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Long-term environmental, climatic and anthropogenic factors affecting subtidal soft-bottom benthic communities, within the Basque coast

Maialen Garmendia*[a], Ángel Borja[a] and Iñigo Muxika[a]

Abstract

Relationships between environmental, climatic, and anthropogenic factors and benthic communities' distribution and structural patterns were studied along the Basque coast, northern Spain. The investigation was undertaken by applying both univariate analysis (Pearson's Common Correlations and Spearman Rank Correlations) and multivariate analysis (Canonical Correspondence Analysis (CCA) and Multidimensional Scaling Ordination (MDS)), to a dataset from the Basque Littoral Monitoring Network, which extends from 1995 to 2006. A total of 674 taxa of benthic organisms were recorded from 19 sampling stations.

Diogenes pugilator, Ampelisca brevicornis, Paradoneis armata, Spiophanes bombyx, Magelona johnstoni, Mediomastus sp., Magelona filiformis, Pisione remota, Edwardsia sp., Urothoe pulchella, Nassarius reticulatus and Nephtys cirrosa present the highest abundances and these 12 species represent more than 25% of the total abundance.

CCA, with forward selection of variables and associated Monte Carlo permutation tests (1000 permutations, $p<0.05$), showed that the considered variables (physico-chemical characteristics, anthropogenic parameters, and environmental or climatic variables) explained 33% of the total inertia. Environmental or climatic variables are the principal factors (17%) explaining differences in benthic communities' structure and distribution; whilst anthropogenic variables explain only 5% of the variability. Univariate analysis revealed that some structural parameters (such as AMBI and biomass) have improved significantly (<0.05) during the last decade, probably due to the sewerage and clean-up works undertaken in the Basque river basins and estuaries.

1. Introduction

New European legislation (see European Water Framework Directive (WFD), in Borja *et al.*, 2004b; Borja, 2005) emphasizes the importance of biological indicators, in order to establish the ecological quality of European coastal and estuarine waters. There are different biological elements which have to be studied, such as phytoplankton, macroalgae, benthos and fishes. Benthic invertebrates are used frequently as bio-indicators of marine status, as various studies have demonstrated that macrobenthos responds relatively rapidly to anthropogenic and natural stress (Pearson and Rosenberg, 1978; Dauer, 1993; Borja *et al.*, 2000, 2006d). In many estuaries and coastal regions close to industrial and urban areas, sediments act as a sink and become the greatest potential source of inorganic and organic contaminants in the marine environment.

In addition to the numerous anthropogenic disturbances that affect coastal environments, leading to habitat modification and changes in ecosystem functioning, these ecosystems are threatened also by global climate change. Changes in climate (e.g. temperature rise, sea level rise, increase in intensity and frequency of floods and droughts) may increase the risk of linear as well as abrupt and non-linear changes in many ecosystems, which would affect their composition, function, biodiversity and productivity (Cardoso *et al.*, 2007). When subjected to climate change, including changes in the frequency of extreme events, ecosystems may be disrupted as a consequence of differences in response times of species (IPCCWGI, 2001). Episodic events such as extreme rain events and flooding can result in the catastrophic deposition of fine sediments with profound influences on the structure and function of macrobenthic communities (Norkko *et al.*, 2002). Studies carried out on benthic populations and community-level processes are required for a holistic and integrative view of the response of an ecosystem to global climate change, preferably over the long time scales associated with such change. However, there are relatively few long time-series of biological measurements in estuarine/marine environments (e.g. Beukema, 1991, 1992; López-Jamar *et al.*, 1995; Beukema *et al.*, 1999; Borja *et al.*, 2006). Responses of biota to these environmental or climatic stressors are the integrated result of both direct and indirect processes which can be manifested as changes in abundance, diversity and fitness of individuals, populations and communities (Adams, 2005). The accelerating rate of biological impoverishment, due to those environmental and climatic stressors, may render ecosystems unable of compensating the loss of biodiversity, thereby

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reducing their resilience to climatic change (Vinebrooke *et al.*, 2004). Distinguishing and integrating the effects of natural and anthropogenic stressors is an essential challenge for understanding and managing coastal biotic resources (Vinebrooke *et al.*, 2004; Paerl, 2006).

1.1. The Basque coast

The Basque coast (150 km long, and orientated E-W, see Figure 1) is of high energy and faces the Bay of Biscay (for a recent description of the coastal features, see Borja and Collins, 2004). The continental shelf (0-200 m water depth) is less than 20 km wide in this region; this is due to the active role played by the north coast of Iberia, during the geological formation of the Bay of Biscay since the Jurassic. Most of the coastline is erosional, with extensive cliffs. Depositional areas associated with recent deposits are confined mostly to estuaries that have formed along lines of structural weakness, such as faults and diapirs. The estuaries are small, with a maximum length of around 20 km. The dominant wind direction and, therefore, wave approach is from the northwest; these cause a sand transport onto the eastern margin of the estuary mouths, where it forms sandy beaches and dunes.

1.1.1. Climate and meteorology

The Basque Country is located within the middle latitudes of the eastern North Atlantic Ocean (for this section, see also Usabiaga *et al.*, 2004). Therefore, there exist influences of the Gulf Stream and the atmospheric westerlies, in the middle and upper troposphere. As a consequence, the annual average air temperature is >10ºC. All the winter months have average temperatures well above -3ºC. So, the climate is temperate, oceanic, with moderate winters and warm summers. It is also rainy, with mean interannual precipitation in the coast between 1,000 and 2,000 mm. Rainfall occurs commonly during the year; even the driest month (July) has half of the rainfall of the wettest month (December). Therefore, according to Köppen´s classification, the area is associated with a Cfb climate (marine west coast-mild). The distribution of winds differs from season to season. Southerly winds occur less frequently during March–August, than in July–December. In autumn and winter, the northwesterly winds are weaker than in spring and summer; they very rarely reach 130 km/h (Usabiaga *et al.*, 2004).

1.1.2. Hidrography of the coastal water masses

Along the Basque coast, as in other temperate areas located at mid-latitudes, the annual cycle of the Sea Surface Temperature (SST) shows a marked seasonality, strongly related to atmospheric temperatures, at least for monthly averaged temperatures (Valencia, 1993; Valencia *et al.*, 1996, 2003; Borja *et al.*, 2000a). There are two clearly defined seasons, winter and summer, which are more or less stable and predictable; and two transitional seasons, spring and autumn, more irregular than the previous ones. In spring and autumn, the gradients of (respectively) increasing and decreasing temperature are very sharp. Hence, the date, week or even the month in which a SST value is reached varies strongly from year-to-year. Reversal in the trend of increasing SST occurs frequently during the spring season. The normal range of monthly averaged SST extends from lower than 12ºC in winter, to higher than 22ºC en summer (Borja *et al.*, 2000a).

The average temperature of the water column over the continental shelf, shows a seasonal cycle slightly more irregular than that of the sea surface temperature (Valencia, 1993). There is a delay of about two months between the extreme values, which indicate the change in trend of local cooling or warming in the sea surface and cooling or warming throughout the whole water column (Valencia, 1993). The

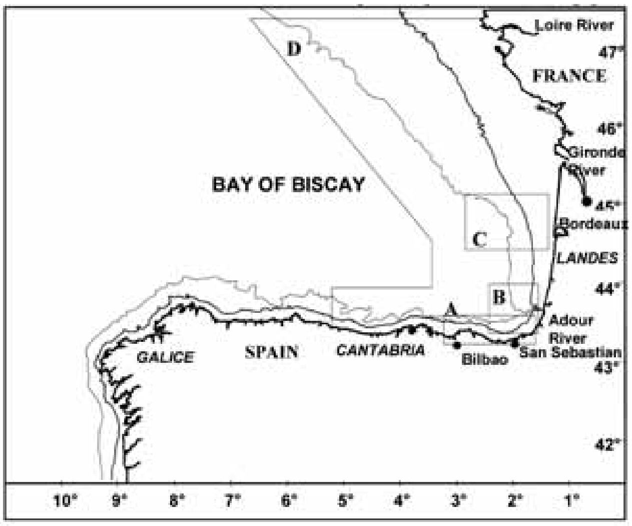

Figure 1: Map of the south Bay of Biscay. Contour lines indicate 100 m and 200 m isobaths. (A): Basque coast (see Figure 2 to distinguish the different estuaries). (B): Cap Breton Canyon and the Landes Plateau area. (C): Gironde River. (D): Adour River. (Figure from Borja and Collins, 2004).

pluriannual average temperature, for the upper 100 m layer of the waters over the continental shelf along the Basque coast is 13.9ºC (Valencia *et al.*, 2004).

Hence, general advection and associated mechanisms of upwelling or downwelling produce additional characteristics in the annual cycle of the shelf waters of the Basque coast. Anomalies are set up, with respect to typical seasonal or annual cycles in a quasistic water mass, in temperate areas. Further, changes in advection and the relative prevalence of upwelling and downwelling mechanisms can be considered in themselves a fundamental part of the annual cycle (Valencia *et al.*, 2004).

1.1.3. Main impacts on the coastal water masses

Due to its geographical placement and morphological peculiarities, the Basque coast forms a discontinuity in the SE part of the Bay of Biscay. Such a feature is formed by the Cap Breton Canyon (see Figure 1), between the flat coast and wide continental shelf on the French side and the rugged coast and narrow continental shelf on the Spanish side. Further, other meteorological and hydrological features (wind-driven transport, rainfall, river run-off, etc.) affect also the characteristics of the coastal water masses, in the southeastern Bay of Biscay. Such influences create anomalous patterns, in comparison with the conventional seasonal and annual cycles of the open oceanic areas, in temperate mid-latitudes.

In general, the shelf waters of the south-eastern Bay of Biscay show an over-continentalisation because of the strong terrestrial influence on the thermal and hydrological balances. In addition to direct land runoff, river inputs (dissolved and particulate loads) have more influence in the properties of the coastal waters, than direct rainfall. Such inputs are represented by the discharges of the large rivers along the French coast (Gironde and Adour) and the cumulative discharges of the numerous small rivers of the Basque coast (Valencia and Franco, 2004).

The rivers are the major routes for the input of dissolved and particulate materials, from the land to the sea. Heavy rainfall episodes are the main mechanism of input of terrestrial dissolved and particulate materials into the estuaries and the adjacent coastal areas. Hence, it is difficult to calculate the loads of various materials, because of the inaccuracy in determining flows and concentrations. Likewise, because of the lack of linearity between the factors in load calculations, especially in small torrential regime-dominated rivers (Lopez, 1986). Nonetheless, the increase in river flow appears to compensate partly the dilution of the concentrations and, finally, some significant correlations between nutrient fluxes and river flows can be estimated (Prego and Vergara, 1998).

As abovementioned, there is a large number of estuarine ecosystems along the Basque coast; these are strongly differentiated by the size of the basin and by hydrological, morphological and dynamic features. Additionally, it exists a high diversity between these systems, from the point of view of the anthropogenic pressure conditions (land uses, urban and industrial pollution, harbour activities, etc.) and characteristics of the water masses of the estuaries (Valencia and Franco, 2004).

The estuaries act as regulators of the inputs to the coastal areas: by trapping particulate materials, by precipitating some dissolved materials, by biological processes of nutrient uptaking; and, in general, by diluting the total concentrations of the different substances (Valencia and Franco, 2004).

Shelf waters are strongly modified by the atmospheric and continental influence, especially above the seasonal thermocline (Valencia, 1993; Valencia *et al.*, 1996). Advection and transport have also a great influence in the properties of the waters. Similarly, the balance between upwelling and downwelling, in turn, affects the ranges and pattern of distribution of multiple properties of the coastal and shelf waters (optical properties, oxygen, nutrients and chlorophyll concentrations, etc.) (Valencia and Franco, 2004).

1.1.4. Characteristics of marine sediments

On the Basque continental shelf muddy sediments appear to be frequently limited to an association with sandy deposits, forming muddy sands or sandy muds. Pure muds (silts and clays) occur infrequently in the coastal environment, but do occur offshore of the continental shelf; indeed, they are well developed at the head of the submarine canyons (Uriarte *et al.*, 2004).

Sandy sediments on the shelf appear in patches, with no real continuity (Iberinsa, 1990, 1992); this is due to the presence of numerous bathymetric irregularities. The principal sand concentrations are found in pronounced embayments and rias. In some other areas, the littoral dynamics contribute to the formation of sandy deposits; this is caused by the occurrence of some geographic obstacles (capes and rivers), where sediment transfer is hindered and sedimentation processes become enhanced. All these factors contribute to the existence of a sedimentary littoral prism, which is limited in terms of its extension and development (Uriarte *et al.*, 2004).

Most of the gravely sediments are associated with rocky outcrops, which determine the individual pebble composition. In some areas of the Cantabrian continental shelf calcareous and biogenic gravels are found; generally, these infill to some small depressions, which appear to have been produced during storm events (Flor, 1978). According to Flor *et al.* (1982), the carbonate content of the sediments on the Cantabrian continental shelf decreases towards the east (Uriarte *et al.*, 2004).

Sampling of superficial coastal sediments along the Basque coast has been carried out under the Littoral Water Quality Monitoring and Control Network (Borja *et al.*, 1996, 1997, 1998, 1999, 2000, 2001, 2002). The sediment along the Basque littoral consists mainly of sand (mean >80%), together with less than 6% (the mean value for all samples) of fine sediments. (Uriarte *et al*., 2004).

1.1.5. Benthic communities

The geographical and hydrodynamical features of the Basque coast determine the distribution of benthic communities. The coast is very steep, dominated by rocky substrata, with vertical cliffs and abrasion platforms, intercalated by sandy beaches.

It is also very exposed, as a result of its orientation towards N and NW (the direction of the dominant winds) and its own physiography and prevailing hydrodynamic regime. The physical setting determines the extent and composition of the communities, living in both the intertidal and the subtidal coastal habitats. The narrow continental shelf causes also the swell to reach the coast, with high strength.

For a description of the most important benthic communities, including estuarine and coastal, hard- and soft-bottom substrata, within the Basque coast, see Borja *et al.* (2004e). However, as this study is focused only in subtidal soft-bottom communities, only these communities are described below.

-*Pontocrates arenarius-Eurydice pulchra* community

This is a typical crustacean-dominated community, present from the low intertidal to 5-10 m water depth, in highly exposed sites, associated with coarse sand and gravel bottoms (Picard, 1965: Bellan and Lagardére, 1971). It is characterised by *Pontocrates arenarius, Haustorius arenarius, Eurydice pulchra, Iphinoe* sp., etc. In the Basque Country, this community appears both in the mouth of small estuaries, with low river flow and along the littoral sandy beaches (Lagardére, 1966; Martínez and Adarraga, 2001). In the latter case, some molluscs of the genus *Donax* and some annelida (*Dispio uncinata, Scolelepis mesnilii*, etc.) can also be found (Borja *et al.*, 2004e).

-*Tellina tenuis*-boreal community

This community was described firstly by Stephen (1930), and appears both in deep estuaries (20-30 m) and in the circalittoral area (60-100 m water depth); it is associated with mixed sediments, dominated by sand and mud (Cornet *et al.*, 1983). This community is very common in the Cantabrian estuaries and littoral sandy beaches (Cadée, 1968; Anadón, 1980; Junoy and Viéitez, 1990; Curras and Mora, 1991), although some changes in species composition are observed; for example, *Nephtys caeca* is replaced by *N. cirrosa* and *N. hombergii*. Along the Basque coast, the core of the community is represented habitually by *Tellina fabula*, more than by *T. tenuis* (Borja, 1989; Borja *et al.*, 1995, 1998). Besides *Tellina* and *Nephtys* species, the other main species are *Spiophanes bombyx*, *Gouldia minima*, *Nucula* sp., *Dentalium dentalis*, *Echinocardium cordatum*, *Dispio uncinata*, *Nephtys cirrosa*, *Cumopsis fagei*, *Diogenes pugilator*, *Glycera* sp., etc. In the intertidal areas, there are other species which are characteristic of the community, such as *Spio martinensis*, *Phyllodoce mucosa*, *Capitella capitata*, etc. (Garcia-Arberas, 1999). *C. capitata* is known widely as an indicator of organic enrichment but in the latter study it is found in much lower densities than in disturbed enviroments. Further, it is considered as a species with a wide ecological spectrum, inhabiting different types of sediments (García-Arberas and Rallo, 2002). In some sandy bottoms characterised as *Tellina* community, one of the main species was either the bivalve *Cerastoderma edule* or the polychaeta *Scoloplos armiger*. Their presence could indicate a transition towards a *Scrobicularia-Cerastoderma* community, or to a *Venus* community, respectively. The distinction between communities is not always clear and overlapping is often observed (Borja *et al.*, 2004e).

-*Venus fasciata* community

This community, described by Ford (1923), Thorson (1957) and Cabioch (1961), is typical of sandy bottoms in 20-40 m water depth. The most characteristic species are *Venus fasciata*, *Venus casina*, and *Chamelea striatula*; but also *Nephtys cirrosa*, *Urothoe brevicornis*, *Bathyporeia elegans*, *Prionospio steenstrupi*, *Echinocardium cordatum*, *Branchiostoma lanceolatum*, *Spisula subtruncata*, etc. This community could be related with *Chamelea gallina* community as described by Thorson (1957) and Pérés (1967); this is associated with the sublittoral zone, between 5 and 40 m water depth, and fine or muddy sand bottoms (Borja *et al.*, 2004e).

In the Basque Country, in sublittoral bottoms, the coexistance of the two latter communities (*Tellina* and *Venus*) demonstrate the close interrelations between them (Borja *et al.*, 1995, 1998; Martínez and Adarraga, 2001); as such, they are sometimes very mixed and indistinguishable (Borja *et al.*, 2004e).

-*Dendrodoa grossularia-Smittina trispinosa* community

This community, described by Cabioch (1961), appears in littoral gravel bottoms, between 25 and 50 m water depth. Besides *Dendrodoa* and *Smittina*, there are other species representative of this community, such as *Diastylis laevis* (Borja *et al.*, 2004e).

-*Amphiura* community

The distribution of this community ranges from 70 to 150 m water depth, in very fine sediments; it was described firstly by Thorson (1957). For the Basque coast, Martínez and Adarraga (2001) found that *Amphiura* (*A. chiajei* and *A. filiformis*) are under-represented (<5 ind.m^{-2}), with the most abundant species being: *Thyasira flexuosa*, *Prionospio fallax*, *Lumbrineris gracilis*, *Ampharete finmarchica*, *Chaetozone setosa*, *Terebellides ströemi*, *Turritella communis*, *Nephtys* sp., *Pulsellum lofotense*, etc. (Borja *et al.*, 2004e).

-*Auchenoplax crinita- Paradiopatra calliopae- Ditrupa arietina* community

This community is characteristic of the southeastern Bay of Biscay (Cornet *et al.*, 1983), in sandy bottoms from 150 to 250 m water depth; it has been identified in the Basque Country by Martínez and Adarraga (2001). The community represents the transition from the continental shelf to the continental slope. Other species that appear in the community are *Onchnesoma steenstrupii*, *Galathowenia oculata* and *Terebellides ströemi*. Within the lower part of the depth range appear *Nothria hispanica*, *Thyasira ferruginosa*, *Abra longicallus*, *Euchone incolor*, etc. (Borja *et al.*, 2004e).

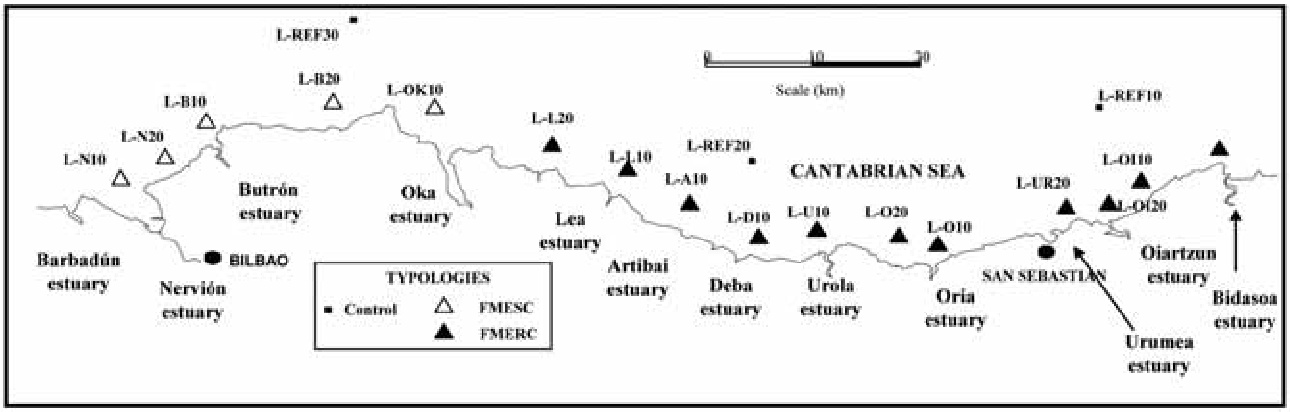

Figure 2 Sampling stations within the Littoral Water Quality Monitoring and Control Network of the Basque Country, at present (FMESC: Full marine exposed, sandy coast; FMERC: Full marine exposed, rocky coast) (Borja *et al.*, 2004b). See also Table 1.

1.2. The littoral water quality monitoring and control network

The Department of Land Action and Environment of the Basque Government, by means of the Littoral Water Quality Monitoring and Control Network (LQM) has monitored the Basque coastal and estuarine water quality since 1994 (Borja *et al.*, 1996; 2003b; 2006c). This network comprises the analyses of both physico-chemical (in water, sediment and biota, the latest only in estuaries) and biological elements (phytoplankton, macroalgae, benthic macroinvertebrates and fishes, the latest only in estuaries).

The LQM series data includes 19 coastal and 32 estuarine sampling stations (Figure 2, Table 1). Only data from the coastal stations have been used in this study.

From 1994 to 2001, the LQM has been used for assessing the evolution of the marine waters quality under the development of various sewerage schemes (Franco *et al.*, 2004; Gorostiaga *et al.*, 2004), contributing to the knowledge of pollutant ranges and backgrounds in different matrices, such as: (i) waters (Belzunce *et al.*, 2004a; Bald *et al.*, 2004); (ii) sediments (Belzunce *et al.*, 2004b; Rodríguez *et al.*, 2006); and (iii) biota (Borja *et al.*, 2004a; Marigomez *et al.*, 2004). Likewise, since 2001, the LQM has been used for the implementation of the European Water Framework Directive (WFD), using the database as a useful tool in the development of new methods in assessing the Ecological Quality Status (Borja *et al.*, 2003c; 2004b). The important relationships between the WFD and benthic communities can be seen in Borja, 2005; Borja *et al.*, 2000, 2004, 2006, 2007; and Muxika *et al.*, 2007.

1.3 Objectives

The objectives of this study deal with the effect of multiple stressors (environmental or climatic, and anthropogenic) on benthic ecosystems of the Basque coast at the community level. In order to improve our knowledge of benthic communities' structure and distribution, the main goals of the present study are: (i) Verify the role of physico-chemical properties of the sediments in the benthic communities' strucure; investigating anthropogenic influence and environmental/climatic changes that affect to the benthic communities. (ii) Investigate inter-annual changes in the community composition, at each of the 12 coastal stations operating from 1995. (iii) Study the tendencies along the data series, to determine trends in the different variables, in relation to climatic changes or anthropogenic influence.

2. Methods

2.1. Sampling stations

As explained before, the LQM includes 32 estuarine and 19 coastal stations; however this work is only focused on the coastal ones (Figure 2, Table 1). From these, three stations are reference or control stations (REF-10, REF-20 and REF-30). The rest of the stations are associated to different river basins. Some of them have 2 stations, and this is the case of Oiartzun (L-OI10; L-OI20), Oria (L-O10; L-O20), Nervión (L-N10; L-N20), Lea (L-L10; L-L20) and Butrón (L-B10; L-B20). Other river basin districts have a unique station (Bidasoa (L-BI10), Deba (L-D10), Oka (L-OK10), Urola (L-U10), Urumea (L-UR10) and Artibai (L-A10)), without taking into account the reference stations. Although there are 19 coastal stations, this study focuses only in the 12 stations operating since 1995.

2.2. Selection of parameters

The LQM comprises the analysis of both physico-chemical (in water, sediment and biota) and biological elements (phytoplankton, macroalgae, benthos and fishes) but only physico-chemical data of sediments and benthos biological elements are used in this study .

Soft-bottom macrobenthic communities are sampled annually, always in winter, using a van Veen grab (see sampling methods in Borja *et al.*, 2003b). The benthic structural parameters which are determined include abundance, biomass, species richness (number of *taxa*), Shannon's diversity index, Pielou's evenness, maximum diversity and AMBI (Borja *et al.*, 2000). Guidelines derived from Borja and Muxika (2005) are used in the calculation of AMBI, using the species list of July 2006. Sediments are sampled also annually, in winter; measured parameters are general variables (grain size, organic matter, Redox potential, C/N, POC (Particulate Organic Carbon) and PON (Particulate Organic Nitrogen), heavy metals (Mn, Ni, Pb, As, Cd, Cr, Cu, Fe, Hg and Zn) and organic compounds (Total PCBs, Alfa HCH, Gamma HCHs, Total HCHs, Total HCBs, Aldrin, Dieldrin, Total DDTs,Trans_ Nona and Total PAHs) . The WFD does not include reference to the sediment physico-chemical characteristics; however, due to their importance, they are taken into account in the LQM in order to assess marine environmental quality.

Finally, some environmental or climatic factors from different sources (Table 2) have been analysed:

- Data series of east-west (u) and north-south (v) geostrophic wind components, from 1995 to 2006, were obtained from NOAA (National Oceans & Atmosphere Administration),

Table 1: Coastal stations sampled in the littoral water quality monitoring and control network and their location. (date= when they started being sampled).

Station	Location	UTMX	UTMY
	LITTORAL		
	1995		
L-B10	Coast of Gorliz	503723	4809563
L-B20	Coast of Bakio	516022	4810728
L-BI10	Coast of Hondarribia	597114	4805780
L-D10	Coast of Deba	552606	4797494
L-L10	Coast of Elantxobe	533700	4805814
L-N10	Coast of the Abra	493466	4803512
L-N20	Coast of Sopelana	498434	4805360
L-O10	Coast of Orio	570211	4795303
L-OI10	Coast of Pasaia	586643	4799066
L-OK10	Coast of Mundaka	524251	4810031
L-U10	Coast of Zumaia	561520	4796532
L-UR20	Coast of Tximistarri	584831	4799191
	1998		
L-A10	Coast of Ondarroa	548545	4798500
	2002		
L-O20	Coast of Getaria	566590	4796395
L-OI20	Coast of Pasaia (Asabaratza)	589907	4801608
L-REF10	Continental Platform (Reference)	587651	4811946
	2003		
L-L20	Coast of Lekeitio	541453	4802562
	2006		
L-REF20	Coast, in front of Deba	556687	4805561
L-REF30	Coast in front of Bakio	516170	4816448
TOTAL			19

FNMOC (Fleet Numerical Oceanographic Centre) and PFEG (Pacific Fisheries Environmental Group). The FNMOC supplies surface pressure fields from one-degree surface, at mean sea level (mb), every 6 h. The following daily data are then derived: wind speed cubed, or turbulence, and the upwelling index along the French and Spanish coasts, as described in Borja *et al.* (1996, 1998b). On the basis of the daily data, monthly and yearly means were derived. The upwelling index (U) is computed by adding the average monthly positive values of upwelling along both Spanish and French coasts from March to July for each year as:

$$U_y = \sum_{m=3}^{7} \left(I_{Fym} + I_{Sym} \right), \ \forall I_{Fym}, I_{Sym} \phi 0 \qquad (1)$$

, where IF and IS are the monthly upwelling values per km of the French and Spanish coasts (respectively) and m refers to the month of the year y.

- The Aquarium of San Sebastián (Oceanographic Society of Gipuzkoa, Spain) has monitored SST since 1947; it constitutes the longest oceanographic series of the southeastern Bay of Biscay.
- Daily sun hours, provided by the 'National Institute of Meteorology' (Observatory of Igeldo, San Sebastián).
- French rivers daily flow (Bordeaux Port Authority, for the Gironde River; and national database on hydrometry
- and hydrology (www.hydro.eaufrance.fr/accueil.html), for the Adour River).
- Basque rivers daily flow (Gipuzkoa County Council www4.gipuzkoa.net/oohh/web/esp/index.asp), for Bidasoa, Oiartzun, Urumea, Oria, Urola and Deba rivers; and Bizkaia County Council (www.bizkaia.net/Ingurugiroa_Lurraldea/Hidrologia/ca_DatosHistorico.htm), for Artibai, Lea, Oka and Nervión rivers).
- Daily rainfall in Gipuzkoa and Bizkaia, provided by the "National Institute of Meteorology" (Observatories in Igeldo (San Sebastián, Gipuzkoa) and in Sondika airport (Bizkaia)).
- North Atlantic Oscillation (NAO) and Eastern Atlantic (EA) pattern data (www.cgd.ucar.edu/cas/jhurrell/indices.data.html#naopcann). The calculation of winter NAO index is based on the difference of the normalised sea level pressure (SLP) between Lisbon (Portugal) and Stykkisholmur/Reykjavik (Iceland).

2.3. Data analysis: Pearson's correlations

In order to investigate correlations between physico-chemical variables (general parameters, organic compounds and heavy metals) and structural parameters and between environmental or climatic variables and structural parameters, Pearson's Common Correlations were run out and two matrices were built up. These matrices show Pearson's correlations between each pair of variables.

- The first matrix shows correlations between benthic communities' structural parameters and some physico-chemical sediment parameters, in order to see if there is any relation between the communities' structure and anthropogenic influences.
- The second matrix shows correlations between the structural parameters and some environmental or climatic variables. It is convenient to point out that, in this case, not all the structural parameters were analysed, but only 5 were used in the analysis: Abundance, Diversity, Biomass, Species Richness and AMBI, as theywere previously determined as those explaining more variability in the community (Muxika *et al.*, 2007). Taking into account the series length, only stations sampled every year were used (see Table 1), as the recently incorporated to the LQM are

Table 2 Description of the dataset of environmental/climatic variables used for the analyses, including source and data frequency.

DATA	AREA	SOURCE	DATA FREQUENCY
Sea Surface Temperature	San Sebastián	Aquarium	daily
Sun hours	San Sebastián	Meteorological Observatory	monthly
Wind vectors	45º N, 2º W	PFEG-NOAA-FNMOC	6 hours
NAO and EA	North Atlantic	Web site	monthly
River Flow	Gironde	Bordeaux Port Authority	daily
River Flow	Adour	HYDRO Database	daily
River Flow	Gipuzkoa county	Web site	daily
River Flow	Bizkaia county	Web site	daily
Precipitation	San Sebastián	Meteorological Observatory	daily
Precipitation	Sondika	Meteorological Observatory	daily

too short to show temporal trends or climatic influence. On the other hand, as the samples were taken in winter (January to March), the correlations were undertaken with climatic factors of the previous year (such as rainfall, river flow, upwelling, etc.), excepting winter NAO or data from the same winter period. This makes only 11 complete cases for each series.

These analyses were carried out using STATGRAPHICS plus 5.0 package.

2.4. Data analysis: Canonical Correspondence Analysis

Studies dealing with numerical descriptions of communities in relation to environmental or climatic factors are important to understand the processes that determine the structure and distribution of communities. Species responses to environmental or climatic conditions cannot be inferred in a causal way from multivariate analysis or any other statistical method; however, these techniques are useful to identify spatial distribution patterns and to assess which of the included environmental or climatic variables contribute most to species variability and which factors should be experimentally tested (Díez *et al.*, 2003).

Multivariate analysis techniques were applied to examine the relationships between species distributions and the distributions of the associated environmental or climatic factors (NAO, EA, SST, rainfall…) and anthropogenic factors (heavy metals and organic compounds). The benthic data set analysed was truncated and a new data set including only 275 species (those species whose frequency was >3% of occurrence) from the total of 674 species recorded was obtained.

The selection of the appropriate type of analysis depends on whether the species response to the environmental or climatic and anthropogenic variables is linear or unimodal (the best performance around some environmental or climatic optima). One way to determine this relationship is to analyze the species data first by Detrended Correspondence Analysis (DCA) and to examine the length of the maximum gradient. If the gradient exceeds 3 sd (sd=standard deviation), the data show unimodal response (Hill and Gauch, 1980). In this case, the gradient exceeded 3 sd and subsequently a unimodal ordination method was carried out. Canonical Correspondence Analysis (CCA) was selected among unimodal methods. This is a direct gradient analysis that displays the variation of benthic community in relation to the included environmental or climatic factors by using environmental or climatic data to order samples (Kent and Coker, 1992). This method combines multiple regression techniques together with various forms of correspondence analysis or reciprocal averaging (Ter Braak, 1986, 1987). The statistical significance of the relationship between the species and the whole set of environmental or climatic variables was evaluated using Monte Carlo permutation tests.

The multivariate analysis was carried out in three steps:

- In the first step influence of physico-chemical properties of the sediments in the benthic communities was studied. Physico-chemical properties that were not significant were eliminated for the next steps.
- In the second step anthropogenic variables (organic compounds and heavy metals) were studied and physico-chemical significant variables were taken into account as covariables. Not significant anthropogenic variables were eliminated for the next step.
- In the third and last step environmental or climatic variables were studied and physico-chemical and anthropogenic significant variables were taken into account in the analysis as covariables.

In the three CCAs carried out, communities' structural parameters were represented in the diagrams as supplementary variables, in order to see their distribution without taking them into account.

Ordination analysis and Monte Carlo tests were carried out using the computer program CANOCO 4.0 (Ter Braak and Smilauer, 1998). The results were represented using the computer program CANODRAW 3.0 (Smilauer, 1992).

2.5. Data analysis: Non Metric Multidimensional Scaling

Inter-annual changes in community composition have been visualised from non-metric multidimensional scaling (MDS) plots based on triangular matrices of Bray-Curtis (B-C) similarities using 4th root transformed species abundance data.

The number of individuals of each species at each station and sampling period was grouped in classes or phylum (Cnidaria, Nemertina, Nematoda, Annelida, Gastropoda, Bivalvia, Crustacea, Sipuncula, Echinodermata, Cephalochordata). These data aggregations were undertaken because of computational limitations in the computer used, and because ordinations undertaken on large matrices are unlikely to reveal any clear and reliable patterns (Clarke, 1993).These transformations were made to prevent abundant species from influencing the B-C similarity measures excessively (Clarke and Green, 1988; Clarke, 1993). Multidimensional scaling (MDS) ordination was subsequently used to plot spatial and temporal relationships in the B-C Similarities for the 12 stations and 11 sampling periods. The computer package PRIMER (Clarke and Gorley, 2001) was employed for all non-metric ordinations in this study, and the final configurations presented were the best solutions (i.e. exhibited the lowest 'stress' values, or least distortion).

Although these plots indicate the relative magnitude of community change between years, and the relative directions of change, they do not in themselves enable to place a value judgement on this change, such as AMBI or diversity. That is why tendencies of the different parameters and variables have been analysed in the next part of the work.

2.6. Data analysis: Goodness-of-fit tests and Correlations

Finally time-series tendency was analysed, in order to see any trend or gradient during the studied period. First of all, each variable was analyzed, by Chi-square and Kolmogorov-Smirnov test, in order to study if it could be adequately modelled by a normal distribution.

Then Spearman Rank Correlations were run out to study the trend of those variables that cannot be modelled by a normal distribution. In contrast to the more common Pearson correlations, the Spearman coefficients are computed from the ranks of the data values rather than from the values themselves. Consequently, they are less sensitive to outliers than the Pearson coefficients. These correlations have been calculated between each variable and the studied time-period. With the variables that could be adequately modelled by a normal distribution Pearson's´ common correlations (abovementioned) were calculated between each variable and the studied time-period.

As abovementioned two matrices were built up:

- One, where physico-chemical (general parameters, heavy metals and organic compounds) were analysed along time, taking into account all the stations.
- Another, where environmental or climatic factors were studied along the 12 years, taking into account only the stations sampled since 1995 (see Table 1).

These analyses were also carried out using STATGRAPHICS plus 5.0 package. Structural parameters with a significant tendency were also represented in MICROSOFT OFFICE EXCEL 2003 in order to visualize the parameters trend along the 12-year period.

Table 3 Top 14 dominant taxa of soft-bottom invertebrates taking into account all the samples in each year, ranked in decreasing order of abundance (in individuals and percentage).

SPECIE	ABUNDANCE	%
Diogenes pugilator	2,967	4.15
Ampelisca brevicornis	2,627	3.67
Paradoneis armata	2,109	2.95
Spiophanes bombyx	1,614	2.26
Magelona johnstoni	1,451	2.03
Mediomastus sp.	1,383	1.93
Magelona filiformis	1,333	1.86
Pisione remota	1,265	1.77
Edwardsia sp.	1,203	1.68
Urothoe pulchella	1,061	1.48
Nassarius reticulatus	766	1.07
Nephtys cirrosa	684	0.96
NEMERTINA	568	0.79
Prionospio steenstrupi	541	0.76
Hippomedon denticulatus	254	0.35
MYSIDA	191	0.27
TOTAL		27.99

Table 4 Structural parameters and dominant taxa for each station (mean values for the whole studied period). N: number of samples.

STATION	N	TOTAL ABUNDANCE (ind)	BIOMASS ($g.m^{-2}$)	SPECIES RICHNESS (n sp)	DIVERSITY ($bit.ind^{-1}$)	AMBI	DOMINANT TAXA (abundance) (%)
L-A10	9	311.4	16.5	26.2	3.9	1.5	52.11
L-B10	12	222.1	0.5	15.7	2.6	1.6	41.96
L-B20	12	188.1	2.4	19.3	3.2	1.1	53.87
L-BI10	12	350.5	2.9	18.7	2.7	1.5	44.22
L-D10	12	412.9	2.7	31.4	3.9	1.5	53.57
L-L10	12	322.9	4.5	34.7	4.2	1.4	52.28
L-L20	4	230.1	1.4	23.3	4.0	1.4	41.50
L-N10	12	135.2	5.3	19.1	3.6	1.4	35.79
L-N20	12	64.4	0.8	10.8	2.9	1.8	47.51
L-O10	12	436.9	3.9	30.5	3.7	1.4	43.63
L-O20	5	286.7	8.7	22.4	3.7	1.3	49.95
L-OI10	12	727.6	12.8	19.2	2.7	2.3	55.84
L-OI20	5	157.0	0.6	12.4	2.6	2.0	39.62
L-OK10	12	206.1	16.6	20.5	3.5	0.9	46.72
L-RF10	5	799.0	24.5	51.6	5.0	1.6	69.81
L-RF20	1	469.3	1.8	44.0	5.0	2.6	66.69
L-RF30	1	960.0	4.9	67.0	4.8	0.9	25.42
L-U10	12	417.9	9.0	30.9	3.6	1.2	47.76
L-UR20	12	1790.3	14.2	20.4	2.4	3.4	89.30

3. Results

3.1 Species and samples composition

A total of 674 invertebrate taxa were identified from the 173 samples. Dominant *taxa* included *Diogenes pugilator* (4.1%), *Ampelisca brevicornis* (3.7%), *Paradoneis armata* (2.9%) and *Spiophanes bombyx* (2.3%). Together, the top 14 dominant taxa, made up more than the 25% of total abundance of all identified species (Table 3).

In Table 4 structural parameters and the dominant taxa, from all stations, are shown. All stations, except L-UR20, have in general low values of AMBI and high species richness, as an indicator of their good quality or status.In addition, in station L-UR20 AMBI equals 3.4, which is relatively high in comparison with the remainder of the stations. L-RF30, L-OK10 and L-B20 show the lowest AMBI values (0.9, 0.9 and 1.1, respectively).

L-UR20 also shows the highest abundance with 1790.3 individuals (mainly Annelida), which suggests that high densities not always indicate good quality or status.

In general, the dominant *phylum* is Annelida, although Crustacea dominates in some stations and Nematoda dominates in one (see Table 4). Other important *taxa* that are not dominant but which are important because of their high abundance are Cnidaria and Bivalvia.

Nemertina, Sipuncula, Echinodermata and Cephalochordata are also present in most of the stations, but in very low relative abundances.

Reference stations (RF) show the highest values of species richness and diversity; some stations show high AMBI values (L-UR20, L-OI10, L-OI20) (probably related to the urban and industrial discharges in the zone).

Abundances for each station and year (Table 5) show that almost all the stations with the highest densities belong to two river basin districts: Oiartzun and Urumea (both with some anthropogenic impacts). However, other stations, such as L-D10 (2000), L-BI10 (2001), L-U10 (2006) and L-O10 (1998) showed also high levels of abundance.

Table 5 Top 14 sampling stations, ranked in decreasing order of abundance (in individuals).

STATION	TOTAL ABUNDANCE
L-UR20 (1997)	9,265
L-UR20 (2001)	4,852
L-OI10 (2001)	3,563
L-UR20 (2006)	1,766
L-OI10 (1996)	1,722
L-BI10 (2001)	1,625
L-UR20 (2000)	1,554
L-D10 (2000)	1,441
L-UR20 (1999)	1,257
L-OI10 (2004)	1,106
L-O10 (1998)	1,049
L-OI10 (1997)	1,045
L-UR20 (1996)	1,016
L-U10 (2006)	946

3.2. Data analysis: Pearson Correlations

As explained in the methodology chapter two matrices were built:

- Physico-chemical variables *vs.* benthic structural parameters.
- Environmental or climatic variables *vs.* benthic structural parameters.

Once both matrices were built, variables correlated with structural parameters were chosen (see Table 6). Hence, the number of variables was reduced. This was the main purpose of this section indeed, since there wasa large number of variables and only those that were correlated with benthic structural parameters were considered interesting for this work.

3.3 Data analysis: Canonical Correspondence analysis

3.3.1 Detrended Correspondence Analysis (DCA)

Table 7 shows a summary of the DCA using detrending-by-segments. The summary has an additional line for the lengths of the gradient. The length of the gradient is a measure of how unimodal the species responses are along an ordination axis. It is the range of the sample scores divided by the average within species standard deviation along the axis.

In this case the DCA revealed an environmental gradient of long length (6.06 standard deviation units), indicating that the species show a unimodal distribution.

Tabla 6 List of variables used in the different analysis after realising a leak of variables with Pearsons' Correlations.

STRUCTURAL PARAMETERS		
Diversity	Density	Richness
AMBI	Biomass	
PHYSICO-CHEMICAL PARAMETERS		
General parameters		
% of Gravel	% of Silt/Clay	POC
% of Sand	% of organic mater	PON
Redox potencial		
Anthropogenic variables		
Mn	As	Cr
Ni	Cd	Cu
Pb	Zn	Fe
Hg	Total PAHs	
ENVIRONMENTAL OR CLIMATIC VARIABLES		
Turbulence	Summer SST	Adour river annual flow
Upwelling	Autumn SST	Winter NAO
Annual SST	Sunshine	Annual precipitation in Gipuzkoa
Winter SST	Annual EA	Annual precipitation in Bizkaia
Spring SST	Gironde river annual flow	Oria river annual flow
Urola river annual flow	Urumea river annual flow	Lea river annual flow
Deba river annual flow	Oiartzun river annual flow	Artibai river annual flow
Nerbioi river annual flow	Oka river annual flow	

Table 7. Summary of the DCA: see that the maximum length of gradient is 6.064.

AXES	1	2	3	4	TOTAL INERTIA
Eigenvalues	0.869	0.586	0.491	0.345	17.809
Lengths of gradient	6.064	5.140	3.954	3.587	
Cumulative percentage variance of species data	4.9	8.2	10.9	12.9	
Sum of all eigenvalues					17.809

3.3.2 First step: General physico-chemical parameters

As described in the Methods the multivariate analysis was carried out in three steps.

The resulting diagram (Figure 3) from the first CCA analysis shows species (or taxa) which represent the dominant patterns in community composition, while each significant ($p<0{,}05$) physico-chemical variable (POC, PON, Redox potential, % of silt/clay, % of gravel and % of sand) across the diagram shows the direction of the change, and its length is proportional to the rate of change. The species triangles and the physico-chemical variables arrows jointly reflect the relative distribution of species along each of the physico-chemical variables. Samples and structural parameters (supplementary variables) are also represented.

Table 8 shows a summary of the second CCA analysis. The eigenvalues measure the importance of each of the axes (values between 0 and 1). The eigenvalues for the two first axes are

0.55 and 0.483. The total inertia is the total variance in the species data, 17.809 in this case.

The species-environment correlation (physico-chemical variables in this case) measures the strength of the relation between species and physico-chemical variables for a particular axis. The species-environment correlations of the first two axes are high: 0.856 and 0.839, respectively.

Species and environmental variables (general physico-chemical variables in this case), based on the first two axes, explain 5.8% of the variance (inertia) in the species data and 52.2% of the variance in the species-environment (general physico-chemical variables) relation.

As explained before, the percentage of variance explained by each variable was calculated. Among significant ($p<0.05$) general physico-chemical parameters, the grain size (including % of sand, % of gravel and % of silt/clay) explains more than 50% of the inertia explained by all the general physico-chemical variables. In contrast, PON explains the minor percentage of inertia (10%) (data not presented).

Before undertake the second step, non significant physico-chemical variables were removed, organic matter in this case.

3.3.3 Second step: Anthropogenic variables

In the second step, another CCA was carried out including anthropogenic variables. The influence of the significant physico-chemical variables was also taken into account, including them as covariables.

In the resulting diagram (Figure 4) from the second CCA analysis, red arrows represent the significant ($p<0,05$) anthropogenic variables, Cr, As, Mn and Fe in this case.

Table 9 shows a summary of the second CCA analysis. The eigenvalues for the two first axes are 0.304 and 0.232. The species-environment correlation (anthropogenic variables in this case) measures the strength of the relation between species and anthropogenic variables for a particular axis. The species-environment correlations of the first two axes are 0.765 and 0.695, respectively.

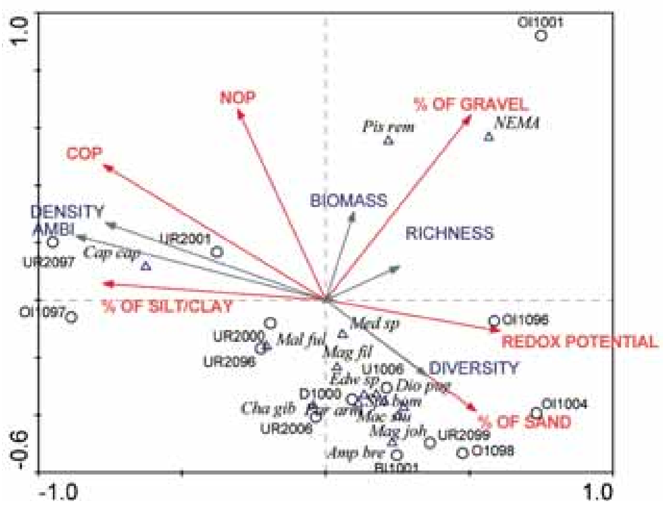

Figure 3 Canonical Correspondence Analysis ordination for 275 taxa with respect to 6 physico-chemical variables (red arrows). Samples (circles), communities structural parameters (blue arrows), species (triangles). Only the most abundant species (14) and the richest stations (14) in abundance are represented.

Table 8 Summary of the CCA with physico-chemical variables (1st step).

AXES	1	2	3	4	TOTAL INERTIA
Eigenvalues	0.55	0.483	0.388	0.272	17.809
Species-environment correlations	0.856	0.839	0.787	0.68	
Cumulative percentage variance:					
of species data	3.1	5.8	8	9.5	
of species-environment relation	27.8	52.2	71.8	85.6	
Sum of all eigenvalues					17.809
Sum of all canonical eigenvalues					1.979

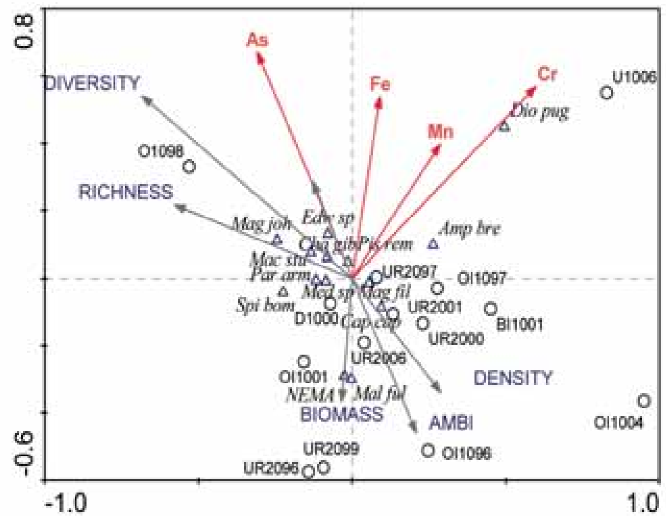

Figure 4 Canonical Correspondence Analysis ordination for 275 taxa with respect to 11 anthropogenic variables (red arrows: only represented the 4 significant ones). Samples (circles), communities structural parameters (blue arrows), species (triangles). Only the most abundant species (14) and the richest stations (14) in abundance are represented.

Species and environmental variables (anthropogenic variables), based on the first two axes, explain 3.4% of the variance (inertia) in the species data and 62.6% of the variance in the species-environment (anthropogenic variables) relation.

Covariables explain 11.1% of the inertia and the anthropogenic variables, 4.8%.

Non significant anthropogenic variables were removed (Cd, Cu, Hg, Ni, Pb AND Zn) and significant ($p<0,05$) anthropogenic variables were included with the physico-chemical variables as covariables for the next CCA (significant anthropogenic variables are: Cr, As, Mn and Fe).

3.3.4 Third step: Environmental and climatic variables

In the third step, environmental and climatic variables such as upwelling, sunshine, flows, etc. were included in the CCA, in order to study their effect on macrobenthic communities. As abovementioned significant physico-chemical and anthropogenic variables were used as covariables, and community structural parameters as supplementary variables. The resulting diagram (Figure 5) from this CCA shows environmental and climatic variables as red arrows.

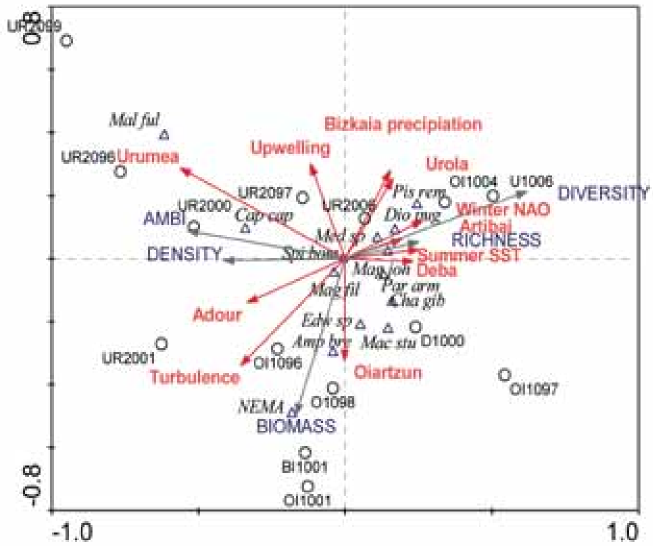

Figure 5 Canonical Correspondence Analysis ordination for 275 *taxa* with respect to 23 environmental or climatic variables (red arrows). Samples (circles), communities structural parameters (blue arrows), species (triangles). Only the richest species (14) and the richest stations (14) in abundance are represented.

Table 9 Summary of the CCA with anthropogenic variables (2nd step).

AXES	1	2	3	4	TOTAL INERTIA
Eigenvalues	0.304	0.232	0.181	0.139	17.809
Species-environment correlations	0.765	0.695	0.748	0.655	
Cumulative percentage variance:					
of species data	1.9	3.4	4.5	5.4	
of species-environment relation	35.5	62.6	83.7	100	
Sum of all eigenvalues					15.830
Sum of all canonical eigenvalues					0.855

Although not all significant ($p<0.05$) environmental and climatic variables are represented in the plot, the significant ($p<0.05$) ones were: Urumea river flow, Oiartzun river flow, Bizkaia rainfall, Winter NAO, Adour river flow, Gironde river flow, EA, Artibai river flow, winter SST, Deba river flow, Turbulence, Sunshine, summer SST, Urola river flow, annual SST and spring SST.

Table 10 summarizes third CCA results. The eigenvalues for the two first axes are 0.431 and 0.349. The species-environment correlations of the first two axes are 0.926 and 0.803, respectively.

Species and environmental variables based on the first two axes, explain 5.2% of the variance (inertia) in the species data and 25.7% of the variance in the species-environment relation.

The covariables explain 15.9% of the inertia and the environmental and climatic variables (removing covariables) explain 17.0%.

Moreover, the percentages of variance explained by each variable were calculated. Considering all the significant ($p<0.05$) environmental variables, all river flows together explain almost 50% of the inertia explained by the environmental variables. Other variables that explain an important percentage of inertia are rainfall (7.2%), Winter NAO (6.9%), Sunshine (6.6%) and EA (5.9%). In contrast the variables that explain the minor percentage of inertia are turbulence (4.6%) and SST 4-5.6%).

Table 10 Summary of the CCA with environmental or climatic variables (3rd step).

AXES	1	2	3	4	TOTAL INERTIA
Eigenvalues	0.431	0.349	0.28	0.246	17.809
Species-environment correlations	0.926	0.803	0.77	0.853	
Cumulative percentage variance					
of species data	2.9	5.2	7.1	8.7	
of species-environment relation	14.2	25.7	34.9	43	
Sum of all eigenvalues					14.974
Sum of all canonical eigenvalues					3.035

3.4 Data analysis: Non-Metric Multidimensional Scaling

A series of MDS ordinations were constructed to investigate the collective nature of temporal trends in community structure along the coast. Only data for stations operating from 1995 were considered in these analyses, as the remainder of the stations were sampled during a short period of time and their inclusion would have resulted in an unbalanced design, and biased estimate of macrobenthic abundance.

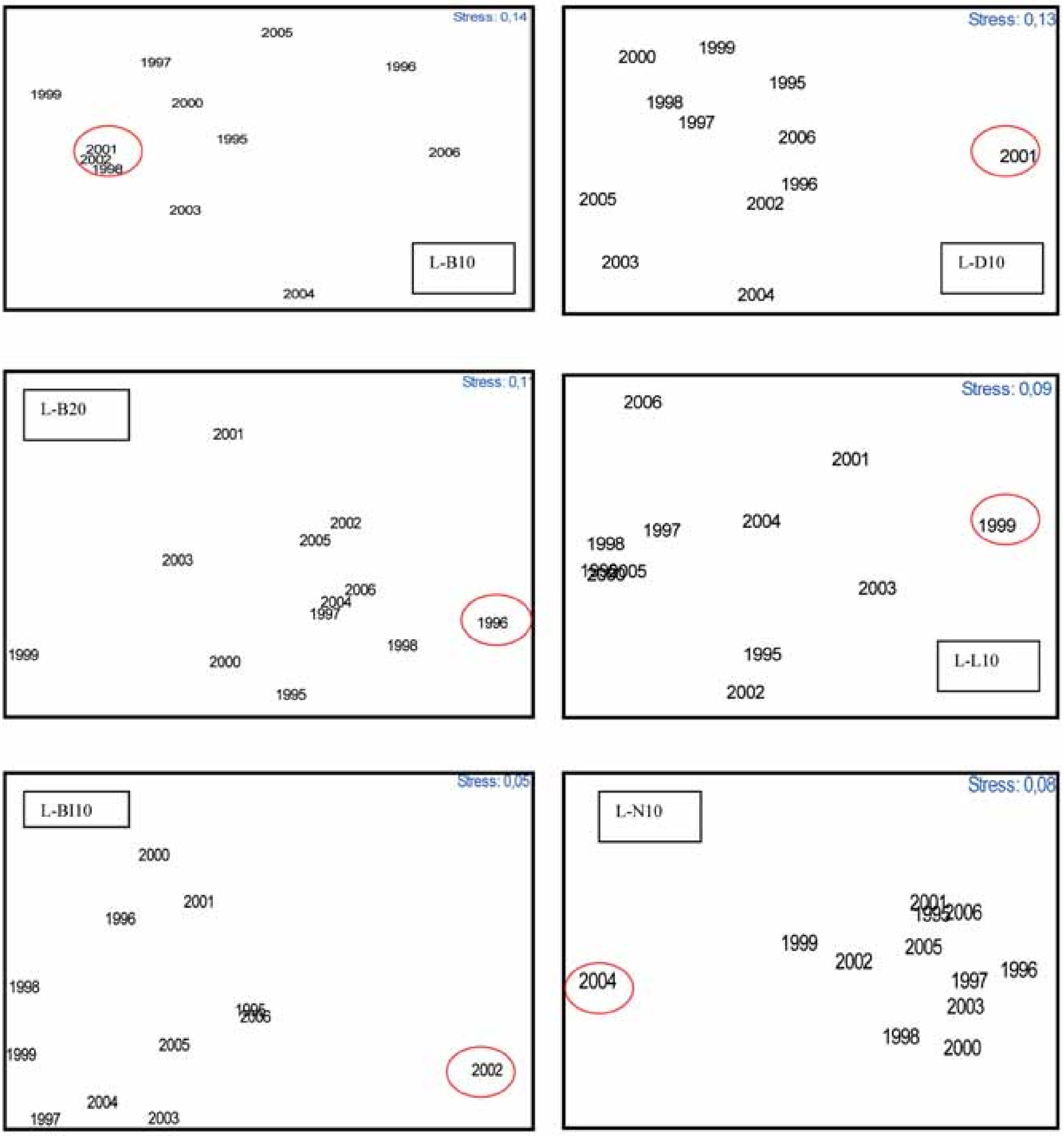

Figure 6 Multidimensional scaling (MDS) ordination plots of Basque coast assemblages, separately for each station (L-B10, L-B20, L-BI10, L-D10, L-L10 and L-N10), over the 11 years of sampling. Points are based on 4th root transformed abundances and Bray-Curtis similarities. Red circles show the years with the lowest abundances.

MDS plots for individual station were not similar one to another (Figures 6 and 7). Most of them do not follow a clear trend. Although these plots are unable to explain if the variables or the conditions are improving, worsening or remaining the same, changes or differences can be easily identified. Some stations present some years that differ from the remainder, e.g.

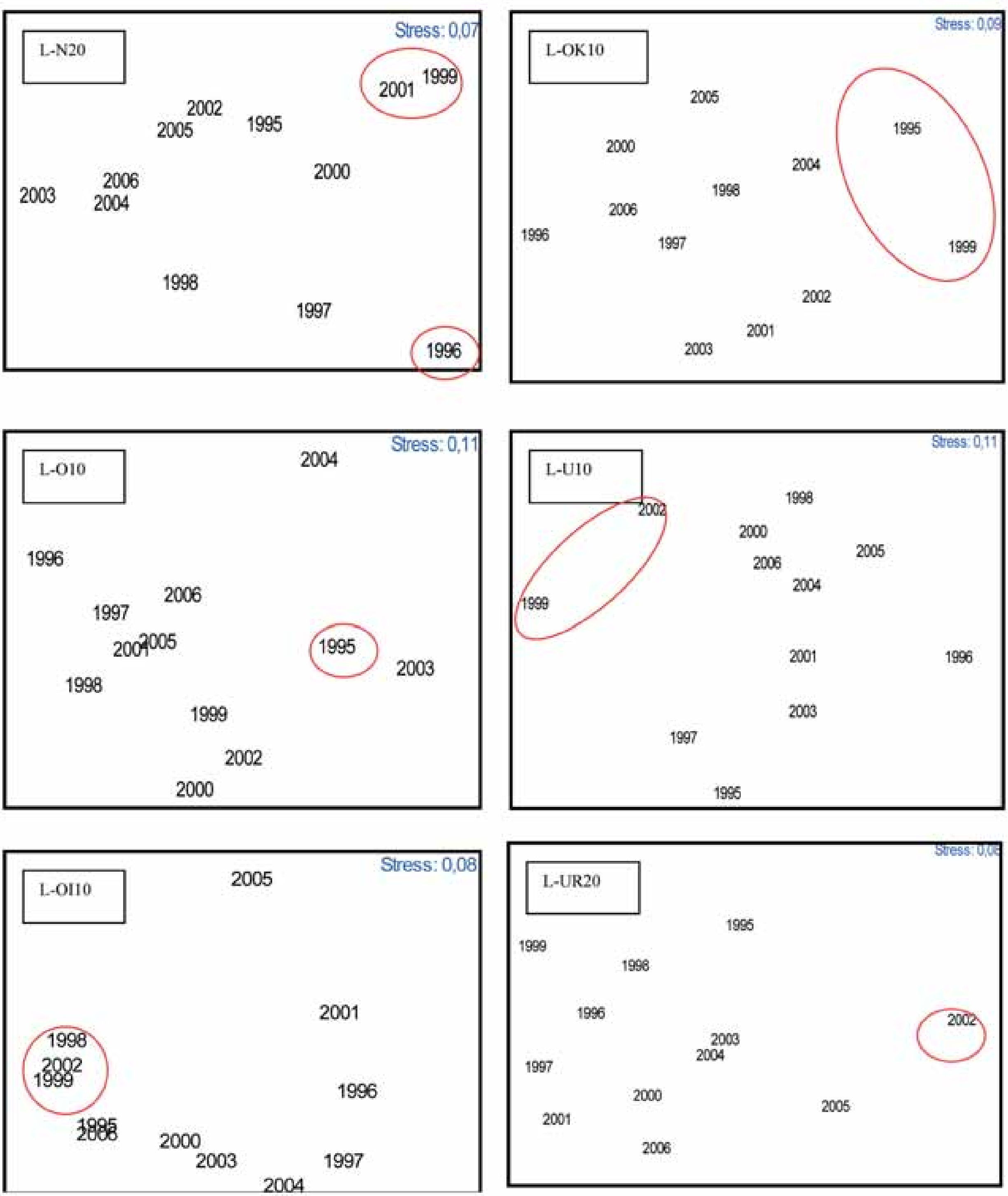

Figure 7 Multidimensional scaling (MDS) ordination plots of Basque coast assemblages, separately for each station (L-N20, L-O10, L-OI10, L-OK10, L-U10 and L-UR20), over the 11 years of sampling. Points are based on 4th root transformed abundances and Bray-Curtis similarities. Red circles show the years with the lowest abundances.

abundances of L-BI10 (2002), L-D10 (2001), L-L10 (1999), L-N10 (2004), and L-UR20 (2002). These differences are explained by a decrease in benthic abundance in that year.

Low abundances in L-BI10 (2002) and L-N10 (2004) were associated to sampling problems (bad meteorological conditions in sampling day). Differences in L-UR20 (2002) could be due to a benthic redistribution just after the water treatment beginning (discharges were removed from that area in 2001). There were no apparent explanations for the rest of the stations.

3.5 Data analysis: goodness-of-fit tests and Correlations

The idea that any of the physico-chemical variables (general and anthropogenic) and structural parameters studied come from a normal distribution can be rejected with 90% or higher confidence. Hence, for these variables Spearman Rank Correlations were run out.

However, for almost all the environmental and climatic variables (except Adour, SSTiwin, SSTsum and Upwelling) we cannot reject the idea that they come from a normal distribution with 90% or higher confidence. In this case, Pearson's Common Correlations were used for the analysis.

Hence, Spearman Rank Correlations, for the physico-chemical, structural and very few environmental or climatic variables, demonstrate that some variables show significant trends during the studied period of time. These variables are summarized in Table 11.

3.5.1 Structural parameters

In Table 11 the structural parameters show a general increase in biomass and a decrease in AMBI (low AMBI values represent unimpacted or low impacted areas, after Borja et al., 2000). Hence, in general these results show that the community quality has improved through time. However, there are some cases were the situation has degraded, such as L-N10, and L-L10.

In Figure 9 these trends are shown. Station L-D10 shows a decrease in AMBI from 2 in 1995 to 0.9 in 2006. Station L-O10 shows a similar trend; AMBI has decreased from 1.8 in 1995 to 1 in 2006.

Table 11 Significant variables after deriving Spearman Rank Correlations for the structural, physico-chemical, anthropogenic parameters vs. time and Pearson's Correlations for the environmental and climatic factors (except Adour, SSTwin, SSTsum and Upwelling) vs. time. R: correlation coefficients; N: number of pairs of data values used to compute each coefficient. P: p-value

PARAMETERS		R	N	P
STRUCTURAL PARAMETERS				
PARAMETERS IN GENERAL	AMBI	-0.1927	174	0.0113
	BIOMASS	0.1495	174	0.0492
PARAMETERS FOR STATIONS	AMBI_L_D10	-0.7273	12	0.0159
	AMBI_L_O10	-0.6853	12	0.023
	DIVERSITY_L_N10	-0.6294	12	0.0369
	DIVERSITY_L_UR20	0.5944	12	0.0487
	SPECIES RICHNESS_L_L10	-0.6	12	0.0466
	SPECIES RICHNESS_L_N10	-0.6014	12	0.0461
GENERAL PHYSICO-CHEMICAL PARAMETERS				
PON		-0.1651	171	0.0314
ANTHROPOGENIC PARAMETERS				
METALS	As	0.2484	170	0.0012
	Cr	0.1801	170	0.0192
	Mn	0.2376	169	0.0021
ENVIRONMENTAL OR CLIMATIC FACTORS				
Oiartzun river annual flow		-0.7853	9	0.0121
Gironde river annual flow		-0.6376	12	0.0257

Although station L-UR20 shows very low diversity in some years, in general it shows a positive trend, with a maximum in 2006 (4.1 bit.ind^{-1}).

In contrast, stations L-N10 and L-L10 show negative trends in species richness. Station L-N10 does not only shows a decrease in diversity from 4.6 (bit.ind^{-1}) in 1995 to 3.4 (bit.ind^{-1}) in 2006; it also shows a decrease in species richness from 38 in 1995 to 18 in 2006. Both parameters show a minimum in 2004.

Finally, station L-L10 shows a decrease in species richness from 60 in 1995 to 19 in 2006 with a minimum in 1999.

3.5.2 General physico-chemical parameters

General physico-chemical parameters hardly show significant trends. Only PON shows a negative significant (p=0.031) trend during the studied time-period (see Table 11).

3.5.3 Anthropogenic parameters

All the heavy metals (As, Cr and Mn) that present a significant (p=0.001; p=0.019 and p=0.002 respectively) trend show an increase during the 12 years (Figure 8).

Although the general trend is of increasing, from 2000-2001 it seems that these metal concentrations are decreasing. The highest concentrations were found between 1998 and 2001.

Cr and Mn show a maximum in 2000 while for As it is in 2001. Afterwards all of them seems to decrease.

3.5.4 Environmental or climatic variables

Pearson’s common correlations for the rest of the environmental and climatic variables show very few significant trends along time.

Only Oiartzun and Gironde flows present significant (p=0.012 and p=0.026 respectively) trends (see Table 11 for more details). Both river flows show a negative trend, which means that in both rivers the flow decreased.

4. Discussion

4.1 General physico-chemical variables

Data presented here show that the general physico-chemical properties have an important influence on the number of species (species richness) and individuals (abundance) of benthic invertebrates within the Basque coast. Hence, this study shows that physico-chemical variables explain 11.1% of the total community inertia, for the whole data set (19 stations, 12 years, and 275 species). The explained variability can be considered high in terms of CCA analysis. Among these variables sediment grain size plays an important role determining benthic composition and structural parameters. Hence, from the variability explained by physico-chemical variables, the percentage of sand, gravel and silt/clay explain 52.5% of the total.

Although general physico-chemical parameters explain an important part of the total inertia in the benthic community, these parameters do not show any significant ($p<0.05$) trend, except PON, which presents a negative significant (p=0.031) trend, probably related with the reduction of urban and industrial discharges to the Basque rivers and estuaries (Borja *et al.*, 2006). This suggests that physico-chemical parameters (grain size, organic matter, etc.) remain the same during the studied period or that conditions have changed slightly, within each of the locations.

Distributional patterns of individual species of benthic fauna are reported to be largely controlled by abiotic factors at the broader scale and biotic factors at finer scales (Snelgrove, 1999). In particular, the distribution and abundance of soft-sediment benthos in natural habitats have been related to sediment particle size structure and depth (Sanders, 1958; Gray, 1981; Butman and Grassle, 1992; Sundberg and Kennedy, 1993; Coleman *et al.*, 1997), as found within the Basque Country (both in this study and previously by Borja *et al.*, 1995, 2004e, 2006d; González-Oreja and Sáiz-Salinas, 1999; Martínez and Adarraga, 2001). In this study, depth did not explain such variability, as all the samples were taken at depths between 25-30 m.

4.2 Anthropogenic variables

Anthropogenic variables explain the lowest percentage of variability, within the Basque coast (4.8%). Among all analysed anthropogenic variables, only Cr (p=0.001), As (p=0.001), Mn (p=0.002) and Fe (p=0.001) are significant.

These variables explain 29.1%; 26.7%; 23.2% and 20.9%, respectively, of the total variability explained by anthropogenic variables.

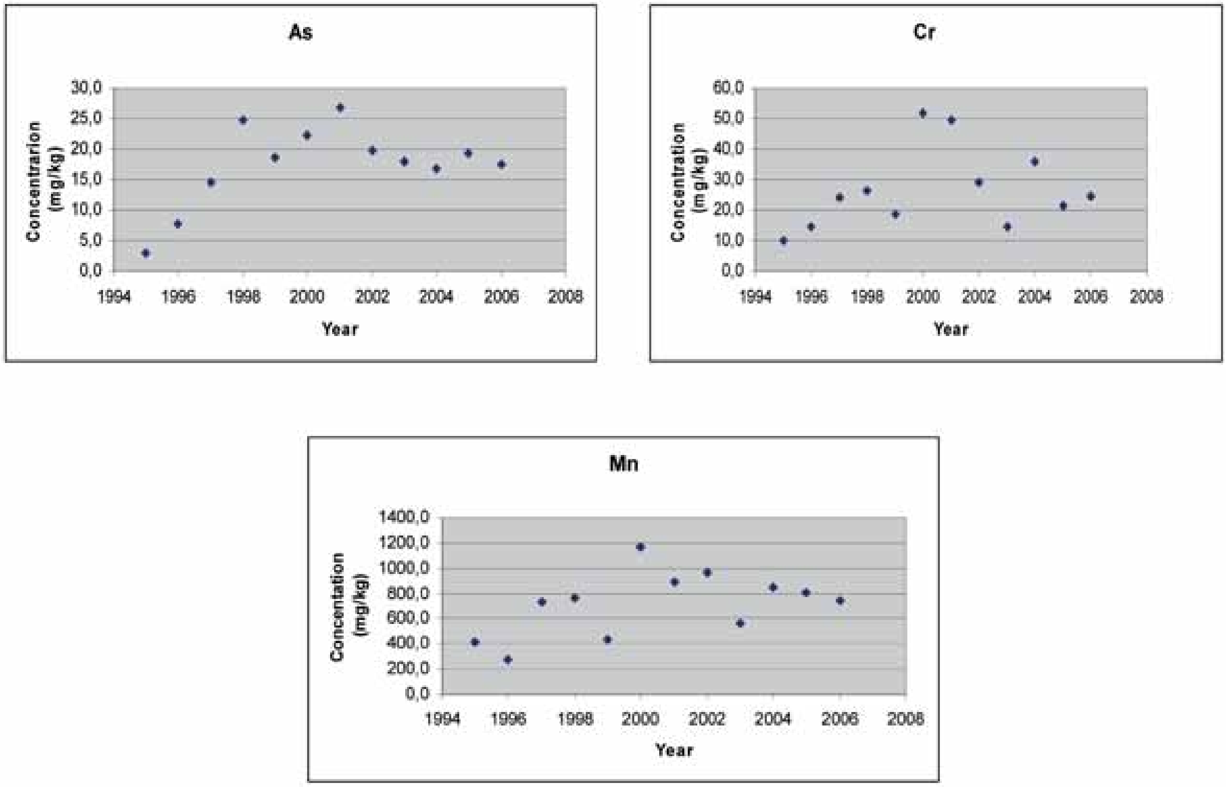

Figure 8 Anthropogenic variables with significant trend (p=0.001 for As, p=0.019 for Cr and p=0.002 for Mn): yearly averages taking into account the 13 stations sampled since 1995.

Although anthropogenic impact is important in Basque estuaries, in coastal areas this influence is more limited (Borja *et al.*, 2006a, d). The industrial and urban discharges arriving to the estuaries are more concentrated, but then they are diluted and dispersed, arriving to the coastal area with low concentration values, being the impact on benthic communities relatively limited, as shown in this study. Hence, only stations near the outfalls (Mompás, in San Sebastián) or historical impacted sites (mouth of Nervión estuary, especially in the 80s and 90s) show structural parameter values indicating anthropogenic impact. However, in these cases, the evolution through time is positive, showing a recovery after the impacts produced in the 20th century.

Moreover, the ecological status (*sensu* WFD) of the coastal water masses is in general good and has experienced a positive evolution in the last decade (Borja *et al.*, 2006c, 2007); this is because anthropogenic variables (in terms of pollutants) have small influence in the coastal benthic communities' structure, within the Basque coast. In addition, some of those metals explaining most of the variability, such as Fe and Mn, are related with the geological composition of the Basque Country (Belzunce *et al.*, 2004b; Uriarte *et al.*, 2004), and, in this way, they could be more related to the general sediment characteristics, because they have not hazard effects over benthic communities.

Only As (p=0.0012), Cr (p=0.0192) and Mn (p=0.0021) show significant positive trend, with increasing concentrations. Although the general trend is positive, the concentration of these metals is decreasing since 2000-2001(see Figure 8). Between 1998 and 2001 their concentrations increased probably due to industrial discharges. However, after the implementation of several environmental protection policies, the improvement in waste treatment systems, and the closure of some major companies during recent periods, the concentration of some metals in water and sediments is decreasing (Belzunce *et al.*, 2004a, b). In addition pollutants remain in sediments more time than in water column, making difficult to show negative trends in pollutants concentration in a short period of time. However, it seems that the current metal concentration trend is to decrease, but more years of monitoring are needed in order to confirm this hypothesis.

4.3 Environmental or climatic variables

The study of long-term variation of subtidal macrofaunal communities usually reveals both general trends and the effect of local disturbances (storms, extremely cold winters, human induced alterations, etc.) (Souprayen *et al.*, 1991). Severe winters may be important in more northern areas (Glémarec, 1979) and mainly in the intertidal habitat, although some important effects of cold winters have been reported in subtidal communities as well, such as in the *Amphiura filiformis* community in the North Sea (Gerdes, 1977). However, these climatic anomalies, related mainly with strong changes in temperature, that may be important in higher latitudes, usually do not affect the benthic communities of the southern European coasts, e.g. the range of winter sea surface temperature in the Basque coast ranges between 9 and 13ºC, from 1947 to 2006 (Á. Borja, AZTI-Tecnalia, pers. comm.).

In areas where the effect of severe winters and storms is not very important, the long-term changes generally are due to longer period phenomena (López-Jamar *et al.*, 1995), such as fluctuations of the sea water temperature, variations in the river flows and changes in NAO, sunshine, and EA.

Results from the present study suggest that these type of climatic phenomena have a significant impact on the benthic fauna of the Basque coast. Hence, climatic and environmental factors explain 17% of the total variability, being these variables more important than physico-chemical and anthropogenic variables in explaining the structure of these communities, for the period 1995-2006.

From the environmental variables explored, river flows, precipitation, winter NAO, sunshine and EA are the principal factors determining coastal benthic structure. They explain 49.2% (taking into account all river flows together), 7.3%, 6.9%, 6.6% and 5.9%, respectively, of the total variability explained by the environmental or climatic variables.

Almost any environmental variable shows significant ($p<0.05$) trend: only Oiartzun and Gironde river flows show negative significant trends.

This is mainly because environmental or climatic variables present long-term cycles and a 12-year study is not long enough to see clear tendencies or inter-annual changes, in terms of climatic variation. This is used to be the main hurdle in this type of studies where effects of environmental or climatic phenomena are studied.

To sum up, the results suggest that different river flows, precipitation, winter NAO, sunshine and EA play an important role in the Basque coastal benthic structures. In addition, although not significant tendencies have been found in relation to these environmental or climatic variables because of temporal limitations, this study suggest that global climatic changes, including alterations in rainfall (and, in consequence, river flows), changes in sunshine or sea level, air pressure, etc. could entail changes in the benthic community and for marine communities in general. That is why long-term monitoring programmes are necessary in order to see how these phenomenon behave and to make possible to study how these variables changes affect to benthic structure.

Atmospheric changes are known to have a significant influence on the population structure of many marine organisms including plankton, pelagic fishes and cetaceans (Shane, 1995; Fromentin and Planque, 1996; Grover *et al.*, 2002). Direct and indirect changes to marine benthic assemblages, as a result of global climatic change, may therefore be anticipated, but are rarely documented (Tunberg and Nelson, 1998; Dippner and Ikauniece, 2001; Nichols, 2003).

The relationships between NAO and benthic communities have been documented elsewhere (Hagberg and Tunberg, 2000; Drinkwater *et al.*, 2002; Schroeder, 2003; Gröger and Rumohr, 2006). The climatic factors can drive other environmental variables (rainfall, river flow, etc.) which finally produce changes in benthic communities. These relationships have been extensively studied in coastal waters and under different environmental variables (Grémare *et al.*, 1998; Hernández-Arana *et al.*, 2003; Gilberto *et al.*, 2004; Lercari and Defeo, 2006; Wysocki *et al.*, 2006).

However, most of the studies in this topic claim about the knowledge gap, which prevails largely because of the absence of long-term benthic data sets (Borja *et al.*, 2006d). In this way, the LQM data set is one of few such comprehensive long-term soft-bottom coastal benthic data sets in the Basque Country, and offers a unique insight into the effect of climate change on coastal benthic communities (although the length of series is too short yet).

4.4 Status of the subtidal soft-bottom benthic community

It seems that the quality of the Basque coast, assessed through benthic communities, is improving (Borja *et al.*, 2006a, 2006d). This has been confirmed in this study, where the results indicate that several structural parameters of the communities have improved during the studied period. Hence, biomass shows a significant ($p=0.049$) increase and AMBI shows a significant ($p=0.011$) decrease. Temporal variations in the different stations are in general due to implementation of environmental protection policies, improvement in waste treatment systems and closure of some major polluting companies during the 90s.

The Basque coast has suffered, since the end of the 19th century, the undesirable effects of pollution as the result of the concentration of industrial-urban centres along rivers, estuaries and other coastal areas. Until the last quarter of the 20th century, practically no waste-water treatments were in operation (Gorostiaga *et al.*, 2004). But due to a major awareness of the problem and waste-water treatment plans, the water quality has improved significantly. In addition benthic communities' structural parameters have also improved as abovementioned. In some estuaries such as Nervión and Oiartzun, the most affected ones, a great effort has been done in order to recover the water quality and faunal assemblages (Gorostiaga *et al.*, 2004; Borja *et al.*, 2006c).

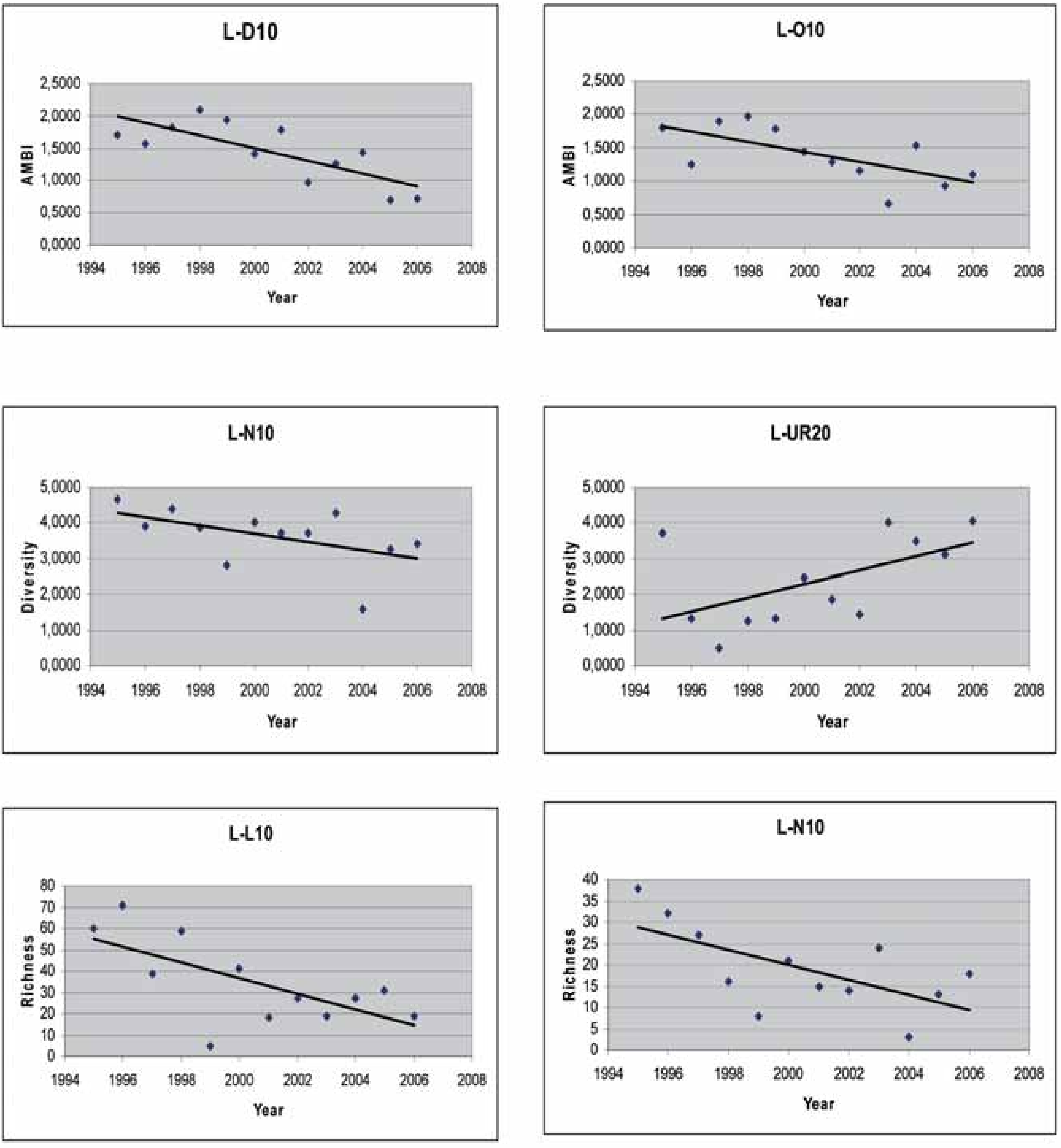

Figure 9 Structural parameters with significant trend. AMBI in stations L-D10 and L-O10, Diversity in stations L-N10 and L-UR20 and Species richness in stations L-L10 and L-N10.

In our data, the positive trend in station L-D10 could be due to industrial waste-water treatments. In the upstream of Deba river there are located some industries which work with heavy metals and have polluted the river and the estuary with these compounds for many decades (Belzunce *et al.*, 2004a). This estuary has been shown to be one of the most affected by heavy metal pollution in the waters indeed. But due to industrial waste-water treatments, the river quality has improved and also the transitional and coastal waters (Borja *et al.*, 2006c). In addition AMBI shows a significant (p=0.016) negative tendency in this station, which means an improvement in the benthic community.

Station L-O10 also shows a positive significant (p= 0.023) trend in AMBI. At the beginning of the studied period, this station was affected by some works (e.g. dyke enlargement, dredging) carried in the Oria estuary. Hence, during the first years, the benthic community was quite influenced. However, the results of this study suggest that the benthic community is adapting to the new conditions.

Species richness in station L-UR20 also shows a significant (p=0.049) positive trend. This station is located in the coastal zone of Mompás (San Sebastián) and, until 2001, benthic communities in this location presented very bad quality, due to the discharges from two outfalls located in Ulia and Murgita. Since 2002 there is a noticeable improvement due to the derivation of the discharges to a submarine outfall constructed in the spring of 2001 and located in Ulia, 1.5 km from the coast and in a water depth of around 50 m (Borja *et al.*, 2006c). However it is necessary to study how this station progress in the next years in order to verify if it evolves satisfactorily.

However, although improvement is the main pattern of evolution, there are two stations (L-N10 and L-L10) which do not follow this trend. L-L10 shows a decrease in species richness values and station L-N10 shows a decrease in species richness and diversity.

The negative trend in station L-N10 could be due to the enlargement of the port, undertaken since 1993, in the outer part of the Nervion estuary (in the Abra). Since 2003 some dredging activities were undertaken near this station, and benthic structural parameters were negatively affected. In addition, as mentioned previously, in 2004 structural parameters show very low values, because of sampling problems (high waves during sampling, which reduced the efficacy of grabs). This seems to be the main reason that explains this negative trend, as generally the benthic quality in this station has not been so bad (Borja *et al.*, 2006c).

Station L-L10 shows significant (p=0.047) negative trend in species richness. However, this station has small or no influence from the estuary, in terms of water plume affection. Even though, the negative result could be related to the heterogeneity of the area and the fact that the communities temporal pattern is quite irregular (Borja *et al.*, 2006c).

5. Conclusions

Summarizing, this work supports that environmental or climatic variables have a high influence in benthic macroinvertebrates structure and distribution, although any trend was significantly identified. This could be due to the fact that environmental or climatic variables have very long cycles and very large time series are required.

Although these variables have a mayor influence than the anthropogenic variables, this study reveals and confirms that the Basque coastal benthic communities show an important recovering in the last decade due to the measures abovementioned (environmental protection polices, wastewaters treatment, etc.). In addition, a mayor awareness and prevention, protection and control (by means of the LQM) and improvements in the future waste-water treatments are necessary tools in order to achieve “good water status for all waters, by 2015” as the WFD requires.

In addition the confirmation of inter-annual changes and trends demands longer studied periods by means of long-term monitoring programmes such as the LQM.

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