Long-Term Trends in Intertidal and Subtidal Benthic Communities in Response to Water Quality Improvement Measures

Paula Chainho · Gilda Silva · Michael F. Lane ·
José L. Costa · Tadeu Pereira · Carla Azeda ·
Pedro R. Almeida · Inês Metelo · Maria J. Costa

Abstract Long-term monitoring studies are needed to understand changes in ecosystem status when restoration measures are implemented. A long-term data series (1996–2007) of the Tagus estuary (Portugal) intertidal and subtidal benthic communities was collected in a degraded area where mitigation measures were implemented. Multivariate analysis was used to analyze spatial and temporal patterns in benthic community composition and trends in five benthic community metrics (i.e., taxonomic richness, density, biomass, Shannon–Wiener diversity and the AMBI index) were also examined. The results revealed a clear separation between intertidal and subtidal assemblages, although they had 50% of taxa in common, including the most abundant. Significant positive trends were found for all metrics showing that both intertidal and subtidal communities responded to the restoration measures implemented. Nevertheless, biotic indices need some adaptation before being universally applied to intertidal and subtidal habitats.

Keywords Benthic quality assessment · Long-term trends · Spatial and temporal patterns · Tagus estuary · AMBI

P. Chainho (✉) · G. Silva · J. L. Costa · T. Pereira · C. Azeda ·
P. R. Almeida · M. J. Costa
Centro de Oceanografia,
Faculdade de Ciências da Universidade de Lisboa,
Campo Grande,
1749-016 Lisbon, Portugal
e-mail: pmchainho@fc.ul.pt

M. F. Lane
Department of Biological Sciences, Old Dominion University,
Norfolk, VA 23529, USA

P. R. Almeida
Departamento de Biologia, Escola de Ciências e Tecnologia,
Universidade de Évora,
Largo dos Colegiais, 2,
7000 Évora, Portugal

I. Metelo
Direcção Municipal de Ambiente Urbano,
Departamento do Ambiente e Espaços Verdes,
Divisão de Controlo Ambiental,
Câmara Municipal de Lisboa,
Av. 24 de Julho, 171C,
1399-021 Lisbon, Portugal

M. J. Costa
Departamento de Biologia Animal,
Faculdade de Ciências da Universidade de Lisboa,
Campo Grande,
1749-016 Lisbon, Portugal

Introduction

Benthic macroinvertebrate communities respond predictably to human impacts and have been widely used as indicators for assessing and monitoring human impacts on aquatic ecosystems (e.g., Bilyard 1987; Alden et al. 1997). Most benthic studies are carried out at one spatial and one temporal scale, and in general, spatial scales have been better addressed than temporal scales primarily because the costs of field operations limits the potential for long-term studies (Rhoads and Germano 1986). A few long-term studies on marine benthic invertebrates have datasets longer than 20 years for the North Sea (Warwick et al. 2002; Frid et al. 2009), Scotland (Pearson et al. 1986), and English Channel (Dauvin 2000), and several others have more than 10 years of monitoring (e.g., Beukema and Essink 1986; Dorjes et al. 1986; Weisberg et al. 1997; Kress et al. 2004; Borja et al. 2009; Patrício et al. 2009). Most of these studies collected samples once a year, although a few conducted

sampling twice a year (Frid et al. 2009) or seasonally (Dorjes et al. 1986). Although some studies have shown that trends in benthic data are consistent across seasons (Alden et al. 1997), others stressed seasonal variability as a constraint in the use of biological indices for assessing benthic status (Reiss and Kröncke 2005; Chainho et al. 2007). In addition to temporal variability, benthic communities show high spatial heterogeneity in estuaries resulting from the influence of natural gradients. Salinity and sediment type are some of the most important factors influencing the spatial distribution of estuarine benthic invertebrates (e.g., Boesch 1977; Mannino and Montagna 1997), but another major gradient occurs between intertidal and subtidal environments, due to different levels of exposure resulting from tidal cycles (Ysebaert et al. 2003). Most studies that encompass data from several years focused only on subtidal (Buchanan and Moore 1986; Dauvin and Ibanez 1986; Pearson et al. 1986; Salen-Picard and Arlhac 2002; Warwick et al. 2002; Kress et al. 2004; Zettler et al. 2007) or intertidal (Beukema and Essink 1986; Ysebaert and Herman 2002; Timsit et al. 2004; Silva et al. 2006; Patrício et al. 2009) assemblages exclusively and only a few sampled both communities (McLusky et al. 1993; Dorjes et al. 1986; Ysebaert et al. 2003; Borja et al. 2009). Although intertidal areas can be highly representative in some estuaries, benthic assessment tools have been developed primarily for subtidal communities and require modified thresholds prior to assessing intertidal benthic status (Blanchet et al. 2008). In Portugal, long-term data sets are available only for the Mondego (e.g., Patrício et al. 2009) and Tagus estuaries (this study) but as a result of the Water Framework Directive (WFD) studies on the ecological status in Portuguese estuaries were encouraged and monitoring programs are being implemented. The Tagus is among the largest European estuaries (320 km^2) and has high human population levels, but also a high diversity of benthic habitat types (Chainho et al. 2008). Although several studies have sampled the intertidal and subtidal invertebrate communities of the Tagus estuary (e.g., Pereira et al. 1997; Rodrigues et al. 2006; Silva et al. 2006; França et al. 2009), only Gaudêncio and Cabral (2007) and Chainho et al. (2008) conducted comprehensive surveys on subtidal communities along the estuarine gradient, and no long-term studies have been conducted. Several measures have been implemented during the last two decades to improve environmental quality throughout the Tagus estuary. The publication of the European Urban Waste Water Directive requiring an appropriate level of treatment of urban waste water discharges impelled the construction and improvement of several wastewater treatment plants in urban areas along the Tagus estuary, which helped reduce organic point source impacts from the area's nearly 2 million inhabitants. Most wastewater treatment plants were built before 2003, but there are still treatment systems being implemented that will collect sewage from 445,000 inhabitants, currently discharged into the Tagus estuary without treatment. These treatment systems are expected to be fully implemented by 2012. In the Parque das Nações area of Lisbon as part of the World Exposition (EXPO'98), several additional management actions were taken. These included the removal of all industrial facilities and contaminated soil, dredging operations, and the improvement of the Beirolas wastewater treatment plant, the construction of a recreational marina, and river margin embankments. These efforts were coupled with measures to clean up the Trancão River, located upstream of the Parque das Nações area (Fig. 1) and included the construction of the Frielas and S. João da Talha wastewater treatment plants (Table 1). Major characteristics of the wastewater treatment plants located nearby the EXPO'98 area are provided in Table 1. Figure 2 shows the results obtained at the discharge area of the Beirolas and Chelas plants for biochemical oxygen demand (BOD) and total nitrogen (Lisbon Municipality, unpublished data), two parameters that are commonly monitored as indicators of a wastewater treatment plant's efficiency for removing organic inputs. A reduction in both parameters was observed during the monitoring period, indicating that treatment efficiency
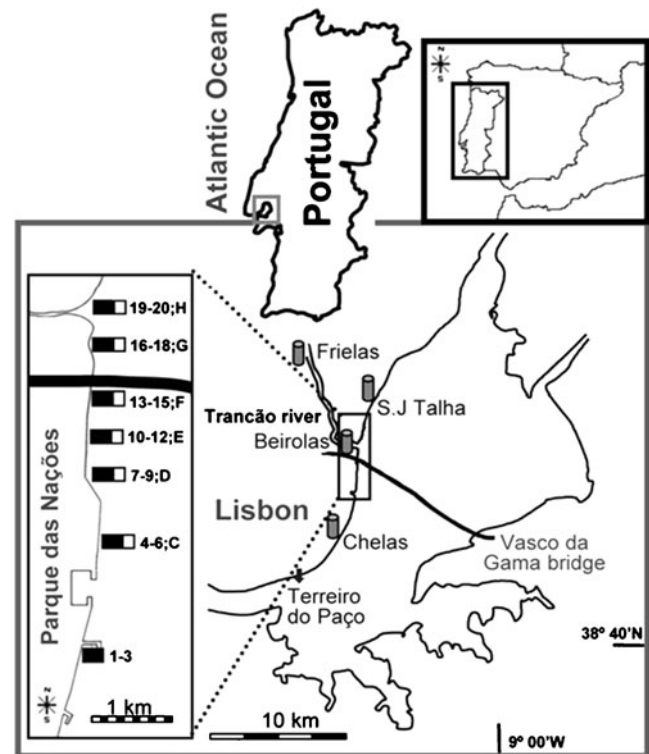
Fig. 1 Study area showing the location of sampling stations in the intertidal (■) and subtidal areas (□) of the Tagus River estuary (*numbers* refer to intertidal stations and letters to subtidal stations) and of major wastewater treatment plants (⬗) located nearby. Terreiro do Paço untreated wastewater discharge location is also indicated (⬇)

Table 1 Major characteristics of wastewater treatment plants located nearby the EXPO'98 area

	Wastewater treatment plants			
	S. João da Talha	Frielas	Beirolas	Chelas
Construction	1998	1999	1989	1989
Improvements	–	–	2000	1999
Population served	130,000	700,000	250,000	255,000

improved. Results obtained for an untreated wastewater discharge area (Terreiro do Paço) of 110,000 inhabitants are also provided for comparison, showing that total nitrogen and BOD loads can be 65% and 90% lower, respectively, when comparing treated with untreated sewage discharges (Fig. 2). The objective of this study was to compare responses of intertidal and subtidal benthic communities of the EXPO'98 intervention area to restoration measures implemented in the Tagus estuary. More specifically, this study examined multivariate patterns in species composition and long-term trends in specific metrics of benthic community health for intertidal and subtidal communities to determine if (1) long-term changes in species composition and benthic community health occurred in response to anthropogenic restoration efforts, (2) different levels of natural stress constrained that response, and (3) variation at different spatial and temporal scales influences the assessment of overall trends in that response.

Materials and Methods

Sampling Design

Data reported in this study were collected as part of the EXPO'98 monitoring program, implemented in 1996. The

objective of the monitoring program was to identify possible changes induced in intertidal and subtidal benthic invertebrate communities by the restoration measures associated with the 1998 World Exposition that took place in the area of Parque das Nações, Lisbon. The intervention area, located on the right bank of the Tagus estuary, extends for nearly 5 km in the polyhaline salinity region of the estuary, with salinities varying between 26 and 31 along the study area and similar variation across seasons (Cabrita and Moita 1995; Ferreira et al. 2003; Chainho et al. 2008). Samples were collected along seven transects consisting of three intertidal and one subtidal station, except for the downstream transect where only intertidal stations were sampled (Fig. 1). Two replicate samples were collected at each station at roughly quarterly intervals from October 1996 to December 2007. Seven seasons could not be sampled, namely Autumn 1997, Winter 1998, 2003 and 2004, Spring 1999 and 2007, and Summer 1999. A total of 38 sampling events were performed, and 1,956 samples were collected.

Benthic invertebrate samples were taken using a modified van Veen LMG grab (0.05 m^2), and grab contents were fixed and preserved with 4% buffered formalin, sieved using a 0.5-mm mesh, and preserved in 70% ethanol. All samples were sorted, identified to the lowest possible taxonomic level, and counted, to determine the number of taxa and their respective abundances. Biomass of species per sample was also determined as dry weight after drying at 60°C for 24 h. At each station, an additional grab sample was taken and frozen for sediment grain size and total organic content (TOC) analysis. Sediment grain size composition was determined using an AFNOR type sieve battery (0.063, 0.250, 0.500, 2.000, and 9.250 mm) after drying the sediment (60°C) for a period of 48 h. TOC was obtained as the difference between dry weight, measured after drying the sample at 60°C during 24 h and ash weight, obtained after ignition at 550°C for a period of 4 h.

Data Analysis

For the purposes of describing long-term changes in community structure, it is often useful to examine patterns at broader spatial scales than those observed at the level of individual stations. For this study, both environmental impacts and restoration efforts were not associated with
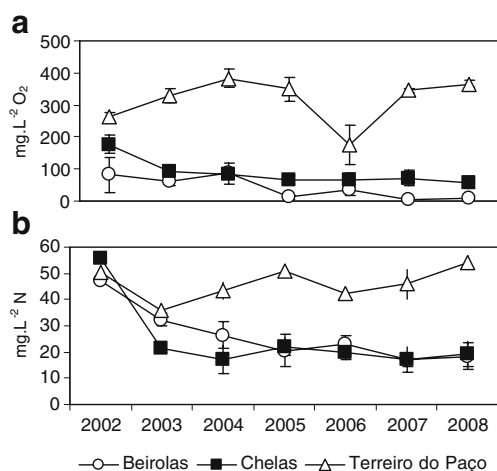
Fig. 2 Annual mean concentrations of biochemical oxygen demand (**a**) and total organic nitrogen (**b**) ± one standard error at the Beirolas and Chelas wastewater treatment plants and at the Terreiro do Paço sewage discharge, for the period of 2002 through 2008 (Lisbon Municipality, unpublished data)

point sources from which gradients of degradation and/or recovery might be expected to originate. As such, any responses observed were not anticipated to be limited to specific stations but rather to operate at broader spatial scales throughout the study area. We made the assumption that benthic communities in the study area with similar species composition would respond to any anthropogenic effects in similar ways. Therefore, prior to any assessment of long-term changes in community structure, we first identified groups of stations (areas) with similar species composition. A two-way crossed analysis of similarity (ANOSIM) procedure was used to determine if there was a significant difference overall between stations and seasons with respect to species composition and to assign stations to areas. The spatial and temporal homogeneity of local environmental conditions was also examined, to account for any possible influence of habitat patchiness and temporal variability in the physical environment on changes in the benthic communities. Taking into account that salinity and sediment type are the most important variables determining the distribution patterns of benthic invertebrate communities (Mannino and Montagna 1997) and that salinity variation across the study area is negligible, we analyzed variations in sediment composition and TOC. Since sediment type in the study area is largely dominated by fine sediments (<0.063 mm), only this fraction was examined for consistency of patterns across stations, seasons, and years. Station, season, and annual means were compared for sediment composition and TOC using an analysis of variance (one-way ANOVA), because the data met the assumptions of normality and non-heterogeneity of variances without transformation. Pairwise multiple comparisons were conducted between all pairs of variables, using a Bonferroni test. Pearson's correlation coefficient was used to test for a significant correlation between fine sediments and TOC, to determine if there were any changes in TOC not related to changes in the sediment composition. A P value of 0.05 was considered for significance of the statistical tests.

Changes in community structure over time at this spatial scale were assessed by describing patterns observed in ordination plots of both species composition and benthic community metrics. The first ordination plot was created using non-metric multidimensional scaling (nMDS) performed on triangular matrices of Bray–Curtis similarity coefficients between square root transformed annual means of abundance values (Clarke and Warwick 1994). A second nMDS was performed on Euclidean distances between samples calculated using the annual means of five benthic community metrics including: (1) number of taxa, (2) density (ind m^{-2}), (3) biomass (g m^{-2}), (4) the Shannon–Wiener diversity index (log$_2$) (Legendre and Legendre 1976), and (5) the AMBI-AZTI Marine Biotic Index (Borja

et al. 2000). All metrics were calculated for each season based on the benthic invertebrate abundance/biomass data of all replicates. Stress values were calculated to assess the validity of the representation of ordinations in two dimensions (Clarke and Warwick 1994). The resulting ordinations represented the relative distances and directions of change between years in both species composition and benthic community health and were also used to delineate groups of years (periods).

Significance testing ($P<0.05$) for differences in community composition among areas, seasons, years, and periods was conducted using ANOSIM randomization tests based on rank similarities of the samples (Clarke 1993). One-way ANOSIM tests ($P<0.05$) were also conducted separately for each area to determine if there were significant differences between seasons, years, and periods. Multiple comparison tests have to be interpreted with some caution because no formal correction is available in the PRIMER routine. To address this problem, not only the P value was considered when determining the significance but also the R value, with only tests with $R>0.25$ considered as significant, as suggested by Clarke and Gorley (2001). Note that for the analyses using annual means, data for 1996 through 1997 and for 1998 through 1999 were pooled due to missing values.

Species were classified with respect to their importance in explaining differences between spatial (areas) and temporal (periods) groups and their importance with respect to frequency of occurrence. Contributions of individual taxa to dissimilarities between spatial and temporal groups were estimated using the similarity percentage breakdown procedure (SIMPER) (Clarke 1993). Importance with respect to frequency of occurrence was measured using the constancy index described by Bachelet and Dauvin (1993) calculated as $C_{ij}=(n_{ij}/n_j)\times100\%$, where n_{ij} is the number of occurrences of species i in station group j, and n_j is the number of stations in station group j using season means as observations. Species were classified as constant ($C>50\%$) and common ($50\%\geq C>25\%$).

Long-term trends in benthic community metrics were identified using the Mann–Kendall test for monotonic trends (Mann 1945; Kendall 1975). Prior to an examination of overall trends for each area-metric combination, homogeneity of trends between seasons was confirmed based on the results of the van Belle and Hughes (1984) test. These particular tests are statistically robust and powerful tools that can be used effectively for data sets characterized by high numbers of missing values or values below method detection limits (e.g., 0 count) and when the data do not conform to any particular distribution (Gilbert 1987). Trend analyses were conducted on seasonal means calculated at the area spatial scale with statistical significance for all tests being evaluated using a criterion of $P<0.05$. Magnitude of
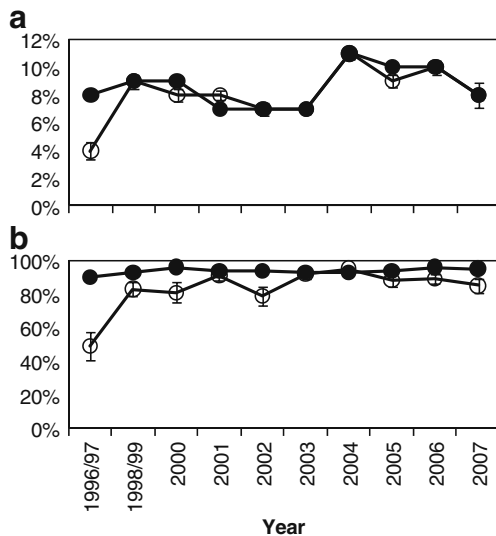
Fig. 3 Annual mean percentage of total organic content ± one standard error (**a**) and fine sediments (<0.063 mm) (**b**) for the intertidal (*filled circles*) and subtidal (*empty circles*) areas for the period of 1996 through 2007

the trends (slope) was calculated as Sen's nonparametric estimator of slope (Sen 1968). Percent change values for all indicators were calculated as (Change/Base)×100%, where Change is the absolute change in the parameter over 12 years and Base is the baseline mean value. The absolute change was the Sen's slope multiplied by the total number of years of monitoring, while the baseline mean values were the means of all data collected in a given area for the first 2 years of monitoring.

All multivariate analyses data were conducted using routines in the PRIMER v5.0 software package (Clarke and Gorley 2001). Pearson correlations and ANOVA tests were performed using SPSS 16.0 software. The AMBI index was calculated using the AMBI 4.1 index software, available at www.azti.es. Trend analyses were conducted with programs developed in PC-SAS (v 9.2) BASE and STAT software.
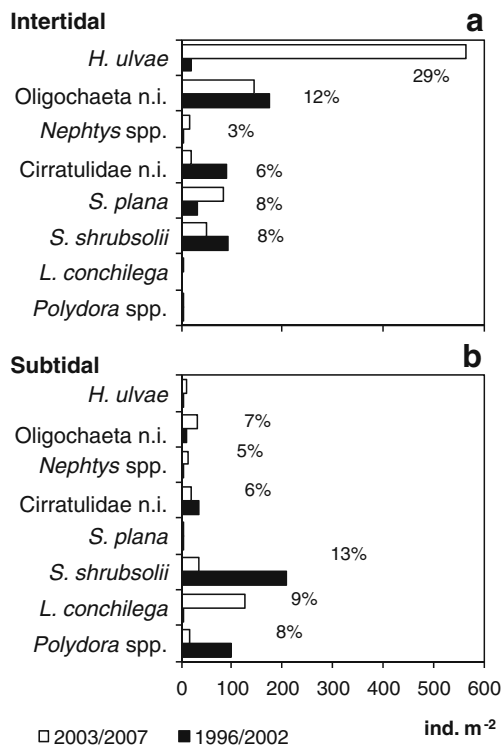
Results

Spatial Patterns

A total of 69,784 macroinvertebrate specimens were collected, and 96 different taxa were identified during the study period. The results of the initial ANOSIM tests showed a significant difference overall between stations ($R=0.463$; $P<0.001$). Pairwise comparisons indicated that, although all intertidal stations were significantly different from all subtidal stations, there were no significant differences between stations within the subtidal portion or intertidal portions of the transects with one exception.

Station 2 in the intertidal was significantly different from all other intertidal stations except station 1. As a result of these findings, stations were classified into two groups: the intertidal and subtidal areas. Spatial differences do not appear to be related to sediment composition since the overall significant differences found between sampling stations with respect to the percentage of fine sediments ($F=11,04$; $df=977$; $P<0.001$) were limited to the occurrence of coarser sediments at one intertidal station (station 10) and two subtidal stations (stations C and G) (Fig. 3).

Multivariate ordination (nMDS) showed a clear separation between intertidal and subtidal invertebrate communities, although both displayed a similar pattern of inter-annual changes in community structure (Fig. 4). Those differences were mainly related to distinct abundances of dominant taxa rather than differences in the taxonomic composition since 50 taxa were common to both areas, and only 22 and 24 taxa were exclusive to the intertidal and subtidal areas, respectively. The taxa exclusive to each area (e.g., Ephydridae n.i. and *Lumbrineris gracilis* in the intertidal; *Aonides oxycephala* and *Phaxas pellucidus* in the subtidal) occurred only rarely and with very low abundances. The SIMPER analysis indicated a dissimilarity of 65% between intertidal and subtidal areas, with eight taxa accounting for 50% of those differences. *Hydrobia ulvae* (12%), Oligochaeta n.i. (10%), *Scrobicularia plana* (6%), Cirratulidae n.i. (5%), and *Nereis diversicolor* (4%) were more abundant in the intertidal area, while the contributions of *Streblospio shrubsolii* (6%), *Lanice conchilega* (5%), and *Polydora* spp. (5%) were due to their higher abundances in the subtidal area (Fig. 5). In the nMDS ordination plot using benthic community metrics, there was no apparent separation between intertidal and subtidal assemblages (Fig. 6),
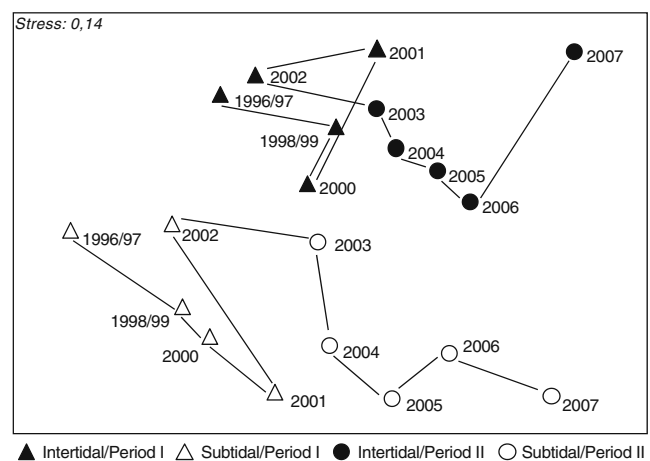
Fig. 4 Non-metric multidimensional scaling plot of Tagus River intertidal and subtidal invertebrate assemblages from 1996/1997 through 2007, with different periods identified. Annual mean abundance values for each species were used and the stress value is indicated. Bray–Curtis distances were used as a similarity measure

Fig. 5 Average density values for dominant taxa for the intertidal (**a**) and subtidal (**b**) areas for each period. Contributions of those taxa (%) to dissimilarities between periods in each area are also shown

and the ANOSIM results confirmed that no significant differences were found in the overall structure of both assemblages. Nevertheless, although the mean number of taxa and Shannon–Wiener diversity were very similar for both assemblages, the mean density and biomass values were higher in the intertidal (808 ind m^{-2}; 11 g m^{-2}) area when compared with the subtidal (419 ind m^{-2}; 9 g m^{-2}).

Temporal Patterns

Ordination plot of the species composition data revealed two distinct temporal groups in both the intertidal and subtidal areas: period I, which extended from the start of monitoring through 2002, and period II from 2003 to 2007 (Fig. 4). There was a gradual divergence from starting conditions during the first 6 years of monitoring followed by a 2-year reversal of this trend during 2001 and 2002. During period II, both intertidal and subtidal areas returned to the previous trend of gradual change in species composition, although the intertidal area exhibited less variation between years when compared with the subtidal area. ANOSIM tests indicated significant differences between periods I and II for both the intertidal ($R=0.424$; $P<0.001$) and subtidal ($R=0.470$; $P<0.001$) areas, but no significant differences were found between seasons ($P>0.05$). Significant differences between periods were also

found for sediment composition ($F=12.37$; $df=977$; $P<0.05$), but multiple comparisons using years as factors revealed that those differences were exclusively related to the presence of much coarser sediments during 1996/97 (Fig. 3), particularly in subtidal stations. No seasonal differences were found in sediment composition. Significant differences between periods ($F=93.91$; $N=978$; $P<0.01$) were also found for TOC, with higher mean TOC percentages during period II (9.4%) than period I (7.9%). This parameter also registered significant differences between seasons ($F=29.53$; $N=978$; $P<0.01$), and the post hoc test revealed differences between all seasons except for autumn and spring. The percentage of fine sediments and TOC were significantly correlated ($R=0.472$; $N=978$; $P<0.01$), indicating that changes in TOC are most likely related to the affinity of organic matter to silt and clay particles rather than any increase in organic inputs. Major benthic community changes in the intertidal area between periods included a significant increase on the density of the gastropod *H. ulvae* that registered an average of 565 ind m^{-2} during period II, contributing to 29% of the difference between periods, as identified by the SIMPER procedure (Fig. 5). Density of the bivalve *S. plana* also increased during period II explaining 8% of the difference between periods, while significant reductions were observed in the average densities of Oligochaeta n.i., *S. shrubsolii* and Cirratulidae n.i., which contributed respectively 12%, 8%, and 6% for those differences. Decreases in population density of *S. shrubsolii* (13%), Cirratulidae n.i. (6%), and *Polydora* spp. (8%) and a significant increase of the polychaete *L. conchilega* (9%) explained most of the
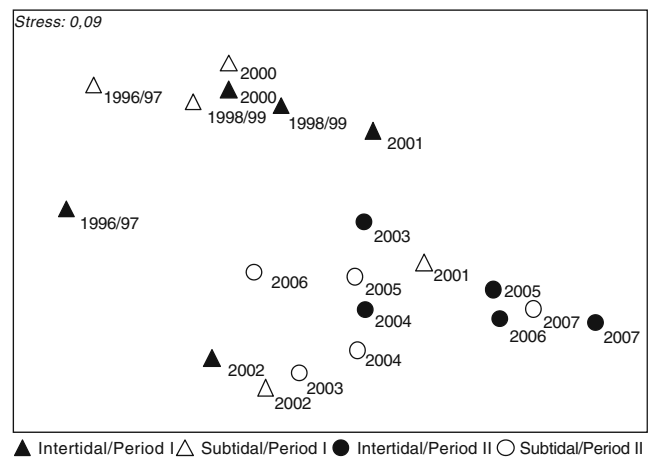
Fig. 6 Non-metric multidimensional scaling plot of Tagus River intertidal and subtidal invertebrate metrics (taxonomic richness, density, biomass, Shannon–Wiener diversity, and AMBI) from 1996/1997 to 2007, with indication of different periods identified. Annual mean abundance values for each metric were used, and the stress value is provided. Euclidean distances were used as a similarity measure

Table 2 Results of the constancy index and respective classifications (Ct—constant; Cm—common) for species that were identified in more than 25% of the observations (seasons) for intertidal and subtidal areas

Taxa	Intertidal		Subtidal	
Oligochaeta n.i.	100	(Ct)	87	(Ct)
Streblospio shrubsolii	100	(Ct)	97	(Ct)
Scrobicularia plana	100	(Ct)	58	(Ct)
Cirratulidae n.i.	97	(Ct)	92	(Ct)
Nereis diversicolor	97	(Ct)	76	(Ct)
Hydrobia ulvae	87	(Ct)	39	(Cm)
Dolichopodidae n.i.	79	(Ct)	–	
Corophium spp.	68	(Ct)	74	(Ct)
Polydora spp.	55	(Ct)	74	(Ct)
Cerastoderma glaucum	68	(Ct)	63	(Ct)
Nephtys spp.	63	(Ct)	58	(Ct)
Cyathura carinata	55	(Ct)	47	(Cm)
Gammaridea n.i.	42	(Cm)	34	(Cm)
Nemertea n.i.	45	(Cm)	–	
Lanice conchilega	–		42	(Cm)
Cossura coasta	37	(Cm)	–	
Barnea candida	–		37	(Cm)
Syllidae n.i.	–		37	(Cm)
Capitellidae n.i.	34	(Cm)	32	(Cm)
Corbula gibba	26	(Cm)	29	(Cm)

species occurred in more than 50% of the observations (i.e., Constant) for the subtidal area, while eight taxa were common within these assemblages (Table 2).

The nMDS ordination plot using benthic community metrics reflected a similar pattern to that shown with the community species composition (Fig. 6), and the results of the ANOSIM tests showed significant differences between years overall, both for intertidal ($R=0.305$; $P<0.05$) and subtidal communities ($R=0.256$; $P<0.05$), as well as between periods I and II ($R=0.417$; $P<0.05$ and $R=0.345$; $P<0.05$, respectively), but no significant differences between seasons were found for both areas ($P>0.05$).

Trend Analysis

The van Belle and Hughes homogeneity test indicated no significant differences in trends between seasons for any of the parameters tested in either the intertidal or subtidal areas (Table 3), therefore allowing for the interpretation of the overall trend results for all parameters in both areas. Significant trends ($P<0.05$) indicative of improving benthic biological communities were detected for all metrics in both areas except for total density in the subtidal area (Table 3). All trends were positive except for the AMBI index since higher scores of this index indicate worse status. Percent changes in the baseline mean (first 2 years) values over the 12-year study for all parameters with significant trends were substantial, ranging from a roughly 33% decrease in the AMBI index in the subtidal area to an increase of nearly 10,000% in total biomass, also in this area (Table 3). Plots of seasonal mean values of most parameters with significant trends showed patterns that were, in general, reflective of the predicated trend lines with the exception of the period between 2001 and 2002 when the improvements made appeared to reverse (Fig. 7). This reversal in community metrics corresponded to the reversal in trends observed in

difference between periods for the subtidal area. This last species was absent from both areas at the beginning of the study, but its abundance increased considerably in later years, reaching an average seasonal maximum density of 7,170 ind m^{-2} in Autumn 2007. The constancy index results identified 12 constant and five common taxa in the intertidal area, three of which (i.e., Oligochaeta n.i., *S. shrubsolii*, and *S. plana*) occurred in all sampling events (Table 2). Nine

Table 3 Long-term trends in the benthic biological indicators for the intertidal and subtidal areas for the period of 1996 through 2007

Provided for each indicator is the P value for the seasonal Kendall test for monotonic trends (P value), the mean of the first 2 years of monitoring (baseline mean), the percent changes in the parameter after 10 years, and the P value for the van Belle and Hughes test for homogeneity of trends between seasons within each area

	P value	Baseline mean	% Change	Homogeneity test P value
Intertidal				
Number taxa	<0.001	1.70	353.93	0.634
Density (ind m^{-2})	<0.001	297.4	515.28	0.402
Biomass (g m^{-2})	<0.001	0.54	3,387.34	0.692
Shannon–Wiener diversity	<0.001	0.48	279.48	0.533
AMBI	<0.001	4.80	−47.65	0.561
Subtidal				
Number taxa	<0.001	2.30	206.39	0.837
Density (ind m^{-2})	0.740	272.5	18.96	0.538
Biomass (g m^{-2})	0.002	0.04	9,291.25	0.707
Shannon–Wiener diversity	<0.001	0.67	206.20	0.783
AMBI	0.014	4.50	−32.59	0.127

Fig. 7 Seasonal mean values of the taxa richness (**a**, **b**), density (**c**, **d**), biomass (**e**, **f**), Shannon–Wiener diversity (**g**, **h**), and AMBI (**i**, **j**) in the intertidal and subtidal areas and their associated trend lines as estimated using Sen's nonparametric estimator of slope. *Dash line* represents the separation between the two periods identified by nMDS analysis
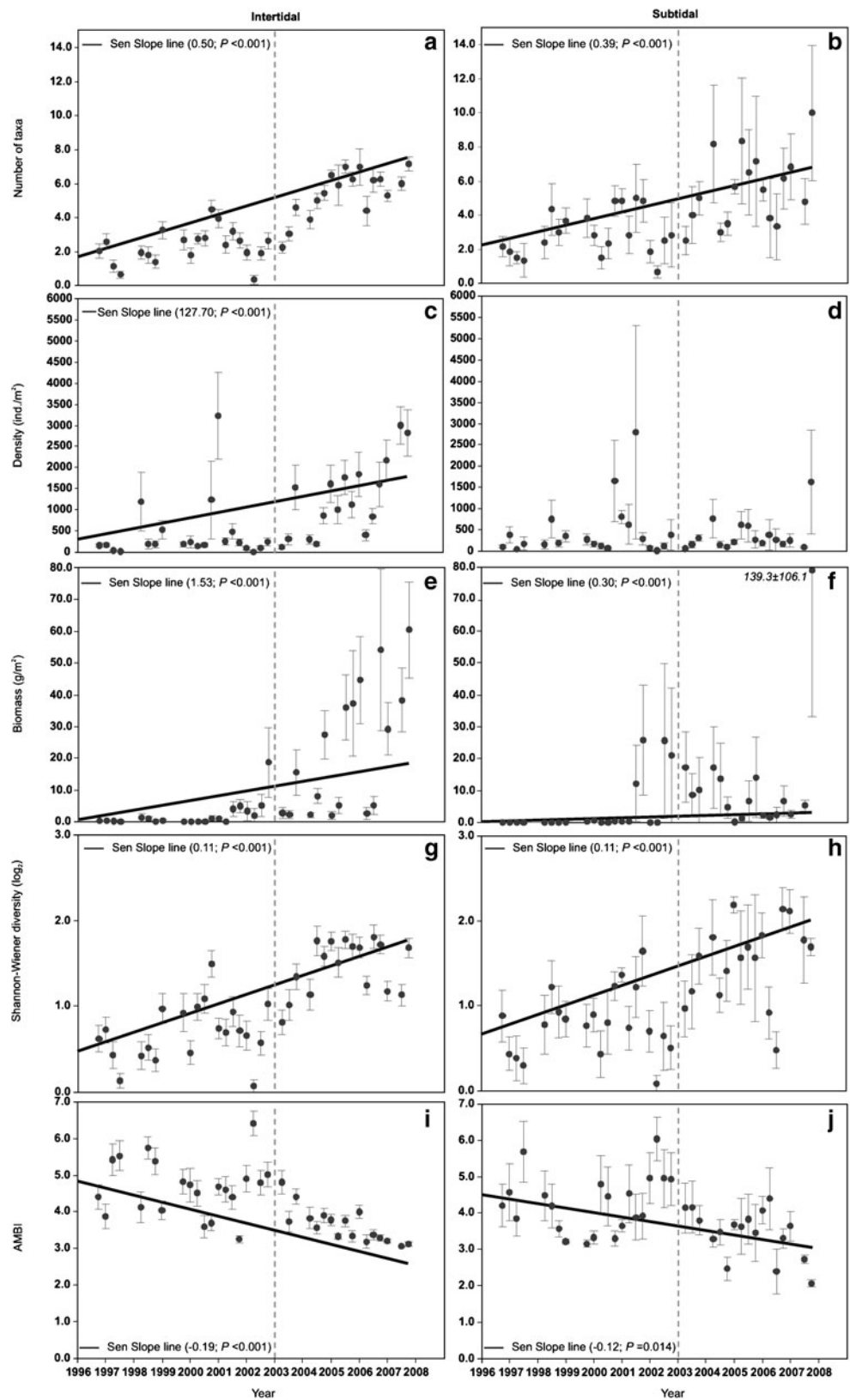
species composition described above for the nMDS (Fig. 4). The exception to this generalization was total biomass for which the trends were due primarily to a large increase that occurred during the last 5 years of the study (Fig. 7e, f). Large bivalve specimens, particularly *S. plana*, accounted for major biomass inputs, except for the high values observed in 2007 that were mainly due to the polychaete *L. conchilega*.

The mean values for the AMBI index showed an improvement in the scores between periods I and II for both intertidal and subtidal areas (Fig. 8; Table 4). In spite of the larger decrease in the scores obtained for the intertidal area, it was classified as having a moderate status in both periods, while subtidal assemblages changed from a moderate to a good status, according to the AMBI thresholds. The relative contributions of each of the ecological groups defined within the AMBI index showed a general decrease in the relative abundance of first- and second-order opportunists (e.g., Oligochaeta n.i., Cirratulidae n.i., and *Polydora* spp.) in both intertidal and subtidal areas (Fig. 8). In the intertidal area, the decrease in the AMBI was due to an increase of the relative abundance of tolerant species, mainly *H. ulvae* and *S. plana*, while the subtidal area experienced an increase in sensitive taxa such as *L. conchilega*, Gammaridea n.i., and Syllidae n.i.

Discussion

Spatial Patterns

Several studies have demonstrated that soft sediment invertebrates show patchy distributions at small spatial scales (Morrisey et al. 1992; Ysebaert and Herman 2002),
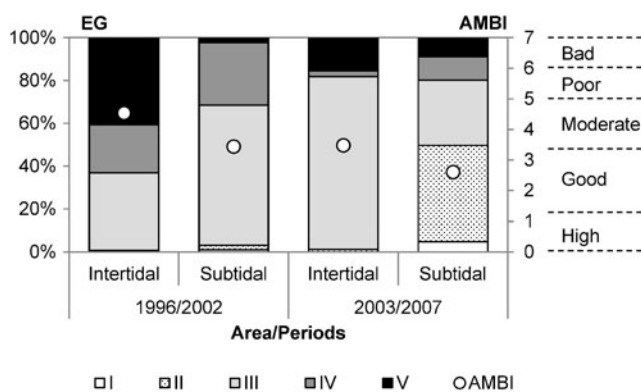
Fig. 8 Mean AMBI values obtained for intertidal and subtidal areas during each period (second axis) and equivalent ecological status (according to Muxika et al. 2005). Percentages of each of the AMBI ecological groups (EG) are shown in the first axis: *I* sensitive, *II* indifferent, *III* tolerant, *IV* second order opportunist, and *V* first order opportunist

Table 4 Mean values for each benthic biological indicators for each period identified by nMDS analysis for the intertidal and subtidal areas

Metrics	Periods	
	1996/2002	2003/2007
Intertidal		
Number taxa	2.51	5.42
Density (ind m^{-2})	521.30	1,269.20
Biomass (g m^{-2})	1.17	21.98
Shannon–Wiener diversity	0.75	1.46
AMBI	4.54	3.48
Subtidal		
Number taxa	2.90	5.54
Density (ind m^{-2})	458.49	369.96
Biomass (g m^{-2})	4.16	15.00
Shannon–Wiener diversity	0.80	1.53
AMBI	3.44	2.61

as well as zonation patterns along the gradient between intertidal and subtidal habitats (Moreira et al. 1993; Ysebaert et al. 2003; Rodrigues et al. 2006; Salgado et al. 2007; França et al. 2009). The spatial resolution and sample number for this study was not adequate to delineate detailed zonation patterns along this gradient, but a clear separation between both assemblages was identified. Differences between intertidal and subtidal stations in close proximity were greater than differences between distant stations within their respective assemblages, and no significant differences between stations within areas were observed. Although there might be some patchiness in the distribution of species within each area, that variation is likely to be observed at smaller spatial scales than could be observed with this study design. The spatial homogeneity within areas is probably related to the uniformity of sediment composition dominated by fine-sized particles and a high organic content throughout the study area.

The average number of taxa identified in each sampling campaign never exceeded 10 different taxa for each assemblage, which is very similar to values found in other regions of the Tagus estuary with similar salinities (Rodrigues et al. 2006; Salgado et al. 2007; França et al. 2009). The dominant taxa (i.e., *H. ulvae*, *L. conchilega*, Oligochaeta n.i., *S. shrubsolii*, Cirratulidae n.i., *N. diversicolor*, *Corophium* spp., Polydora spp., and *S. plana*) are typical of shallow muddy sediments of the polyhaline region of European temperate estuaries (e.g., McLusky et al. 1993; Rodrigues et al. 2006; Chainho et al. 2008; de Paz et al. 2008; Patrício et al. 2009). With respect to other community metrics, higher abundance and biomass were found in the intertidal area when compared with the

subtidal, a pattern typically observed in other estuaries (McLusky et al. 1993; Ysebaert et al. 2003). Differences found between intertidal and subtidal assemblages were related both to differences in the relative abundance of dominant species common to both areas and to the occurrence of rarer species that were exclusive to each area. Higher abundances of *H. ulvae*, Oligochaeta n.i., *S. plana*, Cirratulidae n.i., and *N. diversicolor* in the intertidal area and *S. shrubsolii L. conchilega* and *Polydora* spp. in the subtidal accounted for much of the differences between assemblages as showed by the SIMPER analyses, indicating that distinct habitat conditions exist over short distances. Ysebaert et al. (2003) indicated higher current speeds in subtidal areas as a major factor influencing the intertidal/subtidal gradient, while greater exposure to variable environmental conditions such as low oxygen concentration in fine sediments, fluctuations in salinity, drying and flooding, temperature fluctuations, and covering by ice are major constraints for intertidal communities (e.g., Dorjes et al. 1986; McLusky et al. 1993).

Temporal Patterns

Ordination plots showed a similar pattern of change over time for intertidal and subtidal benthic communities with respect to both species composition and benthic community metrics indicating that, in spite of significant differences between assemblages, their response to the drivers of those changes was comparable. Interpreting the qualitative value of changes in benthic community structure in relation to restoration effects requires an understanding of the ecological significance of those changes. We assumed that increases in taxonomic richness, diversity (Shannon–Wiener index), and pollution sensitive species (AMBI) should be considered positive responses to restoration efforts (see Dauer and Alden 1995 and Borja et al. 2000 for detailed justifications). Positive trends were found for all the benthic metrics in both areas, except for density of subtidal communities and trends were consistent between seasons which, according to our expectations, indicate that an improvement in the benthic ecological status occurred during our study period. These results are consistent with improvements observed in water quality after the implementation of high wastewater treatment levels.

Average density found in the EXPO'98 area during both periods was lower than in other areas of the estuary with similar salinities and sediment type. Nevertheless, density and biomass during the last 4 years were very similar to values found in mudflats located in a nature protection area characterized by minimal human pressure (Rodrigues et al. 2006; Salgado et al. 2007; França et al. 2009), indicating that the community structure might be evolving towards a good ecological status. Organic content is often used as a measure of inputs from domestic sources; nevertheless, in the Tagus estuary, endogenous natural organic inputs are very significant, due to extensive input from saltmarshes (Caçador et al. 2009). The results of the AMBI, developed specifically to measure the benthic response to organic enrichment, indicate an improvement in ecological status between periods in both areas, with the subtidal benthic community changing from a moderate to a good status. These results were due to a gradual replacement of dominant opportunistic taxa such as Oligochaeta n.i., Cirratulidae n.i., and *Polydora* spp. by more typical tolerant estuarine species in the intertidal area (e.g., *H. ulvae* and *S. plana*) and sensitive species in the subtidal areas (e.g., *L. conchilega* and Syllidae n.i.). The colonization of all the study area by the polychaete *L. conchilega* provides additional evidence of improvement of the benthic environment since this species is assigned to the ecological group II of the AMBI, which includes species that tolerate only minor deviations from reference conditions. High densities of this species were registered in the subtidal area in the last 4 years, and data collected in 2008 confirm a continuation of the increasing trend observed for this species in both intertidal and subtidal areas (unpublished data). *L. conchilega* is a tube-building species that forms a complex framework of tubes that provides other species with refuge from predation, competition, and physical stresses, and may also represent an important food resource, critical nursery, and spawning habitat (Rabaut et al. 2007). By changing the surrounding habitat, these polychaetes induce increased species richness and density (Rabaut et al. 2007), resulting in improvements in indicators of benthic health.

Although the trend analyses indicated a clear improvement in all parameters, a reversal of this trend occurred in 2001/2002 due to what seems to have been a catastrophic event. Data collected from other monitoring studies being carried out at the mouth of the Tagus estuary showed a similar decrease in benthic community richness and abundance at the same time (unpublished data), indicating that this phenomenon was more widespread. The Tagus River experienced higher than average flow during 2001 (Costa et al. 2007), but this wet year was followed by a period of extremely low flows during 2002. The combined effect of extreme high flows followed by drought may have induced changes in species recruitment patterns and precipitated the reversal in benthic community conditions observed (Montagna and Kalke 1992; Salen-Picard and Arlhac 2002). A similar impoverishment occurred in the Mondego estuary in 2001 due to a large flood, but the effects on the benthic communities were immediate, mainly related to the physical and physiological stress caused by strong currents and low salinities, respectively (Chainho et al. 2006). The improving trends in Tagus benthic communities in both intertidal and subtidal areas were restored after this temporary

setback, indicating some resilience to natural events of short duration. Seasonal fluctuations and periodic events might result in misleading interpretations of long-term trends when studies are limited to shorter time scales.

Implications for Monitoring

Previous studies indicated that long-term comprehensive monitoring studies are needed to quantify the effectiveness of efforts undertaken to improve ecological quality of aquatic ecosystems, but not many long-term monitoring surveys have been conducted. Complex systems such as estuaries also require reliable estimates of natural variability because ecological responses to natural stress can be similar to those related to human pressure (Rhoads and Germano 1986; Reiss and Kröncke 2005; Chainho et al. 2007; Zettler et al. 2007). A 12-year dataset was used in the present study to understand how benthic invertebrates responded to the implementation of several restoration measures. Although the present study covered only a small extension of the polyhaline region of the Tagus estuary, the dataset resulted from a single monitoring program rather than from the compilation of results from multiple sources as is the case with most studies of long-term variations (e.g., Ysebaert et al. 2003; Borja et al. 2009). Studies with data from multiple sources often suffer from the problem of biased abundance and diversity calculations due to a lack of consistency in sampling techniques and/or devices (e.g., Ysebaert et al. 2003; de Paz et al. 2008). In the EXPO'98 monitoring program, the same collection methods and laboratory procedures were used during the entire study period, thus eliminating methodological effects as a source of variation requiring interpretation. In addition, samples were collected at spatial and temporal intervals that allowed for an adequate assessment of the natural variability inherent in benthic communities. These results confirm the importance of long-term studies conducted with consistent methods and adequate spatial and temporal coverage in understanding changes in the ecological condition of estuarine ecosystems. This study also demonstrated that both intertidal and subtidal communities respond to improvements in environmental conditions in similar ways both with respect to species composition and indicators of benthic community health. However, the level of change with respect to the classification of ecological status was substantially different between the two communities, suggesting that the assessment tools used may require some recalibration before being universally applied to intertidal habitats. The results emphasized the importance of long-term monitoring studies for understanding changes in ecosystem ecological status by demonstrating that periodic events such as floods and droughts can induce temporary changes in community trends that may confound the interpretation of anthropogenic effects. In addition, this study

showed that similar trends were observed between seasons despite the high seasonal variation observed in the benthic community composition. This suggests that reducing sampling effort to specific seasons may not undermine assessments of the effects of restoration measures paralleling results of an earlier study in Chesapeake Bay, USA (Alden et al. 1997). The selection of specific seasons for monitoring purposes would require the development of season specific reference criteria since the available classification methods for transitional waters may produce different status results when applied in different seasons (Chainho et al. 2007).

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