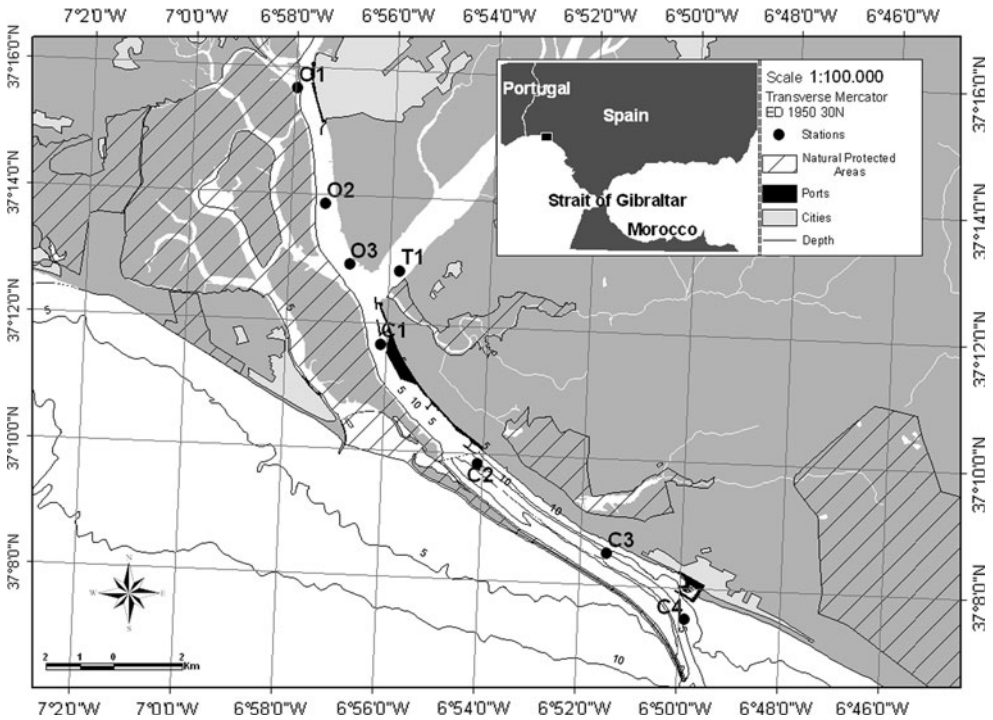


**Fig. 1** The Odiel–Tinto estuary and location of the sampling stations

$I_{geo} = \log_2 (C_n/1.5 \times B_n)$, where $C_n$ is the value of the element $n$, and $B_n$ is the background data of that element. Following Ruiz (2001), the index values were divided into five groups: unpolluted ($I_{geo} < 1$); very low polluted ($1 < I_{geo} < 2$); low polluted ($2 < I_{geo} < 3$); moderate polluted ($3 < I_{geo} < 4$); highly polluted ($4 < I_{geo} < 5$) and very highly polluted ($I_{geo} > 5$).

For water analysis, a water sample per station was obtained close to the bottom by a vertical Alpha Van Dorn-style bottle. The following parameter was measured in situ: temperature, conductivity and salinity by conductivimeter WTW LF-323; pH by pH meter WTW 330i and dissolved oxygen by oximeter WTW OXI-196.

Univariate and multivariate analysis for environmental variables and macrofauna communities were performed using the PRIMER v 5.2.8 software package. Previously, the replicate data were pooled for the multivariate analysis. The macrofauna data were analysed to obtain the total number of taxa, abundance, evenness and Shannon diversity index using neperian logarithms. Spatio-temporal differences for univariate variables were analysed by a two-way ANOVA, after verifying normality (Kolmogorov–Smirnov test) and homogeneity of variances (Barlett test). The data were $\log_{10} (x + 1)$ transformed prior to analysis. Homogenous groups were separated by a Student–Newman–Keuls (SNK) test set at the 5% significance level. Temporal differences for environmental variables were analysed by one-way ANOVA.

Affinities between stations and/or samplings were established using MDS (non-metric multidimensional scaling) analysis with the taxa abundance (transformed by the fourth root). The validity of the ordination was verified with the Kruskal stress coefficient. The differences in community composition were tested with the non-parametric ANOSIM test (Clarke and Green 1988). Percentage of similarity analysis (SIMPER; Clarke 1993) was used to determine the taxa involved in grouping of the different stations and/or samplings. This analysis, based on the matrix of similarity in taxa abundance obtained from the Bray-Curtis index, calculates the contribution of each taxa to either the dissimilarity between groups of stations (discriminatory taxa) or the similarity within a group (typical taxa). Sediment and water variables (transformed by log $x + 1$) were examined using principal components analysis (PCA).

The relationship between the physical environment and macrofauna assemblages was analysed by BIOENV and canonical correspondence analysis (CCA). BIOENV analysis consists of comparing, through the harmonic rank correlation coefficient of Spearman, the rank similarity matrix on species abundance and the rank similarity matrix obtained through Euclidean distances with the abiotic variables (Clarke and Ainsworth 1993). CCA is based on a unimodal response model that constrains the ordination axes to be linear combinations of the environmental variables that maximize the dispersion of sample or taxa scores

(Ter Braak 1986, 1990). In the ordinations, stations were represented as points and statistically significant environmental variables (after a Monte-Carlo permutation procedure) as arrows.

## Results

### Environmental variables

Water and sediment characteristics are showed in Table 1. Water parameters showed the natural trend of estuarine systems, e.g., increase in pH and decrease in salinity from inner to outer points. Sediments were dominated by silt and clay (<0.063 mm) with the exception of the two nearest stations to the mouth (C3 and C4). The values of organic matter in sediment showed a natural trend of decreasing from high estuary until the river mouth. Other parameters such as TOC and metal contents have showed a similar spatial pattern. In relation to metal contents, most of them have showed higher concentrations in the upstream stations, except Cd and Cr, which have showed scarce spatial differences.

To test the differences between sampling events in sediment parameters, one-way ANOVA was used with the station values as replicates (Table 2). There was scarce temporal variation of the composition of the sediments in the entire study zone. However, phosphate values have showed a great increment during the last sampling year (e.g., station O3 with 20.3 and 174.5 ppm in 1998 and 2002, respectively) while total nitrogen values have decreased in the same period (e.g., station O3 with 1,023 and 23.43 ppm in 1998 and 2002, respectively).

In relation to the geoaccumulation index, the stations were classified as unpolluted or very low polluted for Cr ($I_{geo} < 1$ or $1 < I_{geo} < 2$). However, during the all study period, most of stations were highly or very highly polluted by Zn, and very highly polluted by Cu, except stations C3 and C4 in 1998 (unpolluted) and station C4 in 2002 (low polluted).

PCA analysis, based on all measured parameters (water and sediment) and all samplings, is plotted in Fig. 2. The first two principal axes retained 61.6% of the variance (eigenvalues 8.5 and 3.8, respectively). The first principal component discriminated the stations mainly based on a gradient of pH (0.32), % of organic matter (−0.29) and % of sand (0.30) and silt (−0.31), as stated by the eigenvectors. This axis separated stations according to a natural gradient from estuarine to marine environments. The second axis was influenced by other sediment parameters such as fats (0.39), hydrocarbons (0.39) and copper (0.29).

### Macrofauna community

A total of 86 taxa were found in the studied area belonging to Phyla Annelida (27), Arthropoda (27 crustacean taxa), Mollusca (23), Echinodermata (3), Chordata (2), Cnidaria (1), Platyhelminthes (1), Nemertea (1) and Phoronidea (1). Abundance for each station and sampling is presented in Table 6 in Appendix. Polychaetes were the dominant group in all stations and samplings, mainly the abundance of the Spionidae.

Spatial and seasonal variations of number of taxa, Shannon diversity index and Pielou's evenness are plotted in Fig. 3. These univariate parameters have showed a general pattern of increasing toward the channel mouth, except for abundance at upstream stations (O1 to T1) during 2000 sampling (by the contribution of the polychaetes Spionidae) and station O3 during 2002 (2,520 individuals $m^{-2}$ of the molluscs Cardiidae). Taxa number and Shannon diversity have ranged at a wide interval (e.g., 2 and 46 families and 0.96 and 2.84 of diversity index at stations O1 and C4, respectively, during 2002 sampling). The spatio-temporal differences of these univariate parameters were tested by two-way ANOVAs (Table 3). According to the SNK test ($P < 0.05$), there were significant differences between outer stations (stations C3 and C4) and the rest by all the parameters except abundance (this parameter did not show a clear spatial or temporal trend). Besides, there was a pattern of increasing in diversity, evenness and taxa number since 1998–2002 samplings, mainly in the channel stations (C1 to C4).

A global MDS analysis of macrofauna community shows, independently of sampling period, the presence of two groups of stations: channel mouth stations (C3 and C4) and the more upstream points (Fig. 4). The first group is represented by the stations with more influence of the marine areas, sandy sediments and lower contents in sediment parameters. Meanwhile, the second group is comprised by stations with silty sediment and higher contents in the most of sediment parameters. Furthermore, a temporal pattern can be observed in MDS ordination from 1998 to 2002 samplings (it is indicated as a vertical arrow in Fig. 4).

The spatial–temporal differences in community composition were tested using a two-way crossed ANOSIM test. The test for stations gave a value of the global $R = 0.57$ (significance level of 0.1%) so that there were spatial differences across all samplings. Nevertheless, the pairwise test showed no significant differences between stations O2 and O3 ($R = 0.23$, significance level = 3.5%) and between T1 and C1 ($R = 0.14$, significance level = 6.6%). For samplings, there were also global differences ($R = 0.65$, significance level = 0.1%), although $R$ values for pairwise test showed no significant differences between 1998 and 2000 samplings ($R = 0.38$).

**Table 1** Values of sediment and water parameters in each station and sampling period

| Station | Sediment | | | | | | | | | | | | | | | | Water | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fats (ppm) | Hydrocarbon (ppm) | TOC (ppm) | Phosphate (ppm) | N (ppm) | Cd (ppm) | Zn (ppm) | Cu (ppm) | Cr (ppm) | Hg (ppm) | Igeo Zn | Igeo Cu | Igeo Cr | Organic matter (%) | Sand (%) | Silt and clay (%) | Coarse sand (%) | Temp. (°C) | Cond. (µS) | Sal. | pH | Diss. Oxygen (%) | Diss. Oxygen (mg $l^{-1}$) |
| 1998 | | | | | | | | | | | | | | | | | | | | | | |
| O1 | 586.4 | 339.2 | 2.46 | 2.7 | 781 | 7.72 | 1,316.5 | 1,490.1 | 18.81 | 6.27 | 4.49 | 6.26 | −0.74 | 8.06 | 28 | 72 | 0 | 17.2 | 50.4 | 36.8 | 7.77 | 82 | 7.7 |
| O2 | 700.7 | 387.8 | 1.88 | 5.7 | 894 | 8.69 | 1,621.4 | 1,651.5 | 63.19 | 1.88 | 4.79 | 6.40 | 1.00 | 5.06 | 32 | 68 | 0 | 17.7 | 50.2 | 36.8 | 7.79 | 92 | 8.6 |
| O3 | 646.4 | 345.7 | 2.66 | 20.3 | 1,023 | 17.84 | 3,480.3 | 2,447.9 | 168.2 | 5.96 | 5.89 | 6.97 | 2.42 | 7.12 | 27 | 73 | 0 | 18.3 | 49.7 | 36.3 | 7.85 | 93 | 8.3 |
| T1 | 692 | 367.3 | 1.72 | 19 | 789 | 11.35 | 2,015.2 | 2,111.7 | 70.11 | 2.04 | 5.11 | 6.76 | 1.15 | 5.51 | 32 | 68 | 0 | 17.7 | 49.4 | 36 | 7.73 | 81 | 8.6 |
| C1 | 1,308 | 600 | 2.18 | 2.7 | 851 | 8.76 | 1,704.8 | 1,953.5 | 44.42 | 1.75 | 4.87 | 6.65 | 0.50 | 5.24 | 31 | 69 | 0 | 18.2 | 49.7 | 36.4 | 7.91 | 90 | 8.2 |
| C2 | 339 | 177 | 2.25 | 11.3 | 548 | 4.89 | 929.1 | 749.8 | 25.47 | 2.62 | 5.27 | 5.26 | 0.92 | 1.9 | 50 | 50 | 0 | 18.2 | 49.6 | 36.3 | 8.02 | 90 | 8.9 |
| C3 | 620.2 | 290.4 | 0.07 | 1.8 | 110 | 2.13 | 86.74 | 12.91 | 7.6 | 0.08 | 3.27 | 0.52 | −0.24 | 0.21 | 91.24 | 0.55 | 8.21 | 17.2 | 49.7 | 36.4 | 8.2 | 95 | 9.1 |
| C4 | 536.1 | 244.6 | 0.08 | 2.9 | 48 | 1.93 | 71.83 | 7.73 | 8.05 | 0.08 | 3.00 | −0.2 | −0.16 | 0.2 | 99.33 | 0.08 | 0.59 | 17.3 | 49.7 | 36.3 | 8.21 | 95 | 9.3 |
| 2000 | | | | | | | | | | | | | | | | | | | | | | |
| O1 | 199.7 | 53.3 | 4.44 | 2.1 | 3,624 | 10.06 | 2,395.4 | 2,068.3 | 50.32 | 5.63 | 5.36 | 6.73 | 0.68 | 13.77 | 27 | 73 | 0 | 25.3 | 51.9 | 38.6 | 7.58 | 71 | 6.4 |
| O2 | 756.9 | 389.1 | 3.48 | 2.7 | 2,662 | 9.78 | 1,377.2 | 1,060.4 | 120.3 | 8.66 | 4.56 | 5.77 | 1.93 | 13.06 | 23 | 77 | 0 | 25.6 | 50.9 | 37.8 | 7.71 | 75 | 6.5 |
| O3 | 466.4 | 185.8 | 0.63 | 3.28 | 490 | 5.45 | 727.9 | 443 | 27.26 | 27.26 | 3.64 | 4.51 | −0.21 | 7.65 | 26 | 74 | 0 | 25.5 | 50.9 | 37.8 | 7.71 | 80 | 6.2 |
| T1 | 331 | 69.4 | 3.24 | 1.9 | 1,528 | 8.518 | 943.4 | 896.5 | 42.59 | 2.85 | 4.01 | 5.52 | 0.44 | 9.59 | 26 | 74 | 0 | 25.7 | 50.9 | 37.8 | 7.53 | 84 | 6.1 |
| C1 | 356.1 | 72.2 | 2.1 | 1.2 | 520 | 10.45 | 6,377.6 | 901.7 | 52.28 | 0.7 | 6.77 | 5.53 | 0.73 | 13.05 | 15 | 85 | 0 | 25.7 | 50.7 | 37.6 | 7.68 | 83 | 6.6 |
| C2 | 318.3 | 123.8 | 2.8 | 1.82 | 383 | 7.98 | 1,295.4 | 1,117.7 | 48.1 | 1.83 | 4.47 | 5.84 | 0.61 | 6.52 | 42 | 58 | 0 | 25.7 | 50.6 | 37.6 | 7.7 | 80 | 6.7 |
| C3 | 96.1 | 15.2 | 3.5 | 2.65 | 711 | 5.22 | 389.9 | 259.9 | 26.13 | 0.39 | 5.44 | 4.85 | 1.54 | 11.56 | 87.86 | 12.14 | 0 | 25.3 | 50 | 37 | 7.88 | 87 | 7.1 |
| C4 | 152.3 | 15.5 | 0.16 | 1.6 | 500 | 0.5 | 472 | 159 | 31 | 0.4 | 5.71 | 4.14 | 1.78 | 0.56 | 100 | 0 | 0 | 23.7 | 49.7 | 36.8 | 8.06 | 90 | 7.5 |
| 2002 | | | | | | | | | | | | | | | | | | | | | | |
| O1 | 552.3 | 296.2 | 5.88 | 38.8 | 75 | 5 | 1,087.6 | 1,207.7 | 34.94 | 1.47 | 4.22 | 5.95 | 0.15 | 10.29 | 30 | 70 | 0 | 25.6 | 52.1 | 38.8 | 7.32 | 69 | 5.6 |
| O2 | 337.3 | 191.3 | 3.97 | 100.1 | 24.64 | 5 | 1,486 | 1,551.8 | 46.39 | 3.46 | 4.67 | 6.31 | 0.56 | 6.52 | 30 | 70 | 0 | 25.4 | 51.5 | 38.4 | 7.39 | 63 | 5.1 |
| O3 | 305 | 166.2 | 3.23 | 174.5 | 23.43 | 8.69 | 2,382.1 | 1,959.6 | 96.66 | 10.46 | 5.35 | 6.65 | 1.62 | 4.96 | 33 | 67 | 0 | 25 | 50.2 | 37.1 | 7.44 | 67 | 5.7 |
| T1 | 534.9 | 305.8 | 3.57 | 105.1 | 142.6 | 5 | 1,430.4 | 1,653.9 | 40.52 | 2.74 | 4.61 | 6.41 | 0.36 | 8.01 | 27 | 73 | 0 | 24.8 | 50.2 | 37.3 | 7.5 | 74 | 6 |
| C1 | 902.2 | 489.4 | 5.83 | 167.8 | 70.2 | 5 | 2,593.4 | 1,955.1 | 36.08 | 3.02 | 5.47 | 6.65 | 0.20 | 8.65 | 30 | 70 | 0 | 23.9 | 49.9 | 36.8 | 7.52 | 75 | 6.1 |
| C2 | 491.6 | 293.2 | 1.99 | 176.4 | 16.21 | 5 | 1,578.9 | 1,437.2 | 20.2 | 1.91 | 4.75 | 6.20 | −0.64 | 2.90 | 36 | 64 | 0 | 22.5 | 49.5 | 36.5 | 7.87 | 76 | 6.5 |
| C3 | 106 | 69.2 | 0.72 | 117.5 | 8.32 | 5 | 317.9 | 172.2 | 4 | 0.25 | 5.14 | 4.26 | −1.17 | 1.24 | 76.03 | 5.91 | 18.06 | 21.2 | 49.4 | 36 | 8.03 | 90 | 7.8 |
| C4 | 286.4 | 185.5 | 0.33 | 65.23 | 5.19 | 5 | 254.7 | 63.7 | 3 | 0.1 | 4.82 | 2.82 | −1.58 | 0.76 | 98.95 | 0 | 1.05 | 21.1 | 49.2 | 36.2 | 8.14 | 92 | 8.2 |

**Table 2** Results of the one-way ANOVA for the temporal differences of sediment parameters

| Parameter | MS | MS error | *F* | *P* | Homogeneous group |
|---|---|---|---|---|---|
| Organic matter (%) | 0.24 | 0.11 | 2.19 | NS | – |
| Silt and clay (%) | 0.04 | 0.53 | 0.08 | NS | – |
| TOC | 0.06 | 0.06 | 1.11 | NS | – |
| Cd | 0.01 | 0.06 | 0.19 | NS | – |
| Zn | 0.06 | 0.23 | 0.28 | NS | – |
| Cu | 0.12 | 0.49 | 0.24 | NS | – |
| Cr | 0.15 | 0.16 | 0.94 | NS | – |
| Hg | 0.04 | 0.14 | 0.32 | NS | – |
| Fats | 0.26 | 0.06 | 4.26 | <0.03 | 1998 2000 2002 |
| Hydrocarbon | 0.94 | 0.11 | 8.87 | <0.001 | 1998 2002 2000 |
| N | 5.02 | 0.20 | 24.78 | <0.0001 | 1998 2000 2002 |
| Phosphate | 5.22 | 0.06 | 84.08 | <0.0001 | 1998 2000 2002 |

The homogeneous groups according to the SNK test ($P < 0.05$) are indicated with a continuous line. Degrees of freedom = 2

*NS* not significant

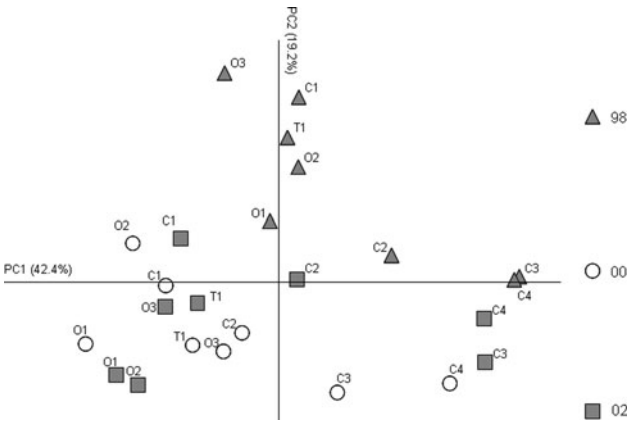


**Fig. 2** PCA analysis plot for all stations and sampling periods from parameters of water and sediment. The percentage of variability explained by the two principal axes is given

One-way ANOSIM tests for each sampling showed spatial differences at a significance level of 0.1% (1998: $R = 0.40$; 2000: $R = 0.45$; 2002: $R = 0.66$). Homogeneous groups according to pairwise test are plotted in Fig. 5. At all samplings, the inner stations could be considered as a homogeneous group, meanwhile outer channel stations (C3 and C4) showed significant differences with the rest.

The SIMPER analysis gave the best discriminating taxa between the groups of stations or sampling periods identified in the multivariate analyses. Table 4 gives the contributions of taxa to discriminate between inner and channel mouth stations. In 1998 sampling, the differences

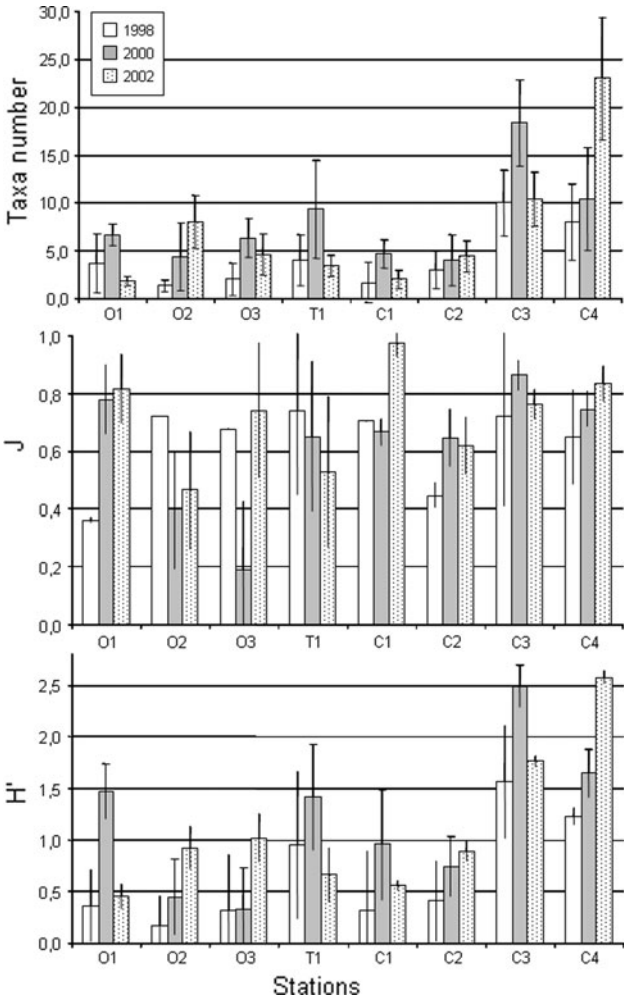


**Fig. 3** Average mean and standard deviation of number of taxa, Pielou's evenness (*J*) and Shannon diversity index (*H*′) of the macrofauna community in each station and sampling period

(average dissimilarity = 85.6%) were based on the presence at channel mouth stations of taxa such as the venerid and mactrid molluscs or the phyllodocid polychaetes, while inner stations were mainly characterised by the high abundance of the spionid polychaetes. In 2000 sampling, the differences (average dissimilarity = 73.3%) were based on similar taxa to 1998 sampling: e.g., presence at channel stations of taxa such as the venerid and corbulid molluscs and orbiniid polychaetes, and again, high abundance of spionids (average abundance of 1,054 individuals) and the anthurid crustaceans at inner areas. In 2002 sampling, the differences (average dissimilarity = 71.2%) were based on a similar pattern to previous samplings again (e.g., high abundance of venerids and mactrids at outer areas), however, more taxa took part in these differences; there were higher abundance of cardiids at inner stations and spionids showed a more

**Table 3** Results of the two-way ANOVA for the spatio-temporal differences of univariate parameters

| Parameter | *df* | MS | MS error | *F* | *P* |
|---|---|---|---|---|---|
| Abundance | | | | | |
| Station | 7 | 0.86 | 0.16 | 5.26 | 0.001 |
| Year | 2 | 1.26 | 0.16 | 7.68 | 0.001 |
| Interaction | 14 | 0.69 | 0.16 | 4.23 | 0.0003 |
| Taxa number | | | | | |
| Station | 7 | 0.56 | 0.33 | 16.64 | 0.00001 |
| Year | 2 | 0.46 | 0.33 | 13.74 | 0.00001 |
| Interaction | 14 | 0.12 | 0.33 | 3.44 | 0.0003 |
| *J* | | | | | |
| Station | 7 | 0.27 | 0.007 | 4.01 | 0.001 |
| Year | 2 | 0.38 | 0.007 | 5.68 | 0.005 |
| Interaction | 14 | 0.008 | 0.007 | 1.27 | NS |
| *H*′ | | | | | |
| Station | 7 | 0.15 | 0.01 | 14.45 | 0.00001 |
| Year | 2 | 0.11 | 0.01 | 10.16 | 0.0001 |
| Interaction | 14 | 0.27 | 0.01 | 2.54 | 0.006 |

*NS* not significant

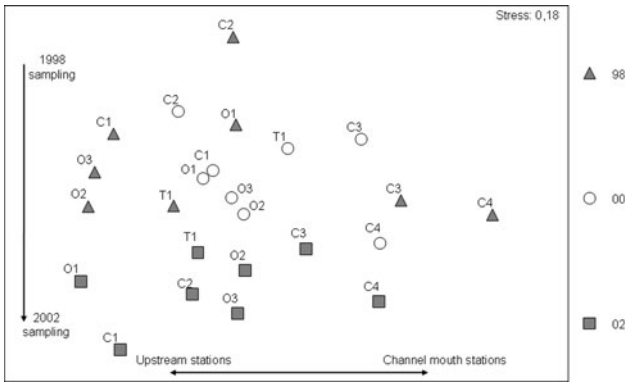


**Fig. 4** MDS ordination for all stations and sampling periods, using Bray-Curtis similarities on taxa abundance. *Horizontal* and *vertical arrows* show the spatial and temporal pattern of station distributions

homogeneous distribution along study zone. In terms of temporal changes (Table 5), SIMPER distinguished only between inner and outer areas. In the first ones, the main differences among sampling periods were based on higher abundance of spionids and anthurids in 2000 (e.g., spionid average abundance of 193, 1,054 and 248 individuals, respectively), and higher abundance of cardiids and the pectinariid polychaetes in 2002. At the outer channel area, the main differences were based on the presence of a greater number of taxa in 2000 and 2002 as opposed to 1998 sampling.

## Relationship between environmental and macrofauna

The results of a global BIOENV analysis (all samplings pooled) indicated that the best correlations always occurred with % sand, organic matter, phosphates and TOC (maximum correlations of 0.44). Separately, a BIOENV with 1998 data showed that the best correlations occurred with others variables such as pH, TOC, Hg or organic matter (maximum correlation of 0.77 with pH, TOC, Hg and Cd). However, in 2000 and 2002 samplings, these best correlations were obtained with the variables related with granulometry and water characteristics (maximum correlations of 0.73 with pH and % sand in 2000 and of 0.79 with temperature, salinity, dissolved oxygen, fats and % silt and clay in 2002).

The global CCA analysis (Fig. 6) indicated a similar distribution of stations to MDS ordination: channel mouth stations (C3 and C4) and the upstream area. The environmental variables that best explained the observed community distributions were pH and % of sand toward channel mouth and heavy metals, % organic matter and salinity toward inner points. The second axis was influenced by hydrocarbons and phosphates and discriminated between 2002 sampling and the others two periods. The Monte-Carlo test was significant for both axes ($P = 0.01$).


**Fig. 5** Homogenous groups of stations according to one-way ANOSIM test in each sampling period

**Table 4** Average abundance (Av. abund) of the most relevant taxa of the stations located in the inner and outer areas in each sampling

| Taxa | Av. abund | | Av. diss | Ratio | Contrib (%) | Cum. (%) |
|---|---|---|---|---|---|---|
| | Inner | Outer | | | | |
| 1998 (average dissimilarity = 85.6) | | | | | | |
| Veneridae | 0 | 426.7 | 8.1 | 2.3 | 9.4 | 9.4 |
| Phyllodocidae | 0 | 320 | 7.6 | 3.4 | 8.9 | 18.3 |
| Mactridae | 0 | 193.3 | 6.2 | 1.8 | 7.2 | 25.5 |
| Orbiniidae | 0 | 36.7 | 4.9 | 5.8 | 5.8 | 31.3 |
| Spionidae | 193.3 | 10 | 4.9 | 1.8 | 5.7 | 37 |
| Capitellidae | 0 | 10 | 3.6 | 5.4 | 4.2 | 41.2 |
| Dexaminidae | 0 | 10 | 3.5 | 7.5 | 4.1 | 45.3 |
| Phoronidea | 3.3 | 23.3 | 3.4 | 1.6 | 3.9 | 49.3 |
| 2000 (average dissimilarity = 73.3) | | | | | | |
| Spionidae | 1,054.4 | 55.3 | 3.4 | 1.9 | 4.6 | 4.6 |
| Orbiniidae | 1.1 | 78 | 2.9 | 2.6 | 4.1 | 8.7 |
| Corbulidae | 0 | 69.3 | 2.8 | 2.1 | 3.8 | 12.5 |
| Serpulidae | 0 | 50 | 2.7 | 6.9 | 3.8 | 16.3 |
| Veneridae | 0 | 208 | 2.7 | 0.9 | 3.7 | 19.9 |
| Mactridae | 2.2 | 42 | 2.4 | 2.7 | 3.3 | 23.3 |
| Leptocheliidae | 2.2 | 50.7 | 2.3 | 2.3 | 3.2 | 26.4 |
| Anthuridae | 132.2 | 30 | 2.2 | 1.4 | 2.9 | 29.4 |
| Oligochaetes | 32.2 | 113.3 | 2.1 | 1.9 | 2.9 | 32.3 |
| 2002 (average dissimilarity = 71.2) | | | | | | |
| Mactridae | 0 | 329.5 | 3.1 | 2.8 | 4.3 | 4.3 |
| Veneridae | 0 | 130.5 | 3 | 7.8 | 4.2 | 8.5 |
| Oweniidae | 0 | 50.5 | 2.9 | 1.6 | 4 | 12.5 |
| Oligochaetes | 0 | 80 | 2.6 | 0.9 | 3.6 | 16.1 |
| Pectinariidae | 94 | 218 | 2.1 | 1.3 | 2.9 | 19.1 |
| Nemertea | 5.3 | 57.5 | 1.9 | 1.6 | 2.7 | 21.8 |
| Hesionidae | 0.7 | 14.5 | 1.9 | 1.6 | 2.7 | 24.5 |
| Corbulidae | 0 | 12 | 1.9 | 6.1 | 2.6 | 27.2 |
| Diogenidae | 0.7 | 29.5 | 1.8 | 2.6 | 2.6 | 29.7 |
| Nassaridae | 0.7 | 27 | 1.8 | 2.6 | 2.6 | 32.3 |
| Cardiidae | 486 | 92.5 | 1.8 | 1.3 | 2.5 | 34.8 |
| Philinidae | 0 | 9.5 | 1.8 | 5.2 | 2.5 | 37.4 |
| Spionidae | 248 | 319 | 1.7 | 1.8 | 2.4 | 39.8 |

Taxa are listed in decreasing order according to its contribution to the average of the dissimilarity (Av. diss) between areas

**Table 5** Interannual variation of the average abundance (Av. abund) of the most relevant taxa of the stations located in the inner and channel mouth stations

| Taxa | Av. abund | Av. abund | Av. diss | Ratio | Contrib (%) | Cum. (%) |
|---|---|---|---|---|---|---|
| Inner stations | | | | | | |
| | 1998 | 2000 | (average dissimilarity = 69.24) | | | |
| Anthuridae | 0.00 | 132.22 | 7.38 | 2.68 | 10.66 | 10.66 |
| Nereididae | 1.11 | 60.00 | 5.84 | 2.65 | 8.43 | 19.09 |
| Spionidae | 193.33 | 1,054.44 | 5.09 | 1.46 | 7.35 | 26.44 |
| Oligochaetes | 88.89 | 32.22 | 4.99 | 1.90 | 7.20 | 33.65 |
| | 1998 | 2002 | (average dissimilarity = 72.78) | | | |
| Cardiidae | 5.56 | 486.00 | 9.82 | 1.59 | 13.49 | 13.49 |
| Pectinariidae | 0.00 | 94.00 | 6.85 | 1.43 | 9.42 | 22.90 |
| Spionidae | 193.33 | 248.00 | 5.24 | 1.61 | 7.19 | 30.10 |
| Nephtyidae | 11.11 | 24.67 | 4.49 | 1.08 | 6.17 | 36.27 |
| | 2000 | 2002 | (average dissimilarity = 69.56) | | | |
| Spionidae | 1,054.44 | 248.00 | 5.86 | 1.43 | 8.42 | 8.42 |
| Nereididae | 60.00 | 0.67 | 5.24 | 2.53 | 7.53 | 15.95 |
| Anthuridae | 132.22 | 4.67 | 5.17 | 1.46 | 7.43 | 23.38 |
| Pectinariidae | 0.00 | 94.00 | 4.64 | 1.48 | 6.67 | 30.05 |
| Capitellidae | 36.67 | 0.00 | 4.20 | 1.45 | 6.03 | 36.09 |
| Oligochaetes | 32.22 | 0.00 | 4.17 | 1.82 | 6.00 | 42.08 |
| Cardiidae | 28.89 | 486.00 | 3.78 | 1.13 | 5.43 | 47.52 |
| Channel mouth stations | | | | | | |
| | 1998 | 2000 | (average dissimilarity = 61.55) | | | |
| Veneridae | 426.67 | 208.00 | 2.55 | 1.48 | 4.14 | 4.14 |
| Corophiidae | 0.00 | 59.33 | 2.46 | 4.46 | 3.99 | 8.13 |
| Leptocheliidae | 0.00 | 50.67 | 2.31 | 3.82 | 3.76 | 11.89 |
| Phyllodocidae | 320.00 | 5.33 | 2.26 | 1.80 | 3.67 | 15.56 |
| Phoronidea | 23.33 | 0.00 | 2.14 | 5.57 | 3.47 | 19.03 |
| Oligochaetes | 0.00 | 113.33 | 1.77 | 0.87 | 2.87 | 21.91 |
| Serpulidae | 3.33 | 50.00 | 1.71 | 1.68 | 2.78 | 24.69 |
| Corbulidae | 23.33 | 69.33 | 1.69 | 1.34 | 2.74 | 27.43 |
| Anomiidae | 0.00 | 8.67 | 1.62 | 11.45 | 2.64 | 30.07 |
| | 1998 | 2002 | (average dissimilarity = 72.88) | | | |
| Pectinariidae | 0.00 | 218.00 | 3.40 | 5.08 | 4.67 | 4.67 |
| Spionidae | 10.00 | 319.00 | 2.65 | 2.19 | 3.63 | 8.30 |
| Cardiidae | 0.00 | 92.50 | 2.58 | 1.44 | 3.54 | 11.84 |
| Oweniidae | 0.00 | 50.50 | 2.28 | 1.58 | 3.13 | 14.97 |
| Phyllodocidae | 320.00 | 4.50 | 2.13 | 1.64 | 2.92 | 17.89 |
| Oligochaetes | 0.00 | 80.00 | 2.00 | 0.87 | 2.75 | 20.64 |
| Phoronidea | 23.33 | 0.00 | 1.96 | 3.13 | 2.69 | 23.33 |
| Hesionidae | 0.00 | 14.50 | 1.76 | 2.09 | 2.42 | 25.75 |
| Corophiidae | 0.00 | 32.00 | 1.76 | 9.02 | 2.41 | 28.16 |
| Diogenidae | 0.00 | 29.50 | 1.74 | 10.13 | 2.38 | 30.54 |

## Discussion

The natural gradients in salinity, granulometry and organic content have been described as the most important factors to explain the distribution and abundance of macrobenthic community in numerous estuarine ecosystems (Wolf 1983; Warwick et al. 1991; Attrill et al. 1996; Rakocinski et al. 1997; Ysebaert et al. 2002; Mucha et al. 2003; Sousa et al. 2006). In general, The Tinto–Odiel system has shown typical water and sediment characteristics of estuarine

**Table 5** continued

| | 2000 | 2002 | (average dissimilarity = 61.23) | | | |
|---|---|---|---|---|---|---|
| Pectinariidae | 0.00 | 218.00 | 2.72 | 6.29 | 4.44 | 4.44 |
| Serpulidae | 50.00 | 0.00 | 1.83 | 3.86 | 2.98 | 7.42 |
| Oweniidae | 0.00 | 50.50 | 1.79 | 1.67 | 2.92 | 10.34 |
| Veneridae | 208.00 | 130.50 | 1.62 | 1.79 | 2.64 | 12.98 |
| Orbiniidae | 78.00 | 5.00 | 1.59 | 1.36 | 2.60 | 15.59 |
| Cardiidae | 4.00 | 92.50 | 1.43 | 1.16 | 2.34 | 17.92 |
| Oligochaetes | 113.33 | 80.00 | 1.42 | 0.91 | 2.33 | 20.25 |
| Capitellidae | 15.33 | 105.00 | 1.36 | 3.36 | 2.22 | 22.47 |
| Spionidae | 55.33 | 319.00 | 1.24 | 1.44 | 2.03 | 24.50 |
| Mactridae | 42.00 | 329.50 | 1.22 | 4.18 | 1.99 | 26.49 |

Taxa are listed in decreasing order according to its contribution to the average of the dissimilarity (Av. diss) between sampling periods

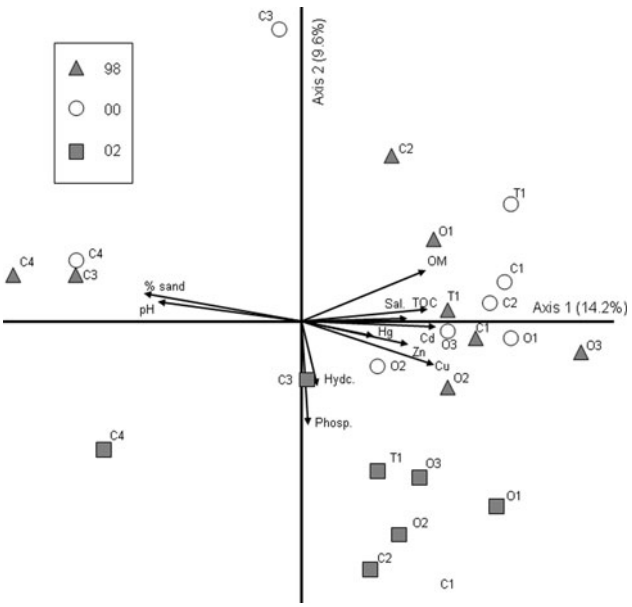


**Fig. 6** CCA analysis plot for all stations and sampling periods from selected parameters of water and sediment: pH, salinity (*Sal*), % of sand, heavy metals (Hg, Zn, Cu and Cd), hydrocarbons (*Hydc.*), phosphates (*Phosp.*), organic matter content (*OM*) and total organic carbon (*TOC*). The percentage of variability explained by the axis is given

areas at the spatial scale. This is corroborated through PCA analysis in which the stations were separated according to a natural gradient from estuarine to marine environment.

A main characteristic of Tinto–Odiel system is the high concentration of some heavy metals in sediment, in comparison with other European rivers (Elbaz-Poulichet et al. 2001). For example, the maximum value of Zn in the present study was 6,378 ppm (Station C1 in 2000 sampling), which is much higher than 169 ppm in the Seine estuary, the most heavily contaminated French river (Dauvin 2008) or >200 ppm in the Douro estuary (Portugal: Mucha et al. 2005). In other geographically near estuary, the Guadiana River, the concentrations only reached maximum values of 149 ppm (Sánchez-Moyano et al. 2003). This last estuary, opposite to Tinto–Odiel, is located in an extensive agricultural zone, with few inhabitants and the main disturbances are moderate urban sewages. The same pattern was observed for other metals such as Cu: 2,448 ppm in Tinto–Odiel system; 44.1 ppm in the Seine; 80 ppm in the Douro and 20 ppm in the Guadiana. For both metals, the geoaccumulation indices showed highly or very highly polluted level (except at the mouth river stations). These data are in agreement with the results obtained in this zone by Ruiz (2001). According to Ruiz et al. (1998), the origin of these heavy metals is industrial discharges (70–80%), acid-mine drainage (20–30%) and minor contributions from urban effluents, while near the mouth, the sediments are periodically dredged and have not had sufficient time to accumulate a high metal content, by which they show very low geoaccumulation indices for all the metals. Furthermore, industrial dumping corrective measures have resulted in remarkable local improvement but have not had a significant effect on the global contamination in the estuary (Sáinz et al. 2003).

In spite of the historical knowledge of the high level of contamination of the Tinto–Odiel system, there is only a single study on the structure of macrofauna communities (Cano and García 1987). All estuaries are characterised by a soft-bottom macrobenthic communities impoverished in relation to those from marine sediments (Wolf 1983; Warwick et al. 1991; Rakocinski et al. 1997; Peeters et al. 2000), and this can difficult the interpretation of the effects of pollution on the structure of the animal communities. However, it has been demonstrated that the human impact increases this impoverishment in species diversity, for example, Marques et al. (1993) observed that the subtidal macrofauna in the Mondego Estuary appeared to be clearly impoverished compared to other Portuguese estuary much less exposed to human impacts.

In the present study, and according to MDS and CCA analysis, the different stations were distributed according to a natural gradient from estuarine to marine environments. Independently of sampling year, the structure of the community has been mainly determined by granulometry and the organic matter content in sediment, which are considered as the major structuring factors for the natural macrobenthic distribution pattern together with salinity (Warwick et al. 1991; Ysebaert et al. 2002; Mucha et al. 2003; Sousa et al. 2006). Community variables such as Shannon Diversity or taxa number showed the typical trend of estuaries, with a progressive increment toward the mouth river, but in a wide interval (e.g., $H' = 0.96$ and only 2 families at O1 until $H' = 2.84$ and 46 families at

C4), which seem to demonstrate a strong stress on communities, at least at inner areas. High concentrations of heavy metals have been associated with low number of taxa and diversity in other estuaries (Mucha et al. 2005). In fact, high concentrations of metals, together with the granulometric composition and water characteristics, has been one of the main factors in the ordinations of stations through Odiel–Tinto system, although it is not possible to determine separately the effect from each one of these factors. The macrobenthic community was dominated by polychaetes and, especially, by small size opportunistic taxa such spionids; while the crustaceans, the most sensitive marine animal group to pollution (Warwick 2001; Dauvin 2008), were the least abundant.

Recovery of marine ecosystems from pollution is inevitably a long-term process (Hawkins et al. 2002). A 4-year sampling period appears to be too short-term to establish definitive conclusions on possible improvements of the system after the implementation of corrective measures. This is particularly true for grossly polluted estuaries, which are organically enriched from sewage discharges, and receive heavy metals and other contaminants (Matthiessen and Law 2002; Essink 2003; González-Oreja and Saiz-Salinas 2003). Compared to results obtained in 1980–1981 from Cano and García (1987), the diversity values or richness were very similar to those obtained in 1998. However, an increase in values of diversity index or number of taxa was observed since 1998–2002, and this "improvement" has been more notable during the last sampling period. The main difference between 1980 and 1981 study and the present work has been a drastic decrease in the nereidid polychaetes (especially *Hediste diversicolor*), which were replaced by small size spionids. *H. diversicolor* is described as a species indifferent to pollution (Pearson and Rosenberg 1978), even in grossly polluted sediment (González-Oreja and Saiz-Salinas 2003), so that it is difficult to attribute some cause to this particular change in the community composition.

Since 2000 sampling, other changes have been a high abundance of the anthurid isopods (exclusively *Cyathura carinata*) and greater richness and abundance of crustaceans. According Warwick and Clarke (1993), the structure of estuarine soft-bottom communities generally shifts with environmental stress to one that is less dominated by crustaceans and more dominated by polychaetes or oligochaetes. Furthermore, pericarid crustaceans appeared to be sensitive to sediment contamination, except some opportunistic amphipod such as *Corophium* (Rakocinski et al. 1997). Parallel, a high abundance of the cardiid molluscs (group represented here exclusively by *Cerastoderma edule*) occurred in the inner zone since 2000 sampling (with peak of abundance in 2002 period). The exclusion or restriction of some species of bivalves, such as *C. edule*, in the Fal estuary (UK) has been attributed in part to a strong copper and zinc pollution (Matthiessen and Law 2002), just as it happened in Odiel–Tinto estuary.

Considering all information, some changes have been noticed along the sampling period. There were (1) slight increment in richness and diversity; (2) higher presence of molluscs and crustaceans in the inner zones; (3) a more homogeneous spatial distribution of opportunistic taxa (e.g., Spionidae); (4) a higher number of taxa characterising the differences among the estuarine sectors. However, the period is too short to conclude that these changes are the consequence of a true improvement of the environment or to natural cycles. In this sense, it would be necessary the establishment of a long-term monitoring programme to study the evolution of the macrofauna communities to state whether the corrective measures could achieve a future remarkable improvement of this environment. This programme should focus on the study of macrobenthic community structure and on selected parameters (e.g., sediment composition, organic content, salinity and Cu and Zn), which have been the major structuring factors for these communities.

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## Appendix

See Table 6.

**Table 6** Abundance (ind. $m^{-2}$) of the taxa identified in the Odiel–Tinto estuary in each station and sampling period

| Taxa | 1998 | | | | | | | | 2000 | | | | | | | | 2002 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | O1 | O2 | O3 | T1 | C1 | C2 | C3 | C4 | O1 | O2 | O3 | T1 | C1 | C2 | C3 | C4 | O1 | O2 | O3 | T1 | C1 | C2 | C3 | C4 |
| CNIDARIA | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PLATYHELMINTHES | | | | | | | | | | | | | | | | | | | | | | | | |
| **Turbellaria** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| NEMERTEA | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 7 | 7 | 0 | 0 | 20 | 0 | 16 | 0 | 24 | 0 | 4 | 0 | 4 | 20 | 95 |
| PHORONIDEA | 13 | 0 | 0 | 7 | 0 | 0 | 13 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ANNELIDA | | | | | | | | | | | | | | | | | | | | | | | | |
| **Oligochaeta** | 220 | 0 | 0 | 0 | 0 | 313 | 0 | 0 | 20 | 0 | 20 | 120 | 7 | 27 | 227 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 160 | 0 |
| **Polychaeta** | | | | | | | | | | | | | | | | | | | | | | | | |
| Capitellidae | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 13 | 167 | 7 | 13 | 13 | 20 | 0 | 27 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 210 |
| Cirratulidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 8 | 28 | 0 | 0 | 8 | 0 | 0 |
| Chrysopetalidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eunicidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glyceridae | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 20 | 4 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 125 |
| Hesionidae | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 24 | 5 |
| Magelonidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 65 |
| Nephtyidae | 20 | 7 | 7 | 7 | 0 | 27 | 33 | 53 | 0 | 0 | 20 | 13 | 0 | 0 | 27 | 28 | 0 | 40 | 44 | 40 | 0 | 24 | 76 | 100 |
| Nereididae | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 33 | 20 | 193 | 27 | 13 | 7 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 |
| Onuphidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 20 | 20 | 0 | 0 | 0 | 0 | 35 |
| Orbiniidae | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 47 | 0 | 0 | 0 | 7 | 0 | 0 | 40 | 116 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 10 |
| Oweniidae | 7 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 5 |
| Paraonidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 13 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pectinariidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 | 16 | 4 | 20 | 416 | 156 | 280 |
| Phyllodocidae | 0 | 0 | 0 | 0 | 0 | 0 | 587 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 |
| Pilargididae | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 113 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Pisionidae | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Poecilochaetidae | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polynoidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 4 | 4 | 0 |
| Sabellidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| Saccocirridae | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serpulidae | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sigalionidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 93 | 0 | 0 | 100 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Spionidae | 533 | 93 | 180 | 153 | 193 | 7 | 20 | 0 | 593 | 873 | 2,567 | 1,540 | 553 | 200 | 107 | 4 | 32 | 876 | 4 | 464 | 8 | 104 | 148 | 490 |
| Syllidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Terebellidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CRUSTACEA | | | | | | | | | | | | | | | | | | | | | | | | |
| **Amphipoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| Ampeliscidae | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 7 | 73 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 10 |
| Aoridae | 20 | 0 | 7 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 180 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caprellidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 8 | 5 |
| Corophiidae | 20 | 0 | 0 | 33 | 13 | 0 | 0 | 0 | 0 | 0 | 13 | 267 | 7 | 0 | 107 | 12 | 0 | 12 | 4 | 0 | 0 | 0 | 4 | 60 |
| Dexaminidae | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gammaridae | 7 | 0 | 0 | 13 | 133 | 0 | 27 | 0 | 7 | 20 | 7 | 240 | 20 | 0 | 0 | 8 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 5 |
| Haustoridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ischyroceridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 12 | 0 |
| Oedicerotidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| **Cumacea** | | | | | | | | | | | | | | | | | | | | | | | | |
| Bodotriidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| **Decapoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| Alpheidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Crangonidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 8 | 0 | 20 | 8 | 4 | 4 | 0 |
| Diogenidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 55 |

**Table 6** continued

| Taxa | 1998 | | | | | | | | 2000 | | | | | | | | 2002 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | O1 | O2 | O3 | T1 | C1 | C2 | C3 | C4 | O1 | O2 | O3 | T1 | C1 | C2 | C3 | C4 | O1 | O2 | O3 | T1 | C1 | C2 | C3 | C4 |
| Dorippidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Grapsidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hippolytidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Penaeidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Portunidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Processidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Upogebiidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Xanthidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Isopoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| Anthuridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 240 | 73 | 7 | 327 | 140 | 7 | 60 | 0 | 0 | 20 | 4 | 4 | 0 | 0 | 8 | 0 |
| Gnathiidae | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chaetiliidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sphaeromatidae | 27 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Mysidacea** | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Tanaidacea** | | | | | | | | | | | | | | | | | | | | | | | | |
| Leptocheliidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 93 | 8 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 15 |
| ECHINODERMATA | | | | | | | | | | | | | | | | | | | | | | | | |
| **Echinoidea** | | | | | | | | | | | | | | | | | | | | | | | | |
| Lovenidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| **Ophiuroidea** | | | | | | | | | | | | | | | | | | | | | | | | |
| Amphiuridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Ophiuridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| MOLLUSCA | | | | | | | | | | | | | | | | | | | | | | | | |
| **Bivalvia** | | | | | | | | | | | | | | | | | | | | | | | | |
| Anomiidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cardiidae | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 100 | 20 | 33 | 13 | 7 | 0 | 0 | 8 | 92 | 2,520 | 276 | 8 | 12 | 8 | 180 | 5 |
| Corbulidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 132 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 20 |
| Donacidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 180 |
| Glycimerididae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hiatellidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mactridae | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 380 | 0 | 13 | 0 | 0 | 0 | 0 | 40 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 655 |
| Montacutidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 |
| Mytilidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Ostreidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 173 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pandoridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Pharidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Semelidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 50 |
| Tellinidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 190 |
| Thracidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Venereidae | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 813 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 416 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 245 |
| **Gastropoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| Acteonidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Nassaridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 50 |
| Philinidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 15 |
| Ringiculidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 |
| Scaphandidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| Turridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| Turritellidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CHORDATA | | | | | | | | | | | | | | | | | | | | | | | | |
| **Tunicata** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Cephalochordata** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 85 |

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