Assessment of the climate and human impact on estuarine water environments in two estuaries of the Bay of Biscay

by

Arantza Iriarte*,[1], Fernando Villate[2], Ibon Uriarte[1], Santiago Arranz[2]

[1] *Laboratory of Ecology, Department of Plant Biology and Ecology,*
Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Paseo de la Universidad 7, 01006 Gasteiz, Spain

[2] *Laboratory of Ecology, Department of Plant Biology and Ecology,*
Faculty of Science and Technology, University of the Basque Country (UPV/EHU), PO Box 644, 48008 Bilbao, Spain

* Corresponding author: *arantza.iriarte@ehu.es*

Abstract

The effect of climate variability on estuarine water environments was assessed in two systems of the Bay of Biscay, the estuaries of Bilbao and Urdaibai, with contrasting morphology, hydrodynamic features and anthropogenic influence. To that purpose, the main time scales of variability in relevant environmental factors were established along spatial salinity gradients and, using a combination of multivariate and regression analyses, the contribution of each factor to the total variability, as well as the influence of climate factors in the seasonal and inter-annual estuarine environment variations were assessed. The major seasonal modes of variability in the water environments of both estuaries were accounted for by water temperature together with salinity stratification and/or chlorophyll *a*. This seasonal variability was associated with climate variability, as shown by the relationship with air temperature and river flow. The major inter-annual modes of variability were also accounted for by water temperature, along with dissolved oxygen and/or chlorophyll *a* in Bilbao, and chlorophyll *a* and/or transparency in Urdaibai. These were also associated with climate variability, in this case summer air temperatures. Water quality variables, such as dissolved oxygen and transparency, were found to be sensitive to reveal the effect of long term anthropogenic activities.

Key words: estuaries, environmental variability, temperature, climate, anthropogenic impact, Bay of Biscay

Introduction

Knowledge of environmental variability patterns and factors that govern these patterns is essential to understand the functioning of ecosystems. The impact of environmental variability on biological processes is complex and difficult to disentangle, but in order to address this challenge we first need to know and understand the environmental variability (Cyr & Cyr 2003). Furthermore, we also need to determine the scales of variability, since the scale of observation affects the description of the pattern. In addition, dominant drivers of the pattern can be different at different scales. Indeed, one of the central problems in ecology is the problem of pattern and scale (Levin 1992). Climate factors are important drivers of environmental variability, governing processes that are linked either to short-term weather patterns or to the annual climate cycle (Cloern & Jassby 2010), or even to long-term environmental changes in response to large-scale changes in the climate system (Stenseth et al. 2004). In terrestrial systems, environmental variability is large in both short and long-term periods, but in the sea, short-term variability is largely reduced by the very large heat capacity of the ocean (Steele 1985). Therefore, the pace of climate change and the response time to climate are also different in aquatic and terrestrial systems (Burrows et al. 2011). Furthermore, due to the differences in e.g. depth, circulation pattern, and influence of land-derived processes we can expect that different types of marine ecosystems have different sensitivity to changes in climate factors. Temperature is claimed to be a master parameter governing changes in oceanic and coastal systems (Scavia et al. 2002; Goberville et al. 2010). It is well known that increases in temperature can cause a stronger thermal stratification (Harley et al. 2006). Rising temperatures can also affect metabolic rates, e.g. primary production and respiration. Therefore, they have a strong influence on organisms' activities, distribution, ecosystem net metabolism and dissolved oxygen concentrations (Revilla et al. 2000; Nydhal et al. 2013). Estuaries are systems of transitional waters connected to both land and sea, and therefore other climate factors (such as rainfall through its effect on the river flow) could potentially have a significant impact on the water environment through the effect on salinity, haline stratification, turbidity and inputs of land-derived nutrients and pollutants (Harley et al. 2006). Furthermore, in addition to climate effects, many estuaries are subject to anthropogenic impact other than the recent human-induced climate change. Consequently, estuaries are amongst the most fertile, but also the most polluted marine systems (McLusky & Elliot 2004). Disentangling the influence of natural and anthropogenic forcing on ecosystems is a major challenge in ecology (Goberville et al. 2010). Furthermore, the dominance of climate or anthropogenic drivers of estuarine environmental variability may vary as a function of the observation time scale. For instance, in the case of near-shore coastal and estuarine chlorophyll *a* patterns, disturbances from human activities are mainly reflected in a large year-to-year variability. In the case of climate drivers, strong seasonal patterns develop where the governing processes are linked to the annual climate cycle, whereas shifts in the climate system give rise to a large inter-annual variability (Cloern & Jassby 2010).

The assessment of responses of ecological systems to climate variability may yield uncertainties in regional projections and an accurate understanding of how changes in climate are manifested at the level of individual ecosystems is needed for purposes of environmental and resource management (Preston 2004).

The objectives of the present study were a) to compare the inter-annual, seasonal and event scales of variability of the main water environment factors between systems, salinity zones and depth layers in two estuaries of the Bay of Biscay with contrasting morphologies, hydrodynamic features, and degrees of anthropogenic impact, i.e. the estuary of Bilbao and the estuary of Urdaibai, and b) to assess the sensitivity of these estuarine water environments to two major climate drivers, i.e. temperature and rainfall (and related hydrological variable, i.e. river flow).

Materials and methods

Study area

A) Estuary of Bilbao

The estuary of Bilbao (also known as the Ibaizabal-Nerbioi estuary or Nervión estuary) is located within 43°23'N to 43°14'N and 3°07'W to 2°55'W. Two areas can be distinguished: the 15 km long, narrow and relatively shallow (2-9 m) tidal channel, and the wider and deeper (on average 5 km wide; up to 30 m deep) Abra Bay (Fig. 1). The main river discharging at the head of the estuary is the Ibaizabal-Nerbioi, although four other minor streams (Kadagua, Asua, Galindo and Gobelas) discharge into the main channel. It is a macro-mesotidal estuary, partially mixed in the outer part and highly stratified in the inner part. Except for short periods of high river discharge, euhaline waters (salinity >30) dominate within the estuary (see Iriarte

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et al. 2010; Intxausti et al. 2012).

Anthropogenic activities heavily impacted water and sediment quality. By the 1970s, the estuary had extremely low oxygenation, together with high organic matter and heavy metal concentrations (Cearreta et al. 2004), as well as low biomass and low biodiversity (González-Oreja & Sáiz-Salinas 1998). The implementation of the Comprehensive Plan for
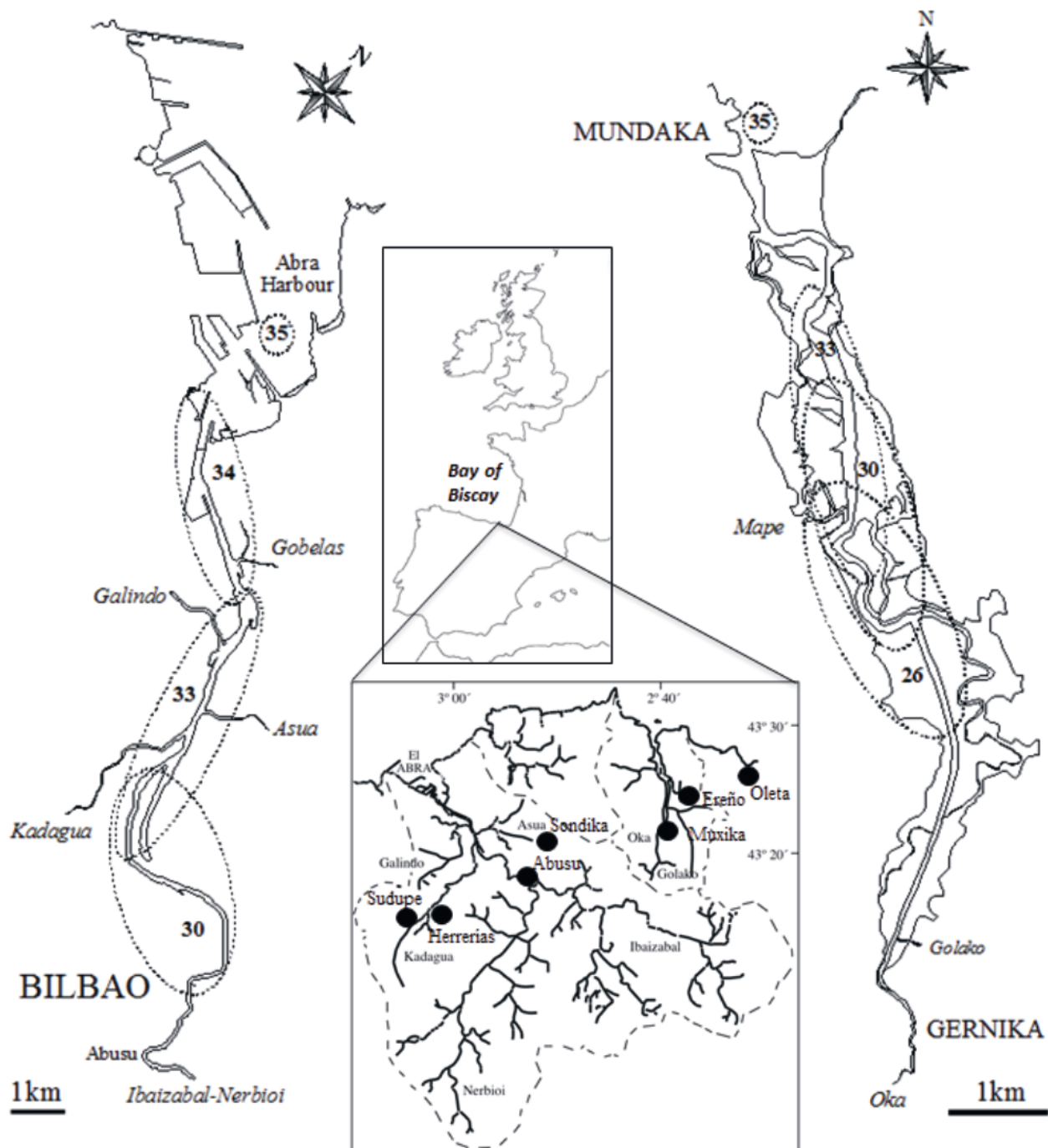

Figure 1

Maps of the Bilbao and Urdaibai estuaries, showing the usual extent of water masses of 35, 34, 33, 30 and 26 salinity below the halocline during neap tides and high water. The map of the river catchment areas, showing the location of the meteorological and hydro-meteorological stations is also shown.

the Sanitation of the Metropolitan Area of Bilbao in 1979 (García-Barcina et al. 2006), new environmental protection policies and the closure of several industries have resulted in a gradual rehabilitation of the estuarine environment, with a significant decrease in heavy metal concentrations and organic matter loading, and an increase in oxygenation and biodiversity (Borja et al. 2010; Pascual et al. 2012; Villate et al. 2013).

B) Estuary of Urdaibai

The estuary of Urdaibai (also known as the Oka estuary or Mundaka estuary) (43°22′N, 02°43′W) is shorter (12.5 km) and shallower (mean depth of 3 m). It has undergone lesser physical modification than the estuary of Bilbao, although the upper estuary is an artificial channel that reaches the town of Gernika (Fig. 1). The outer half of the estuary remains well mixed most of the time, the inner half being partially stratified. Most of the estuary is marine-dominated, with high salinity waters in the outer half and a stronger axial gradient of salinity toward the head, where it receives the freshwater inputs from the Oka river (Villate et al. 2008).

It is part of a biosphere reserve, but it is not a pristine estuary. The main sources of pollution are an old waste water treatment plant discharging in the upper reaches (Franco et al. 2004) and industrial activities (Irabien & Velasco 1999).

Sampling design and data set acquisition

Monthly environmental data were collected at high tide during neap tides in selected salinity zones (30, 33, 34 and 35 in the estuary of Bilbao and 26, 30, 33 and 35 in the estuary of Urdaibai) from 1998 to 2008 (Fig. 1). At each sampling site, the Secchi disk depth (SDD) was measured and vertical profiles (every 0.5 m) of salinity, water temperature (WT) and percentage saturation of dissolved oxygen (DOS) were obtained *in situ* using a Multi-Parameter Water Quality Meter. Water samples were collected with a Niskin bottle for chlorophyll *a* analysis from below the halocline.

Water samples were filtered through Whatman GF/C filters. Chlorophyll *a* was extracted in 90% acetone and measured spectrophotometrically according to the monochromatic method with acidification (Jeffrey & Mantoura 1997).

Salinity stratification was calculated as the maximum difference in salinity at 0.5 m depth intervals. This calculation has been put forward as an index to reflect the sharpening of the salinity gradient associated with the narrowing of the halocline

layer. This index was found to explain seasonal and inter-annual variability of DOS below the halocline in the estuary of Bilbao better than the stratification index calculated from the difference between bottom and surface salinities (Villate et al. 2013).

Hydro-meteorological data were obtained from diverse sources. For air temperature, ERA-Interim reanalysis data were accessed through the European Centre for the Medium-Range Weather Forecasts (ECMWF) data server (http://apps.ecmwf.int/datasets/) as monthly means from daily mean temperature values at 2 m above sea level for an area located between 43°15′N and 43°30′N and between 3°15′W and 2°30′W, using a grid of 0.25° × 0.25°. Rainfall (Sondika, Abusu, Muxika stations) and river flow (Ibaizabal-Nerbioi, Kadagua and Oka rivers) data were obtained from the Provincial Council of Bizkaia and the State Meteorological Agency.

Data analyses

Missing values were filled in with the mean value for the whole period of the corresponding month. The number of missing values for each water environment variable in each estuary and salinity zone ranged from 5 to an exceptional case of 26, but in most cases (>70% of the series) fewer than 12 values were missing (<10% of the values). The exception was with meteorological and river flow data from stations managed by the Provincial Council of Bizkaia (Kadagua and Oka rivers), which were lacking at the beginning of some of the series. In this case, missing values were filled in with values obtained using regression models performed with data from the nearest station with a complete series. Regarding the river flow data collection for the Kadagua river, the Herrerías station started the collection in April 2000 and the Sudupe station in February 2001. The missing values were filled in using regression models with data from the Balmaseda station for the Sudupe river flow ($y = 1.5758x - 0.5347$; $R^2 = 0.7729$) and for the Herrerías river flow ($y = 0.9282x - 0.8767$; $R^2 = 0.8922$). In the case of the estuary of Urdaibai, the Muxika hydro-meteorological station started collecting rainfall data in December 1998. The missing values were filled in using regression models with data from the Ereño station for Muxika rainfall ($y = 0.807x - 0.0569$; $R^2 = 0.8267$) and from the Oleta station for the Oka river flow ($y = 0.3892x$; $R^2 = 0.9333$)

Since salinity stratification differed between estuaries and within estuaries, a different number of water layers were established, and we used the average values of salinity, water temperature and DOS for each layer. In most cases we distinguished

two layers, the halocline and below the halocline. In the 30 salinity zone of the estuary of Bilbao, however, three consistent layers were distinguished: above the halocline (without a significant salinity gradient), within the halocline (the maximum salinity gradient was recorded here) and below the halocline. In the 35 salinity zone of the estuary of Urdaibai, the whole water column mean values were used, because the water column was well mixed.

In order to examine patterns of variability over time scales from events to years, the multiplicative model described by Cloern & Jassby (2010) to extract inter-annual, seasonal and residual components of variability from the time-series was used. This model is defined by the following expression:

$$c_{ij} = Cy_i m_j \varepsilon_{ij}$$

where for a monthly data series of several years c_{ij} is the value of the variable in year i and month j; C is the whole series mean; y_i is the annual effect in the ith year (where the annual effect is the annual mean Y_i/C and the annual mean Y_i is the sum of monthly data in the ith year divided by 12); m_j is the seasonal (monthly) effect in the jth month (where m_j is the mean over all years of M_{ij}/Y_i and M_{ij} is the value for month j in year i, and Y_i is again the annual mean for year i); and ε_{ij} is the residual effect due to residual events (where the residual series is defined by $\varepsilon_{ij} = c_{ij}/Cy_i m_j$). For individual series, the standard deviations of the annual component (y), the seasonal component (m) and the event component (ε) are taken as measures of inter-annual, seasonal and residual variability, respectively (Cloern & Jassby 2010).

To extract the main inter-annual and seasonal patterns of the water environmental factors, principal component analyses (PCAs) were performed with the SPSS software on the inter-annual (y_i) and seasonal (m_j) variability.

To detect change points in the inter-annual and seasonal components of variability, the cumulative sum graphs of PCA scores (PC1 and PC2) were computed. The interpretation was based on the sign and steepness of the slopes, which reflect the deviation of a period from the mean value (Ibañez et al. 1993).

To assess the relationship between hydro-climatic factors and water environment variability, Spearman rank order correlations were performed between seasonal and inter-annual variability of hydro-climatic factors (air temperature, rainfall and river flow) and PC1 and PC2 of the seasonal and inter-annual variability of the water environment variables.

Results

## Scales of variability of hydro-climatic factors

The seasonal variability was clearly higher than the inter-annual variability for all the hydro-climatic factors under study, i.e. air temperature, rainfall and river flows (Fig. 2). For air temperature it was the dominant scale of variability, while for rainfall – the event-scale variability was dominant.

## Distribution and scales of variability in water environment variables

Salinity stratification was much higher and Secchi disk depth (SDD) smaller in the estuary of Bilbao than in Urdaibai (Fig. 3A, 3B, 3C, 3D). Stratification increased and SDD decreased from high to low salinity sites in both estuaries. Residual variability was dominant for stratification and SDD. For stratification, unlike for SDD, residual variability was much higher in Urdaibai than in Bilbao. In most cases, stratification showed higher seasonal than inter-annual variability. It is noteworthy, however, that SDD showed higher inter-annual than seasonal variability at the intermediate 34 and 33 salinity sites in the estuary of Bilbao, but not at the other salinity sites or in Urdaibai.

On average, water temperature was higher in the estuary of Urdaibai than in Bilbao at all depth layers (Fig. 4A, 4B, 4C, 4D). The seasonal component of variability was largely dominant and the inter-annual component was the least dominant. In both estuaries, seasonal variability was higher in the halocline than below the halocline and increased from the outer to the inner estuarine zones in both layers. The highest water temperature variability occurred above the halocline at the 30 salinity site in Bilbao and the lowest values were recorded below the halocline in the estuary of Bilbao; in the estuary of Urdaibai, the differences in the seasonal variability between within and below the halocline were smaller.

The highest and fairly similar dissolved oxygen (DOS, %) was recorded at the highest salinity sites in both estuaries, but it showed a much sharper landward decrease in the estuary of Bilbao than in Urdaibai, both within and below the halocline waters (Fig. 5A, 5B, 5C, 5D). The highest variability in DOS was, in general, the residual in both estuaries. Overall, the three components of DOS variability were higher in the estuary of Bilbao than in the estuary of Urdaibai. They increased with decreasing salinity in both estuaries and depth layers; the sharpest increase was observed in waters below the halocline in the estuary of Bilbao. An important exception was the inter-annual
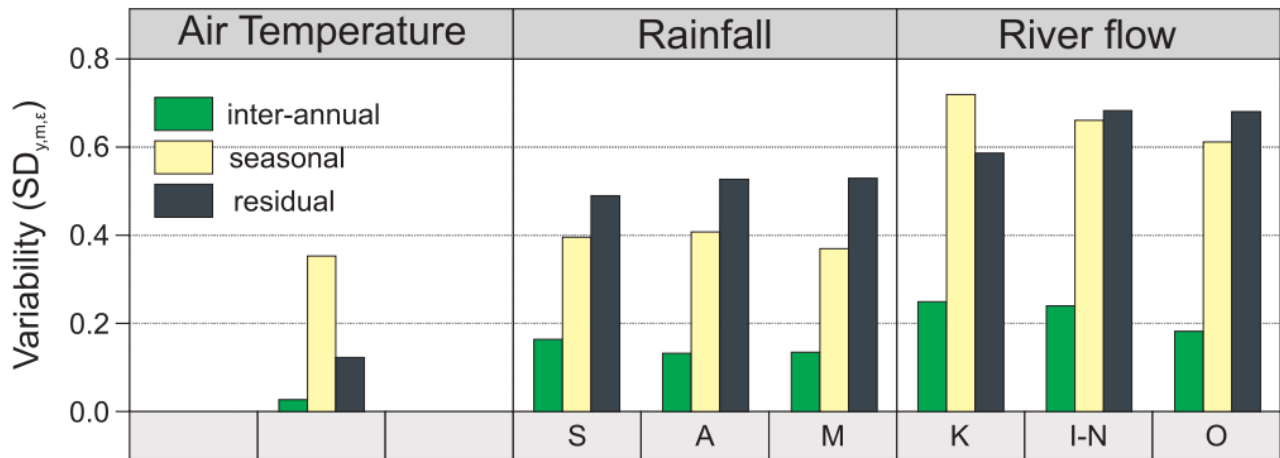
Figure 2

Averaged inter-annual, seasonal and residual variability ($SD_{y,m,\varepsilon}$) of air temperature, rainfall for the sites of Sondika (S), Abusu (A) and Muxika (M) and river flow for the Kadagua (K), Ibaizabal-Nerbioi (I-N) and Oka (O) rivers
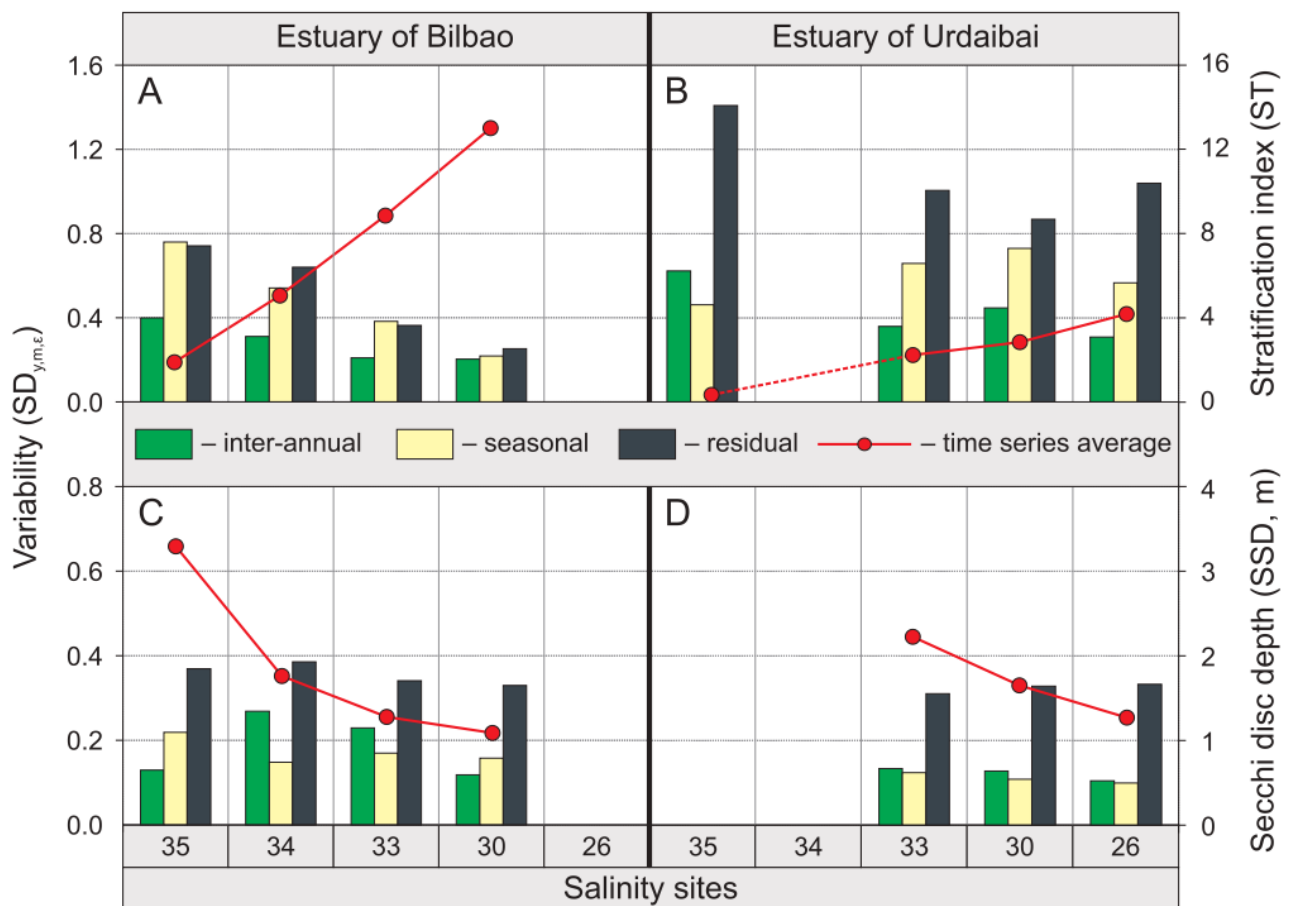
Figure 3

Averaged inter-annual, seasonal and residual variability ($SD_{y,m,\varepsilon}$), and time-series average values of the stratification index (ST) and Secchi disk depth (SDD, m) in the estuaries of Bilbao (A and C) and Urdaibai (B and D)
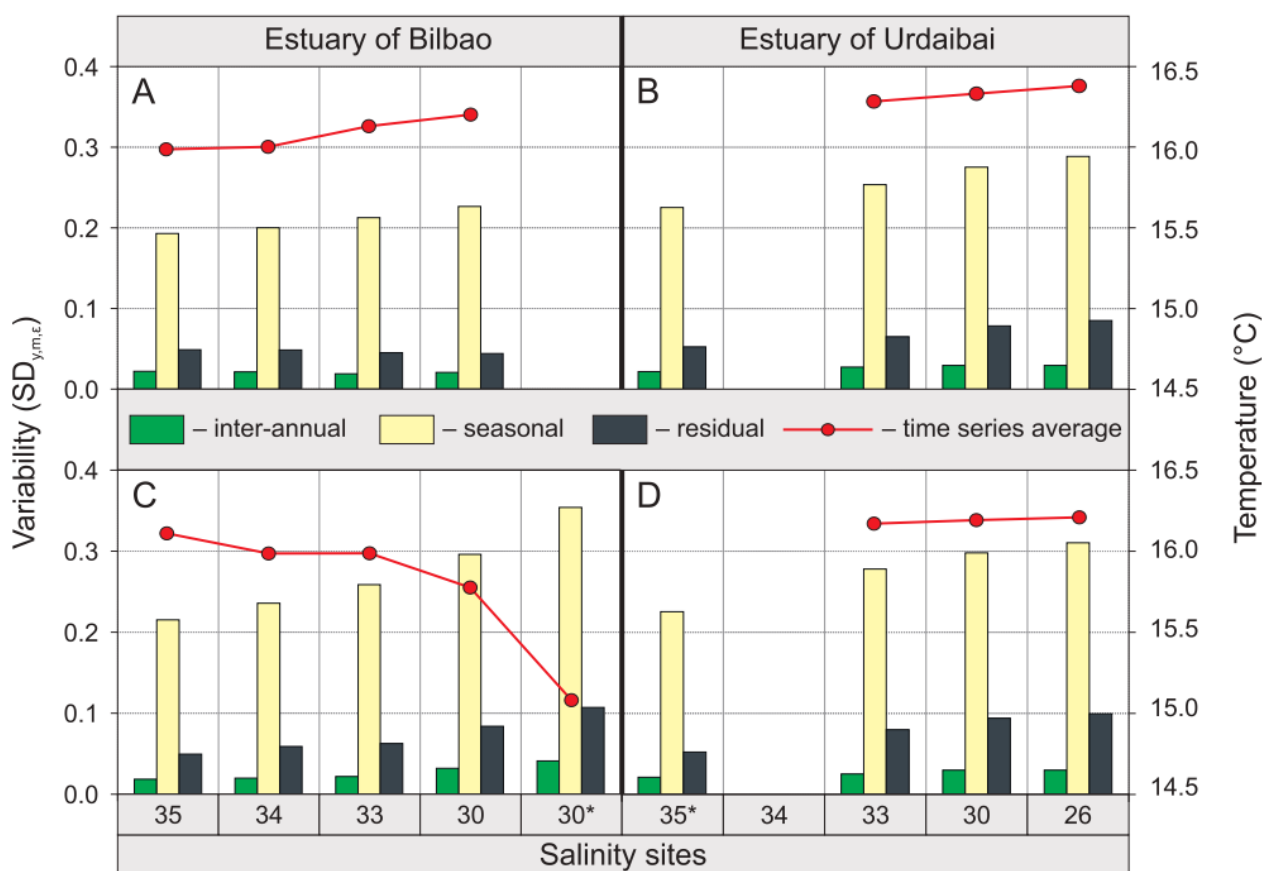
Figure 4

Averaged inter-annual, seasonal and residual variability ($SD_{y,m,\varepsilon}$), and time-series average values of temperature below the halocline (A and B) and in the halocline (C and D) in the estuaries of Bilbao and Urdaibai. 30* – above the halocline and 35* – the whole water column

component of variability, which was higher at the 33 salinity site than at the 30 salinity site in the estuary of Bilbao. In addition, the seasonal component of variability was higher or similar to the inter-annual component in all cases except the 33 salinity site.

Chlorophyll *a* concentration increased with decreasing salinity in both estuaries (Fig. 6A 6B). The seasonal and residual components were substantially higher than the inter-annual component in all cases, with the seasonal components being higher in the estuary of Bilbao than in Urdaibai.

**Seasonal patterns of water environment variability**

The PCA results of the seasonal water environment variability are shown in Figure 7. The first component (PC1) explained 46-75% and 46-55% of the variance at Bilbao and Urdaibai sites respectively, and the second one (PC2) 14-32% and 26-34% respectively. The PC1 was mainly accounted for by water temperature and

salinity stratification, which were negatively related to each other at all salinity sites in both estuaries. In most cases, the PC2 was mainly accounted for by DOS. The cumulative sum plot of the PC1s (Figs 8A and B) illustrates (in both estuaries) progressive delays in the seasonal cycles from the inner to the outer estuary. These annual cycles mainly reflected the seasonal pattern of water temperature variability (Fig. 9A, B, C, and D), where the summer peak exhibited a small delay seaward, and both warming and cooling processes occurred faster in the inner estuary than in the outer estuary (and even faster in above halocline waters at the 30 salinity site in Bilbao). The cumulative sum plot of PC2s showed greater differences between estuaries and between salinity sites than that of the PC1s (Fig. 8A, B, C and D).

Correlation analyses showed that the PC1s were positively correlated with seasonal anomalies of air temperature ($p<0.001$) and negatively correlated with those of rainfall (generally $p<0.001$) and river flows
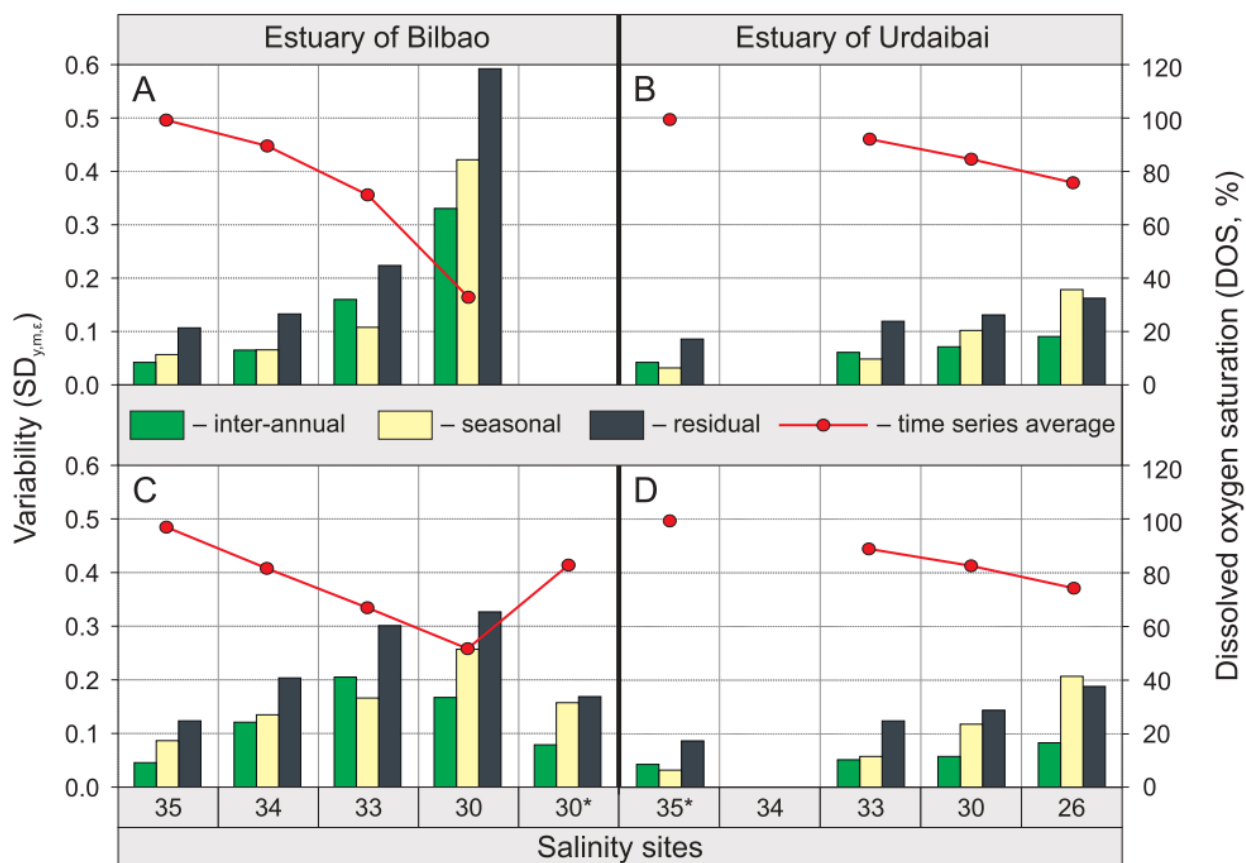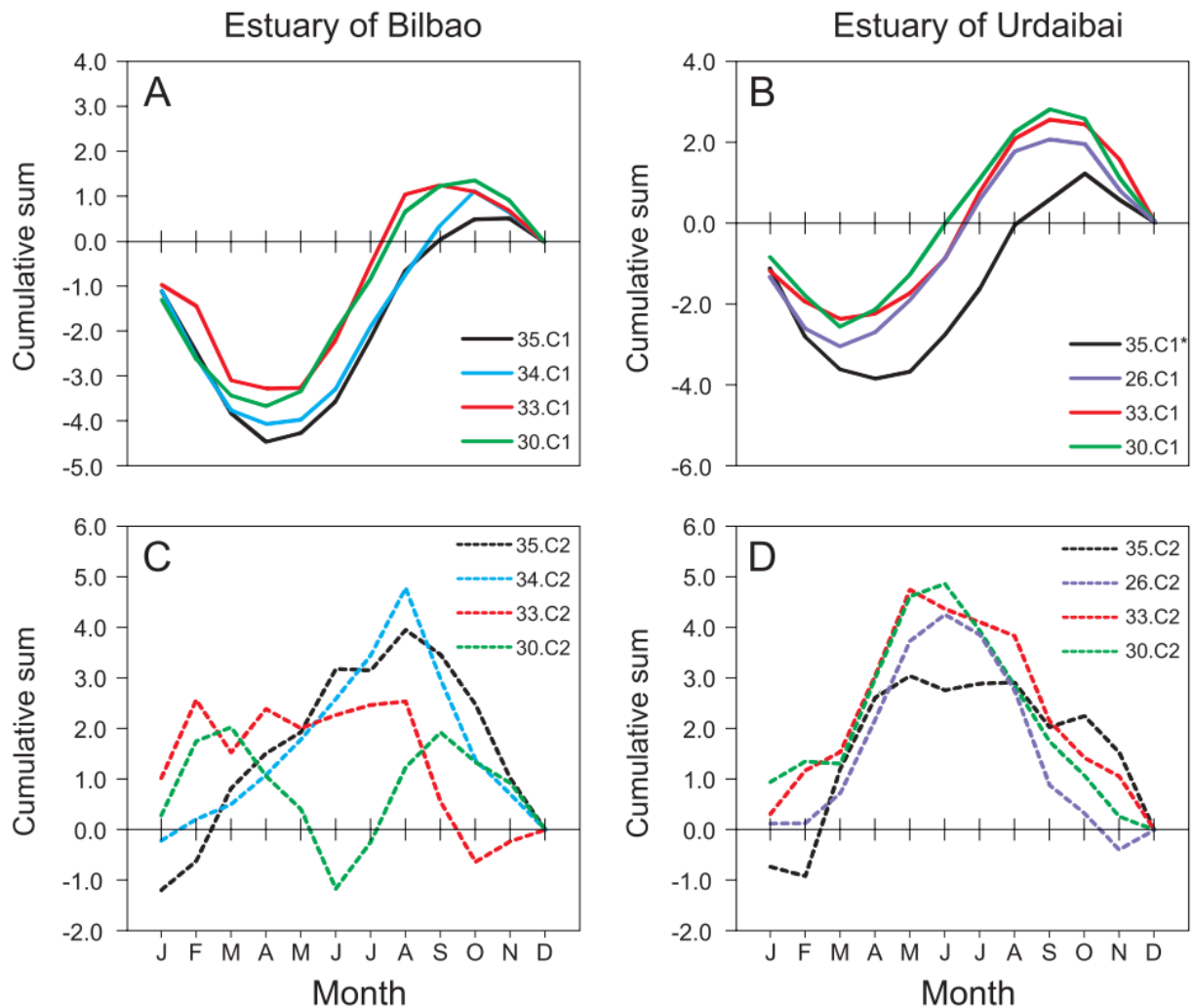
Figure 5

Averaged inter-annual, seasonal and residual variability (SD$_{y,m,\varepsilon}$), and time-series average values of DOS below the halocline (A and B) and in the halocline (C and D) in the estuaries of Bilbao and of Urdaibai. 30* – above the halocline and 35* – the whole water column
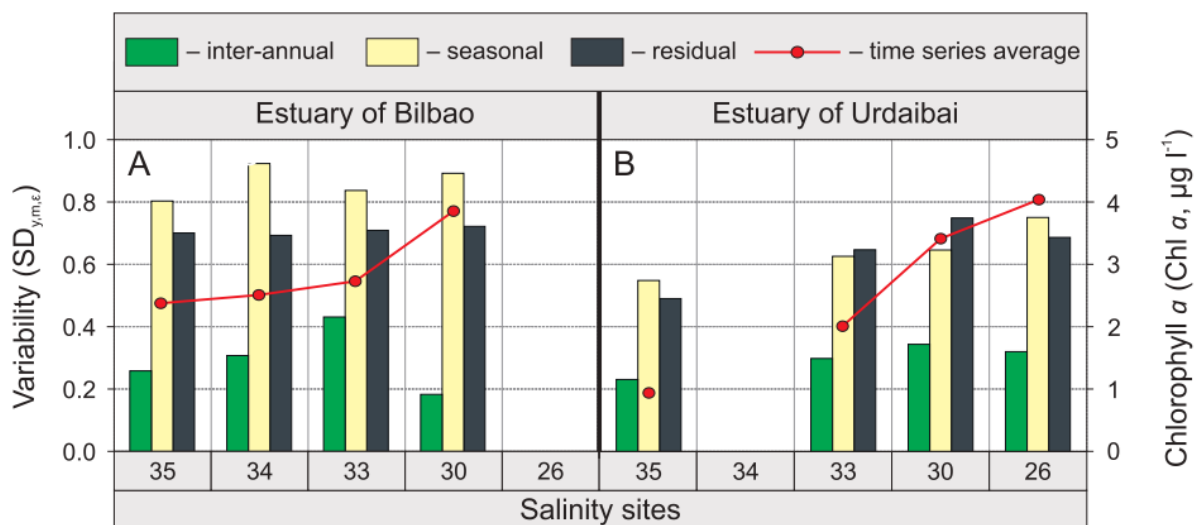
Figure 6

Averaged inter-annual, seasonal and residual variability (SD$_{y,m,\varepsilon}$), and time-series average values of chlorophyll a in the estuaries of Bilbao (A) and Urdaibai (B)
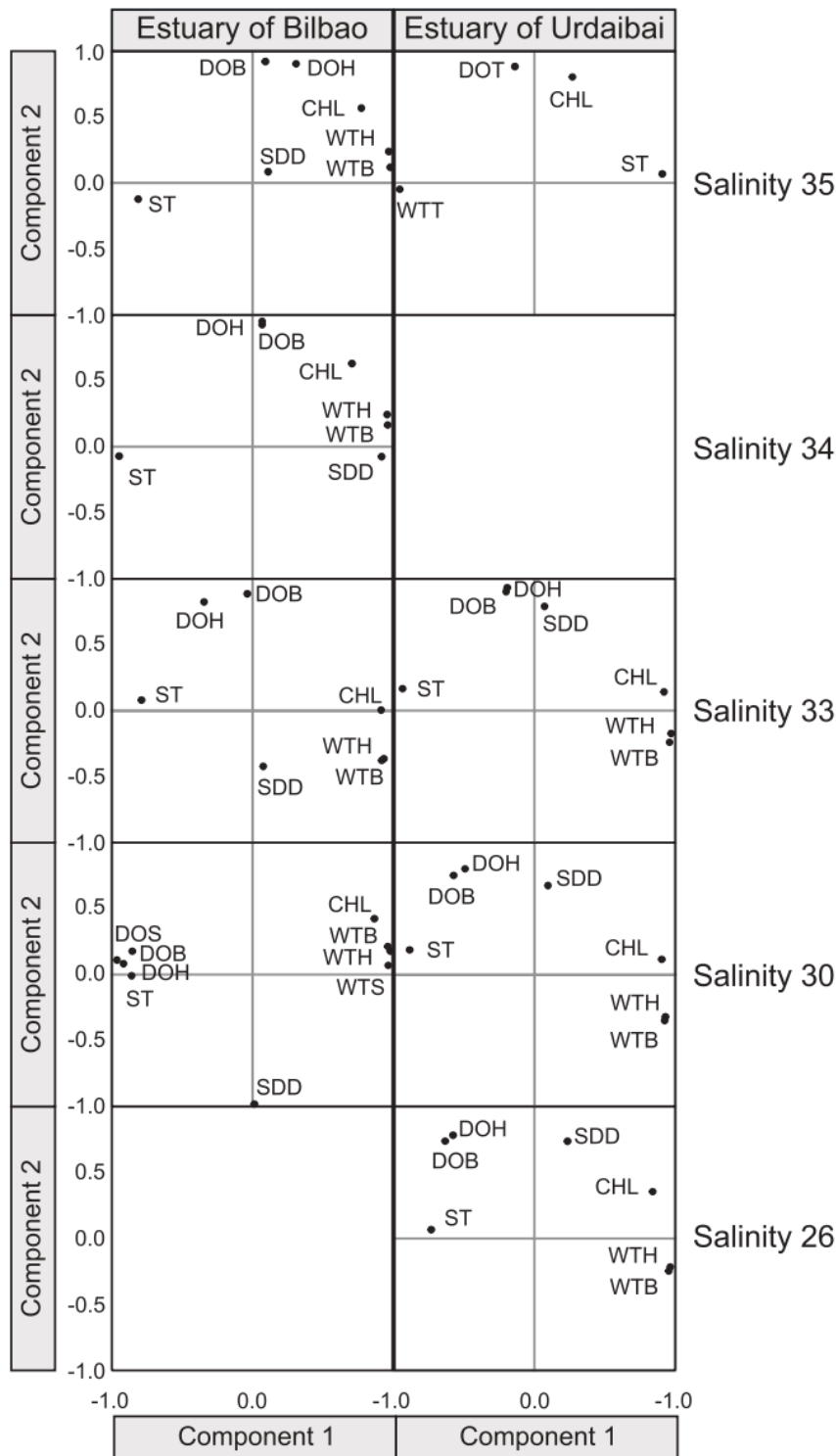
Figure 7

Principal component analysis of the seasonal changes in the water environmental variables (ST: Stratification index; SDD: Secchi disk depth; WTT: Water temperature for the total water column; WTB: Water temperature below the halocline; WTH: Water temperature in the halocline; WTS: Water temperature in the surface layer above the halocline; DOT: Dissolved oxygen for the total water column; DOB: Dissolved oxygen below the halocline; DOH: Dissolved oxygen in the halocline; DOS: Dissolved oxygen in the surface layer above the halocline; CHL: Chlorophyll *a* at different salinity sites of the Bilbao and Urdaibai estuaries

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Figure 8

Cumulative sums of month scores from January to December in PC1 and PC2 for different salinity sites of the Bilbao (A and C) and Urdaibai (B and D) estuaries. *Inverse values: positive values are reported as negative values and vice versa.

(p<0.001) in all salinity zones of both estuaries. At the 35 salinity site in Urdaibai, correlations (p<0.001 for river flow and p<0.05 for rainfall) were of the opposite sign due to the fact that water temperature and stratification were positioned at the negative and positive end of the PC1 axis, respectively. The PC2s showed no significant correlation with any of the hydro-climatic factors under study in neither of the two estuaries.

Inter-annual patterns of water environment variability

The PCA results of the inter-annual water environment variability are shown in Figure 10. The PC1

explained 32.0-36.6% and 40.2-53.1% of the variance in the estuarine sites of Bilbao and Urdaibai respectively, while the PC2 explained 23.1-35.2% of the variance in Bilbao and 21.5-38.4% in Urdaibai. In most cases, water temperature significantly contributed to the PC1. Chlorophyll *a* also contributed significantly to PC1 at the 35 salinity site of Urdaibai, where it was negatively related to water temperature. It also contributed at 33, 30 and 26 salinity sites of Urdaibai and the 33 salinity site of Bilbao, where it was positively related to water temperature. SDD and DOS also contributed to PCs (mainly PC2s), but with differences between salinity sites and estuaries. In essence, the intermediate salinity sites of both estuaries displayed similar modes of inter-annual variability, while each of the highest and
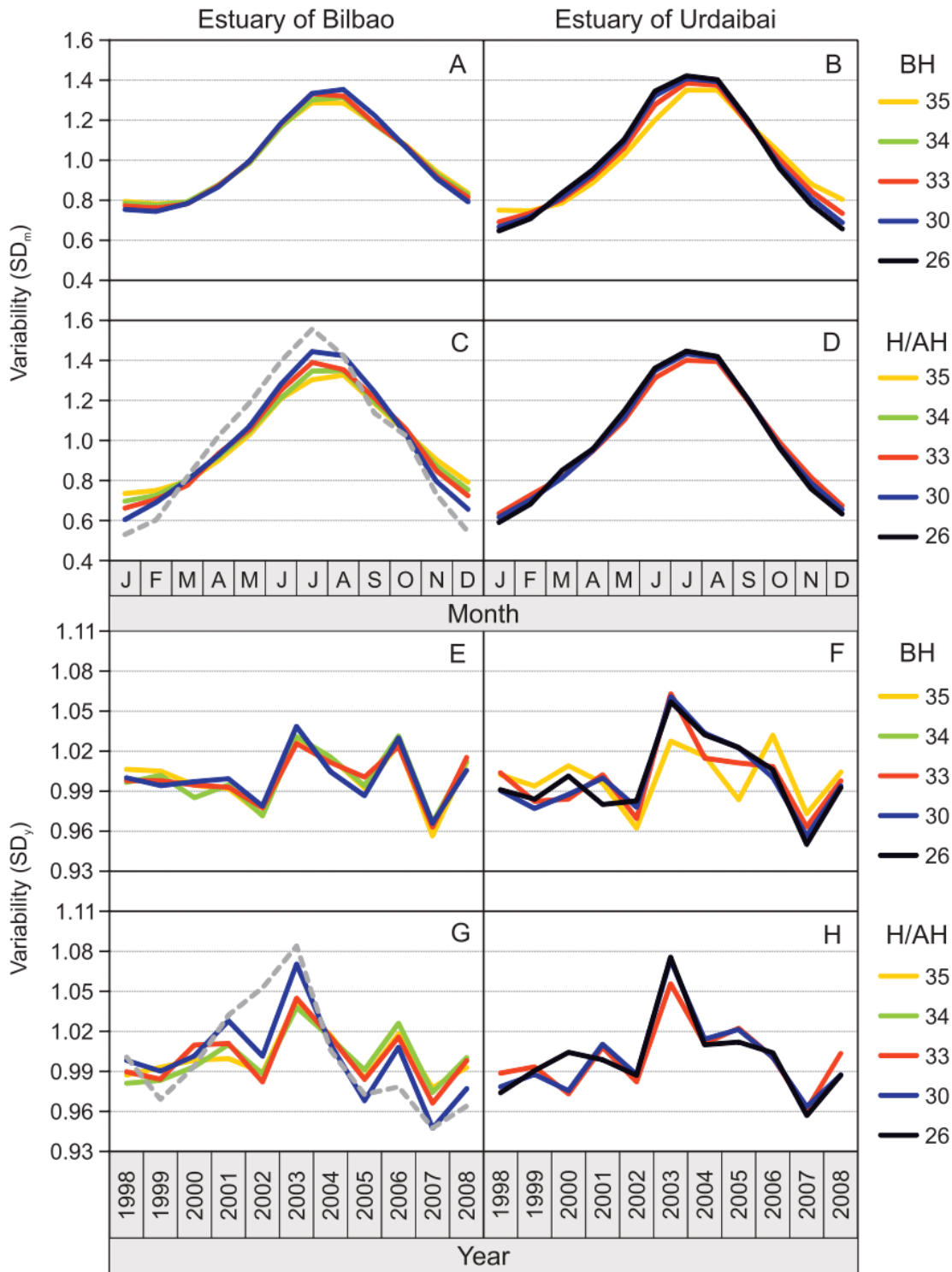
Figure 9

Patterns of seasonal (SD$_m$) and inter-annual (SD$_y$) variability of water temperature at 35, 34, 33, 30 and 26 salinity sites in the estuaries of Bilbao (A, C, E and G) and Urdaibai (B, D, F and H). BH and H indicate below halocline and halocline waters, respectively. The dashed line for the above halocline layer at the 30 salinity site in the Bilbao estuary. At 35 salinity in the estuary of Urdaibai, the data are provided for the whole water column.
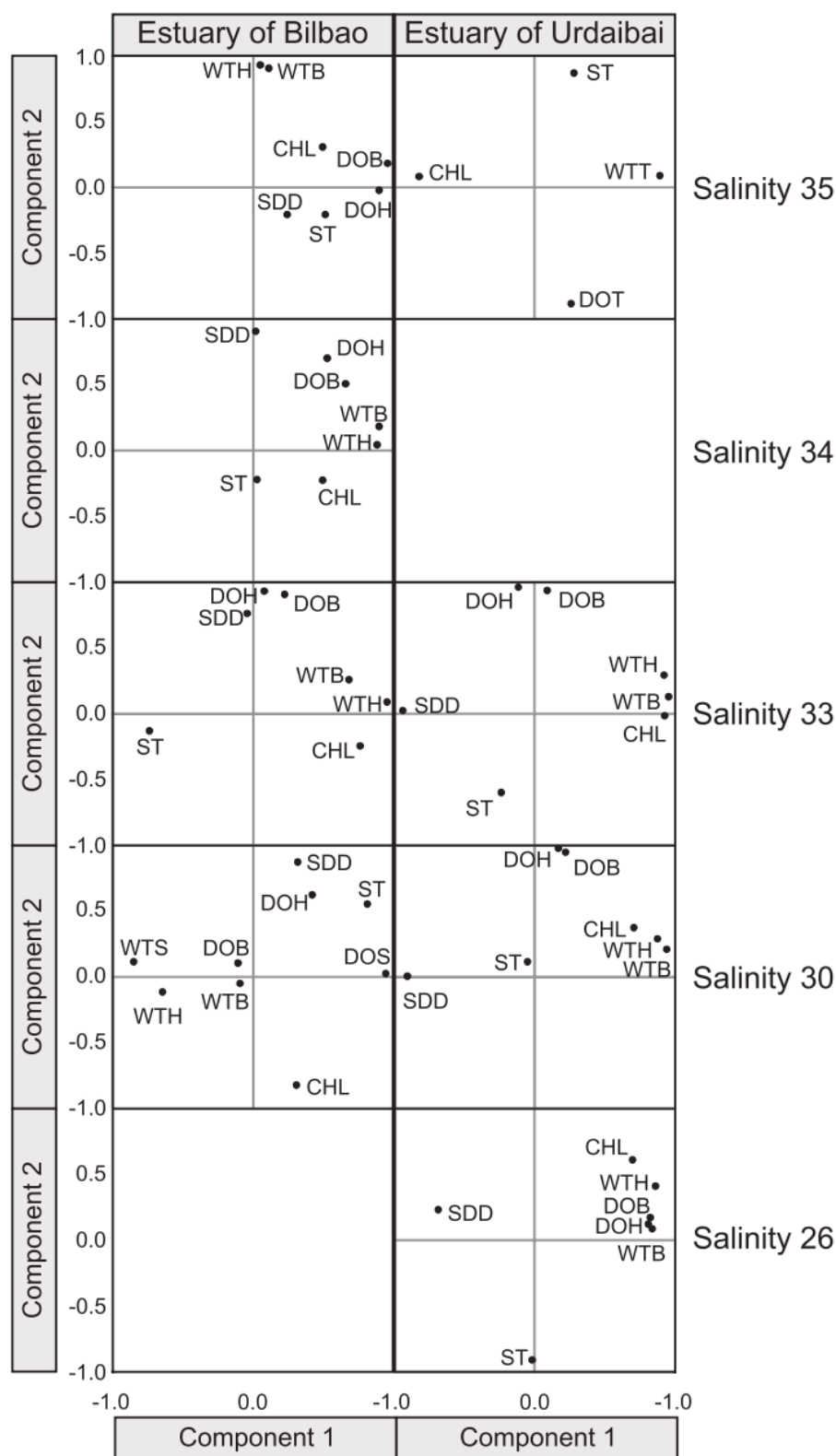
Figure 10

Principal component analysis of inter-annual changes in water environmental variables (acronyms as in Figure 7) at different salinity sites in the estuaries of Bilbao and Urdaibai

lowest salinity sites exhibited its own distinct mode.

The cumulative sum plots of the year-to-year changes in the PC1 and PC2 values of different salinity zones in the two estuaries are shown in Figures 11A, B, C and D. The modes of variation in the estuary of Bilbao, which were mainly accounted for by the inter-annual variations in water temperature (PC2 of the 35 salinity site and PC1s of 34 and 33 salinity sites), showed the major break point in 2002, when values started to be higher than the time-series average, and the second peak in 2006. The rest of PCs from the estuary of Bilbao, which were mainly associated with variations in DOS and SDD, reflected that DOS and SDD were generally higher than the time-series average in the second half of the series. In the cumulative sum plots of the Urdaibai estuary, the PC1s of the salinity

sites accounted for mainly by water temperature, chlorophyll *a* and SDD also showed the initial break point in 2002 and then changed to a steep increasing slope from 2002 to 2004 (35 and 26 salinity sites) or to 2006 (33 and 30 salinity sites). The cumulative sum plots of the inter-annual modes of variability, which were accounted for mainly by water temperature, reflect differences in the inter-annual trends of water temperature variability (Fig. 9E, F, G, H). In halocline and below halocline waters of the high-intermediate salinity (35-34) sites of Bilbao, as well as in waters of the highest salinity (35) site of Urdaibai, two temperature peaks of similar magnitude were observed in 2003 and 2006. On the other hand, at the other salinity sites of Urdaibai (both in halocline and below halocline waters), as well as in halocline and above halocline
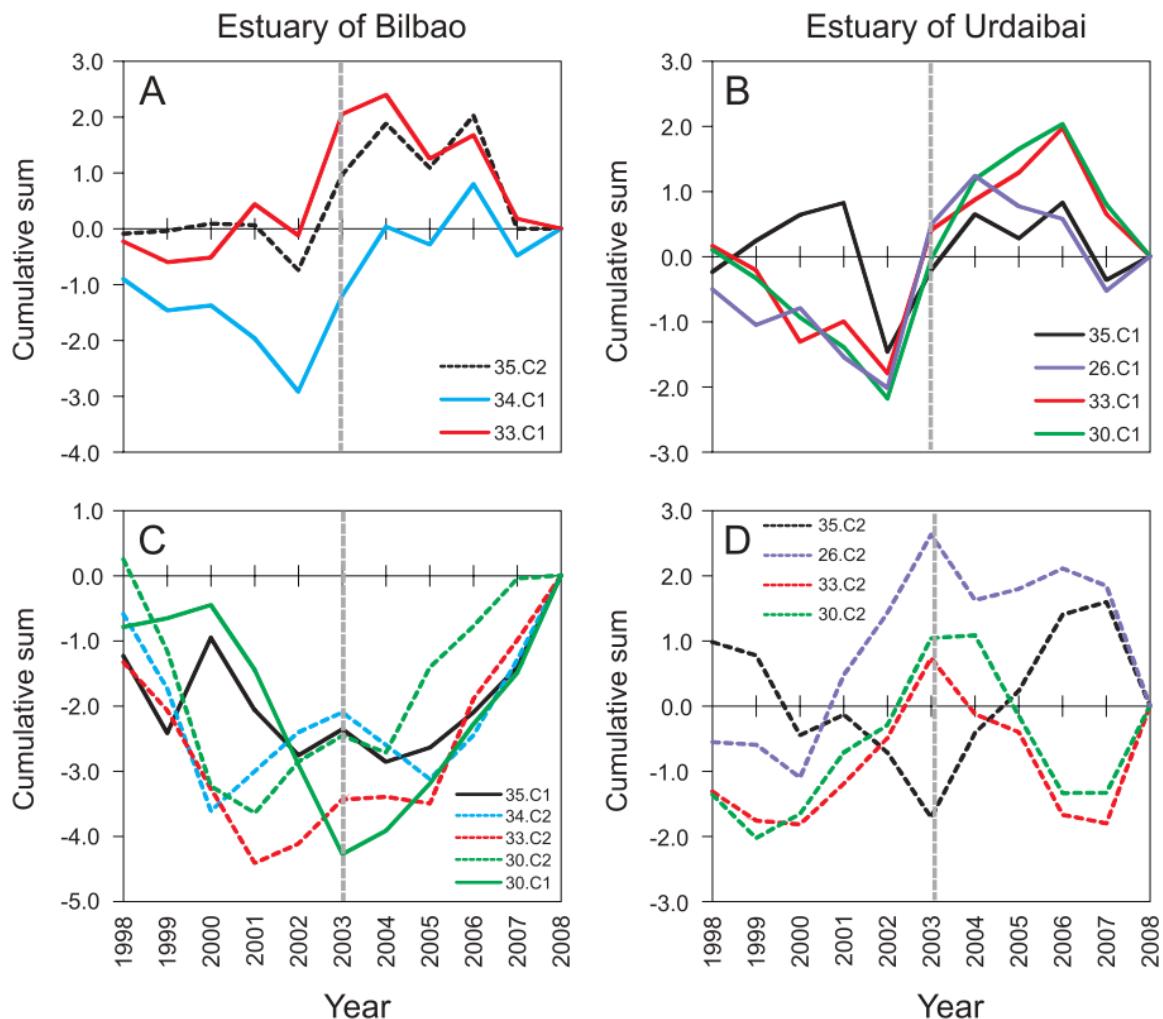

Figure 11

Cumulative sums of year scores from 1998 to 2008 in PC1 and PC2 for different salinity sites in the estuaries of Bilbao (A and C) and Urdaibai (B and D)

waters of the 30 salinity site in Bilbao, the 2003 peak was much more pronounced and no peak was observed in 2006 or the peak was much smaller.

Correlation analyses between the PC1 and PC2 scores of the water environment variables and the annual air temperature anomalies did not show a clear-cut pattern. However, the annual anomalies of summertime air temperatures (Table 1) showed the latter to be positively correlated with most of those principal components in which water temperature had a high loading (with positive scores) such as the PC2 of the 35 salinity site and the PC1s of 33 and 34 salinity sites in Bilbao and the PC1s of 35, 33 and 30 salinity sites in Urdaibai. Rainfall showed no significant correlation with any of the PCs in the estuary of Bilbao. The Kadagua river flow showed a positive correlation with the PC1 of the 30 salinity site of Bilbao; the Oka river flow and rainfall showed a significant negative correlation with the PC2 of the 26 salinity site in Urdaibai.

Discussion

Scales of variability

The seasonal variability of air and water temperature was clearly higher than the interannual variability in both estuaries. If we consider sensitivity as the magnitude of change of a component of the system in response to changes in external driving forces (Najjar et al. 2010), our results showed that water temperature sensitivity to air temperature fluctuations increased from the outer to the inner estuary and from bottom to surface layers in both estuaries. However, in the deeper and more strongly stratified estuary of Bilbao, spatial differences in water temperature sensitivity to air temperature were higher than in the shallower and less stratified estuary of Urdaibai.

Unlike temperature, rainfall and related hydrographical parameters, i.e. river flow and salinity stratification, showed a residual variability usually as high as or even higher than the seasonal variability. Precipitation is regarded as a chaotic process (Rodríguez-Puebla et al. 1998) and, despite the underlying seasonal cycle on the Basque coast, its episodic nature affected the river flow and salinity stratification variability. Salinity stratification is also influenced by other factors varying at time scales shorter than the seasonal one, such as tides and winds (Kennish 1986). This was particularly apparent in the outer, highly exposed zone of the Urdaibai estuary, where stratification was absent or minimal (Iriarte et al. 2010), but the residual variability was very high. It would also explain the increase in variability from the inner, more sheltered and strongly

Table 1

Results of (Spearman rank) correlation analyses of the first (C1) and second (C2) components of PCA with the annual anomalies in rainfall, Kadagua and Oka river flows (RF) and summertime air temperature anomalies from different salinity sites (35, 34, 33, 30, 26) of the Bilbao (B) and of Urdaibai (U) estuaries. Only the p-values of significant correlations (in bold) are shown.

Site	PCA component	Air temperature	Rainfall	Kadagua (RF)	Oka (RF)
B35	C1	-0.036	0.459	0.066	
	C2	**0.882** (p<0.001)	-0.041	-0.005	
B34	C1	**0.736** (p<0.01)	0.166	0.107	
	C2	-0.409	0.139	-0.158	
B33	C1	**0.609** (p<0.05)	-0.230	-0.358	
	C2	-0.018	0.283	0.066	
B30	C1	-0.027	0.472	**0.674** (p<0.05)	
	C2	-0.327	0.251	0.110	
U35	C1	**0.891** (p<0.001)	0.096		0.093
	C2	0.227	-0.318		-0.353
U33	C1	**0.700** (p<0.05)	-0.041		-0.201
	C2	-0.364	0.271		0.263
U30	C1	**0.773** (p<0.01)	0.057		-0.048
	C2	-0.109	0.042		0.040
U26	C1	0.491	0.295		0.184
	C2	0.100	**-0.718** (p<0.05)		**-0.748** (p<0.01)

stratified zone to the outer, less sheltered and less stratified zone in the estuary of Bilbao. Similarly, in all salinity zones of the open and irregular basin of the Urdaibai estuary, residual values were higher than in the channelized estuary of Bilbao.

Residual variability was also generally higher than seasonal or interannual variability for dissolved oxygen (DOS). Variations in DOS are the result of complex interactions between physical and biogeochemical factors that operate at different time scales, including

those other than the monthly or annual scales (see Kemp et al. 2009) and they can contribute to an increase in the residual variability (Cloern & Jassby 2010). In our case, DOS variability was generally higher in the moderately polluted estuary of Bilbao, which accumulates higher organic loads in sediments due to its long history of organic pollution (Cotano & Villate 2006). In addition, DOS variability increased toward the inner, low salinity zones of both estuaries and so does the organic pollution, since the major inputs of anthropogenic organic compounds (main tributary streams and waste water treatment plants) are located in the upper reaches of both estuaries, as well as in the middle reaches of the estuary of Bilbao. All this indicates that anthropogenic disturbance can be an important source of differences in DOS variability, both between estuaries and within estuaries. It is interesting to note that the intermediate zone of the Bilbao estuary (33 salinity), which has shown the greatest recovery in DOS following the pollution abatement (Villate et al. 2013), was the only one with an inter-annual variability higher than the seasonal. This suggests that the change to the dominance of inter-annual variability over seasonal variability can be related to an increasing response to human actions. This is consistent with findings by Cloern & Jassby (2010) who analyzed patterns and scales of chlorophyll variability in estuaries and near shore coastal systems.

As for DOS, the interannual variability of the Secchi disk depth (SSD) was much higher than the seasonal one at the intermediate salinity sites of Bilbao. This may also be attributable to the improvement in water transparency related to sewage and industrial pollution abatement (Borja et al. 2010), thus reinforcing the view that long-term environmental changes that have occurred in the estuary of Bilbao in the last two decades or so are primarily man-induced changes which manifest themselves mainly at the inter-annual scale. However, the largest variability in water transparency was observed at the event-scale, both in the estuaries of Bilbao and Urdaibai, in agreement with the wide variety of drivers (e.g. river discharge, wind-driven and tide-driven re-suspension, sewage and industrial discharges, phytoplankton growth) involved in the presence of particulate materials in the water column, and with the episodic occurrence of some of them.

Chlorophyll *a* showed strong seasonal and residual components and a much weaker inter-annual component of variability. This is consistent with findings by Cloern & Jassby (2010) who, after analyzing a vast number of estuarine sites, concluded that inter-annual variability falls within a narrow range at most sites and the largest component of variability is

generally the residual. It is important to note, however, that all the salinity zones of the Bilbao estuary were characterized by a stronger seasonal than inter-annual component of variability, which suggests a marked sensitivity of chlorophyll *a* variability to the seasonal cycle of residence time, which, in turn, is linked to the river flow seasonality (Uriarte et al. 2014).

Seasonal variability of the water environment

The major mode of water environment variability at the seasonal scale in the estuaries of Bilbao and Urdaibai was mainly accounted for by water temperature and stratification, and correlated with air temperature, rainfall and river flow, which indicates that hydro-climatic factors were the dominant drivers of the seasonality of the water environment in both estuaries. Other works have also claimed temperature to be a master parameter governing the environmental changes in the coastal environment (Beaugrand 2009; Goberville et al. 2010) but in our case, both temperature and river flow contributed to the reinforcement of the same mode of seasonal water environment variability because of their strong seasonal co-variation. Chlorophyll *a* was also close to the environmental mode of variation governed by temperature and river flow/stratification in both estuaries, except for the outer estuary of Urdaibai. This was due to the fact that chlorophyll *a* showed minima in winter and maxima in summer (except for the outer estuary of Urdaibai), which is the most general pattern in Basque estuaries (Iriarte et al. 1997; Butrón et al. 2009) and in a large number of temperate estuaries (Boynton et al. 1982). The case of the outer Urdaibai estuary was different because chlorophyll *a* shows a bimodal seasonal pattern, with peaks in spring and late summer/early autumn and lower values in midsummer, which is attributed to nutrient depletion in this zone in summer (Iriarte et al. 1997; Villate et al. 2008). Thus the well-flushed outer estuary of Urdaibai reflects water environment and plankton dynamics in shelf waters; while the water environment and phytoplankton seasonal dynamics in the outer estuary of Bilbao remain similar to those in nutrient-rich intermediate and inner estuarine zones because of the presence of the estuarine plume (Ferrer et al. 2009).

The important water quality variables such as DOS and water transparency showed a weaker association with hydro-climatic variables. However, the contribution of DOS to the major mode of variability, and therefore its relationship with hydro-climatic variables increased landward in the estuary of Urdaibai, because of a stronger relationship between DOS and the river flow inputs upstream and the tidal exchange and

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turbulence downstream (Iriarte et al. 2015). River discharge and dissolved oxygen were also found to be significantly and positively correlated in the inner zone of the Gironde estuary (Lanoux et al. 2013). In any case, the highest contribution of DOS to the major mode of variability and, therefore, the strongest relationship with hydro-climatic variables was found at the lowest salinity zone of Bilbao. Here, in addition to river flow inputs in surface and halocline layers, seasonal dynamics of DOS also seems to be governed by temperature through the enhancement of respiration rates during the summer (Villate et al. 2013). This has been shown in other estuaries too (Nydhal et al. 2013). The outer estuary of Bilbao diverges from this pattern, because seasonal DOS dynamics is determined more by autotrophic oxygen production than respiratory oxygen consumption (Villate et al. 2013).

The clear seasonal delay in the major seasonal mode of water environment variability from the inner to the outer estuary and from surface to bottom layers indicates a clear relationship with the temperature cycle. This was more evident in the highly stratified estuary of Bilbao. These differences between estuaries and depth layers in terms of timing of seasonal peaks, the speed of seasonal warming and cooling as well as the range of variability, are indicative of different, i.e. inland *versus* coastal, types of seasonality of water temperature (*sensu* Lemos et al. 2007). Thus, a more coastal type of seasonal signal for below halocline waters than for halocline waters (for halocline waters than for above halocline waters in the case of the low salinity zone) was observed in the estuary of Bilbao. The existence of spatial differences in terms of inland and coastal types of seasonality of water temperature in estuaries has also been described elsewhere and can be explained in terms of the buffering action of the ocean (Lemos et al. 2007). In the estuary of Urdaibai, all depth layers and salinity zones showed a more inland type of seasonality and this is because it is shallower and less stratified than the estuary of Bilbao. Our results corroborate the theory that shallow zones respond more quickly to changes in air temperature than deeper zones (Shellenbarger & Schoellhamer 2011), and the increased stratification advances the phase of the seasonal cycle at the surface, while delaying its phase at the bed of the estuary (Uncles & Stephens 2001).

Inter-annual variability of the water environment

As for the seasonal scale, a high contribution of water temperature to the major mode of water environment variability was observed in most zones at the inter-annual scale. In addition, chlorophyll *a* was correlated with water temperature in the intermediate salinity zones in the estuary of Bilbao and in the entire estuary of Urdaibai. This correlation between water temperature and chlorophyll *a* was positive in nutrient-rich estuarine zones, probably due to temperature enhancement of metabolic rates and thermal stratification, which would improve the phytoplankton cells' light quota (Richardson & Schoeman 2004; Villate et al. 2008). However, the correlation between temperature and phytoplankton biomass at the inter-annual scale was negative in the outer Urdaibai estuary, which suggests that the enhanced thermal stratification would reduce even more the phytoplankton biomass in summer (Richardson & Schoeman 2004). Furthermore, water environment variability showed important break points during years of considerable temperature change, such as the cold summer of 2002, the warm summers of 2003 and 2006 and the cold winters of 2005 and 2006. They have also been observed in the sea surface temperature of other nearby coastal sites of the Basque coast (Fontán et al. 2008; Goikoetxea et al. 2009) as well as in the eastern Bay of Biscay (Hermant et al. 2010). It is interesting to observe that, unlike the major mode of seasonal variability, the major mode of inter-annual variability was found to be primarily connected with year-to-year anomalies of summer temperature. Extreme events in air temperatures on the Basque coast are in line with (at least in terms of the year of winter minima and the year of summer maxima) observations on the Atlantic coast of the Iberian Peninsula (Gameiro et al. 2007).

In the low-salinity zones of both estuaries, the river flow also showed significant correlations with the inter-annual water environment variability. Interestingly, except for the lowest salinity zone, hydro-climatic factors seemed not to make a high contribution to the PC2. This PC2 was accounted for by important water quality factors such as DOS in the estuary of Urdaibai and DOS and SDD in the estuary of Bilbao (except for the highest salinity site of Bilbao where DOS had a high contribution to the PC1 variability). The year-to-year increases in DOS and SDD observed in Bilbao, an estuary in the rehabilitation phase (Borja et al. 2010), can be mainly explained by the implementation of sewage pollution abatement measures and the industrial decline in the metropolitan area of Bilbao, although DOS variations in the outer estuary are determined more by autotrophic oxygen production (Villate et al. 2013). Long-term improvements in dissolved oxygen following the reductions in sewage pollution have been documented for other estuaries too (e.g. Soetaert et al. 2006). Water transparency is affected by river flow (Iglesias et al. 2014), but years with comparable

flows may show very different fluxes of particulate matter (Schäfer et al. 2002). Turbidity can be affected by sewage pollution too. Sewage discharges can reduce water transparency (Laws & Redalje 1979) and sewage pollution abatement can result in increases in water transparency (Xu et al. 2011). The latter is a likely reason for the general increases in SDD observed in the estuary of Bilbao. In the estuary of Urdaibai, no such year-to-year trend in SDD increase was observed because this estuary had not been exposed to a pollution abatement process. Instead, SDD appeared in opposition to chlorophyll *a* and contributed to the major mode of environmental variability, which suggests that inter-annual variations in SDD were associated with variations in chlorophyll *a.*

Acknowledgements

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