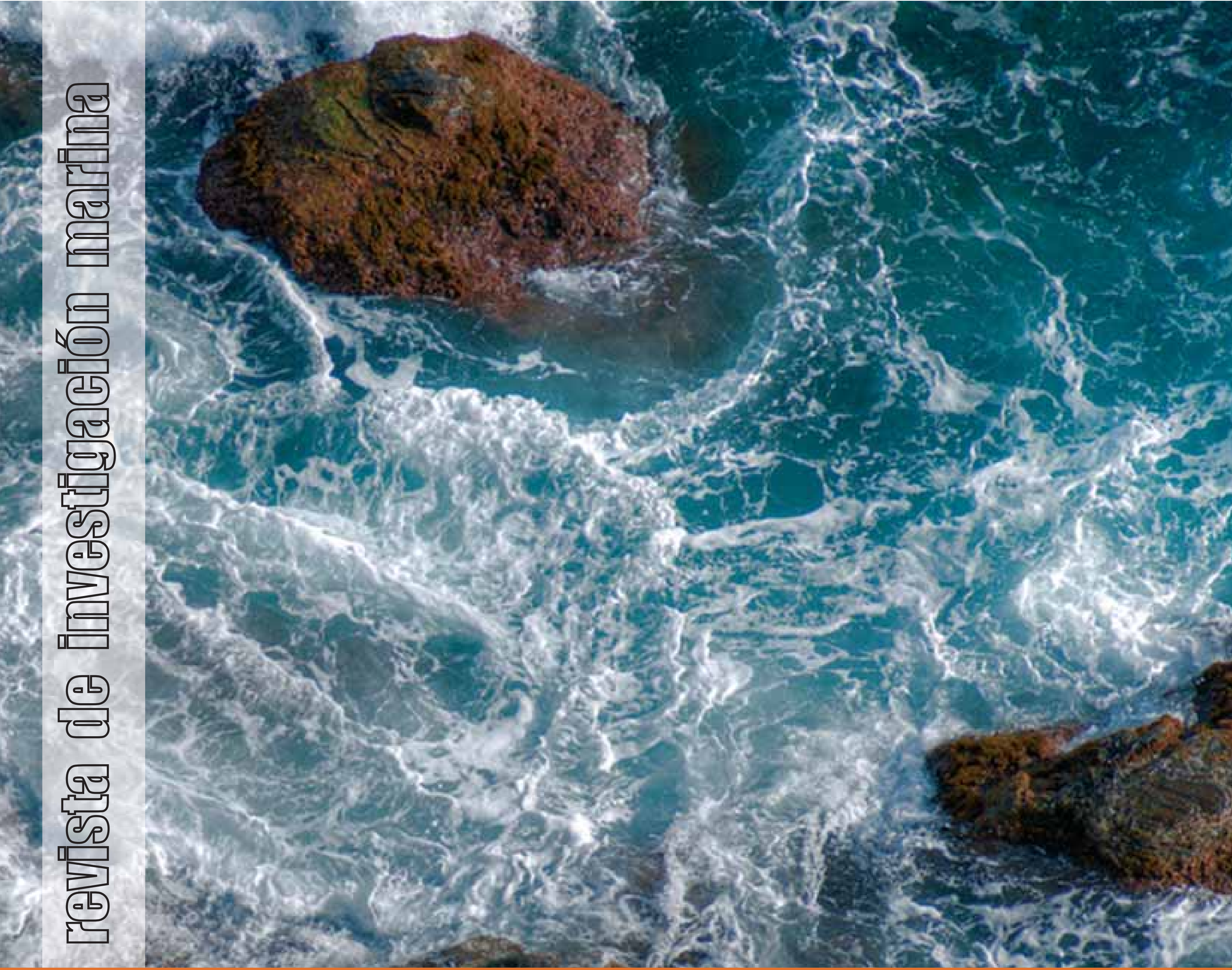

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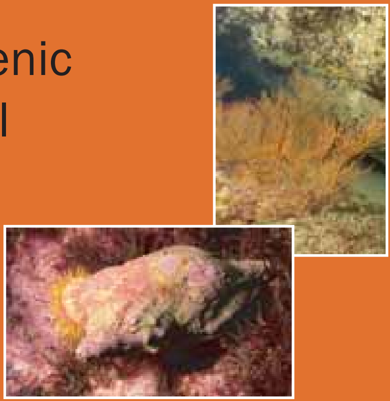

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Long-term environmental, anthropogenic and climatic factors explaining spatial and temporal distribution of soft-bottom benthic communities within the Basque estuaries

Laura Pérez[a,b], Ángel Borja[a], J. Germán Rodríguez[a] and Iñigo Muxika[a]

Abstract

In response to extensive industrial development that took place in the 19th Century in the Basque Country, human activities damaged considerably the ecological status of the Basque estuaries. The Water Framework Directive (WFD) emphasises the need of implementing monitoring programmes, providing a new view of water resources management in Europe, based mainly upon ecological elements, being the benthos one of these. The Litoral Water Quality Monitoring and Control Network (LQM) has been monitoring the Basque coastal and estuarine water quality, since 1994; data gathered in this programme has been used here. The aim of the present study is to determine, by performing multivariate analysis (Canonical Correspondence Analysis and Redundancy Analysis), the variability in Basque estuarine soft-bottom macrofaunal communities explained by anthropogenic, climatic and sedimentological factors. Thus, this study deals with data on general physico-chemical characteristics of the sediment and with data on pollutants, present within this sediment. Moreover, some oceanic and meteorological variables have been taken into account. Furthermore, temporal trends in all the variables have been analyzed by performing univariate analysis (Spearman Rank Correlations). Multivariate analysis has revealed that the general physico-chemical characteristics of the sediment are of relevance in explaining the variability in the species densities (17.2%), whereas anthropogenic variables (16.9%) explain this variability a higher extent than the climatic variables (15.4%). Thus, assemblages of species such as the *Scrobicularia plana-Cerastoderma edule* community mixed with *Capitella capitata* and found in some estuaries, reflect the low oxygen saturations and the high concentrations of heavy metals. However, increasing trends in benthic status parameters and oxygen saturation have been found, suggesting that the closure of major industries and the implementation of water treatment schemes are leading to a gradual recovery of the environmental status of the Basque estuaries.

Introduction

Estuaries are considered to be one of the most productive and valuable systems in the world (Costanza *et al.*, 1997). They are transitional environments between rivers and the sea that are characterized by widely varying and frequently unpredictable climatic, hydrological, morphological, and chemical conditions (Ysebaert *et al.*, 2003).

Climatic classification of the Basque Country and its interannual variability

The Basque Country (Figure 1) is located within the mid-latitudes of the eastern North Atlantic Ocean. Therefore, there exist influences of the Gulf Stream and the atmospheric westerlies. As a consequence, the annual mean temperature is >10ºC. The climate is temperate, oceanic, with moderate winters and warm summers; it is also wet with over 1,500 mm of rainfall each year (Usabiaga *et al.*, 2004). The Basque Country is located within latitudes that are under the influence of the North Atlantic Oscillation (NAO),and the East Atlantic Pattern (EA). Both are patterns of low- frequency variability of the atmosphere and are depicted often as sea level pressure or geopotential height anomalies; they have influence on some climatological phenomens in terms of rainfall and temperature and, consequently, on ecological communities for which these climatic factors are limiting. In fact, the NAO has been shown to be one of the most important climatic factors that affect marine species in the North Atlantic (Borja *et al.*, 2008b). In this way, it has been proposed to influence benthic populations, by bottom-up control acting through influences on primary production: an increase in primary production would result in more food for the benthos (Drinkwater *et al.*, 2003). In turn, the EA Pattern is connected with temperature variability, ocean-atmosphere heat fluxes and winter sea-surface temperature (SST) evolution; their effects occur over the southern part of the Bay of Biscay (Sáenz *et al.*, 2001b). Regarding its potential influence on marine organisms, the recruitment of the Bay of Biscay anchovy has been related to the EA Pattern. However, it is relevant that the variability associated with the EA Pattern over the Bay of Biscay is somewhat higher than that associated with the NAO (Borja *et al.*, 2008b).

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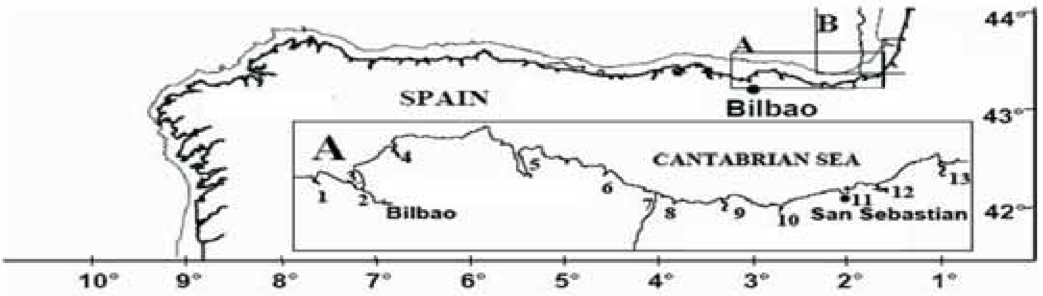

Figura 1. Basque coast including the 12 main estuarine systems: (A, see next list): 1-Barbadún; 2-Nervión; 3-Abra of Bilbao; 4-Butrón; 5-Oka; 6-Lea; 7-Artibai; 8-Deba; 9-Urola; 10-Oria; 11-Urumea; 12-Oiartzun and 13-Bidasoa. (B): Cap Breton Canyon and the Landes Plateau area (figure from Borja and Collins, 2004c).

Hydrographical description of the Basque estuaries

Within the Basque coast, there are 12 estuarine systems that are mainly differentiated by geomorphological and hydrological characteristics (Table 1; see Figure 1 for location). Whereas the deepest are Nervión and Oiartzun, the other estuaries can be classified as being shallow systems. Residence times are also very different as well, with the shortest being that of the Deba estuary and the longest that of the Nervión estuary. Apart from being defined by the previous characteristics, the estuarine systems are affected by meteorological features, such as the rainfall (Valencia *et al.*, 2004a).

Human impacts along the Basque coast over the last two centuries

Human activities derived from the industrial development resulted in a high demographic pressure on the coastal area and above all, the estuaries. These human activities were established in areas occupied previously by natural habitats. Globally, about 45% of the original total surface of the Basque estuaries (following the post-Flandrian retreat) (Cearreta *et al.*, 2004) has been lost. Moreover, the large wastewater discharges reduced the quality of the water and the sediments of the estuaries, whilst impoverishing the flora and the fauna (Franco *et al.*, 2004). Additionally, the necessity of constructing ports together with facilities for navigation has involved the disposal of large amounts of dredged materials into the sea (Belzunce *et al.*, 2004). The situation described is exemplified clearly by the Nervión and Oiartzun estuaries, which have been receiving wastewaters from industries and large populations; their ports, Bilbao and Pasaia have been extensively dredged (Uriarte *et al.*, 2004). Nervión estuary suffers, at present, from extremely low concentrations of dissolved oxygen and a high content of organic matter and heavy metals (Seebold et al, 1982; Swindlehurst and Johston, 1991; Sáiz-Salinas *et al.*, 1996; Belzunce *et al.*, 2001; Borja *et al.*, 2002; Raposo *et al.*, 2008; Prieto *et al.*, 2008). In turn, the Oiartzun estuary also suffers at present from hypoxic or even anoxic waters (Franco *et al.*, 2004). In general, the Basque estuaries retain large amounts of contaminants (Fernández *et al.*, 2008; Tueros *et al.*, 2008). These are related mainly to heavy metals (smelting products, metal treatments, steel production, chromium industries, chemical products, plastics, etc…) and to organic compounds that have been produced by urban areas, the petroleum and paper industries (Belzunce *et al.*, 2004). Nowadays, intensive actions are being undertaken towards the treatment and general management of wastewaters and thus, the quality of the estuaries has improved in recent years (Borja *et al.*, 2009; Tueros *et al.*, 2009).

Table 1. Main geomorphological and hydrological characteristics of the Basque estuaries. Current surfaces, percentages of current surfaces with respect to the surfaces in the Post-Flandriense and percentages of subtidal and intertidal surfaces (data obtained from Valencia *et a*l., 2004a).

ESTUARY	Basin area (km^2)	Riverflow (m^3 s^{-1})	Estuary lenght (km)	Estuary depth (m)	Estuary volume (m^3 10^6)	Estuary volume/ riverflow (days)	Current surface (km^2)	% with respects to the Post-flandriense	% of sutidal surface	% of intertidal surface
Barbadún	127	2.9	4.4	5	-	-	0.44	19	44	56
Nerbioi	1755	36	22.0	30	200	65.0	24	69	100	0
Butroi	174	4.7	8.0	10	0.7	1.7	1.17	63	90	10
Oka	178	3.6	12.5	10	3.3	10.6	7.65	71	30	70
Lea	84	1.8	2.0	5	-	-	0.43	85	45	55
Artibai	101	2.5	3.5	10	-	-	0.25	58	100	0
Deba	534	14	5.5	5	0.35	0.3	0.40	55	100	0
Urola	364	8.0	5.7	10	-	-	0.81	43	85	15
Oria	888	26	11.1	10	2.1	0.9	1.06	41	100	0
Urumea	279	17	7.7	10	-	-	0.45	12	100	0
Oiartzun	87	4.8	5.5	20	-	-	0.97	45	100	0
Bidasoa	700	29	11.1	10	9.7	3.9	2.50	39	100	0

Soft-bottom fauna communities of the Basque estuaries

The distribution of benthic communities in the estuaries is connected to several important environmental variables; these show high variation along both the upstream-downstream and the tidal level gradient in terms of salinity, dissolved oxygen, redox potential, grain size, organic matter, Particulate Organic Carbon (POC), and water depth. As a consequence, there are differences between nearby estuaries regarding the species composition of their fauna and the abundance and biomass of individual invertebrate species (Warwick *et al.*, 1991). Moreover, in highly disturbed estuaries, some of these environmental factors can reduce their importance due to the strong presence of pollutants. Some of the invertebrates that inhabit soft-bottom areas are key species within estuarine food webs. Borja *et al.* (2004c) describe the most important benthic communities, within the Basque coast including estuarine and coastal, hard- and soft-bottom substrata. The estuarine soft-bottom communities are described below.

Scrobicularia plana – Cerastoderma edule community

This community was described firstly by Petersen (1913, 1918) and Thorson (1957). It is found in the inner and middle of the estuaries of the Basque Country, usually in muddy sand flat bottoms and well-oxygenated waters. The most important species are euryhaline, such as the polychaetes *Hediste diversicolor, Streblospio shrubsolii, S. benedicti* and *Heteromastus filiformis*, the prosobranch *Hydrobia ulvae,* the group Oligochaeta and the crustaceans *Cyathura carinata, Carcinus maenas* and *Corophium* sp. Differences in species density can be found, even within the same estuary, constituting facies of the community; these are characterised by different sand and gravel percentages, redox potential and distribution along the middle part of the estuaries (e.g. *Tapes decussata*).

Abra alba community

This community was described originally by Petersen (1918) and Thorson (1957). It appears in areas which are submerged permanently, in sediments with high content of organic matter and mud, and appears generally within the middle part of the estuaries, for example, in Bidasoa, Oiartzun and Nervión. The main species are the molluscs *Abra alba, Abra prismatica, Corbula gibba* and *Thyasira flexuosa.* Other accompanying species are *Pectinaria koreni, Mysella bidentata*, etc.

Pontocrates arenarius – Eurydice pulchra community

Being a typical crustacean-dominated community, it is present in highly exposed sites and is associated with coarse sand and gravel bottoms (Picard, 1965; Bellan and Lagardère, 1971); it is characterised by *Pontocrates arenarius, Haustorius arenarius, Eurydice pulchra, Iphinoe* sp., etc. This community can be found in the mouths of the small estuaries with low river flows (e.g. Barbadún).

Tellina tenuis Lusitanian-boreal community

This community was described for the first time by Stephen (1930); it appears in deep estuaries (20-30 m), associated with mixed sediments, dominated by sand and mud (Cornet *et al.*, 1983). Along the Basque coast, the core of the community is formed by species of *Nephtys* and *Tellina*. 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, other characteristic species are *Spio martinensis, Phyllodoce mucosa, Capitella capitata*, etc (García-Arberas, 1999).

Venus fasciata community

This community was described by Ford (1923), Thorson (1957) and Cabioch (1961); it is typical of sandy bottoms in 20-40 m depth. The most important species are Venus fasciata, *Venus casina*, and *Chamelea striatula*. The species *Nephtys cirrosa, Urothoe brevicornis, Bathyporeia elegans, Prionospio steenstrupi, Echinocardium cordatum, Branchiostoma lanceolatum, Spisula subtruncata*, etc., are also very common.

The patchy distribution of benthic organisms in the marine soft sediment has been recognised for a long time and, further, the distribution of other variables such as pollutants and sediment particle size, which are likely to be heterogeneous (Morrisey *et al.*, 1992). Warwick *et al.* (1991) have pointed out the importance of dynamic processes (such as tidal range) and static factors (such as the sediment grain size and organic content) when determining the structure of the community of macrobenthos. A series of studies (Hall, 1994; Herman *et al.*, 2001) have demonstrated that there exists a complex interaction between the hydrodynamics, sediment dynamics and benthic biology, in structuring the patterns in which the benthos is distributed (Ysebaert. *et al.*, 2003). On the other hand, soft-sediment populations and communities are described frequently as a mosaic of patches of varying spatial scales, that are in different stages of recovery from human and natural disturbances. The necessity for a quantitative description and the study of several spatial and/or temporal scales in estuaries arises, to achieve a sound implementation of integrated management; it permits improved predictions about future environmental changes, due to anthropogenic impacts (Ysebaert and Herman, 2002).

Benthic organisms as indicators of ecological quality

Within the European Water Framework Directive (WFD; 2000/60/EC), a framework for the protection of estuarine and coastal waters is provided (Borja *et al.*, 2004b; Borja, 2005). The ecological status, which is probably the central concept in the WFD, is based upon the status of biological, hydromorphological and physico-chemical quality elements (Franco *et al.*, 2004), with the biological elements being particularly important (phytoplankton, macroalgae, benthos and fishes). When assessing the quality status of the environment, the benthic invertebrates are a well established target. A series of studies have proved that the response of the macrobenthos to anthropogenic and natural stress is relatively rapid (Pearson and Rosenberg, 1978; Dauer, 1993). The capability of benthos, to reflect the anthropogenic and natural gradients, is a consequence of: (1) their sedentary life-style and subsequent inability to avoid adverse conditions (Dauvin *et al.*, 2007); (2) their relatively long life-spans, which enables them to integrate water and sediment quality conditions over time, therefore indicating temporal and chronic disturbances (Reiss *et al.*, 2005); (3) and their taxonomic diversity, that includes a wide range of responses towards environmental stressors (Coelho *et al.*, 2007). The consequences of these disturbances include changes in diversity, biomass, abundance of the benthic species that are tolerant or sensitive to stress, and the trophic or functional structure of the benthic community (Pearson and Rosenberg, 1978; Kaiser *et al.*, 2000). Several authors have reviewed the use of biotic indexes in evaluating benthic environment (see Diaz *et al.*, 2004; and Pinto *et al.*, 2009), with one of them being the AMBI (Azti´s Marine Biotic Index) (Borja *et al.*, 2000, 2003c, 2004b).

The Basque monitoring network

The necessity to establish comprehensive monitoring programmes is stated in the WFD (Franco *et al.*, 2004). The Department of Land Action and Environment of the Basque Government, by means of the *Littoral and Transitional Water Quality Monitoring and Control Network* (hereafter, LQM), has been monitoring the Basque coastal and estuarine water quality since 1994 (Borja *et al.*, 1996, 2003b). The overall aim of this programme is to contribute to an improved management of the water resources and aquatic environments (Franco *et al.*, 2004). This network comprises the analysis of physico-chemical elements in water, sediment and biota, and the analysis of biological elements (Borja *et al.*, 2004b). The long-term monitoring of benthic communities is considered to be an efficient, accurate and useful tool when aiming to detect the effects of pollution. However, long-term studies are scarce (Gorostiaga *et al.*, 2004). Given the fact that there is information on bioindicator properties at different levels (species, population and communities), obtained over the last two decades, a comparative study of the factors affecting benthic communities within the Basque estuaries can be carried out. Hence, a study dealing with the data obtained from the 19 coastal stations, sampled annually within the LQM, has already been undertaken (Garmendia *et al.*, 2008), whilst the present investigation focusses on data recorded from the 32 estuarine stations established under the LQM.

Objectives

The main objective of this study is to determine the contribution of three main groups of variables: 1) anthropogenic; 2) climatic; and 3) sedimentological. Such variables are used to explain spatial and temporal variability in the composition of the soft-bottom macrofauna, from the Basque estuaries. In order to achieve this objective, several steps have been accomplished: (i) benthic, sediment and contaminant series data from the LQM (1995-2007) have been used together with hydrological and oceano-meteorological variables from other sources; (ii) multivariate analysis has been performed on all variables, to determine which one(s) explain most of the variability in density and structural parameters of the benthic community; (iii) furthermore, partitioning of the variance in the species densities was carried out determining the percentage of the variability explained by the anthropogenic, climatic and sedimentological variables likewise, in establishing the percentage explained by interactions between the groups of variables, in each water body and globally.

Moreover, time trends in all of the variables have been determined and in this way, the evolution of the ecological status of the Basque estuaries has been assessed.

Methods

2.1. Sampling stations

The present study focuses upon the transitional (=estuarine) waters of the Basque Country, sampled within the LQM. 14 water bodies (*sensu* the WFD: for details see Borja, 2005) within 12 estuaries, have been studied. The 14 transitional water bodies are distributed amongst 3 typologies (see ‘delimitation criteria’ in Borja *et al.*, 2004a): (i) Type I, small river-dominated estuaries (Urumea and Deba); (ii) Type II, estuaries with extensive intertidal flats (Barbadún, Butrón, Oka, Lea, Artibai, Urola and Oria); and (iii) Type III, estuaries with extensive subtidal areas (Nervión, Oiartzun and Bidasoa) (Table 2, Figure 2).

The LQM network comprises 32 estuarine sampling stations within these water bodies, sampled from 1995 to 2007 (Borja *et al.*, 2008a). Data used in this study include some water variables (e.g. oxygen), sediment characteristics (e.g. grain size, organic matter, concentration of heavy metals, etc.) and data on soft-bottom benthos. Water was monitored using a CTD probe, whilst sediment and soft-bottom macrobenthic communities were sampled (three replicates) annually, in winter, using a van Veen grab (0.07 m^2) in sublittoral locations, and squares (0.5 x 0.5 m) sampled by hand at intertidal locations (see Sampling Methods, in Borja *et al.*, 2003b, 2007). However, not all the 32 stations have been sampled since the beginning; some of them were incorporated to the monitoring programme, some years later (in 2002).

2.2. Variables and parameters

The Basque Country is a mountainous region, dominated by rocky shores and estuaries. The lithology is characterised by materials ranging from Palaeozoic to Quaternary in age, with an absence of Oligocene materials. The area is characterised by sedimentary rocks, with a higher proportion of sandstones and lutites in the eastern part of the region; there are more marls and limestones towards the west (Pascual *et al.*, 2004). Data from the LQM on structural parameters of the soft-bottom communities have been considered; these are density (ind. m^{-2}), biomass (g. m^{-2}) , species richnes (number of taxa), Shannon-Wiener diversity index, Pielou´s evenness, AMBI (AZTI´s Marine Biotic Index) (Borja *et al.*, 2000, 2003c, 2004b) and Multivariate-AMBI (M-AMBI) (Muxika *et al.*, 2007; Borja *et al.*, 2008c).

This study deals with three main groups of variables, which are summarized in Table 3: i) anthropogenic variables (pollutants present in the sediments); ii) general physico-chemical characteristics of the sediment; and iii) climatic variables (see below).

(i) Anthropogenic variables: most of the estuaries have been affected historically by urban, industrial wastewaters and/or mineral ores; of special relevance are Zn, Pb and Fe (Cearreta *et al.*, 2000,

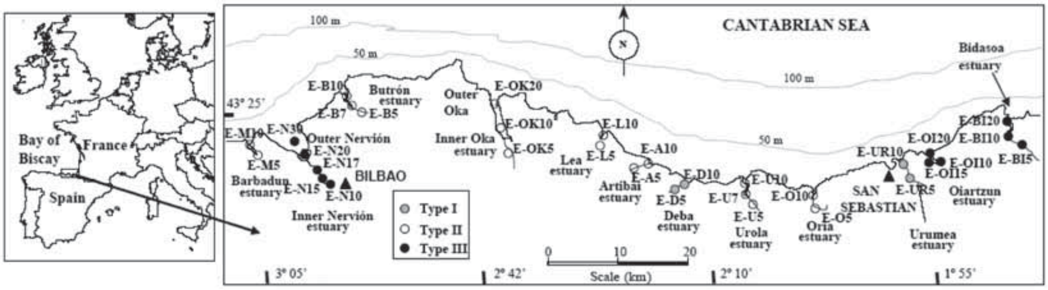

Figura 2. Basque coast including the 14 transitional water bodies and their typologies (figure from Borja *et al.*, 2009).

Table 2. Stations, estuaries and water bodies sampled within the Basque Monitoring Network, with their geographical location. The typology and the year of first sampling are included.

Station	Estuary	Water body	Typology	Location	Start of sampling (year)	UTMX	UTMY
E-M5	Barbadún	Barbadún	II	Muskiz (Petronor)	2002	490982	4797919
E-M10	Barbadún	Barbadún	II	Pobeña (puente)	1995	490251	4799550
E-N10	Nervión	Inner Nervión	III	Bilbao (puente de Deusto)	1995	505054	4790971
E-N15	Nervión	Inner Nervión	III	Barakaldo (puente de Rontegui)	2002	502217	4793792
E-N17	Nervión	Inner Nervión	III	Leioa (Lamiako)	2002	500291	4796070
E-N20	Nervión	Outer Nervión	III	Abra Interior	1995	497919	4798586
E-N30	Nervión	Outer Nervión	III	Abra Exterior	1995	496435	4801048
E-B5	Butroe	Butroe	II	Plentzia (Abaniko)	2002	506252	4805033
E-B7	Butroe	Butroe	II	Plentzia (campo de futbol)	2002	504624	4805212
E-B10	Butroe	Butroe	II	Plentzia (puerto)	1995	504454	4806293
E-OK5	Oka	Inner Oka	II	Gernika (salida de la depuradora)	2002	527165	4798891
E-OK10	Oka	Outer Oka	II	Murueta (astillero)	1995	525704	4801567
E-OK20	Oka	Outer Oka	II	Sukarrieta (txatxarramendi)	1998	524863	4804781
E-L5	Lea	Lea	II	Lekeitio (astillero)	2002	540241	4800773
E-L10	Lea	Lea	II	Lekeitio (molino)	1995	540707	4801147
E-A5	Artibai	Artibai	II	Ondarroa (Errenteria)	2002	545242	4796940
E-A10	Artibai	Artibai	II	Ondarroa (Embarcadero)	1995	547056	4796710
E-D5	Deba	Deba	I	Deba (campo de fútbol)	2002	551707	4793803
E-D10	Deba	Deba	I	Deba (puente)	1995	552251	4793703
E-U5	Urola	Urola	II	Zumaia (Bedua)	2002	560799	4792287
E-U8	Urola	Urola	II	Zumaia (puente del ferrocarril)	2002	561356	4793724
E-U10	Urola	Urola	II	Zumaia (puente Narrondo)	1995	560435	4794201
E-O5	Oria	Oria	II	Orio (rampa)	2002	571498	4792034
E-O10	Oria	Oria	II	Orio (puente de la autopista)	1995	570562	4792779
E-UR5	Urumea	Urumea	I	Donostia (Loiola)	2002	583703	4796437
E-UR10	Urumea	Urumea	I	Donostia (puente de Santa Catalina)	1995	582962	4796743
E-OI10	Oiartzun	Oiartzun	III	Lezo	1995	588984	4797454
E-OI15	Oiartzun	Oiartzun	III	Pasaia de San Pedro (Dársena de Herrera)	2002	586773	4797378
E-OI20	Oiartzun	Oiartzun	III	Pasaia (San Pedro)	1995	587571	4797829
E-BI5	Bidasoa	Bidasoa	III	Irún (Behobia)	2002	600444	4799966
E-BI10	Bidasoa	Bidasoa	III	Hondarribia (Amute)	1995	598063	4800851
E-BI20	Bidasoa	Bidasoa	III	Hondarribia (Txingudi)	1995	598131	4802793

2002, 2004; Belzunce *et al.*, 2001). Hence, overall, the region supports a high number of pressures and impacts (Borja *et al.*, 2005). Contaminants derived from the urban and industrial discharges have been shown to accumulate in estuarine sediments, reaching concentrations that are potentially harmful for the biota (Morrisey *et al.*, 2003). On the one hand, heavy metal (Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) concentrations within the sediment are included, as the concentrations of trace metals are usually higher in aquatic systems near urban or industrial areas (Long *et al.*, 1998). Moreover, it is well-known that metal pollution which results from mining has a negative effect on the biota (Malmqvist and Hoffsten, 1999). In this way, the Effects-Range Median (ERM) guidelines, from Long *et al.* (1995), can be used in quality assessment; if heavy metals in the sediments exceed the ERM, this can result in potential toxic effects to the biota (Calabretta and Oviatt, 2008). On the other hand, persistent organic pollutants (POPs) have been used extensively in agriculture and industry (Borga and Di Guado, 2005).

In this study, data on the sums of PCBs (polychlorinated biphenyls), PAHs (polycyclic aromatic hydrocarbons) and DDT (dichloro diphenyl trichloroethane) measured in the sediment at each sampling station and determined within the LQM, have been included.

Coastal eutrophication, caused by riverine runoff of fertilizers and urban discharges, leads to a decrease in the dissolved oxygen (DO) levels in the bottom waters (Díaz and Rosenberg, 2008). Furthermore, its deficiency can be the most important factor that leads to the localized mortality of benthic macrofauna (Diaz and Rosenberg, 1995). Oxygen saturation measured within the LQM has also been included, by obtaining a mean of the values between the bottom oxygen saturation, during high tide and low tide at each sampling station.

ii) General physico-chemical characteristics of the sediment. The variables considered within this group are: grain size (percentages of sand, silt-clay, gravel); redox potential; particulate organic nitrogen (PON) and particulate organic carbon (POC); N/C; and water depth. Moreover, several studies have proven that the organic enrichment of marine sediments can lead to the gradual reduction of macrofauna abundance and species richness; besides, it can result in a significant reduction of diversity in highly disturbed environments (Swartz *et al.*, 1985; Frouin, 2000, among others). Therefore, organic matter has been also included as a variable within this group.

iii) Climatic variables: these are variables relating to the hydrography of the estuaries, and other oceanic and meteorological ones (hereafter called, climatic variables), likely to explain patterns of spatial and temporal distribution of the benthos. The frequency of the data and the source of each variable are summarized in

Table 3. Variables considered within each main group of variables. The source and the frequency of each variable are included.

Group of variables	Variable	Source	Frequency
CLIMATIC VARIABLES	RAINFALL	Provincial Administration of Bizkaia and Gipuzkoa	a) Annual sum of every day´s registered rainfall (from January to December) b) Sum of the three months prior to the winter sampling (November, December and January)
	RIVER FLOW	Provincial Administration of Bizkaia and Gipuzkoa	a) Annual sum of monthly mean values (from January to December) b) Annual sum of the monthly means of November, December and January
	SUN HOURS	National Institute of Meteorology (Observatory of Igeldo)	Annual sum of the daily sun hours
	SST (Sea Surface Temperature)	Aquarium of San Sebastián	Annual mean bottom temperature
	NAO (North Atlantic Oscillation)	ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/tele_index.nh	a) Annual mean for the year previous to the winter sampling (from January to December)
		ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/tele_index.nh	b) Mean of December, January, February and March, of the previous year to the winter sampling
	EA (East Atlantic Pattern)	ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/tele_index.nh	Annual mean of the EA of the year prior to the winter sampling
GENERAL PHYSICO-CHEMICAL CHARACTERISTICS OF THE SEDIMENT	GRAIN SIZE, REDOX POTENTIAL, PON (Particulate Organic Nitrogen), POC (Particulate Organic Carbon), N/C (Nitrogen/Carbon), ORGANIC MATTER, DEPTH	LQM	Annual (Winter)
ANTHROPOGENIC VARIABLES	HEAVY METAL CONCENTRATIONS, ΣPCBs, ΣPAHs, DDT, OXYGEN SATURATION	LQM	Annual (Winter)

Table 3. They include: the sum of the rainfalls registered every day; the mean of the river flow, per month and estuary; the annual sum of the daily sun hours; the annual mean of the sea surface temperature; the EA Pattern; and the NAO Index. In the water bodies of Barbadún, Butrón and Urola, data on river flows were not available. In the remaining water bodies, when there was a lack of data on river flow on one particular day, a mean between the available data registered the same days of other years was used.

2.3. Statistical analysis

2.3.1. Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA)

Two separated analysis have been performed, by means of the CANOCO software, version 4.5: (i) CCA for the data on the species densities; and (ii) RDA for data on the total structural parameters of the benthic communities. After each analysis, a plot representative of the model has been obtained, by means of the drawing programme CanoDraw 4.0 (Ter Braak and Smilauer, 2002).

i) CCA: the CCA is a direct gradient ordination technique that searches specifically for faunal patterns that correspond to external factors. This type of analysis has been selected since it is considered that the species response fits best to an unimodal shape and the species data have many zeros. Species data were transformed by taking logarithms whilst the rare species were downweighted to reduce their influence on the analysis. As there are three main groups of independent variables, the CCA was run in three consecutive steps, together with a final model. In each step, one particular group of independent variables, plus the densities of the species (dependent variables), were introduced. The objective of this study is to determine which variables, anthropogenic or climatic, explain most of the spatial and temporal variability in benthic communities. Therefore, two separated CCAs were performed (CCA-pollutants and CCA-climatic): both CCAs included in Step 1 the sedimentological variables (it is assumed beforehand that sedimentary variables explain, to some extent, the species distribution); the CCA-pollutants had the anthropogenic variables introduced in Step 3 whilst CCA-climatic included the climatic variables in Step 3.

A 'forward selection' procedure was used to rank the variables in order of importance, and to identify a subset of significant variables related maximally to species distribution. The statistical significance of the selected variables was assessed using a Monte-Carlo permutation test, under 1999 permutations. The resulting significant variables obtained in each step were included as covariables in the following step; thus removing the variance explained by them.

Final models were obtained for both CCAs: final CCA-pollutants included only the significant anthropogenic variables as independent variables (obtained in Step 3), species densities as dependent variables, and the significant sedimentological and climatic variables (obtained in Steps 1 and 2), as covariables; conversely, final CCA-climatic included only the significant climatic variables as independent variables (obtained in Step 3), species densities as dependent variables, and the significant sedimentological and anthropogenic variables (obtained in Steps 1 and 2), as covariables. In both final models, a Global Permutation Test was performed and a plot obtained, when significant. The Global Permutation test was evaluated with a Monte-Carlo permutation test, under 1999 permutations (full model, with the significance of canonical axes together).

ii) RDA: the RDA is a constrained linear ordination method, useful in determining the independent variables that most explain the variability in the structural parameters of the benthic communities. In order to stabilize the variance of the series and to make the distribution closer to normal, Box-Cox power transformation was applied to all the time-series by means of Statgraphics plus 5.0 software. Data on structural parameters of the communities were centred and standardized, then included as dependent variables. Except for this difference (in CCA, the dependent variables are the species densities), exactly the same procedure as with the CCA was followed: as well, two RDAs were performed separately (RDA-pollutants and RDA-climatic), obtaining plots for the significant final models.

2.3.2. Variance partitioning

It is possible to determine the percentage of variance in the species densities data, explained by anthropogenic, sedimentological, and climatic variables. However, it is likely that the these groups of variables are interacting with each other. Thus, part of the variance in the species densities data would be explained by the interactions among groups of variables. In order to identify the *pure* variance explained by each of the three groups, a "variance partitioning" procedure was carried out (based on Bocard *et al.*, 1992 ; and Legendre *et al.*, 2005), as outlined below.

a) One CCA for each of the 3 groups of variables was carried out, including all of the variables within each group. In this way, the 4 variables of each group that explained the highest percentage of variance in the species densities were identified.

b) Another CCA for each of the 3 groups of variables was performed. This time, apart from including all the variables of each group in each CCA, 8 covariables were introduced. As covariables, the 8 variables of the other two groups (i.e. 4 plus 4, identified in the previous section) that explained the highest percentage of variance in the species densities, were included. As a result, the *pure* variances in the species densities explained, by each of the 3 groups of variables were obtained.

c) A global CCA was run, including 12 variables (4 variables of each of the 3 main groups of variables, identified as explained above). In this way, a value that was the variance explained by the 3 main groups of variables, overall, plus the variance explained by the interactions occurring between each group with the other 2 groups, was obtained.

d) Substracting the *pure* variance explained by the 3 groups of variables, overall, to the value of the variance explained by the three groups (which includes the occurring interactions among groups), the percentage of variance in the species densities explained by the interactions among variables was determined. Moreover, performing a simple calculation, the percentage of variance incapable of being explained by any of the groups was also determined.

2.3.3. Spearman rank correlations

Temporal trends in all of the data series (groups of variables and structural parameters of the community) were analysed. As the data did not fit a normal distribution, Spearman rank correlations were run. Regarding the climatic, anthropogenic and sedimentological variables (variables regarding the grain size were not included), the mean values at each water body were analysed. With regards to the structural parameters, Spearman rank correlations were run for each station in each water body, and for the mean value of each parameter in each water body.

Results

3.1. CCA

The results of the CCA-pollutants and CCA-climatic analysis, for each water body, are lined in Tables 4 and 5.

Each of the locations is now described (see below).

Barbadún: The densities of macrofaunal species were not explained significantly ($p>0.05$) by the climatic or anthropogenic variables, once the variance explained by the characteristics of the sediment is removed. Of the characteristics of the sediment, only the percentage of sand was significant ($p<0.05$), explaining 19.2% of the variance in the density.

Outer Nervión: The densities of the soft-bottom macrofauna were not explained significantly ($p>0.05$) by climatic or anthropogenic variables, once the variance explained significantly by the characteristics of the sediment, was removed. Of the characteristics of the sediment, the organic matter, POC, and C/N were significant ($p<0.05$), explaining 23.22% of variance in the density.

Inner Nervión: The densities of the soft-bottom macrofauna were not explained significantly ($p>0.05$), by climatic or anthropogenic variables, once the variance explained significantly ($p<0.05$) by the characteristics of the sediment was removed. Of the characteristics of the sediment, C/N and depth were significant ($p<0.05$), explaining 25.91% of the variance in the density.

Table 4. CCA-climatic for all of the water bodies, composed of 3 steps, including climatic variables in the last step and final model and its significance. p values of the significant ($p<0.05$) variables are included. (-)=variable not significant ($p<0.05$). Final model CCA-climatic significant ($p<0.05$) for Oiartzun. OM=Organic Matter; POC=Particulate Organic Carbon and PON=Particulate Organic Nitrogen (mol/kg); C/N=Carbon/Nitrogen; Depth (m); Heavy metals=mg/kg; O2 SAT=oxygen saturation (%); Annual rainfall (mm); Winter and annual river flows (m^3/s). For locations, see Figure 2.

	Covariables (sediment)							Covariables (pollutants)							
Water Body	**%OM**	**POC**	**PON**	**C/N**	**%Sand**	**%Silt**	**Depth**	**Mn**	**Cd**	**Pb**	**Cu**	**02 SAT**	**Fe**	**Ni**	**Zn**
Barbadún	-	-	-	-	0.004	-	-	-	-	-	-	-	-	-	-
Inner Nervión	-	-	-	0.042	-	-	0.0005	-	-	-	-	-	-	-	-
Outer Nervión	0.006	-	0.031	0.0005	-	-	-	-	-	-	-	-	-	-	-
Butrón	-	-	-	-	0.033	0.0005	-	0.0225	-	-	-	-	-	-	-
Outer Oka	-	-	-	0.002	0.0005	-	-	-	-	-	-	-	-	-	-
Inner Oka	-	-	-	-	-	-	-	-	0.0105	-	-	-	-	-	-
Lea	-	-	-	-	0.004	-	-	-	-	-	-	-	-	-	-
Artibai	0.018	-	-	-	0.0200	-	-	0.019	-	-	0.0235	-	0.0325	-	-
Deba	-	-	-	-	-	-	-	-	-	-	-	0.0045	-	-	-
Urola	0.03	-	-	0.0005	-	-	-	-	-	-	-	-	-	0.0165	0.041
Oria	-	0.003	-	-	-	-	-	0.0355	-	-	-	-	-	-	-
Urumea	-	-	-	0.0005	-	-	-	0.0205	-	0.0025	-	-	-	-	0.001
Oiartzun	-	-	-	0.007	-	0.0005	0.012	-	-	-	-	-	-	-	-
Bidasoa	-	-	-	0.0005	-	-	-	-	-	-	0.028	0.03	-	-	-

	Variables (climatic)				Final model			
Water Body	**Annual Rainfall**	**Winter Riverflow**	**Annual Riverflow**	**Sun hours**	**F-ratio**	**p**	**Sum of all eigenvalues**	**Sum of all canonical eigenvalues**
Barbadún	-	-	-	-				
Inner Nervión	-	-	-	-				
Outer Nervión	-	-	-	-				
Butrón	-	-	-	-				
Outer Oka	-	-	-	-				
Inner Oka	-	-	-	-				
Lea	-	-	-	-				
Artibai	-	-	-	-				
Deba	-	-	-	-				
Urola	-	-	-	-				
Oria	-	-	-	-				
Urumea	-	-	-	-				
Oiartzun	0.0075	0.0085	0.0005	0.023	2.127	0.0005	1.847	0.0533
Bidasoa	-	-	-	-				

Table 5. CCA-climatic for all of the water bodies, composed of 3 steps, including climatic variables in the last step and final model and its significance. p values of the significant ($p<0.05$) variables are included. (-)=variable not significant ($p<0.05$). Final model CCA-climatic significant ($p<0.05$) for Oiartzun. OM=Organic Matter; POC=Particulate Organic Carbon and PON=Particulate Organic Nitrogen (mol/kg); C/N=Carbon/Nitrogen; Depth (m); Heavy metals=mg/kg; O2 SAT=oxygen saturation (%); Annual rainfall (mm); Winter and annual river flows (m^3/s). For locations, see Figure 2.

Water Body	Covariables (sediment)							Covariables (Climatic)							
	%Sand	%Silt	Depth	POC	PON	%OM	C/N	Annual Riverflow	Sun hours	Winter Riverflow	Annual rainfall	02 SAT	Fe	Ni	Zn
Barbadún	0.004	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Outer Nervión	-	-	0.0005	-	-		0.042	-	-	-	-	-	-	-	-
Inner Nervión	-	-	-	-	0.031	0.006	0.0005	-	-	-	-	-	-	-	-
Butrón	0.033	0.0005	-	-	-	-	-	-	-	-	-	-	-	-	-
Outer Oka	0.0005	-	-	-	-	-	0.002	-	-	-	-	-	-	-	-
Inner Oka	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lea	0.004	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Artibai	0.02	-	-	-	-	0.018	-	-	-	-	-	-	0.0325	-	-
Deba	-	-	-	-	-	-	-	-	-	-	-	0.0045	-	-	-
Urola	-	-	-	-	-	0.03	0.0005	-	-	-	-	-	-	0.0165	0.041
Oria	-	-	-	0.003	-	-	-	-	-	-	-	-	-	-	-
Urumea	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	0.001
Oiartzun	-	0.0005	0.012	-	-	-	0.007	0.0005	0.023	0.0085	0.0075	-	-	-	-
Bidasoa	-	-	-	-	-	-	0.0005	-	-	-	-	0.03	-	-	-

Water Body	Variables (pollutants)								Final model			
	Cd	Mn	Zn	Pb	Fe	%O2 Sat	Cu	Ni	F-ratio	p	Sum of all eigenvalues	Sum of all canonical eigenvalues
Barbadún	-	-	-	-	-	-	-	-				
Outer Nervión	-	-	-	-	-	-	-	-				
Inner Nervión	-	-	-	-	-	-	-	-				
Butrón	-	0.0225	-	-	-	-	-	-	2.139	0.0175	2.139	0.207
Outer Oka	-	-	-	-	-	-	-	-				
Inner Oka	0.0105	-	-	-	-	-	-	-	2.843	0.0005	1.218	
Lea	-	-	-			-	-	-				
Artibai	-	0.019	-	-	0.0325	-	0.0235	-	2.031	0.0005	0.924	0.295
Deba	-	-	-	-	-	0.0045	-	-	2.851	0.0005	1.036	0.149
Urola	-	-	0.041	-	-	-	-	0.0165	1.891	0.008	0.796	0.127
Oria	-	0.0355	-	-	-	-	-	-	2.265	0.0085	1.618	0.212
Urumea	-	0.0205	0.001	0.0025	-	-	-	-	3.621	0.0005	1.361	0.594
Oiartzun	-	-	-	-	-	-	-	-				
Bidasoa	-	-	-	-	-	0.0325	0.0205	-	1.785	0.0045	2.71	0.306

Butrón: Regarding the two CCAs, only the CCA-pollutants was significant ($p=0.0175$). Hence, 9.7% of the variance in the soft-bottom macrofaunal species density was explained significantly ($p<0.05$) by the anthropogenic variables (Mn concentration), once the variance explained by the sediment characteristic (sand percentage and silt-clay percentage) was removed. None of the climatic variables were significant ($p>0.05$).

Outer Oka: The densities of the soft-bottom macrofauna were not explained significantly ($p>0.05$) by climatic or anthropogenic variables, once the variance explained significantly ($p<0.05$) by the characteristics of the sediment was removed. Regarding the characteristics of the sediment, C/N and sand percentage were significant ($p<0.05$), explaining 26.23% of the variance in the density.

Inner Oka: Only the CCA-pollutants was significant ($p=0.0005$). Hence, 41.5% of the variance in the soft-bottom macrofauna species density was explained significantly ($p<0.05$) by the anthropogenic variables (Cd concentration), once the variance explained by the sediment characteristic (sand percentage and silt-clay percentage) was removed.

Lea: The densities of the soft-bottom macrofauna were not explained significantly ($p>0.05$) by climatic or anthropogenic variables, once the variance significantly ($p<0.05$) explained by the characteristics of the sediment was removed. Of the characteristics of the sediment, only sand percentage was significant ($p<0.05$), explaining 15% of the variance in the density.

Artibai: Only the CCA-pollutants was significant ($p= 0.0005$). Hence, 25.3% of the variance in the soft-bottom macrofaunal species density was explained significantly ($p<0.05$) by the anthropogenic variables (Mn, Fe and Cu concentration), once the variance explained by the sediment characteristic (percentage of organic matter and percentage of sand) was removed.

Deba: Only the CCA-pollutants was significant ($p=0.0005$). Hence, 14.28% of the variance of the soft-bottom macrofaunal

Table 6. RDA-climatic for all of the water bodies, composed of 3 steps, including climatic variables in the last step and final model and its significance. p values of the significant ($p<0.05$) variables are included. (-)=variable not significant ($p<0.05$). Final model RDA-climatic significant ($p<0.05$) for Barbadún, outer Oka, Lea, Artibai, Deba and Oiartzun. Redox potential (mV); OM=Organic Matter; POC=Particulate Organic Carbon and PON=Particulate Organic Nitrogen (mol/kg); C/N=Carbon/Nitrogen; Mean grain size (mm); Depth (m); Heavy metals (mg/kg); O2 SAT=oxygen saturation (%); Σ PAHs=sum of Polycyclic Aromatic Hydrocarbons (µg/kg); Annual rainfall (mm); Annual river flow (m^3/s); Annual EA=annual East Atlantic Pattern. For locations, see Figure 2.

	Covariables (sediment)									Covariables (Pollutants)						
Water Body	**Redox Pot.**	**%OM**	**Depth**	**CN**	**POC**	**PON**	**% Sand**	**%Silt**	**% Gravel**	**Mn**	**Ni**	**Cu**	**Fe**	**%O2 Sat**	**PAHs**	**Hg**
Barbadún	-	-	-	-	-	-	-	0.0005	-	-	-	-	-	-	-	-
Outer Nervión	-	-	0.0005	-	-	-	0.0345	-	-	-	-	-	-	-	-	-
Inner Nervión	-	-	-	-	-	-	-	0.0005	0.023	-	-	-	0.0205	-	-	-
Butrón	0.0315	0.046	-	-	-	0.0315	-	0.001	-	-	-	-	-	0.035	-	-
Outer Oka	-	0.001	-	0.004	-	-	-	-	-	-	-	-	-	-	-	-
Inner Oka	-	-	-	-	-	-	-	-	-	-	-	-	0.0435	-	0.022	-
Lea	-	0.004	-	-	-	-	-	-	-	-	-	-	-	0.017	-	-
Artibai	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	0.015
Deba	-	-	-	-	-	-	0.0175	-	-	-	0.001	0.0335	-	-	-	-
Urola	-	-	-	0.0295	-	-	-	-	-	-	-	-	-	-	-	-
Oria	-	-	-	-	-	-	-	0.016	-	-	0.0075	-	-	-	-	-
Urumea	-	0.0025	-	-	0.0295	-	0.0015	-	-	-	-	-	-	-	-	-
Oiartzun	-	0.034	0.005	0.0125	0.0365	0.001	0.0005	-	-	-	-	-	-	-	-	-
Bidasoa	-	-	-	-	-	0.0025	-	-	-	0.0425	-	-	-	-	-	-

	Variables (climatic)					Final model			
Water Body	**Sun Hours**	**Annual Rainfall**	**Annual Riverflow**	**Annual EA**	**Temperature**	**F-ratio**	**p**	**Sum of all eigenvalues**	**Sum of all canonical eigenvalues**
Barbadún	-	-	-	-	0.0035	12.622	0.0005	0.874	0.385
Outer Nervión	-	-	-	-	-				
Inner Nervión	-	-	-	-	-				
Butrón	-	-	-	-	-				
Outer Oka	-	-	-	0.043	-	2.929	0.0325	0.604	0.081
Inner Oka	-	-	-	-	-				
Lea	0.045	-	-	0.003	-	3.739	0.004	0.341	0.131
Artibai	-	-	-	0.0275	-	3.734	0.0105	0.583	0.116
Deba	0.002	-	0.014	-	-	3.795	0.0065	0.541	0.264
Urola	-	-	-	-	-				
Oria	-	-	-	-	-				
Urumea	-	-	-	-	-				
Oiartzun	-	0.039	-	-	-	3.138	0.034	0.225	0.026
Bidasoa	-	-	-	-	-				

species density was explained significantly ($p<0.05$) by the anthropogenic variables (only by the oxygen saturation). Of the climatic variables and the sediment related variables, none of them were significant ($p>0.05$).

Urola: Regarding the two CCAs, only the CCA-pollutants was significant ($p=0.045$). Hence, 12.14% of the variance in the soft-bottom macrofauna species densities was explained significantly ($p<0.05$) by the anthropogenic variables (Ni and Zn concentration), once the variance explained by the sediment characteristic (percentage of organic matter and C/N) was removed.

Oria: Only the CCA-pollutants was significant ($p=0.0085$). Hence, 11.65% of the variance of the soft-bottom macrofauna species densities was explained significantly ($p<0.05$) by the anthropogenic variables (Mn concentration), once the variance explained by the sediment characteristic (POC) was removed.

Urumea: Only the CCA-pollutants was significant ($p=0.0005$). Hence, 34.85% of the variance in the soft-bottom macrofauna species density was explained significantly ($p<0.05$) by the anthropogenic variables (Mn, Zn and Pb concentration), once the variance explained by the sediment characteristic (C/N) was removed.

Oiartzun: Only the CCA-climatic was significant ($p=0.0005$). Hence, 23.06% of the variance of the soft-bottom macrofauna species density was explained significantly ($p<0.05$) by the climatic variables (annual rainfall, winter river flow, annual river flow and sun hours), once the variance explained by the sediment characteristic (C/N, silt-clay and depth) was removed.

Bidasoa: Only the CCA-pollutants was significant ($p=0.0045$). Hence, 9.86% of the variance in the soft-bottom macrofauna species density was explained significantly ($p<0.05$) by the anthropogenic variables (Cu concentration and oxygen saturation), once the variance explained by the sediment characteristic (C/N) was removed.

Table 7. RDA-pollutants for all of the water bodies, composed of 3 steps, including climatic variables in the last step and final model and its significance. p values of the significant ($p<0.05$) variables are included. (-)=variable not significant ($p<0.05$). Final model RDA-pollutants significant ($p<0.05$) for outer Nervión, inner Oka, Artibai, Deba, Oria and Bidasoa. OM=Organic Matter; POC=Particulate Organic Carbon and PON=Particulate Organic Nitrogen (mol/kg); C/N=Carbon/Nitrogen; Mean grain size (mm); Depth (m); Redox potential (mV); Annual river flow (m^3/s); Annual EA=annual East Atlantic Pattern; Winter NAO=winter North Atlantic Oscillation Index; Temp=temperature (Cº); Annual rainfall (mm); Heavy metals (mg/kg); ΣPAHs=sum of Polycyclic Aromatic Hydrocarbons (µg/kg)). For locations, see Figure 2.

	Covariables (sediment)										Variables (climatic)					
Water Body	Depth	Redox pot	POC	PON	CN	% OM	% Silt	% Sand	% Gravel	Mean grain size	Annual riverflow	Annual EA	Winter NAO	Temp.	Sun hours	Annual Rainfall
Barbadún	-	-	-	-	-	-	0.043	-	-	-	-	-	-	0.0035	-	-
Outer Nervión	0.0005	-	-	-	-	-	-	0.0345	-	-	-	-	-	-	-	-
Inner Nervión	-	-	-	-	-	-	0.0005	-	0.023	0.019	-	-	-	-	-	-
Butrón	-	0.0315	-	0.0315	-	0.046	0.001	-	-	-	-	-	-	-	-	0.041
Outer Oka	-	-	-	-	0.004	0.001	-	-	-	-	-	0.0485	-	-	-	-
Inner Oka	-	-	-	-	-	-	-	-	-	-	-	-	0.048	-	-	-
Lea	-	-	-	-	-	0.004	-	-	-	-	-	0.0025	-	-	0.008	-
Artibai	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-	-
Deba	-	-	-	-	-	-	-	0.0175	-	-	0.021	-	-	-	0.0465	-
Urola	-	-	-	-	0.019	-	-	-	-	-	-	-	-	-	-	-
Oria	-	-	-	-	-	-	0.014	-	-	-	-	-	-	-	-	-
Urumea	-	-	0.031	-	-	0.003	-	0.0015	-	-	-	-	-	-	-	-
Oiartzun	0.003	-	0.031	0.0005	0.0135	0.028	-	0.0005	-	-	-	-	-	-	-	0.0445
Bidasoa	-	-	-	0.0045	-	-	-	-	-	-	-	-	-	-	-	-

	Variables (pollutans)							Final model			
Water Body	Hg	Mn	Fe	Ni	Cu	Pb	PAHs	F-ratio	p	Sum of all eigenvalues	Sum of all canonical eigenvalues
Barbadún	-	-	-	-	-	-	-				
Outer Nervión	-	-	-	-	-	-	-				
Inner Nervión	-	-	0.024	-	-	-	-	3.819	0.022	0.442	0.068
Butrón	-	-	-	-	-	-	0.0415				
Outer Oka	-	-	-	-	-	-	-				
Inner Oka	-	-	-	-	-	0.033	-	4.974	0.0005	0.484	0.302
Lea	-	-	-	-	-	-	-				
Artibai	0.0165	-	-	-	-	-	-	4.528	0.01	0.748	0.165
Deba	-	-	-	0.0015	0.016	-	-	5.872	0.0015	0.575	0.273
Urola	-	-	-	-	-	-	-				
Oria	-	-	-	0.0045	-	-	-	4.332	0.0015	0.827	0.176
Urumea	-	-	-	-	-	-	-				
Oiartzun	-	-	-	-	-	-	-				
Bidasoa	-	0.045	-	-	-	-	-	3.253	0.046	0.823	0.083

Summarizing, CCA-climatic was significant ($p<0.05$) for Oiartzun, whilst CCA-pollutants was significant for Butrón, inner Oka, Artibai, Deba, Urola, Oria, Urumea and Bidasoa.

3.2. RDA

The results of the RDA-pollutants and RDA-climatic analysis for each water body are shown in Tables 6 and 7.

Barbadún: Only RDA-climatic was significant ($p=0.0005$). Hence, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the climatic variables (temperature), once the variance explained by the sediment characteristic (silt-clay percentage) was removed (Table 6).

Outer Nervión: Only the RDA-pollutants was significant ($p=0.022$) (Table 7). Hence, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the anthropogenic variables (Fe concentration), once the variance explained by the sediment characteristic (silt-clay percentage, gravel percentage and mean grain size) was removed.

Inner Nervión: The structural parameters of the soft-bottom macrofauna were not explained significantly ($p>0.05$) by climatic or anthropogenic variables, once the variance significantly ($p<0.05$) explained by the characteristics of the sediment was removed. Within the characteristics of the sediment, only sand percentage and water depth were significant ($p<0.05$).

Butrón: Regarding the RDA-climatic, the structural parameters of the macrofaunal communities were not explained significantly ($p>0.05$) by the climatic variables, once the variance explained by the characteristics of the sediment (PON, redox potential, organic matter and silt-clay) and the anthropogenic variables (oxygen saturation) was removed. With regard to the RDA-pollutants, the structural parameters of the macrofaunal communities were not explained significantly ($p>0.05$) by the anthropogenic variables, once the variance explained by the characteristics of the sediment (PON, redox potential, organic matter and silt-clay), and the climatic variables (annual rainfall) was removed.

Outer Oka: Only the RDA-climatic was significant ($p=0.032$). Hence, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the climatic variables (annual EA), once the variance explained by the sediment characteristic (organic matter and C/N) was removed.

Lea: Only the RDA-climatic was significant ($p=0.004$). Hence, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the climatic variables (sun hours and annual EA), once the variance explained by the sediment characteristics (organic matter) and by the anthropogenic variables (oxygen saturation) was removed.

Artibai: Both RDAs were significant ($p<0.05$). Regarding the RDA-climatic, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the climatic variables (annual EA), once the variance explained by the sediment characteristics (organic matter) and by the anthropogenic variables (Hg concentration) was removed. Regarding the RDA-pollutants, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p=0.01$) by the anthropogenic variables (Hg concentration), once the variance explained by the sediment characteristics (organic matter) was removed.

Deba: Both RDAs were significant ($p<0.05$). Regarding the RDA-climatic, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p=0.002$) by the climatic variables (sun hours and annual river flow), once the variance explained by the sediment characteristics (sand percentage) and by the anthropogenic variables (Cu and Ni concentrations) was removed. Regarding the RDA-pollutants, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p=0.0015$) by the anthropogenic variables (Cu and Ni concentrations), once the variance explained by the sediment characteristics (sand percentage) and by the climatic variables (sun hours and annual river flow) was removed.

Urola: The structural parameters of the soft-bottom macrofauna were not explained significantly ($p>0.05$) by climatic or anthropogenic variables, once the variance explained significantly ($p<0.05$) by the characteristics of the sediment was removed. Of the characteristics of the sediment, only C/N was significant ($p<0.05$).

Oria: Only the RDA-pollutants was significant ($p=0.0015$). Hence, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the anthropogenic variables (Ni concentration), once the variance explained by the sediment characteristics (silt-clay percentage) was removed.

Urumea: The structural parameters of the soft-bottom macrofauna were not explained significantly ($p>0.05$) by climatic or anthropogenic variables, once the variance explained significantly ($p<0.05$) by the characteristics of the sediment was removed. Of the characteristics of the sediment, POC, organic matter and sand were significant ($p<0.05$).

Oiartzun: Only the RDA-climatic was significant ($p=0.034$). Hence, the variance of the structural parameters in the macrofaunal communities was explained significantly ($p<0.05$) by the climatic variables (annual rainfall), once the variance explained by the sediment characteristics (C/N, depth, POC, PON, organic matter and sand) was removed.

Bidasoa: Only the RDA-pollutants was significant ($p=0.045$). Hence, the variance in the structural parameters of the macrofaunal communities was explained significantly ($p<0.05$) by the anthropogenic variables (Mn concentration), once the variance explained by the sediment characteristic (PON) was removed.

Summarizing, RDA-climatic was significant ($p<0.05$) in Barbadún, outer Oka, Lea and Oiartzun, whereas RDA-pollutants was significant in outer Nervión, inner Oka, Oria and Bidasoa. Further, both RDA-climatic and RDA-pollutants were significant in Deba and Artibai.

3.3. Partition of the variance

On average, the characteristics of the sediment explained 17.2% of the variance in the macrofaunal species density ($p<0.05$) (Table 8). The pollutants explained 16.9% whilst the climatic variables explained 15.4% of the variance. Further, on average, 12.4% of the variance in the species density is explained by the interactions between variables and, finally, there is a 38% of the variance that cannot be explained.

Within each group of variables, the sediment-related variables explained the highest variance in the species density in Urumea (21.9%), Barbadún (21.5%), and outer Oka (18%).

The anthropogenic variables explained the highest percentage of variance in Barbadún (21.1%), Urumea (21%), Butrón (19.8%),

Table 8. Partition of the variance. Percentage of variance in the densities (ind. m^{-2}) of the macrofaunal species explained significantly ($p< 0.05$) by each group of variables; percentage of variance explained by interactions between the variables; and percentage of variance not explained. Inner Oka and Deba are not included.

	Barbadún	Outer Nervión	Inner Nervión	Butrón	Outter Oka	Lea	Artibai	Urola	Oria	Urumea	Oiartzun	Bidasoa	AVERAGE
Climate	21.8	13.2	11.9	12	15.4	16	19	13.5	13	17.2	21.8	10.6	15.4
Sediment	21.5	15.7	17.6	16.2	18	17.7	16.8	17.5	13.4	21.9	15.1	15.1	17.2
Pollutants	21.1	13.8	16.6	19.7	15.5	13.3	19.5	18.2	14.2	21	12.9	16.5	16.9
Interaction	12.8	11.6	26.9	14.7	8.7	3.7	19.6	13.9	1.5	20.3	9.1	6.1	12.4
Not explained	22.7	45.7	26.9	37.2	42.3	49.3	24.9	37	57.9	19.5	41.2	51.7	38

and Artibai (19.5%), whilst the climatic variables explained the highest percentage of variance in Barbadún (21.9%), Oiartzun (21.8%), Artibai (19.04%), Urumea (17.2%) and Lea (16.03%). The highest percentage of interactions between groups of variables occurred in the inner Nervión (26.9%), Urumea (20.3%) and Artibai (19.6%), whilst the highest percentage of variance not explained occurred in Oria (57.9%), Bidasoa (51.7%), Lea (49.3%), and outer Nervión (45.7%).

The "variance partitioning" procedure could not be undertaken for inner Oka and Deba, probably due to a lack of data; E-OK5 and E-D5 have been sampled only since 2002.

3.4. Spearman rank correlations

All of the significant correlations ($p<0.05$) between variables and sampling years are lined in Tables 9, 10, 11, 12 and 13; these are summarized below.

Table 9. Spearman rank correlations between the sampling year and the structural parameters of the different stations of each water body (R=correlation; N=sample size; p=p value).

	Structural parameters for stations			
		R	N	P
Barbadún	Benthic density-EM5	-0.9429	6	0.035
Outer Nervión	Diversity-EN20	0.6492	13	0.0245
Inner Nervión	AMBI-EN10	-0.7885	13	0.0063
	Biomass-EN10	0.7705	13	0.0076
	Benthic density-EN10	0.7466	13	0.0097
	Evenness-EN17	-0.9429	6	0.035
Butrón	Benthic density-EB10	0.6006	13	0.0375
	AMBI-EB5	0.9429	6	0.035
Outer Oka	-	-	-	-
Inner Oka	-	-	-	-
Lea	-	-	-	-
Deba	Biomass-ED5	-0.9429	6	0.035
Artibai	AMBI-EA10	-0.5989	13	0.038
	Biomass-EA10	0.8791	13	0.0023
	Diversity-EA10	0.7802	13	0.0069
	Evenness-EA10	0.6429	13	0.026
Urola	Benthic density-EU8	0.8857	6	0.0476
Oria	-	-	-	-
Urumea	Species richness-EUR5	0.8827	6	0.0484
Oiartzun	AMBI-EOI10	-0.674	13	0.0195
	Biomass-EOI10	0.8122	13	0.0049
	Benthic density-EOI10	0.6188	13	0.0321
	Diversity-EOI10	0.8327	13	0.0039
	Evenness-EOI10	0.6999	13	0.0153
	Species richness-EOI10	0.9212	13	0.0014
	Species richness-EOI15	0.8857	6	0.0476
	Diversity-EOI20	0.5915	13	0.0405
	Evenness-EOI20	0.6713	13	0.0201
Bidasoa	AMBI-EBI20	-0.6758	13	0.0192
	Benthic density-EBI20	0.7637	13	0.0082
	Species richness-EBI20	0.6077	13	0.0353

Table 10. Spearman rank correlations undertaken between the mean values of the structural parameters at each water body and the sampling year (R=correlation; N=sample size; p=p value).

	Structural parameters (General)			
		R	N	P
Barbadún	-	-	-	-
Outer Nervión	-	-	-	-
Inner Nervión	AMBI	-0.4711	25	0.021
	Benthic density	0.5625	25	0.0059
	Diversity	0.4781	25	0.0192
	Evenness	0.5195	25	0.0109
	Species richness	0.5972	25	0.0034
Butrón	AMBI	0.453	25	0.0265
	Benthic density	0.6529	25	0.0014
Outer Oka	-	-	-	-
Inner Oka	-	-	-	-
Lea	-	-	-	-
Artibai	-	-	-	-
Deba	-	-	-	-
Urola	-	-	-	-
Oria	Benthic density	0.5702	19	0.0156
	Species richness	0.7541	19	0.0014
Urumea	Species richness	0.4918	19	0.0369
Oiartzun	Evenness	0.5042	32	0.005
Bidasoa	-	-	-	-

- Structural parameters: AMBI values decreased (low AMBI values represent unimpacted or low impacted areas, after Borja *et al.*, 2000) in the innermost station of inner Nervión (E-N10), Artibai (E-A10), Oiartzun (E-OI10), and Bidasoa (E-BI20) (Table 9). In turn, an increase in the innermost station of Butrón (E-B5) was detected. Species richness increased in Urumea (E-UR5), Oiartzun (E-OI10, E-OI15), and Bidasoa (E-BI20). The benthic density decreased in Barbadún (E-M5) and increased in the inner Nervión (E-N10), Butrón (E-B10), Urola (E-U8), Oiartzun (E-OI10) and Bidasoa (E-BI20). Diversity showed an increase in the outer Nervión (E-N20), Artibai (E-A10), and Oiartzun (E-OI10, E-OI20). Biomass increased in the inner Nervión (E-N10), Artibai (E-A10) and Oiartzun (E-OI10), and decreased in Deba (E-D5). Finally, evenness increased in Artibai (E-A10) and Oiartzun (E-OI10 and E-OI20), and decreased in the inner Nervión (E-N17). With regard to the correlations between water body and sampling year, in general, the AMBI decreased in the inner Nervión and increased in Butrón (Table 10). Benthic density showed an increase in the inner Nervión, Butrón and Oria, whilst species richness showed an increase in the inner Nervión, Oria and Urumea. Finally, diversity showed an increase in the inner Nervión, whilst evenness increased in the inner Nervión and Oiartzun.

- General physico-chemical characteristics of the sediment: The percentage of organic matter showed a decreasing trend in the outer Nervión and in Deba (Table 11). The redox potential decreased in the inner Nervión, Butrón and Urola, whilst the depth decreased also in the inner Nervión.

In Butrón, the POC showed a decreasing trend, whilst the PON increased. In turn, the C/N decreased in Butrón and in Urola.

Table 11. Spearman rank correlations between mean values of the sediment related variables of each water body and the sampling period (R=correlation; N=sample size; p=p value).

General physico-chemical characteristics of the sediment				
		R	**N**	**P**
Barbadún	-	-	-	-
Outer Nervión	Organic matter%	-0.4208	26	0.0354
Inner Nervión	Redox Potential	-0.4701	20	0.0405
	Depth	-0.5348	25	0.0088
Butrón	CN	-0.5766	23	0.0068
	POC	-0.6074	23	0.0044
	PON	0.3985	23	0.0616
	Redox Potential	-0.6693	21	0.0028
Outer Oka	-	-	-	-
Inner Oka	-	-	-	-
Lea	-	-	-	-
Artibai	-	-	-	-
Deba	Organic matter%	-0.5932	18	0.0145
Urola	Redox Potential	-0.4732	21	0.0343
	C/N	-0.4851	25	0.0175
Oria	-	-	-	-
Urumea	-	-	-	-
Oiartzun	-	-	-	-
Bidasoa	-	-	-	-

- <u>Anthropogenic variables</u>: Oxygen saturation decreased in Barbadún, the outer and inner Oka, Lea, Urola and Oria; it increased in the outer and inner Nervión (Table 12). The organic compounds (mainly ΣPAHs) showed increasing trends in Barbadún, Butrón, Artibai, Urola, Oria, Urumea, Oiartzun and Bidasoa. Amongst the heavy metals, only Zn, Mn and Cr showed significant trends ($p<0.05$): Zn decreased in both the inner and outer Oka; Mn increased in Artibai and Bidasoa; and Cr increased in Oiartzun.

- <u>Climatic variables</u>: Winter and annual rainfalls, together with winter and annual river flows showed significant ($p<0.05$) trends (Table 13): Both annual river flow and annual rainfall showed a decrease in Oiartzun, whereas winter and annual river flows showed a decrease in Bidasoa. On the other hand, winter rainfall showed an increasing trend in the outer Oka.

Table 12. Spearman rank correlations between mean values of the anthropogenic variables of each water body and the sampling period (R=correlation; N=sample size; p=p value).

Anthropogenic variables				
		R	**N**	**P**
Barbadún	O2 saturation	-0.371	23	0.0819
	ΣPAHs	0.6165	17	0.0137
Outer Nervión	O2 saturation	0.6034	26	0.0026
Inner Nervión	O2 saturation	0.745	25	0.0003
Butrón	ΣPAHs	0.678	24	0.0011
Outer Oka	Zn	-0.4455	22	0.0412
	O2 saturation	-0.371	23	0.0819
Inner Oka	Zn	-0.8857	6	0.0476
Lea	O2 saturation	-0.6183	19	0.0087
Artibai	ΣPAHs	0.5629	19	0.0169
	Mn	0.502	19	0.0332
Deba	O2 saturation	-0.6182	19	0.0086
	O2 saturation	-0.4599	25	0.0243
Urola	ΣPAHs	0.6726	25	0.001
	ΣPCBs	0.4137	25	0.427
Oria	ΣPAHs	0.6245	18	0.01
	O2 saturation	-0.5262	19	0.0256
Urumea	ΣPAHs	0.5707	17	0.0224
Oiartzun	Cr	0.3923	31	0.0317
	ΣPAHs	0.5566	26	0.0054
Bidasoa	Mn	0.4931	32	0.006
	ΣPAHs	0.4983	32	0.0055

Table 13. Spearman rank correlations between mean values of the climatic variables at each water body and the sampling period (R=correlation; N=sample size; p=p value).

Climatic variables (General)				
		R	**N**	**P**
Barbadún	-	-	-	-
Outer Nervión	-	-	-	-
Inner Nervión	-	-	-	-
Butrón	-	-	-	-
Outer Oka	Winter rainfall	0.633	18	0.0091
Inner Oka	-	-	-	-
Lea	-	-	-	-
Artibai	-	-	-	-
Deba	-	-	-	-
Urola	-	-	-	-
Oria	-	-	-	-
Urumea	-	-	-	-
Oiartzun	Annual riverflow	-0.6152	26	0.0021
	Annual rainfall	-0.4566	32	0.0086
Bidasoa	Annual riverflow	-0.4732	30	0.0108
	Winter riverflow	-0.5178	28	0.0071

Discussion

The anthropogenic variables for the Basque estuaries explain the variance in the species densities of the soft-bottom macrofauna, to a higher extent than the climatic variables. 16.9% of the variance is explained significantly ($p<0.05$) by anthropogenic variables, compared to 15.4% explained by climatic variables. This pattern is in accordance with the high amount of wastes (mineral sluicing, industrial wastes and urban effluents) that Basque estuaries have been receiving for the last 150 years, resulting in degradation of the environmental quality (Cearreta *et al.*, 2000). In turn, in the Basque coast, climatic variables explain much more variance in the soft-bottom communities density than the anthropogenic variables (Garmendia *et al.*, 2008). This observation is related to the different level of anthropogenic pressure supported by these estuaries (high) and coasts (low) (Borja *et al.*, 2005). Moreover, this study has shown that the general physico-chemical characteristics of sediment are relevant, when explaining the variance in the density of macrofaunal species of the Basque estuaries (17.2% of the variability explained).

4.1. General physico-chemical characteristics of the sediment

The physico-chemical properties of the sediment (grain size composition, organic matter content, PON and POC, and redox potential) determine the conditions for the sediment to act as a sink, or as a carrier of contaminants (Belzunce *et al.*, 2004). The inputs of dumping of pollutants and organic matter, through wastewater or sewage sludge inputs, can contribute to increasing the organic carbon content characteristics of an area, and the proportion of silts and clays that reach bottom communities (Cardell *et al.*, 1999). Furthermore, the above mentioned variables, plus water depth, influence the species composition of the fauna and the abundance and biomass of individual invertebrate species (Warwick *et al.*, 1991).

The characteristics of the sediment explained most of the variability in the species density, for the inner Nervión (17.6%) and outer Nervión (15.7%). Within this group of variables, organic matter is of high importance in the outer Nervión, as benthic organisms can react to increasing gradients of organic enrichment (Sáiz-Salinas, 1997). In the outer Nervión, organic matter explained the variability in the species densities; it showed also a significant decreasing trend, with time. The organic matter content plays an important role in the transport, mobility and availability of contaminants. It is associated usually with fine-grained particles and transported with suspended particulate matter. This factor enables the association of contaminants with the fine particulate material, leading to the transport, accumulation and incorporation of contaminants into the bottom sediments (Bubb and Lester, 1991). Thus, organic matter is an indicator of potential contamination (Uriarte *et al.*, 2004). Hence, the decreasing trend in the organic matter content suggests an improvement of the environmental quality of the sediments, in the outer Nervión. This significant decrease in the flux of organic matter in the Nervión is explained by: the implementation of environmental protection policies; the improvement in water-treatment systems; and the closure of some major pollutant companies (Borja *et al.*, 2006; Borja *et al.*, 2009).

On the other hand, in the outer Nervión, the densities of the macrofaunal species were explained by PON and C/N; in the inner Nervión, by C/N. Dissolved oxygen concentrations are lowered usually when organic matter is degraded by aerobia bacteria: anoxic and hypoxic conditions may develop under stratified conditions. Decomposition rates of organic matter increase as N contents increase (Enriquez *et al.*, 1993), and as C/N ratio decreases (Thomann, 1972).

4.2. Anthropogenic variables

Spatial and temporal variability in the density of soft-bottom macrofaunal species

In Bidasoa, the variability in species density was explained by oxygen saturation and Cu concentration. In this water body, most of the species belonged to the *Scrobicularia plana - Cerastoderma edule community* (Figure 3), *sensu* Borja *et al.* (2004c) such as: the bivalves *C. edule* and *Scrobicularia plana*; the polychaetes *Hediste diversicolor* and *Heteromastus filiformis*; the prosobranch *Hydrobia ulvae*; and the oligochaeta and the crustacean *Cyathura carinata*, mixed with the pollution indicator species *Capitella capitata* polychate. The presence of this pollution indicator species, within the *Scrobicularia - Cerastoderma* community, shows a transition to pollution environments, due to the high concentrations of several heavy metals (such as Cu) and organic compounds, associated with muddy sediments (Borja *et al.*, 2004c). Similar groups of species were found in Deba, Urola and Artibai (Figures 4, 5 and 6), indicating the same circumstances. In Bidasoa (Figure 3), a clear discrimination between species related to sediments with high Cu concentrations and low oxygen saturations (e.g. *Spio martinensis*, *Hydrobia ulvae* and *Heteromastus filiformis*) and species related to sediments with lower Cu contents and higher oxygen saturations (e.g. *Paphia rhomboides* and *Tellina* sp.) was observed in the ordination plot. In Deba (Figure 4), species such as *Pachygrapsus marmoratus* and *Upogebia pusilla* were characteristis of sediments with high oxygen saturations, whilst an species such as *Hediste diversicolor* was related to sediments with low oxygen saturations. In turn, a species such as *Tapes* sp. was characteristic of sediments with low Cu and Ni concentrations in Urola (Figure 5). In Artibai (Figure 6), the opportunistic species *Polydora ciliata* was situated in the right-hand side of the ordination biplot, whereas the sensitive species *Liocarcinus* sp. was situated in the left-hand side of the biplot.

It is important to point out that in certain water bodies, such as outer and inner Nervión, that globally support "high pressure" (Borja *et al.*, 2004d), the variability explained by anthropogenic variables should be higher than that found in this study. However, this inbalance could be explained by the interactions occurring between the main groups of variables. Thus, in the outer and inner Nervión, there is 11.6% and 27%, respectively, of variance explained by the interactions between the groups of variables (Table 8). The Nervión estuary is the deepest of all the Basque estuaries (up to 30 m depth); as such, it is a complex estuary where many physico-chemical processes take place. This complexity results in the high number of interactions occurring between all of the studied variables, masking the effect of the anthropogenic variables.

Overall, the explaining anthropogenic variables were Mn, Fe, Cu, Zn, Pb and Ni concentrations, together with oxygen saturation. In fact, in the Deba water body, a significant decreasing trend in the oxygen saturation, throughout the sampling period, has been observed. Similarly, in the Barbadún, outer Oka, Lea and Oria, suggesting a worsening in the water quality within these estuaries. Oxygen limitation is a key factor in estuarine functioning, being capable of causing complete defaunation over large areas (Sáiz-Salinas, 1997).

Conversely, in the outer and inner Nervión, oxygen saturation has been shown to follow an increasing trend; this is due to more than 15 years of water cleansing within the sewerage scheme, approved for the area by the *Consorcio de Aguas Bilbao-Bizkaia*. According to González-Oreja and Sáiz-Salinas (1998), oxygen is the key environmental factor explaining the distribution of benthos in the Nervión estuary. After physico-chemical and biological water treatment, the oxygen values in the inner Nervión, in 2003, reached almost 70%. In turn, in the middle and outer reaches, the mean values increased from 70 to 80%, in the 1990s, and 90 to 100%, in the 2000s (Borja *et al.*, 2006). Indeed, Borja *et al.* (2009) have found recently a positive trend in the benthic status in the Nervión estuary, due to a decrease in the nutrient discharges and an increase in dissolved oxygen.

Although in the Basque Country the highest levels of organic compounds can be found in the Oiartzun (Franco *et al.*, 2001) and Nervión estuaries (Cotano and Villate, 2006), high levels can be found also in Deba (Borja *et al.*, 2007), Urola and Bidasoa (Belzunce *et al.*, 2004). In fact, in the present study, PAHs showed an increasing trend in Barbadún, Butrón, Artibai, Urola, Urumea, Oiartzun and Bidasoa, whilst the PCBs increased in Urola. Nonetheless, this study did not find any association between the variance in the species density and the high levels of organic compounds.

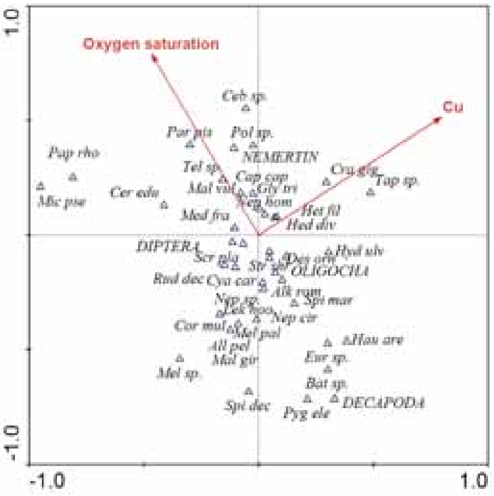

Figure 3. CCA-pollutants biplot for benthic invertebrates densities and environmental variables for Bidasoa. Environmental variables -Cu concentration and oxygen saturation- were plotted on the ordination as arrows. The importance of each environmental variable is indicated by its length. Environmental axes extend increasingly in the direction of the arrow. They can be extrapolated in the opposite direction from the origin to depict a decreasing trend in the variables. Taxa represented by triangles. Only those species whose fit to the diagram is >17% are shown. Full name of abbreviated invertebrate taxa: Arthropoda: *Pinnotheres pisum, Lekanesphaera hookeri, Allomelita pellucida, Haustorius arenarius, Eurydice sp., Melita sp., Bathyporeia sp., Corophim multisetosum, Melita palmata, Allomelita pellucida;* Mollusca*: Cerastoderma edule, Hydrobia ulvae, Paphia rhomboides, Tapes sp., Tellina sp., Crassostrea gigas, Scrobicularia plana, Ruditapes decussatus;* Annelida*: Capitella capitata, Hediste diversicolor, Cerebratulus sp., Malacoceros vulgaris, Nephtys hombergii, Glycera tridactyla, Heteromastus filiformis, Mediomastus fragilis, Streblospio shrubsolii, Desdemona ornata, Nephtys sp., Spio martinensis, Nephtys cirrosa, Malacoceros girardii, Alkmaria romijni, Pygospio elegans, Spio decoratus, Polydora sp., Microphthalmus pseudoaberrans*.

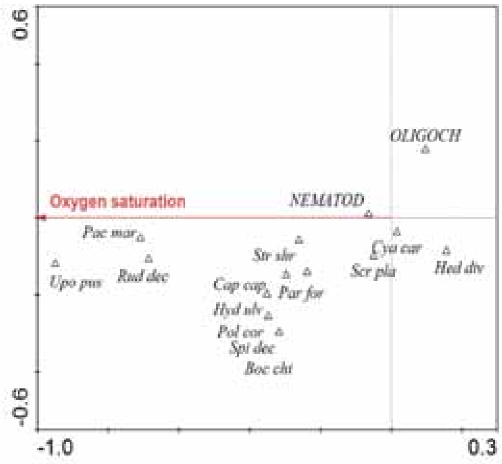

Figure 4. CCA-pollutants biplot for benthic invertebrates densities and environmental variables for Deba. The environmental variable -oxygen saturation- was plotted on the ordination as an arrow (see Figure 3 for explanation). Only those species whose fit to the diagram is >80% are shown. Full name of abbreviated invertebrate taxa: Arthropoda: *Pachygrapsus marmoratus, Upogebia pusilla, Cyathura carinata, Paragnathia formica;* Mollusca*: Ruditapes decussatus, Scrobicularia plana, Hydrobia ulvae;* Annelida: *Streblospio shrubsolii, Capitella capitata, Hediste diversicolor, Polydora cornuta, Spio decoratus, Boccardia chilensis.*

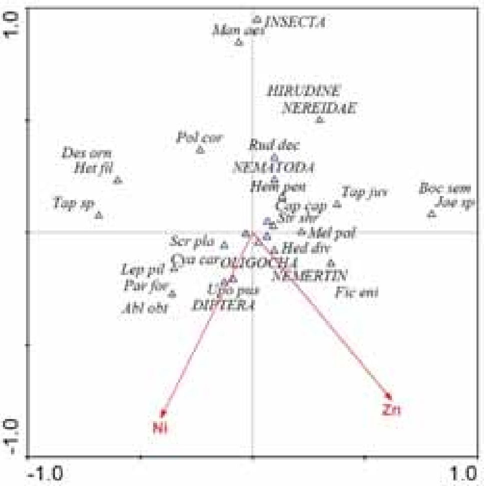

Figure 5. CCA-pollutants biplot for benthic invertebrates densities and environmental variables for Urola. The environmental variables –Ni and Cu concentrations- were plotted on the ordination as arrows (see Figure 3 for explanation). Only those species whose fit to the diagram is >17% are shown. Full name of abbreviated invertebrate taxa: Arthropoda: *Hemigrapsus penicillatus, Jaera sp., Melita palmata, Upogebia pusilla, Cyathura carinata, Paragnathia formica, Abludomelita obtusata, Leptocheirus pilosus;* Mollusca: *Ruditapes decussatus, Tapes sp., Scrobicularia plana;* Annelida: *Manayunkia aestuarina, Polydora cornuta, Desdemona ornata, Streblospio shrubsolii, Capitella capitata, Hediste diversicolor, Spio decoratus, Boccardia semibranchiata, Ficopomatus enigmaticus.*

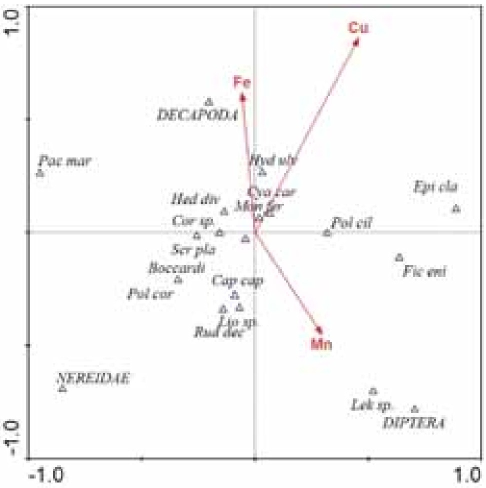

Figure 6. CCA-pollutants biplot for benthic invertebrates densities and environmental variables for Artibai. The environmental variables –Fe, Cu and Mn concentrations- were plotted on the ordination as arrows (see Figure 3 for explanation). Only those species whose fit to the diagram is >26% are shown. Full name of abbreviated invertebrate taxa: Arthropoda: *Pachygrapsus marmoratus, Corophium sp., Liocarcinus sp., Cyathura carinata, Lekanesphaera sp.;* Mollusca: *Ruditapes decussatus, Scrobicularia plana, Hydrobia ulvae, Epilepton clarkiae, Montacuta ferruginosa;* Annelida: *Capitella capitata, Hediste diversicolor, Polydora ciliata, Polydora cornuta, Ficopomatus enigmaticus, Boccardia sp..*

Spatial and temporal variability in the structural parameters of the soft-bottom macrofaunal communities

Sediments of the Basque estuaries are enriched generally in heavy metals well above background levels (Franco *et al.*, 2004, Rodríguez *et al.*, 2006). This result is in accordance with our study, where the variability in the structural parameters of the soft-bottom communities is explained by anthropogenic variables and, in particular, by Hg, Mn, Fe, Ni, Cu and Pb concentrations, in the outer Nervión, inner Oka, Artibai, Deba, Oria and Bidasoa. In fact, the Nervión and Deba estuaries are considered to be amongst the most polluted estuaries of the Basque Country, in terms of heavy metals (Borja *et al.*, 2004d, 2007, 2008a; Franco *et al.*, 2004). Franco *et al.* (2001) in Nervión, and Legorburu and Cantón (1991), and Franco *et al.* (2002), in Oiartzun, found decreasing trends of heavy metals, with time, reflecting the industrial recession over the past years (Franco *et al.*, 2004). However, the present study did not find any significant trend regarding heavy metal concentrations throughout the monitoring period.

Healthy benthic communities can be characterized by high species richness. In fact, habitats *a priori* classified as stressed, show a pattern of low values of species richness and undergo community composition changes from dominance by long-lived equilibrium species, typical of unstressed situations, to dominance by short-lived opportunistic species (Dauer *et al.*, 1993). With increased organic matter load, biomass, number of species and number of individuals decline dramatically as anaerobiosis ensue (Pearson and Rosenberg, 1978). Besides, highly impacted sites generally show high values of AMBI, as they are characterized mainly by species tolerant to the pollution and by opportunist species (Borja *et al.*, 2003a). Deba (Figure 7) is an example of these patterns, because AMBI followed closely the increasing gradient of Cu and Ni concentrations, whilst the species richness increased with decreasing gradients of these heavy metals.

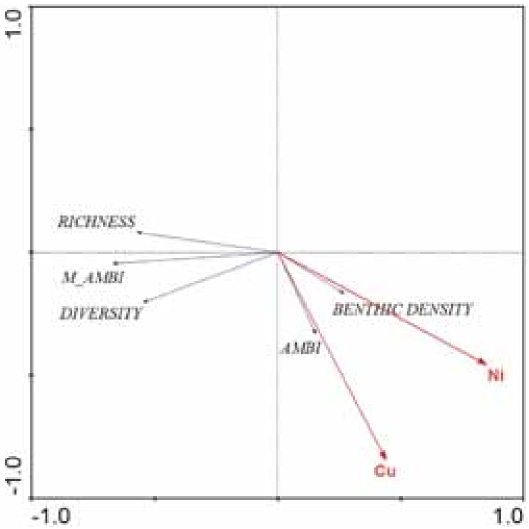

Figure 7. RDA-pollutants biplot for benthic invertebrates structural parameters and environmental variables for Deba. The environmental variables – Cu and Ni concentrations- were plotted on the ordination as arrows (see Figure 3 for explanation).

In the inner Nervión and Oiartzun water bodies, positive trends in the benthic status were observed (Tables 9 and 10), due mainly to a reduction in nutrient discharges in latter years and to an increase in dissolved oxygen. Furthermore, a close association can be observed between the evolution of oxygen saturation and AMBI, in the inner Nervión. The gradual improvement in the benthic quality of the Artibai water body, reflected in its structural parameters (Table 9) could be caused by the partial treatment that began in the late 1990s. A similar response is observed in Bidasoa, which has benefited from several water treatment programmes between 1995 and 2003. The quality in the external part of this water body (station E-BI20), as shown in Table 9, has improved over time (Borja *et al.*, 2009).

4.3. Climatic variables

Although the Oiartzun supports generally a "high pressure" (Borja *et al.*, 2004d), in this water body, no anthropogenic variables were found to be significant in explaining the variability in the macrofaunal species densities or in the structural parameters. Conversely, the climatic variables explained the variability, in both density and structural parameters. Research should be undertaken here to provide an explanation to this fact.

The difference in the relative influence of the river flow is one of the main environmental factors that affects estuarine hydrology, geochemistry and biology. Residence time is a measure of the relative importance of the river flow (Valencia and Franco, 2004b). Thus, for similar loads, the regulation (or pre-dilution) in estuaries with high residence times is greater, than in shallow canalised estuaries with rapid exportation rates (Valencia *et al.*, 2004a). Interestingly, in the Deba, which is the estuary with the smallest residence time, the spatial and temporal variability in the structural parameters of the soft-bottom communities is explained by the annual river flow. Therefore, with lower river flows, the residence time can be expected to increase; thus, interactions between the pollutants and the biota could also increase.

In this study, annual EA explained most of the variability in the structural parameters in the outer Oka, Lea and Artibai, being these water bodies all geographically oriented towards the North-East. Borja *et al.* (2008b) reported an increase in the mortality of the Bay of Biscay anchovy, due to an associated downwelling over the continental shelf, following the positive phase of EA since 1998. In this sense, although it is yet unproven, the EA Pattern could explain the spatial and temporal variability of the soft-bottom macrofaunal communities in those north-eastern oriented estuarie, through processes of upwelling and downwelling over the continental shelf and the subsequent availability of food.

Conclusions

Basque estuaries have suffered very serious anthropogenic impacts which are reflected in the environmental quality of their sediments and their benthic communities reflect it. Overall, the anthropogenic variables explain more spatial and temporal variability in the distribution of the soft-bottom benthic communities, than the climatic variables, which explain more variability in the Basque coast. This outcome is because the Basque estuaries support high pressures, whilst the Basque coast supports low pressures. Furthermore, the general physico-chemical characteristics of the sediment in water bodies, together with the interactions between these variables and the pollutants present within the sediments, are of particular importance in explaining the spatial and temporal distribution of the soft-bottom communities. The combination of both determine the benthic community associated with each water body.

Long-term effects of industrial development and anthropogenic pressures on the Basque estuaries, as well as recovery processes in the environmental quality of the sediments and benthic communities, resulting from the implementation of measures, are possible to assess by means of studies that deal with long-term data series, such as the present study. In this sense, monitoring programmes such as the LQM are necessary tools, to provide data on a regular basis.

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