Seasonal and interannual variability of mesozooplankton in two contrasting estuaries of the Bay of Biscay: Relationship to environmental factors

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ARTICLE INFO

ABSTRACT

Seasonal and interannual variations of total mesozooplankton abundance and community variability were assessed for the period 1998–2005 at 3 salinity sites (35, 33 and 30) of the estuaries of Bilbao and Urdaibai (southeast Bay of Biscay). Spatial differences in mesozooplankton seasonality were recognized, both within and between estuaries, related to differences between sites in hydrodynamic features and anthropogenic nutrient enrichment that drive phytoplankton biomass seasonal cycles. The within estuary seasonal differences in mesozooplankton community were mainly shown through seaward time-advances in the seasonal peak from summer to spring along the salinity gradient, linked to differences in phytoplankton availability during the summer, in turn, related to nutrient availability. These differences were most marked in the estuary of Urdaibai, where zooplankton seasonal pattern at 35 salinity (high tidal flushing) resembled that of shelf waters, while at 35 of the estuary of Bilbao zooplankton showed an estuarine seasonal pattern due to the influence of the estuarine plume. Cirripede larvae contributed most to the mesozooplankton seasonal variability, except at the outer estuary of Bilbao, where cladocerans and fish eggs and larvae were the major contributors, and the inner estuary of Urdaibai, where gastropod larvae contributed most. Total mesozooplankton increased at 30 salinity of the estuary of Bilbao and 35 salinity of the estuary of Urdaibai. Interannual variability of mesozooplankton at the lowest salinity of the estuary of Bilbao was mainly accounted for by copepods due to the introduction of non-indigenous species during estuarine rehabilitation from intense pollution. However, bivalve larvae and gastropod larvae showed the highest contributions at 35 salinity of the estuary of Urdaibai. At the rest of sites, the opposite interannual trends of polychaete larvae and hydromedusae generally made the highest contribution.

1. Introduction

Mesozooplankton are the fundamental link between primary producers and upper trophic level consumers in marine food webs, and play a relevant role in the biogeochemical cycles in the ocean (Dam 2013). Moreover, they are ectotherms with short generation times, capable of responding fast to environmental changes (Dam 2013). Indeed, zooplankton live in ever changing ecosystems, under the influence of multiple stressors. Hydro-climatic changes are important drivers of marine ecosystem variability (Hewitt et al. 2016) and zooplankton variability (Beaugrand and Ibanez 2004; Marques et al. 2014), but direct anthropogenic factors can also have paramount effects on marine ecosystems (Blaber et al. 2000; Islam and Tanaka 2004), in general, and on zooplankton in particular (Verity and Borkman 2010). This is most evident in nearshore coastal and estuarine systems that are subject to high human pressures, such as port activities, industrial and domestic waste disposal, dredging, land reclamation or fisheries and aquaculture (McLusky and Elliott 2004), and even to remediation actions in disturbed areas (Borja et al. 2010), which drive variations of biological communities at different temporal scales. In temperate waters zooplankton show marked seasonality (Miller 1983), but they can also show long-term trends and regime shifts (Beaugrand and Ibanez 2004; Beaugrand et al. 2014; Uriarte et al. 2016). However, despite the potential of zooplankton to be exceptional beacons of environmental change (Richardson 2008; Beaugrand et al. 2009), knowledge about the impact of multi-stressors on coastal and estuarine zooplankton is still scant (Vieira et al. 2015), partly because of the scarcity of long-term time series (Buttay et al. 2015), which are particularly limited in southern Europe (Vieira et al. 2015).

Besides temporal changes, estuaries also show strong spatial variations across and within systems. Estuaries are characterized by a marked heterogeneity across systems due to differences in latitude,

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climate, origin, geomorphology, hydrological regime, degree and type of anthropogenic impact, amongst others. Also, since estuaries are transition systems between continents and oceans, they show high spatial variations along the longitudinal axis, mainly in salinity, but also in hydrodynamic features, sediment characteristics, nutrients, pollutants and others (Knox 1986). These environmental variations can be responsible for spatial differences in zooplankton distribution (Uriarte and Villate 2004; Marques et al. 2007) and, as a consequence, temporal variations of zooplankton can also differ between salinity zones across and within estuaries.

To the best of our knowledge, on the Iberian coast of the Bay of Biscay, the only two estuaries for which zooplankton time series derived from regular monitorings along their longitudinal axes exist are the estuaries of Bilbao and Urdaibai. These are systems located near each other on the Basque coast that share the same climate, but have contrasting geomorphology, hydrodynamic characteristics, degree of anthropogenic influence and ecological status (Franco et al. 2004). These similarities and differences make them ideal systems to distinguish the effects of climate factors from direct anthropogenic driving forces.

Comparisons of temporal and spatial variations in the distribution of major groups of mesozooplankton between these two estuaries have been carried out in previous studies. Uriarte and Villate (2004) showed a clear influence of salinity and pollution on the spatial distribution and abundance of most mesozooplankton groups when they compared different salinity sites along the longitudinal axis of these two estuaries, but they made no comparisons of temporal variations. Albaina et al. (2009) dealt with spatial and temporal variations, but only for a period of < 2 years, over which they detected initial signs of recovery of the mesozooplankton community in the estuary of Bilbao in response to the improvement in water quality. Therefore, a need for investigating in greater detail the temporal (both seasonal and interannual) variations in mesozooplankton at different salinity sites in these estuaries has been identified.

Taking these considerations into account, the aims of the present work were to assess at different fixed salinity sites along the longitudinal axis of the estuaries of Bilbao and Urdaibai (i) the seasonal and interannual variations of the mesozooplankton community, (ii) the contribution of the various holoplankton and meroplankton groups to each type of temporal variability in the mesozooplankton community and (iii) the influence of environmental factors on the main temporal variations of the mesozooplankton community.

2. Material and methods

2.1. Study area

The estuaries of Bilbao (also known as Ibaizabal-Nerbioi estuary or Nervión estuary; 43°23′N, 03°07′W) and Urdaibai (also known as Gernika estuary, Mundaka estuary or Oka estuary; 43°22′N, 02°43′W) are located near to each other (Fig. 1), and share the same temperate-oceanic climate with moderate winters and warm summers. However, they differ largely in morphology, hydrodynamic features and water quality.

2.1.1. Estuary of Bilbao

The estuary of Bilbao is ca. 23 km long and it is divided in two areas: the intermediate-inner channelized area constituted by a 15 km long, narrow (50–150 m) and 2–9 m deep man-made channel and a wider (ca. 3.8 km) and deeper (10–25 m) outer area called the Abra harbour. The estuary is partially mixed in the outer area and highly stratified in the inner area. High salinity waters (> 30) usually penetrate as far as the upper reaches at the bottom, while freshwater flows seaward at surface and is progressively mixed with seawater. The main rivers flowing into this estuary are the Ibaizabal and Nerbioi. Except for short periods of high river discharge, euhaline waters (salinity > 30)

dominate within the estuary (Intxausti et al. 2012; Villate et al. 2013). The natural features of the estuary were dramatically modified by urban, industrial and port developments. Due to land reclamation and channelization works, the estuary has lost most of its original intertidal areas (Cearreta et al. 2004). By the 1970s, extremely low oxygenation together with high organic matter and heavy metal concentrations characterized the estuary (Cearreta et al. 2004), which gave rise to extensive azoic benthic areas (González-Oreja and Saiz-Salinas 1998). At present the estuary is in a rehabilitation process, as a result of the implementation of a Comprehensive Plan for the Sanitation of the Metropolitan Area of Bilbao since 1979, new environmental protection policies and the industrial decline in the area surrounding the estuary, which have caused a significant decrease in heavy metal concentrations, ammonia and organic matter loading, and an increase in oxygenation and biodiversity (García-Barcina et al. 2006; Borja et al. 2010; Pascual et al. 2012; Villate et al. 2013). In the middle reaches, the estuary receives discharges from a waste water treatment plant where secondary treatment began to be applied in 2001 (Franco et al. 2004).

2.1.2. Estuary of Urdaibai

The estuary of Urdaibai, with a maximum and minimum width of 1.2 km and < 20 m in the outer and inner areas respectively, is shorter (12.5 km), shallower (mean depth of 3 m) and physically much less modified than the estuary of Bilbao. The central channel is bordered by salt marshes and muddy intertidal areas at its upper and middle reaches and by relatively extensive intertidal flats (mainly sandy) at its lower reaches. The watershed area is small and river inputs are usually low when compared to the tidal prism. As a consequence, most of the estuary is seawater-dominated at high tide, with high salinity waters in the outer half and a stronger axial gradient of salinity towards the head, where it receives the freshwater inputs from its main tributary, the Oka river (Villate et al. 2008). In the outer zone, tidal flushing is so high that waters of salinities > 34 are flushed out of the estuary with each tidal cycle. The outer half of the estuary remains well mixed most of the time, and the inner half is partially stratified. In the upper reaches, the estuary receives relatively large amounts of nutrients and organic matter from an old primary waste water treatment plant (Franco et al. 2004).

2.2. Data collection

Samplings were carried out monthly at high tide during neap tides for the period 1998–2005 at the sites of 35, 33 and 30 salinity in the estuaries of Bilbao (B35, B33 and B30) and Urdaibai (U35, U33 and U30). The changing spatial zonation of salinity in the estuaries of Bilbao and Urdaibai due to the effect of tides and river discharge was the reason for not sampling at fixed points, but at fixed salinity zones, using thus a lagrangian type of sampling strategy, as in other estuarine zooplankton studies (e.g. Kimmerer et al. 1998). At each site vertical profiles of salinity, temperature and dissolved oxygen were obtained using a WTW multi-parameter water quality meter, but only data from the depth of zooplankton sampling have been used in the present study. Salinity stratification index (unitless) was calculated as the maximum difference in salinity at 0.5 m depth intervals as in previous studies (Villate et al. 2013; Iriarte et al. 2015).

Zooplankton were collected from mid depth, below the halocline (usually at 3–5 m), by horizontal tows of a 200 μm mesh size ring net (25 cm of mouth diameter; 100 cm long) equipped with a digital flow meter. Water samples were taken with a Niskin bottle for chlorophyll *a* measurements at the same depth of zooplankton collection. Secchi disk depths were also measured at each sampling site.

Zooplankton samples were preserved in 4% borax buffered formalin seawater solution. Identification and counting to the lowest possible taxonomical level was performed using a microscope, but only the main coarse holoplankton (i.e. copepods, appendicularians, cladocerans, chaetognaths, siphonophores, doliolids and *Noctiluca*) and meroplank-
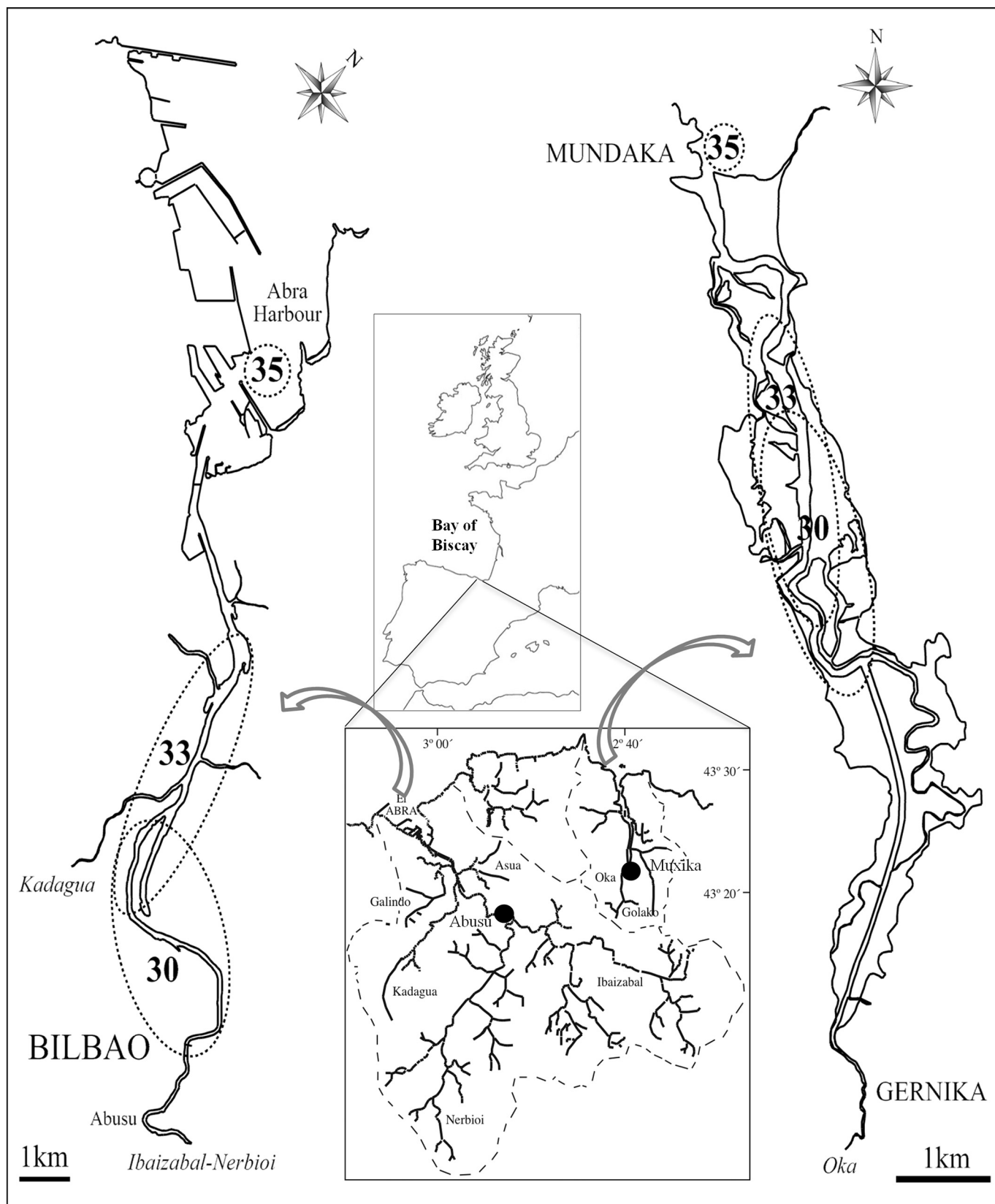
Fig. 1. Map of the estuaries of Bilbao and Urdaibai, showing the location of hydro-meteorological stations and the salinity zones where samples were taken.

ton (i.e. cirripede larvae, gastropod larvae, bivalve larvae, polychaete larvae, decapod larvae, hydromedusae and fish eggs and larvae) categories were used in this study. For chlorophyll *a* determination water samples were filtered through Whatman GF/C filters, pigments were extracted in 90% acetone and chlorophyll *a* was measured spectrophotometrically according to the method described by Lorenzen (1967).

River flow (Ibaizabal-Nerbioi, Kadagua and Oka rivers) data were

obtained from the Provincial Council of Bizkaia.

2.3. Data analysis

Occasional missing values of environmental and biotic variables from each estuary and salinity site were filled with the corresponding monthly mean value for the whole series, and abundance data of zooplankton were log (x + 1) transformed prior to statistical analyses. Exception was with river flow data from stations managed by the Provincial Council of Bizkaia that were lacking at the beginning of some of the series. In this case missing values were filled with values obtained using regression models performed with data from the nearest station with a complete series (see Iriarte et al. 2016 for details).

Multivariate ordination methods were used to examine the main modes of seasonal and interannual variability of mesozooplankton and their relationship to environmental variables using CANOCO software package version 4.5 (ter Braak and Šmilauer 2002). In order to assess whether a unimodal model such as the Canonical Correspondence Analysis (CCA) or a linear model such as the Redundancy Analysis (RDA) best fit the data sets, we conducted Detrended Correspondence Analyses (DCA). Given that the highest value for the longest gradient lengths obtained in these DCAs was < 3, we opted for performing RDA analyses in all cases, as recommended by ter Braak and Šmilauer (2002). We assessed the temporal patterns by performing RDAs for each salinity site and estuary separately. The seasonal variability was analysed conducting partial RDAs using month as categorical explanatory variable and year as categorical covariable (thus removing from the analysis the effect that year may have on abundances). Likewise, the interannual variability was analysed performing partial RDAs using year as categorical explanatory variable and month as categorical covariable (thus removing from the analysis the effect that month may have on abundances). For covariables all dates were used. Monte Carlo tests were performed with 999 permutations under reduced model (ter Braak and Šmilauer, 2002). The permutations were unrestricted and the blocks defined by covariables.

Between-site correlation analyses (Spearman rank) were performed for the month and year scores in order to test the similarity in the seasonal and interannual patterns.

In order to assess the relationship between zooplankton community variations and environmental factors at the seasonal and interannual scales, Spearman rank correlation analyses were conducted between environmental factors (abiotic factors and chlorophyll *a*) and month/year scores on axis 1 obtained from the RDAs. All correlations were carried out using SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY).

Because samplings were usually carried out towards the end of each month, the meteorological seasons have been used, where spring is March, April and May, summer is June, July and August, autumn is September, October and November, and winter is December, January and February.

3. Results

Fig. 2 shows that densities of total mesozooplankton were markedly higher in the estuary of Bilbao than in the estuary of Urdaibai at the 35 and 33 salinity sites, while at B30 maximum annual density was substantially lower than at U30. Annual maxima were delayed from early spring (estuary of Urdaibai) or late spring (estuary of Bilbao) at high salinity sites to summer at low salinity sites. The plot of the month scores on the first axis obtained from the RDA analysis carried out using month as categorical explanatory variable and year as categorical covariable (Fig. 3) showed that the main mode of seasonal variability of the mesozooplankton was quite similar at all salinity sites in the estuary of Bilbao, where the main differences in the community were found between early summer and late autumn-early winter. In the estuary of Urdaibai, however, clear differences along the salinity

gradient were observed, the main ones being detected between the late autumn-early winter community and communities of early spring at U35, late spring at U33 and early summer at U30. Correlation analysis showed statistically significant between-site relationships for the month scores, except between the B30 and U35 sites (Table 1). Within estuaries the least similar pattern between the different salinity sites was also that from B30 in the estuary of Bilbao and that from U35 in the estuary of Urdaibai. The seasonal pattern of chlorophyll *a* (Fig. 4) resembled this pattern since it also showed similar seasonal changes all along the salinity gradient of the estuary of Bilbao, with maxima in summer, and different seasonal patterns in the estuary of Urdaibai, with a delay of the annual maximum from early spring at U35 to summer at U30 and U33. The latter one also showed a secondary peak in spring. However, month to month variations of chlorophyll *a* and zooplankton density or community structure did not always coincide; the highest coincidence being observed at the 30 salinity site of both estuaries.

Cladocerans and cirripede larvae made the highest contributions to the main mode of mesozooplankton community variability in the estuary of Bilbao, together with bivalve larvae at B30 and together with fish eggs and larvae at B33 and B35 (Fig. 5A, B and C). In the estuary of Urdaibai the major mode of seasonal variability was accounted for mainly by cirripede larvae, together with gastropod larvae at U30 and U33, and together with cladocerans at U35 (Fig. 5D, E and F).

The seasonal pattern of abundance of mesozooplankton groups that made the most relevant contribution to the mesozooplankton community seasonal pattern (Fig. 6) showed that the timing of the annual maximum of cirripede larvae differed between salinity sites in both estuaries, the difference being larger in the estuary of Urdaibai than in the estuary of Bilbao. In the estuary of Urdaibai cirripede larvae peaked in early spring at U35 and in late summer at U33 and U30, while in the estuary of Bilbao they peaked in spring at B35 and in early summer at B33 and B30. For cladocerans, the differences in the timing of the annual maxima were greater between estuaries than within estuaries, since they reached maxima earlier at all salinity sites in the estuary of Urdaibai (early spring) than in the estuary of Bilbao (late spring-early summer). Gastropod larvae and fish eggs and larvae showed maxima in late spring-early summer at all salinity sites in the estuary of Bilbao, but gastropod larvae peaked progressively later from spring to summer with decreasing salinity in the estuary of Urdaibai. Bivalve larvae showed more complex seasonal patterns within estuaries because annual peaks were not related to the salinity gradient in any estuary, but, in general, they reached annual maxima earlier in the year in the estuary of Urdaibai (spring) than in the estuary of Bilbao (summer-autumn).

Interannual variations in mean annual density of total mesozooplankton (Fig. 7) showed trends of increase at B30 and at U35. The scores of years on the first axis of the RDA carried out using year as categorical explanatory variable and month as categorical covariable (Fig. 8) showed that the mesozooplankton community had a more unidirectional change in the estuary of Urdaibai than in the estuary of Bilbao. Within estuaries, interannual mesozooplankton changes at different salinity sites were more similar in the estuary of Urdaibai than in the estuary of Bilbao (Table 2). As for the seasonal pattern, the most dissimilar interannual variations between the different salinity sites were those from B30 in the estuary of Bilbao and those from U35 in the estuary of Urdaibai (Table 2). In fact, between-site correlations of year scores showed no significant correlation between variations at U35 and the rest of sites, nor between B30 and the rest of sites (Table 2).

Polychaete larvae and hydromedusae (in most cases siphonophores too) made the highest contribution to the main mode of variability of the mesozooplankton community at B35, B33, U33 and U30 (Fig. 9). At these sites polychaete larvae showed marked increases in the last period (peaks in 2003 or 2004), whereas the gelatinous predators (mainly hydromedusae but also siphonophores in most cases) showed a decreasing tendency (Fig. 10). At B30 copepods made the highest
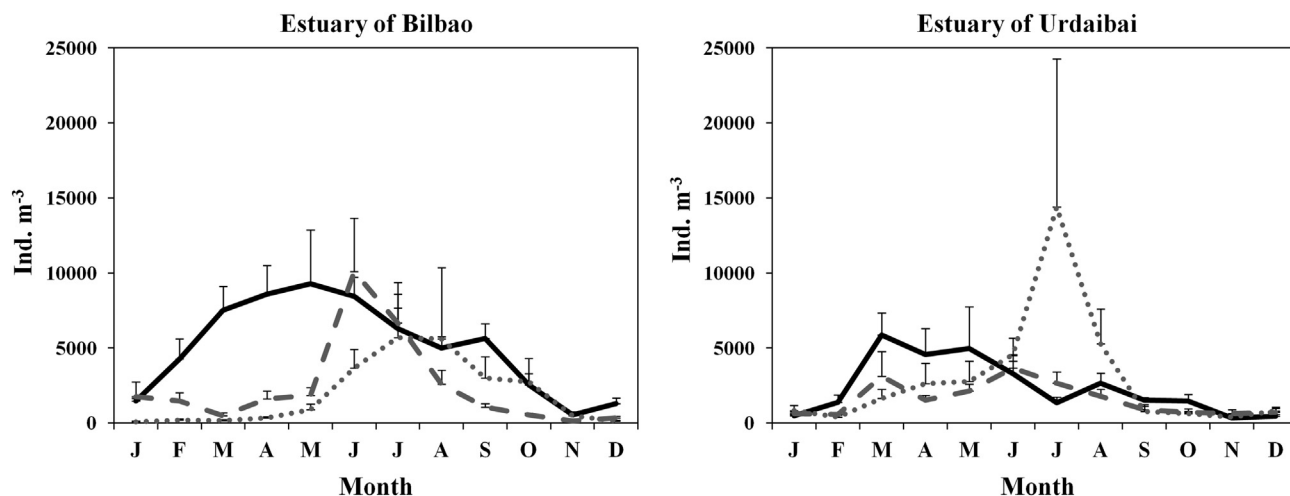
Fig. 2. Time-series mean (+ SD) densities of total mesozooplankton for each month at the 30 (dotted line), 33 (dashed line) and 35 (continuous line) salinity sites of the estuary of Bilbao (left) and Urdaibai (right).

contribution and showed the largest increase in abundance during the study period (Fig. 9C; Fig. 10), although high contributions and increasing tendencies were also observed from an array of taxa, i.e. appendicularians, decapod larvae, cladocerans, cirripede larvae and chaetognaths (Fig. 9C). At U35 bivalve and gastropod larvae showed the highest contributions (Fig. 9D). Both bivalve and gastropod larvae increased in abundance during the study period at all salinities in both estuaries, except at U30. In addition, copepods showed increasing trends not only at B30, but also at U35 throughout the study period (Fig. 10).

The percentage variation of mesozooplankton explained by months increased from the inner to the outer estuary of Bilbao (Fig. 11A) and that explained by years increased from the outer to the inner estuary (Fig. 11B). No such pattern along the longitudinal axis was evident in the estuary of Urdaibai (Fig. 11C and D), where the percentage variation of mesozooplankton explained by months was lower than in the estuary of Bilbao and that explained by years was higher than in the estuary of Bilbao, at salinity sites of 35 and 33 in both cases.

Results of correlation analyses of month scores along axis 1 with abiotic variables and chlorophyll *a* have been shown in Table 3. Chlorophyll *a* was the variable with the highest correlation at B35, B33, U33 and U30. Water temperature had significant positive correlations only at the inner salinity sites of both estuaries, where chlorophyll *a* concentration best correlated with temperature. River flow (particu-

Table 1

Correlation coefficients and *p*-values (in parentheses) of between-site Spearman rank correlation analyses of month scores on Axis 1 obtained in RDAs. Significant correlations in bold.

	B33	B30	U35	U33	U30
B35	**0.979** (< 0.001)	**0.874** (0.001)	**0.734** (0.007)	**0.895** (< 0.001)	**0.916** (< 0.001)
B33		**0.860** (< 0.001)	**0.671** (0.017)	**0.881** (< 0.001)	**0.888** (< 0.001)
B30			0.385 (0.217)	**0.643** (0.024)	**0.748** (0.005)
U35				**0.832** (0.001)	**0.776** (0.003)
U33					**0.902** (< 0.001)

larly of the Ibaizabal-Nerbioi river) showed significant negative correlation at all salinity sites under study in the estuary of Bilbao, but not in the estuary of Urdaibai.

Correlations of year scores along axis 1 with abiotic variables and chlorophyll *a* have been shown in Table 4. Results showed DOS to be the only variable with significant correlation at B33, and stratification index and river flow (with negative correlations) at B30. No significant correlations were observed at B35 or at any of the salinity sites of the
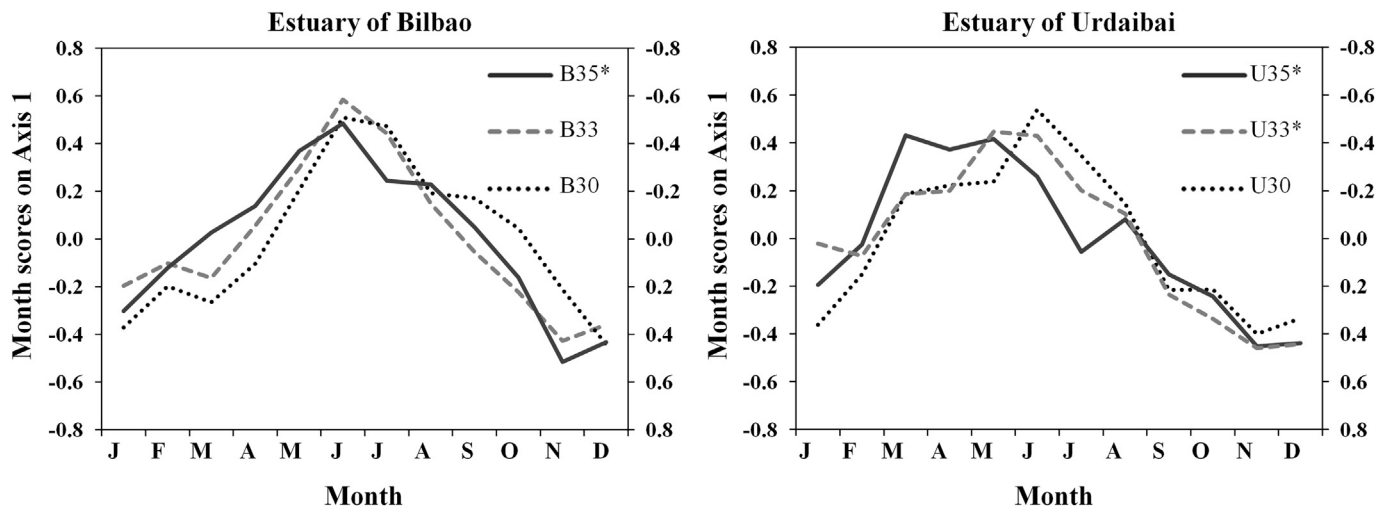

Fig. 3. Month scores on Axis 1 obtained from RDA analyses performed with month as categorical explanatory variable and year as categorical covariable for the 30 (dotted line), 33 (dashed line) and 35 (continuous line) salinity sites of the estuary of Bilbao (left) and Urdaibai (right). *On right axis.
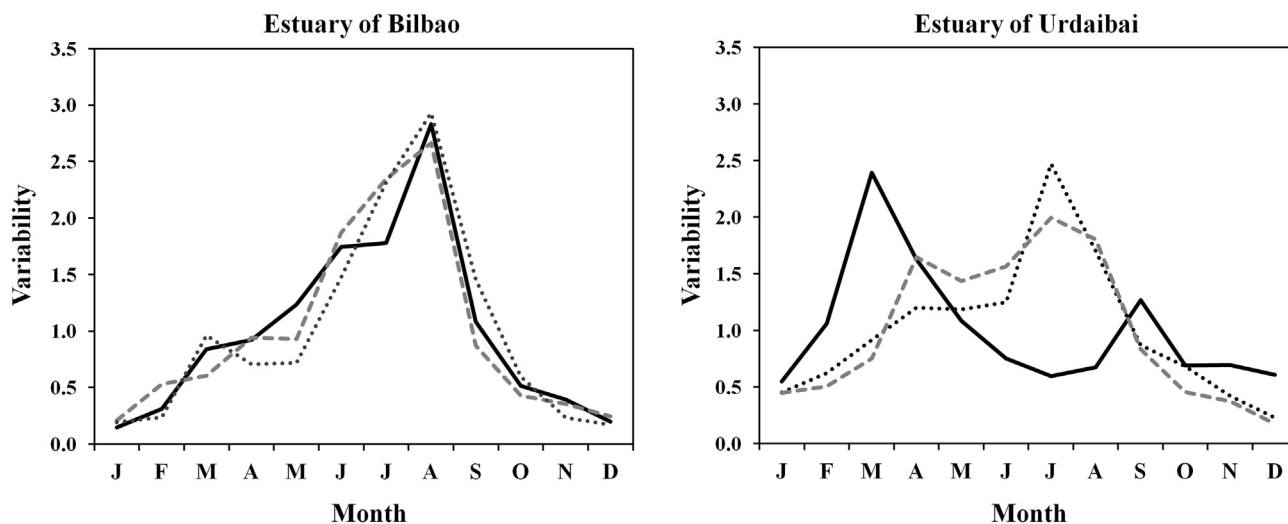
Fig. 4. Patterns of seasonal variability of chlorophyll *a*, calculated according to the multiplicative model described in Cloern and Jassby (2010), for the 30 (dotted line), 33 (dashed line) and 35 (continuous line) salinity sites of the estuary of Bilbao (left) and Urdaibai (right).

estuary of Urdaibai.

4. Discussion

4.1. Seasonal variations

In both of the estuaries under study, with the exception of U35, total mesozooplankton abundance showed maximum values in the warm season, in late spring-summer, as in most temperate estuarine systems (Knox 1986). Temperature can enhance zooplankton production and hatching of dormant eggs in some species (Ambler et al. 1985), and it has been shown to exert a relevant influence on zooplankton dynamics in estuaries (Modéran et al. 2010; Marques et al. 2014). However, it has to be born in mind that for most zooplankton the seasonal cycle is basically triggered by the specific life-history traits (Ribera d'Alcalà et al., 2004). Furthermore, temperature may indirectly control food availability of zooplankton (Mackas et al. 2012), and this seems to be the primary driver of the main mode of mesozooplankton seasonal variability in the estuaries of Bilbao and Urdaibai, since chlorophyll *a* was the variable with the highest correlation in most salinity sites of both estuaries. Exception was at U35 and B30, where chlorophyll *a* showed the second best correlation. At U35, DOS had the highest correlation, but DOS has been shown to be mainly related to phytoplankton production at this site (Iriarte et al., 2015). At B30 river flow was the variable that showed the highest correlation with the main mode of mesozooplankton variability. Longer residence times due to low river flows during summer, as it has been reported for the estuary of Bilbao (Uriarte et al. 2014), can also contribute to the elevated mesozooplankton numbers during the warm season through reduced flushing. Our data also showed earlier occurrences of the annual peak density of total mesozooplankton along the salinity gradient from 30 to 35 salinities in both estuaries. In fact, at U35 the annual cycle of total mesozooplankton abundance differed clearly from the estuarine pattern and showed an early spring maximum followed by a secondary peak in late summer with a decline in between. The latter pattern resembles those of shelf areas of the southern Bay of Biscay, particularly of the Xixón site (Bode et al. 2013b). Clear differentiation of the 35 salinity site of the estuary of Urdaibai from the rest of sites, was also evident by the earlier occurrence of maxima of quantitatively important mesozooplankton groups such as cladocerans, cirripede larvae and bivalve larvae at this zone, when compared to the same salinity site of the estuary of Bilbao and to the lower salinity sites of both estuaries. At U35, the annual maxima of total mesozooplankton and many relevant groups coincided with the annual maximum of chlorophyll *a*, and both

mesozooplankton densities and chlorophyll *a* showed declines in summer. Therefore, as stated above, this decrease of mesozooplankton in summer could be related to the lower phytoplankton (food) availability, which is, in turn, linked to the summer depletion of nutrients (Iriarte et al. 1997b; Villate et al. 2008). Similarly, in shelf waters of the Bay of Biscay high correlation between phytoplankton and zooplankton has been observed, indicating direct transfer of biomass via the classical herbivore food web (Stenseth et al. 2006). At B35, the seasonal pattern of mesozooplankton abundance and community variability differed clearly from the shelf pattern observed at U35, and was more similar to the estuarine pattern observed in lower salinity sites. The 35 salinity site of the estuary of Bilbao is greatly affected by the estuarine plume (Ferrer et al. 2009) and consequently no (or little) limitation of phytoplankton growth by nutrients can be expected in summer. Therefore, differences between estuaries in axial variations of the seasonal pattern of mesozooplankton community could be related to differences in hydrodynamic features (related to tidal flushing) between estuaries, which affect residence times, nutrient availability and phytoplankton standing stocks. Axial variations in zooplankton abundance have also been found to be related to residence times and phytoplankton standing crops in other estuaries (Badylak and Phlips 2008).

Cirripede larvae were one of the most abundant groups in the estuaries of Bilbao and Urdaibai, in agreement with findings for other coastal-estuarine areas of the Bay of Biscay (Castel and Courties 1982) and other temperate estuaries elsewhere in the North Atlantic (Muxagata et al. 2004), and they made a high contribution to the main mode of seasonal variability of the mesozooplankton community at all salinity sites in both estuaries. Cirripede larvae were responsible to a great extent for the differentiation of the community annual cycle at U35, since they showed a clearly defined seasonal maximum in early spring at the highest salinity site, but seasonal maxima in summer at lower salinity sites, which matched the seasonal cycles of chlorophyll *a* at each salinity site. This suggests that, the abundance of cirripede larvae is related to the timing of phytoplankton increase. In agreement, maximal abundance of barnacle larvae was found to be synchronized with the spring bloom in Avacha Inlet (Korn and Kulikova 1995) and coastal waters of the western English Channel (Highfield et al. 2010). In the estuary of Bilbao, however, the timing of the annual maxima of cirripede larvae only changed from mid spring to early summer with decreasing salinity, in accordance with the seasonal maxima recorded in early summer in the inner estuary of Southampton Water (Muxagata et al. 2004). Although annual maxima of cirripede larvae did not coincide in time with chlorophyll *a* annual maxima in the estuary of Bilbao, they also occurred under conditions of high chlorophyll *a*
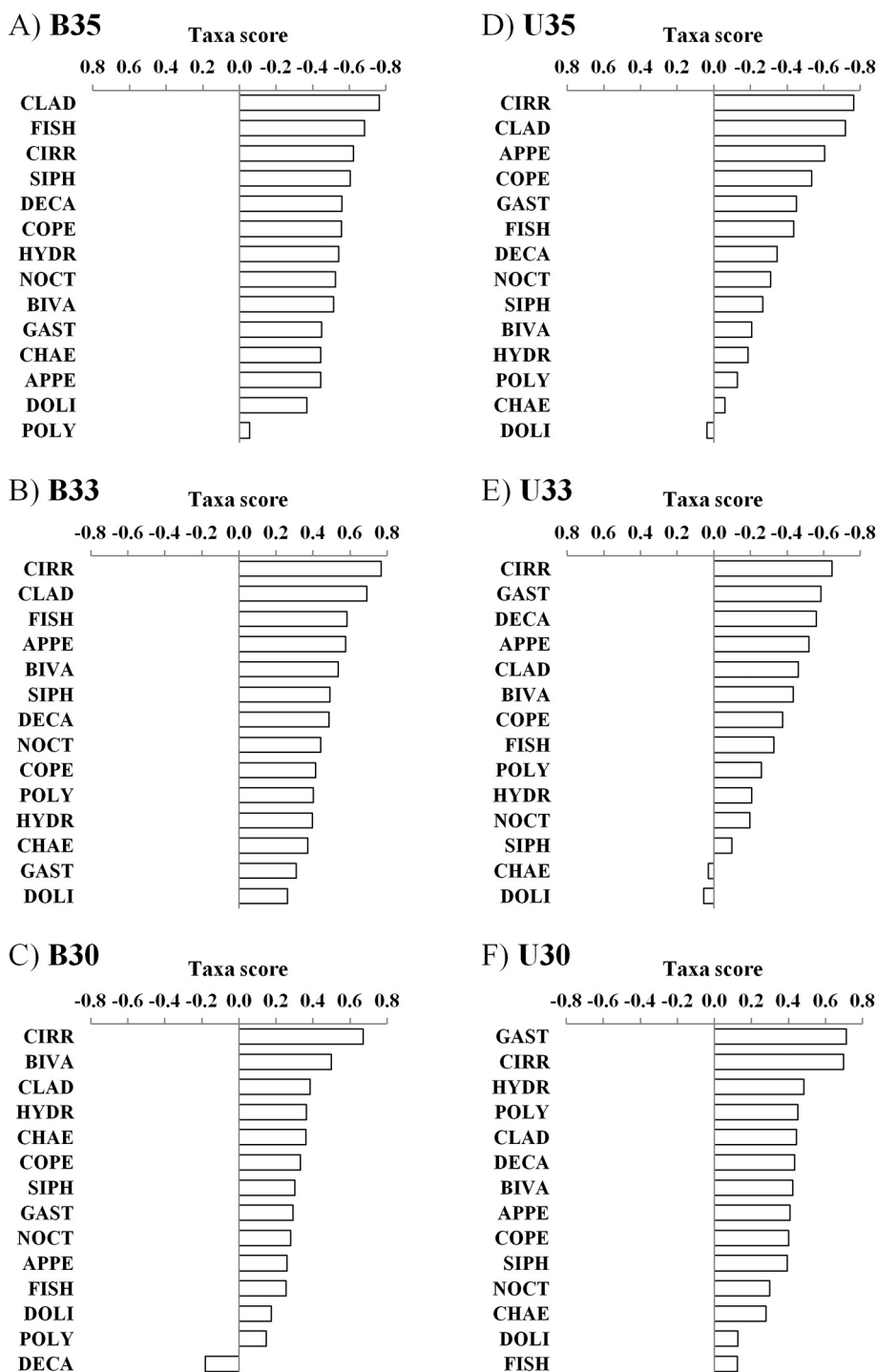
Fig. 5. Mesozooplankton taxa scores on Axis 1 from RDA analyses performed with month as categorical explanatory variable and year as categorical covariable for the 30, 33 and 35 salinity sites of the estuary of Bilbao (left) and Urdaibai (right). APPE: appendicularians, BIVA: bivalve larvae, CHAE: chaetognaths, CIRR: cirripede larvae, CLAD: cladocerans, COPE: copepods, DECA: decapods larvae, DOLI: doliolids, FISH: fish eggs and larvae, GAST: gastropod larvae, HYDR: hydromedusae, NOCT: *Noctiluca*, POLY: polychaete larvae, SIPH: siphonophores.

concentrations.

Cladocerans also made a marked contribution to the seasonality of mesozooplankton community in the outer estuarine sites, but this contribution decreased towards the lower salinity sites, particularly in the estuary of Urdaibai, because the two main species (*Evadne nordmanni* and *Podon intermedius*) are neritic (Alcaraz 1981) and penetrate in the estuary presumably as far as environmental constraints such as salinity allow them to do so (Uriarte and Villate 2004). In the estuary of Urdaibai cladoceran maxima were observed in early spring, whereas in the estuary of Bilbao maxima occurred in late spring-early summer. These two cladoceran species showed annual maxima in spring in an area 20 km off the coast in the Bay of Biscay (d'Elbée et al. 2014) and in spring and summer in the nearby estuary of Plentzia (Villate and Orive 1981). *Evadne nordmanni* has been reported to peak in summer at higher latitudes in the White Sea (Usov et al. 2013) an in winter at lower latitudes in the Alboran Sea (De Souza et al. 2011). Differences between the estuaries of Bilbao and Urdaibai in the seasonality of phytoplankton maxima in the outer estuary could
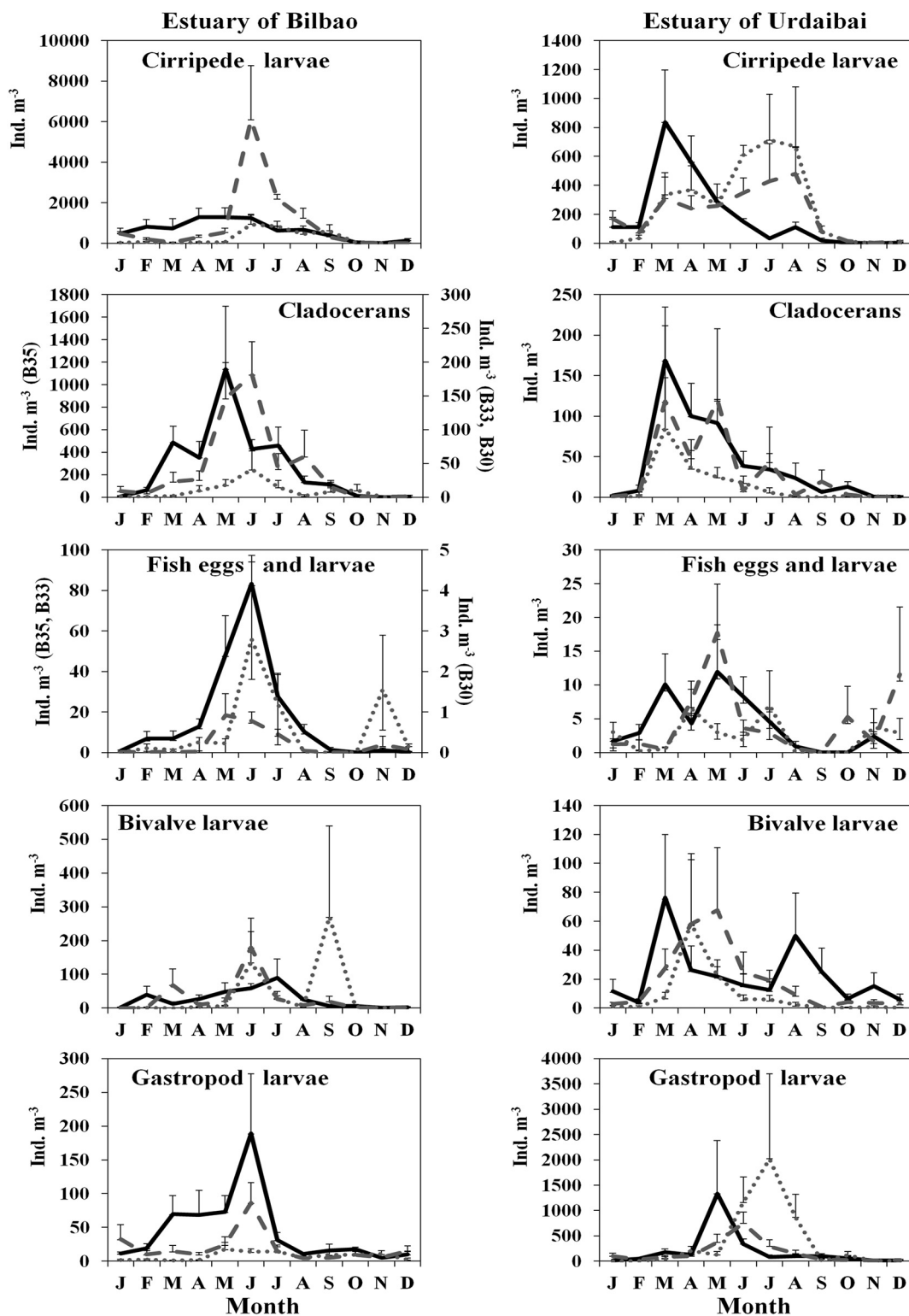
Fig. 6. Time-series mean (+ SD) densities for each month of selected mesozooplankton taxa that made a relevant contribution to the seasonal pattern of mesozooplankton community (holoplankton and meroplankton) at the 30 (dotted line), 33 (dashed line) and 35 (continuous line) salinity sites of the estuary of Bilbao (left) and Urdaibai (right).

contribute to the differences in the seasonality of cladocerans observed in the present work. Being filter feeders, most cladoceran species feed on algae as the principal food because of their suitable size, motility and nutritional value (Brown et al. 1997), although they have been shown to consume also components of the microbial food web (Katechakis and Stibor 2004).

Fish eggs and larvae made a substantial contribution in the outer estuary of Bilbao and much lower in the outer estuary of Urdaibai. Eggs and larvae of marine fishes, mainly pilchard (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*), penetrate in these estuaries from shelf waters (Villate, 1989–1990; Uriarte and Borja 2009) and they are less abundant and show a decrease in the contribution to the estuarine mesozooplankton seasonality in the landward direction, as salinity decreases. Also, pollution, which increases towards the inner estuary,
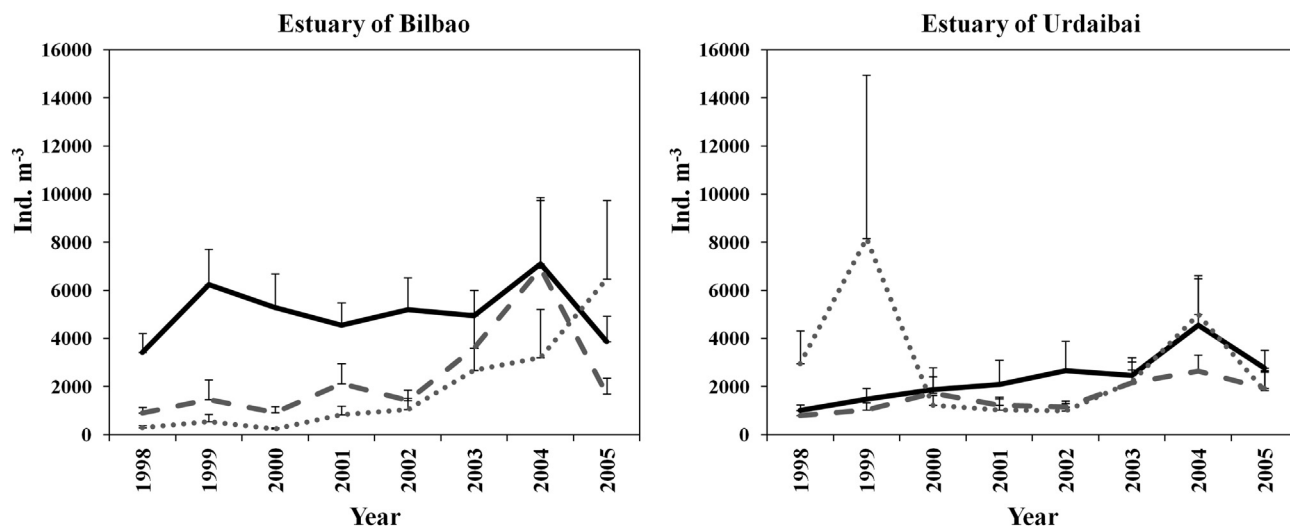
Fig. 7. Mean annual (+ SD) densities of total mesozooplankton at the 30 (dotted line), 33 (dashed line) and 35 (continuous line) salinity sites of the estuary of Bilbao (left) and Urdaibai (right).

and which was particularly strong in the estuary of Bilbao (e.g. low oxygen, high ammonia, Iriarte et al. 1997a; Villate et al. 2013), can compromise the fitness and survival of fish, including eggs and larvae (Bardon-Albaret and Saillant 2016). The lower presence and lower contribution to the main mode of mesozooplankton seasonal pattern of fish eggs and larvae in the estuary of Urdaibai, even in the outer estuary, could be related to the hydrologically less stable conditions in this system than in the estuary of Bilbao, which has been shown to hinder retention in other estuaries too (Whitfield 2005).

The contribution of bivalve larvae to the main mode of seasonal variability of mesozooplankton in the estuary of Bilbao increased relative to other taxa towards the inner estuary. This may be because other taxa which are more relevant in the outer estuary, such as cladocerans, cnidarians, or fish eggs and larvae, decrease their abundance in the inner estuary, whereas bivalve larvae do not show significant changes in density along the salinity gradient, in agreement with findings in a previous work (Uriarte and Villate 2004).

Gastropod larvae made a significant contribution to the main mode of seasonal mesozooplankton variability in the inner and intermediate estuary of Urdaibai, but not in the estuary of Bilbao. This difference between estuaries is due to gastropod larvae (mainly *Peringia ulvae*, reported previously as *Hydrobia ulvae*) being more abundant and

Table 2

Correlation coefficients and *p*-values (in parentheses) of between-site Spearman rank correlation analyses of year scores on Axis 1 obtained in RDAs. Significant correlations in bold.

	B33	B30	U35	U33	U30
B35	**0.762 (0.028)**	0.429 (0.289)	0.310 (0.456)	**0.857 (0.007)**	**0.881 (0.004)**
B33		0.071 (0.867)	0.571 (0.139)	**0.929 (0.001)**	**0.952 (< 0.001)**
B30			− 0.119 (0.779)	0.190 (0.651)	0.119 (0.779)
U35				0.690 (0.058)	0.548 (0.160)
U33					**0.952 (< 0.001)**

representative in the estuary of Urdaibai than in the estuary of Bilbao, as was also found by Uriarte and Villate (2004), due to the much higher availability of soft sediment intertidal areas in the former estuary. *Peringia ulvae* has been found to reach the highest densities on muddy-sandy sediments in a broad belt at approximately mid-tide level (Newell 1962), but the estuary of Bilbao has lost almost all of its

Fig. 8. Year scores on Axis 1 obtained from RDA analyses performed with year as categorical explanatory variable and month as categorical covariable for the 30 (dotted line), 33 (dashed line) and 35 (continuous line) salinity sites of the estuary of Bilbao (left) and Urdaibai (right). *On right axis.
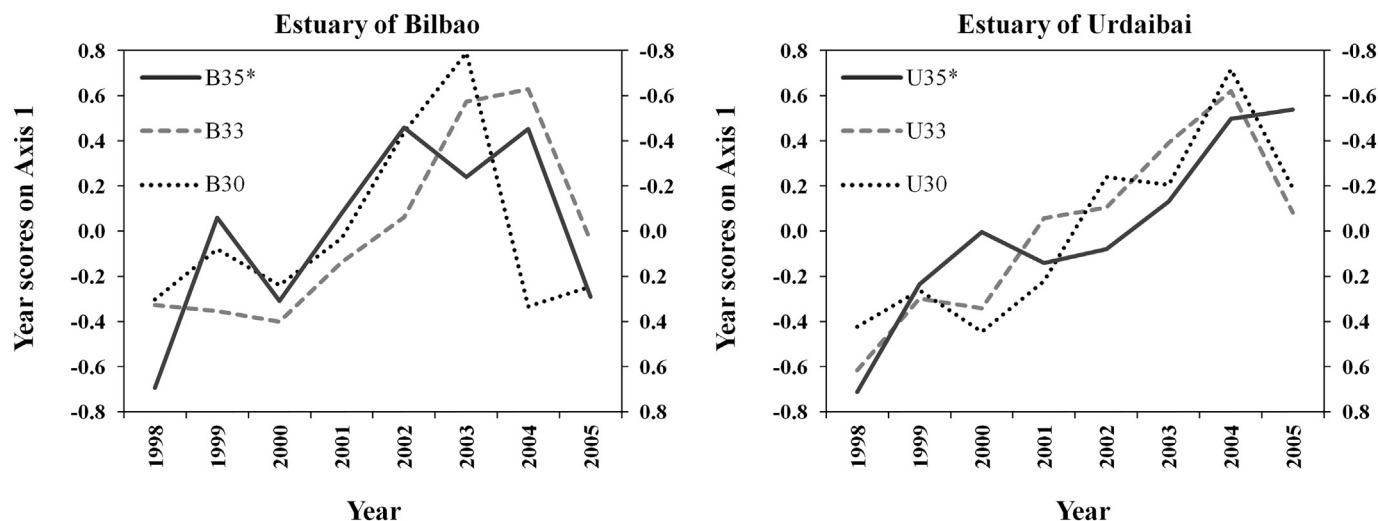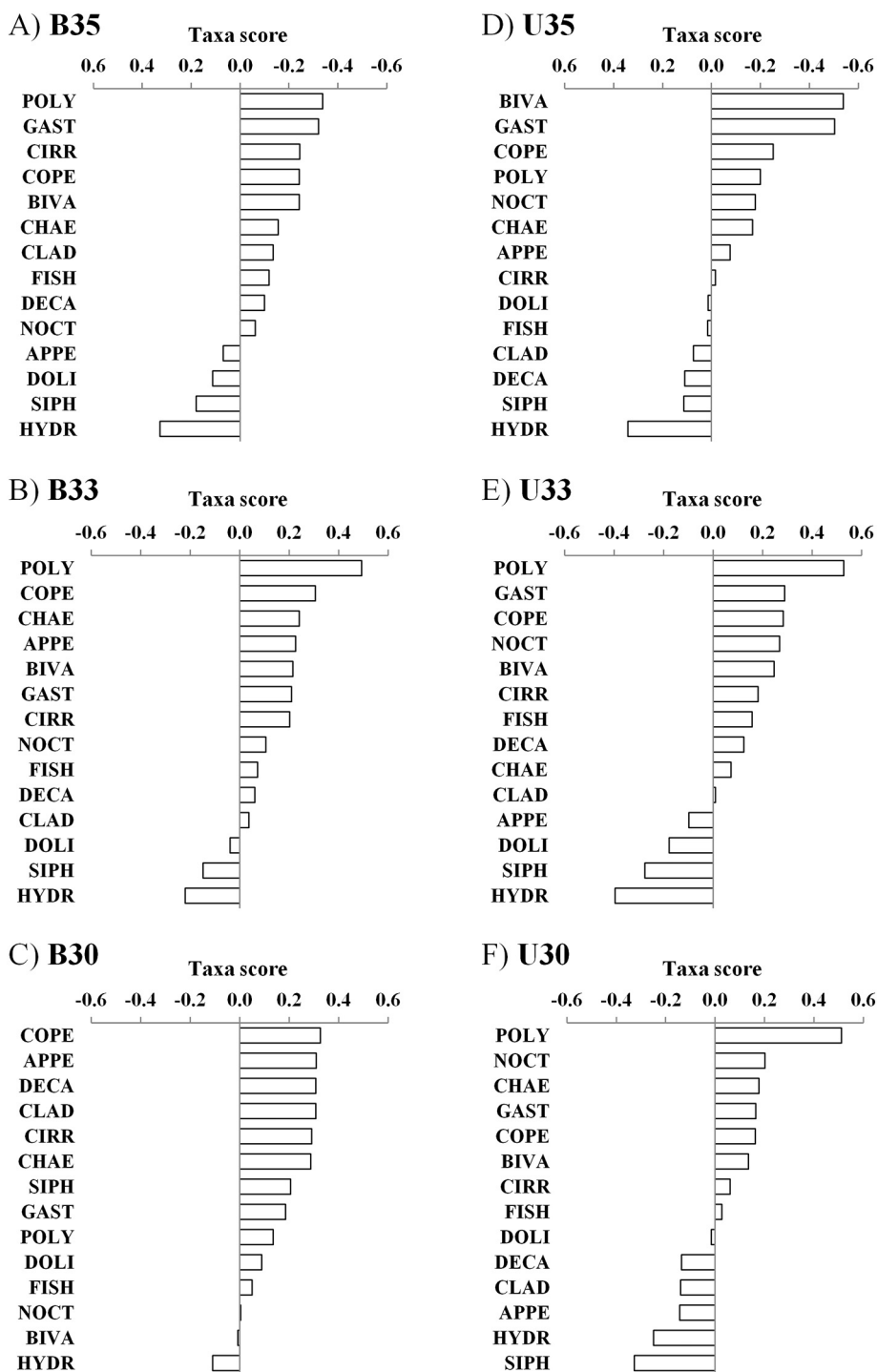
Fig. 9. Mesozooplankton taxa scores on Axis 1 obtained from RDA analyses performed with year as categorical explanatory variable and month as categorical covariable for the 30, 33 and 35 salinity sites of the estuary of Bilbao (left) and Urdaibai (right). Taxa abbreviations as in Fig. 5.

original intertidal areas due to land reclamation and channelization of its watercourse (Cearreta et al. 2004).

4.2. Interannual variations

Regarding interannual variations, mesozooplankton density increased during the study period in the highest salinity site of the estuary of Urdaibai. Overall, increasing trends of zooplankton density for this period, albeit with differences in the specific year-to-year variations, have also been reported in other coastal areas, e.g. outer Ría de Vigo in the Iberian peninsula (Buttay et al. 2015) and L4 station in

the English Channel (Reygondeau et al. 2015). This would suggest the influence of an external driving force acting at regional or supra-regional scales. In fact, no significant correlation with any of the environmental factors under study was observed. Zooplankton have already been shown to be responding to hydro-climatic variables that act at the regional to oceanic scale (Beaugrand et al. 2009). However, in the highest salinity site of the estuary of Bilbao no such increase in density was observed, presumably because of a larger influence of the estuarine plume, and therefore, of local factors.

There were clear differences between estuaries and salinity sites in the contribution of taxa to the main mode of interannual variability of
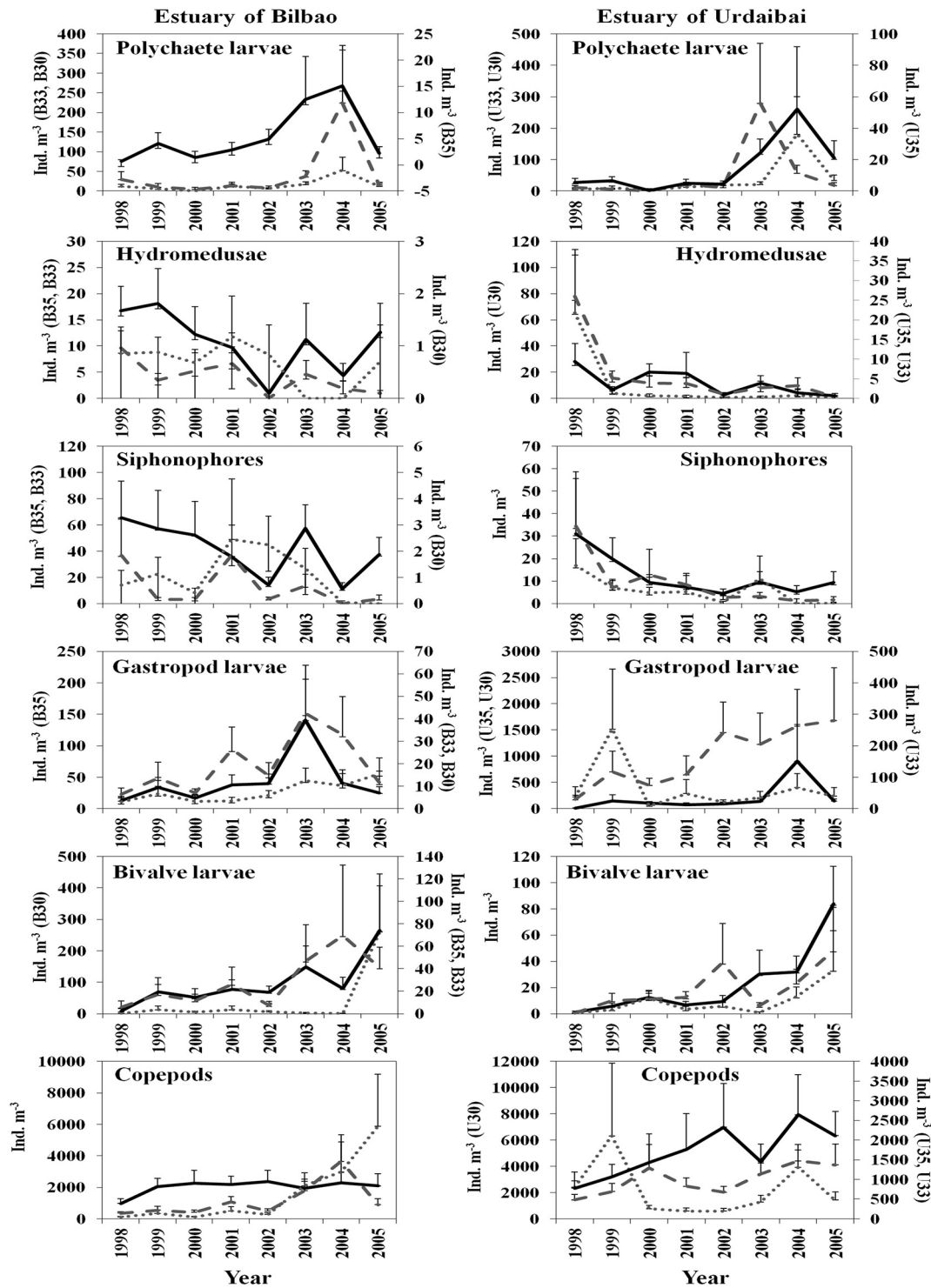
Fig. 10. Mean (+ SD) annual densities of selected mesozooplankton taxa that made a relevant contribution to the year-to-year variations pattern of mesozooplankton community in the estuaries of Bilbao (left) and Urdaibai (right).

mesozooplankton community. In most estuarine sites meroplankton taxa contributed most, polychaete larvae showing positive trends and hydromedusae showing negative trends. These two taxa were also shown to contribute highly to mesozooplankton variability in the estuary of Bilbao in a previous study covering 3 years (Intxausti et al. 2012). The occurrence of gelatinous zooplankton in the marine environment, in general, appears to be pulsed, (see Boero et al. 2008). Alternation of irregular low and high abundance phases with variable lags seems to be common (Boero et al. 2008), which would

agree with the fact that in later years the trends observed in the gelatinous predators were not maintained in the estuaries of Bilbao and Urdaibai (unpublished data). In some studies a large variability in trends of gelatinous zooplankton between relatively nearby areas has been observed (Bode et al. 2013a), however, in the estuaries of Bilbao and Urdaibai the decreasing trend was observed at all stations along the salinity gradient, although such trend was not so clear at B30. In Jiaozhou Bay (China) in different habitats both inside and outside the bay the same unidirectional variation patterns in small jellyfish were
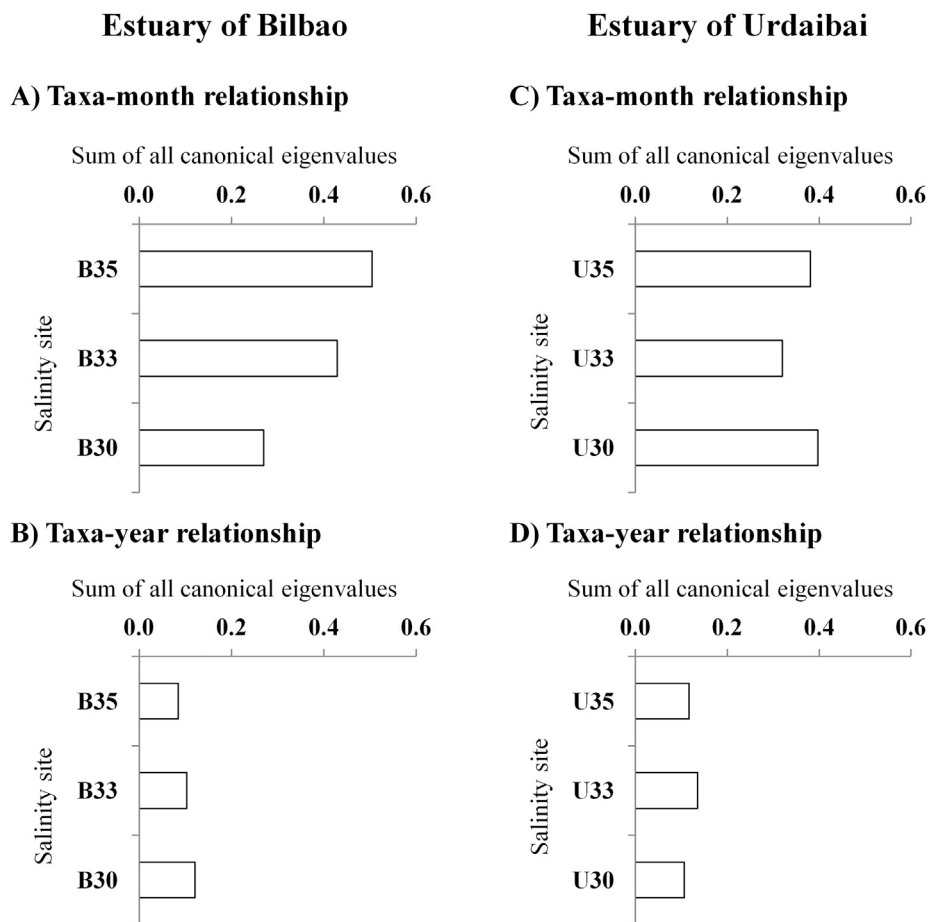
Estuary of Bilbao

A) Taxa-month relationship

Estuary of Urdaibai

C) Taxa-month relationship

B) Taxa-year relationship

D) Taxa-year relationship

Fig. 11. Sum of all canonical eigenvalues obtained from: (i) RDA analyses performed with month as categorical explanatory variable and year as categorical covariable (A, C); (ii) RDA analyses performed with year as categorical explanatory variable and month as categorical covariable (B, D) for the 30, 33 and 35 salinity sites of the estuary of Bilbao (left) and Urdaibai (right).

observed (Sun et al. 2012). Interestingly, in general, ordination analysis showed opposite signs for copepods and cnidarians in the estuaries of Bilbao and Urdaibai. This type of inverse relationship has also been noted in other coastal areas of the Iberian Peninsula (Bode et al. 2013a) and elsewhere (e.g. Molinero et al. 2008). It may be that in these Basque coast estuaries, copepod growth is favoured under low gelatinous predator abundances, because of lower predation, as suggested for other areas (Molinero et al. 2008). The outer estuary of Urdaibai and the inner estuary of Bilbao, however, showed marked deviations from the above described general pattern of taxa contribution. In the outer estuary of Urdaibai, together with hydromedusae, bivalve larvae and gastropod larvae, instead of polychaete larvae, made the highest contribution to the interannual pattern. Polychaete larvae in the

estuaries of Bilbao and of Urdaibai include mainly spionid larvae (Uriarte and Villate 2004), which are known for their high tolerance to pollution (Fritzsche and von Oertzen 1995) and, accordingly, they were far less abundant in the outer well flushed zone of Urdaibai than in the intermediate and inner zones, as observed also by Villate (1991). In addition, as stated above, gastropod larvae were more abundant in the estuary of Urdaibai than in the estuary of Bilbao because of the larger intertidal areas covered with soft sediments in the former. The increasing pattern of gastropod larvae in the estuary of Urdaibai might be related to the fact that intertidal gastropod larvae are responding to the effect of global warming along the Iberian Peninsula (Rubal et al. 2013), but data for more years should be obtained to confirm this hypothesis.

Table 3
Spearman correlation coefficients and *p*-values (in parentheses) between the month scores along axis 1 obtained in RDA analyses and abiotic factors and chlorophyll *a*. Significant correlations in bold.

	B35	B33	B30	U35	U33	U30
WT	0.566 (0.055)	0.552 (0.063)	**0.783 (0.003)**	0.105 (0.746)	0.433 (0.159)	**0.650 (0.022)**
Chl*a*	**0.832 (0.001)**	**0.867 (< 0.001)**	**0.804 (0.002)**	**0.671 (0.017)**	**0.774 (0.003)**	**0.741 (0.006)**
DOS	**0.776 (0.003)**	0.014 (0.966)	− **0.790 (0.002)**	**0.741 (0.006)**	0.417 (0.178)	− 0.007 (0.983)
STR	− 0.573 (0.051)	− **0.629 (0.028)**	− **0.788 (0.002)**	− 0.404 (0.192)	− 0.522 (0.082)	− 0.517 (0.085)
SDD	− 0.182 (0.572)	− 0.127 (0.695)	0.200 (0.534)	–	0.515 (0.087)	− 0.273 (0.391)
RF$_{in}$	− **0.650 (0.022)**	− **0.622 (0.031)**	− **0.846 (0.001)**	–	–	–
RF$_k$	− **0.580 (0.048)**	− 0.545 (0.067)	− **0.783 (0.003)**	–	–	–
RF$_o$	–	–	–	− 0.238 (0.457)	− 0.403 (0.194)	− 0.517 (0.085)

WT: water temperature, Chl*a*: concentration of chlorophyll *a*, DOS: dissolved oxygen saturation, STR: salinity stratification index, SDD: Secchi disk depth, RF$_{in}$: Ibaizabal-Nerbioi river flow, RF$_k$: Kadagua river flow, RF$_o$: Oka river flow.

Table 4
Spearman correlation coefficients and *p*-values (in parentheses) between the year scores along axis 1 obtained in RDA analyses and abiotic factors and chlorophyll *a*. Significant correlations in bold. Abbreviations as in Table 3.

	B35	B33	B30	U35	U33	U30
WT	− 0.095 (0.823)	0.476 (0.233)	− 0.119 (0.779)	0.190 (0.651)	0.524 (0.183)	0.381 (0.352)
Chl*a*	0.048 (0.911)	0.452 (0.260)	− 0.143 (0.736)	0.084 (0.844)	0.467 (0.243)	0.524 (0.183)
DOS	− 0.095 (0.823)	**0.786 (0.021)**	0.548 (0.160)	0.262 (0.531)	0.286 (0.493)	0.119 (0.779)
STR	− 0.429 (0.289)	− 0.190 (0.651)	**− 0.810 (0.015)**	− 0.048 (0.911)	− 0.429 (0.289)	− 0.048 (0.911)
SDD	0.286 (0.493)	0.548 (0.160)	0.000 (1.000)	–	− 0.214 (0.610)	− 0.143 (0.736)
RF$_{in}$	− 0.071 (0.867)	0.405 (0.320)	− 0.595 (0.120)	–	–	–
RF$_{k}$	− 0.667 (0.071)	− 0.238 (0.570)	**− 0.714 (0.047)**	–	–	–
RF$_{o}$	–	–	–	0.286 (0.493)	− 0.071 (0.867)	− 0.071 (0.867)

The inner estuary of Bilbao showed the most dissimilar interannual pattern, since copepods made the highest contribution, together with many other taxa with similar high contributions. This can be explained by the process of recolonization by mesozooplankton (which were virtually absent under the highly polluted past conditions) that has taken place in the inner estuary of Bilbao (Uriarte et al. 2016), following improvement in estuarine sediment and water quality (Borja et al. 2010; Villate et al. 2013). In this recolonization process, the introduction and settlement (attaining high abundances) of non-indigenous copepod species, such as *Acartia tonsa* and *Oithona davisae*, has been notorious (Aravena et al. 2009; Uriarte et al. 2016). The lack of a significant correlation between DOS (the most important stress factor in the highly polluted estuary of Bilbao, see González-Oreja and Saiz-Salinas 1998) and mesozooplankton in the inner estuary of Bilbao may partly be explained by the facts that *A. tonsa* can adapt well to low and variable concentrations of dissolved oxygen (Kimmel et al. 2009) and marine biota respond to multiple environmental drivers which cause non-linear changes in the performance of organisms (Boyd and Brown 2015).

4.3. Contribution of months and years to mesozooplankton variability

The percentage variation of mesozooplankton explained by months and years showed differences between estuaries. Overall, months explained more of the variability in the estuary of Bilbao (at B35 and B33), where the seasonal pattern of zooplankton along the estuary was more uniform, whereas years explained more of the variability in the estuary of Urdaibai (at U35 and U33), likely due to the larger differences in the seasonal pattern along the estuary and the high influence of shelf water dynamics in the year to year variations in the outer estuary.

Furthermore, the percentage variation of mesozooplankton explained by months and years showed different trends along the salinity gradient of the estuary of Bilbao. The decrease of the percentage variation explained by months and the increase of the percentage variation explained by years from the outer to the inner estuary indicated less stable seasonal patterns and stronger interannual variations of mesozooplankton in the inner estuary, due to some extent to the recolonization by native zooplankton and the arrival and settlement of non-native species (Uriarte et al. 2016).

5. Conclusions

There were spatial differences in the mesozooplankton seasonality, both within and between estuaries, linked to differences in hydrodynamic features and anthropogenic nutrient enrichment which drive seasonal patterns of phytoplankton biomass. The within estuary seasonal differences in mesozooplankton community were mainly shown through a seaward advance in the seasonal peaks from summer to spring along the salinity gradient, in relation to differences in food (phytoplankton) availability during the summer, in turn, related to inorganic nutrient availability. This inner-outer seasonal variation was

mainly observed in the estuary of Urdaibai, where the mesozooplankton of the outermost zone showed a bimodal cycle with a maximum in early spring (related to the spring phytoplankton bloom) and a drop in summer by nutrient depletion, which resembles patterns in shelf waters. In fact, the main differences in the seasonal pattern of mesozooplankton between estuaries was found at the highest salinity zone, because in the outer estuary of Bilbao the seasonal pattern was more similar to that observed for intermediate and inner zones (the estuarine pattern), likely due to the influence of the estuarine plume providing nutrients to the outer estuary, and allowing relatively high phytoplankton standing stocks in summer.

Cladocerans and cirripede larvae made a high contribution to the seasonal patterns of mesozooplankton community in both estuaries, but there were also relevant differences between estuaries, which were attributable to local factors, partly linked to man-made physical modifications. Fish eggs and larvae made a higher contribution to the seasonal mesozooplankton pattern at the outer estuary of Bilbao than at the outer estuary of Urdaibai, likely related to the low hydrological stability of the latter one that hinders larval retention. Gastropod larvae, however, made a higher contribution to the seasonal mesozooplankton community pattern in the estuary of Urdaibai because of their much higher abundance in this estuary, as a result of the presence of large areas of soft substrate benthic habitats, which have been enormously reduced by land reclamation and channelization of the watercourse in the estuary of Bilbao.

Differences in interannual variations of mesozooplankton were also apparent between and within estuaries. Clear unidirectional trends of mesozooplankton increase and community variability were observed at the inner estuary of Bilbao and at the outer estuary of Urdaibai, respectively. In the first case, the observed increase of total mesozooplankton and several groups like copepods, bivalve larvae and gastropod larvae was a response to the estuarine rehabilitation process that has taken place during the study period and which has allowed the penetration of native zooplankton and the settlement and massive growth of non-indigenous copepod species. In the case of the outer estuary of Urdaibai, it may be the response to some extent to forces that act at regional scales on marine systems. In the other zones of both estuaries, in general, the taxa that contributed most to the interannual variations in mesozooplankton community were polychaete larvae and hydromedusae, with increasing and decreasing trends respectively.

Acknowledgements

References

d'Elbée, J., Lalanne, Y., Castège, I., Bru, N., D'Amico, F., 2014. Response of planktonic cladocerans (Class: Branchiopoda) to short-term changes in environmental variables in the surface waters of the Bay of Biscay. Deep. Res. Part II Top. Stud. Oceanogr. 106, 87–98. http://dx.doi.org/10.1016/j.dsr2.2013.09.030.

Ribera d'Alcalà, M., Conversano, F., Corato, F., Licandro, P., Mangoni, O., 2004. Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. Sci. Mar. 68, 65–83. http://dx.doi.org/10.3989/scimar.2004.68s165.

Albaina, A., Villate, F., Uriarte, I., 2009. Zooplankton communities in two contrasting Basque estuaries (1999–2001): Reporting changes associated with ecosystem health. J. Plankton Res. 31, 739–752.

Alcaraz, M., 1981. Ciclo anual de los cladóceros y ostrácodos planctónicos en la plataforma continental de Vizcaya (Punta Endata). Investig. Pesq. 45, 3–16.

Ambler, J.W., Cloern, J.E., Hutchinson, A., 1985. Seasonal cycles of zooplankton from San Francisco Bay. Hydrobiologia 129, 177–197. http://dx.doi.org/10.1007/BF00048694.

Aravena, G., Villate, F., Uriarte, I., Iriarte, A., Ibañez, B., 2009. Response of *Acartia* populations to environmental variability and effects of invasive congenerics in the estuary of Bilbao, Bay of Biscay. Estuar. Coast. Shelf Sci. 83, 621–628.

Badylak, S., Phlips, E.J., 2008. Spatial and temporal distributions of zooplankton in Tampa Bay, Florida, including observations during a HAB event. J. Plankton Res. 30, 449–465. http://dx.doi.org/10.1093/plankt/fbn010.

Bardon-Albaret, A., Saillant, E.A., 2016. Effects of hypoxia and elevated ammonia concentration on the viability of red snapper embryos and early larvae. Aquaculture 459, 148–155. http://dx.doi.org/10.1016/j.aquaculture.2016.03.042.

Beaugrand, G., Ibanez, F., 2004. Monitoring marine plankton ecosystems. II: Long-term changes in North Sea calanoid copepods in relation to hydro-climatic variability. Mar. Ecol. Prog. Ser. 284, 35–47. http://dx.doi.org/10.3354/meps284035.

Beaugrand, G., Luczak, C., Edwards, M., 2009. Rapid biogeographical plankton shifts in the North Atlantic Ocean. Glob. Chang. Biol. 15, 1790–1803. http://dx.doi.org/10.1111/j.1365-2486.2009.01848.x.

Beaugrand, G., Harlay, X., Edwards, M., 2014. Detecting plankton shifts in the North Sea: A new abrupt ecosystem shift between 1996 and 2003. Mar. Ecol. Prog. Ser. 502, 85–104. http://dx.doi.org/10.3354/meps10693.

Blaber, S.J.M., Cyrus, D.P., Albaret, J.-J., Ching, C.V., Day, J.W., Elliott, M., Fonseca, M.S., Hoss, D.E., Orensanz, J., Potter, I.C., Silvert, W., 2000. Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. ICES J. Mar. Sci. 57. http://dx.doi.org/10.1006/jmsc.2000.0723.

Bode, A., Álvarez-Ossorio, M.T., Miranda, A., Ruiz-Villarreal, M., 2013a. Shifts between gelatinous and crustacean plankton in a coastal upwelling region. ICES J. Mar. Sci. 70, 934–942. http://dx.doi.org/10.1093/icesjms/fss193.

Bode, A., Bueno, J., Lopez-Urrutia, A., Villate, F., Uriarte, I., Iriarte, A., Alvarez-Ossorio, M.T., Miranda, A., Casas, G., Dos Santos, A., Santos, A.M.P., Chicharo, M.A., Cruz, J., Ben-Hamadou, B., Chicharo, L., 2013b. Zooplankton of the Bay of Biscay and western Iberian shelf. In: O'Brien, T.D., Wiebe, P.H., Falkenhaug, T. (Eds.), ICES Zooplankton Status Report 2010/2011. ICES Cooperative Research Report No. 318, (p. 208).

Boero, F., Bouillon, J., Gravili, C., Miglietta, M.P., Parsons, T., Piraino, S., 2008. Gelatinous plankton: Irregularities rule the world (sometimes). Mar. Ecol. Prog. Ser. 356, 299–310. http://dx.doi.org/10.3354/meps07368.

Borja, Á., Dauer, D.M., Elliott, M., Simenstad, C.A., 2010. Medium- and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. Estuar. Coasts 33, 1249–1260. http://dx.doi.org/10.1007/s12237-010-9347-5.

Boyd, P.W., Brown, C.J., 2015. Modes of interactions between environmental drivers and marine biota. Front. Mar. Sci. 2, 9. http://dx.doi.org/10.3389/fmars.2015.00009.

ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO Reference manual and CanoDraw for Windows User's guide: software for Canonical Community Ordination (version 4.5). In: Microcomputer Power, (500pp. Ithaca, NY, USA).

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315–331. http://dx.doi.org/10.1016/S0044-8486(96)01501-3.

Buttay, L., Miranda, A., Casas, G., González-Quirós, R., Nogueira, E., 2015. Long-term and seasonal zooplankton dynamics in the northwest Iberian shelf and its relationship with meteo-climatic and hydrographic variability. J. Plankton Res. 38, 106–121. http://dx.doi.org/10.1093/plankt/fbv100.

Castel, J., Courties, C., 1982. Composition and differential distribution of zooplankton in Arcachon Bay. J. Plankton Res. 4, 417–433. http://dx.doi.org/10.1093/plankt/4.3.417.

Cearreta, A., Irabien, M.J., Pascual, A., 2004. Human activities along the Basque coast during the last two centuries: geological perspective of recent anthropogenic impact on the coast and its environmental consequences. In: Borja, Á., Collins, M. (Eds.), Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, Amsterdam, pp. 27–50. http://dx.doi.org/10.1016/S0422-9894(04)80054-0.

Cloern, J.E., Jassby, A.D., 2010. Patterns and scales of phytoplankton variability in estuarine-coastal ecosystems. Estuar. Coasts 33, 230–241. http://dx.doi.org/10.1007/s12237-009-9195-3.

Dam, H.G., 2013. Evolutionary adaptation of marine zooplankton to global change. Annu. Rev. Mar. Sci. 5, 349–370. http://dx.doi.org/10.1146/annurev-marine-121211-172229.

De Souza, C.S., Mafalda, P., Sallés, S., Ramirez, T., Cortés, D., Garcia, A., Mercado, J., Vargas-Yañez, M., 2011. Seasonal changes in the distribution and abundance of marine cladocerans of the northwest Alboran Sea (Western Mediterranean), Spain. Braz. Arch. Biol. Technol. 54, 543–550.

Ferrer, L., Fontán, A., Mader, J., Chust, G., González, M., Valencia, V., Uriarte, A., Collins, M.B., 2009. Low-salinity plumes in the oceanic region of the Basque Country. Cont. Shelf Res. 29, 970–984. http://dx.doi.org/10.1016/j.csr.2008.12.014.

Franco, J., Borja, Á., Valencia, V., 2004. Overall assessment—human impacts and quality status. In: Borja, Á., Collins, M. (Eds.), Oceanography and Marine Environment of the Basque Country. Elsevier Oceanography Series, Amsterdam, pp. 581–597. http://dx.

doi.org/10.1016/S0422-9894(04)80054-0.

Fritzsche, D., von Oertzen, J.A., 1995. Bioenergetics of a highly adaptable brackish water polychaete. Thermochim. Acta 251, 1–9. http://dx.doi.org/10.1016/0040-6031(94)01964-I.

García-Barcina, J.M., González-Oreja, J.A., De La Sota, A., 2006. Assessing the improvement of the Bilbao estuary water quality in response to pollution abatement measures. Water Res. 40, 951–960. http://dx.doi.org/10.1016/j.watres.2006.01.004.

González-Oreja, J.A., Saiz-Salinas, J.I., 1998. Exploring the relationships between abiotic variables and benthic community structure in a polluted estuarine system. Water Res. 32, 3799–3807. http://dx.doi.org/10.1016/S0043-1354(98)00167-5.

Hewitt, J.E., Ellis, J.I., Thrush, S.F., 2016. Multiple stressors, nonlinear effects and the implications of climate change impacts on marine coastal ecosystems. Glob. Chang. Biol. 22, 2665–2675. http://dx.doi.org/10.1111/gcb.13176.

Highfield, J.M., Eloire, D., Conway, D.V.P., Lindeque, P.K., Attrill, M.J., Somerfield, P.J., 2010. Seasonal dynamics of meroplankton assemblages at station L4. J. Plankton Res. 32, 681–691. http://dx.doi.org/10.1093/plankt/fbp139.

Intxausti, L., Villate, F., Uriarte, I., Iriarte, A., Ameztoy, I., 2012. Size-related response of zooplankton to hydroclimatic variability and water-quality in an organically polluted estuary of the Basque coast (Bay of Biscay). J. Mar. Syst. 94, 87–96.

Iriarte, A., De La Sota, A., Orive, E., 1997a. Seasonal variation of nitrification along a salinity gradient in an urban estuary. Hydrobiologia 362, 115–126. http://dx.doi.org/10.1023/A:1003130516899.

Iriarte, A., De Madariaga, I., Diez-Garagarza, F., Revilla, M., Orive, E., 1997b. Primary plankton production, respiration and nitrification in a shallow temperate estuary during summer. J. Exp. Mar. Biol. Ecol. 208, 127–151. http://dx.doi.org/10.1016/S0022-0981(96)02672-X.

Iriarte, A., Villate, F., Uriarte, I., Alberdi, L., Intxausti, L., 2015. Dissolved oxygen in a temperate estuary: the influence of hydro-climatic factors and eutrophication at seasonal and inter-annual time scales. Estuar. Coasts 38, 1000–1015. http://dx.doi.org/10.1007/s12237-014-9870-x.

Iriarte, A., Villate, F., Uriarte, I., Arranz, S., 2016. Assessment of the climate and human impact on estuarine water environments in two estuaries of the Bay of Biscay. Oceanol. Hydrobiol. Stud. 45, 505–523. http://dx.doi.org/10.1515/ohs-2016-0043.

Islam, M.S., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Mar. Pollut. Bull. 48, 624–649. http://dx.doi.org/10.1016/j.marpolbul.2003.12.004.

Katechakis, A., Stibor, H., 2004. Feeding selectivities of the marine cladocerans *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*. Mar. Biol. 145, 529–539. http://dx.doi.org/10.1007/s00227-004-1347-1.

Kimmel, D., Boicourt, W., Pierson, J., Roman, M., Zhang, X., 2009. A comparison of the mesozooplankton response to hypoxia in Chesapeake bay and the northern Gulf of Mexico using the biomass size spectrum. J. Exp. Mar. Biol. Ecol. 381, 65–73. http://dx.doi.org/10.1016/j.jembe.2009.07.012.

Kimmerer, W.J., Burau, J.R., Bennett, W.A., 1998. Tidally-oriented vertical migration and position maintenance of zooplankton in a temperate estuary. Limnol. Oceanogr. 43, 1697–1709. http://dx.doi.org/10.4319/lo.1998.43.7.1697.

Knox, G.A., 1986. Estuarine Ecosystems: a Systems Approach. CRC Press Inc., Boca Raton, Florida.

Korn, O.M., Kulikova, V.A., 1995. Seasonal species composition and distribution of barnacle larvae in Avacha Inlet (Kamchatka). J. Plankton Res. 17, 221–234. http://dx.doi.org/10.1093/plankt/17.2.221.

Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346.

Mackas, D.L., Greve, W., Edwards, M., Chiba, S., Tadokoro, K., Eloire, D., Mazzocchi, M.G., Batten, S., Richardson, A.J., Johnson, C., Head, E., Conversi, A., Peluso, T., 2012. Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. Prog. Oceanogr. 97–100, 31–62. http://dx.doi.org/10.1016/j.pocean.2011.11.005.

Marques, S.C., Pardal, M.A., Pereira, M.J., Gonçalves, F., Marques, J.C., Azeiteiro, U.M., 2007. Zooplankton distribution and dynamics in a temperate shallow estuary. Hydrobiologia 213–223. http://dx.doi.org/10.1007/s10750-007-0682-x.

Marques, S.C., Primo, A.L., Martinho, M., Azeiteiro, U.M., Pardal, M.A., 2014. Shifts in estuarine zooplankton variability following extreme climate events: a comparison between drought and regular years. Mar. Ecol. Prog. Ser. 499, 65–76. http://dx.doi.org/10.3354/meps10635.

McLusky, D.S., Elliott, M., 2004. The Estuarine Ecosystem: Ecology, Threats and Management. Oxford University Press, New York (214 pp.). http://dx.doi.org/10.1093/acprof:oso/9780198525080.001.0001.

Miller, C.B., 1983. The zooplankton of estuaries. In: Ketchum, B.H. (Ed.), Estuaries and Enclosed Seas. Ecosystems of the World. 26. Elsevier Scientific, Amsterdam, pp. 103–149 (500 pp.).

Modéran, J., Bouvais, P., David, V., Le Noc, S., Simon-Bouhet, B., Niquil, N., Miramand, P., Fichet, D., 2010. Zooplankton community structure in a highly turbid environment (Charente estuary, France): spatio-temporal patterns and environmental control. Estuar. Coast. Shelf Sci. 88, 219–232. http://dx.doi.org/10.1016/j.ecss.2010.04.002.

Molinero, J.C., Ibanez, F., Souissi, S., Buecher, E., Dallot, S., Nival, P., 2008. Climate control on the long-term anomalous changes of zooplankton communities in the Northwestern Mediterranean. Glob. Chang. Biol. 14, 11–26. http://dx.doi.org/10.1111/j.1365-2486.2007.01469.x.

Muxagata, E., Williams, J.A., Sheader, M., 2004. Composition and temporal distribution of cirripede larvae in Southampton Water, England, with particular reference to the secondary production of *Elminius modestus*. ICES J. Mar. Sci. 61, 585–595. http://dx.doi.org/10.1016/j.icesjms.2004.03.015.

Newell, R.C., 1962. Behavioural aspects of the ecology of *Peringia* (= *Hydrobia*) *ulvae*

(Pennant) (Gastropoda, Prosobranchia). Proc. Zool. Soc. London 138, 49–75. http://dx.doi.org/10.1111/j.1469-7998.1962.tb05687.x.

Pascual, M., Borja, A., Franco, J., Burdon, D., Atkins, J.P., Elliott, M., 2012. What are the costs and benefits of biodiversity recovery in a highly polluted estuary? Water Res. 46, 205–217. http://dx.doi.org/10.1016/j.watres.2011.10.053.

Reygondeau, G., Molinero, J.C., Coombs, S., MacKenzie, B.R., Bonnet, D., 2015. Progressive changes in the Western English Channel foster a reorganization in the plankton food web. Prog. Oceanogr. 137, 524–532. http://dx.doi.org/10.1016/j.pocean.2015.04.025.

Richardson, A.J., 2008. In hot water: Zooplankton and climate change. ICES J. Mar. Sci. 65, 279–295. http://dx.doi.org/10.1093/icesjms/fsn028.

Rubal, M., Veiga, P., Cacabelos, E., Moreira, J., Sousa-Pinto, I., 2013. Increasing sea surface temperature and range shifts of intertidal gastropods along the Iberian Peninsula. J. Sea Res. 77, 1–10. http://dx.doi.org/10.1016/j.seares.2012.12.003.

Stenseth, N.C., Llope, M., Anadón, R., Ciannelli, L., Chan, K.-S., Hjermann, D.Ø., Bagøien, E., Ottersen, G., 2006. Seasonal plankton dynamics along a cross-shelf gradient. Proc. Biol. Sci. 273, 2831–2838. http://dx.doi.org/10.1098/rspb.2006.3658.

Sun, S., Li, Y., Sun, X., 2012. Changes in the small-jellyfish community in recent decades in Jiaozhou Bay, China. Chin. J. Oceanol. Limnol. 30, 507–518. http://dx.doi.org/10.1007/s00343-012-1179-7.

Uriarte, A., Borja, A., 2009. Assessing fish quality status in transitional waters, within the European Water Framework Directive: setting boundary classes and responding to anthropogenic pressures. Estuar. Coast. Shelf Sci. 82, 214–224. http://dx.doi.org/10.1016/j.ecss.2009.01.008.

Uriarte, I., Villate, F., 2004. Effects of pollution on zooplankton abundance and distribution in two estuaries of the Basque coast (Bay of Biscay). Mar. Pollut. Bull. 49, 220–228.

Uriarte, I., Villate, F., Iriarte, A., Duque, J., Ameztoy, I., 2014. Seasonal and axial variations of net water circulation and turnover in the estuary of Bilbao. Estuar. Coast. Shelf Sci. 150, 312–324.

Uriarte, I., Villate, F., Iriarte, A., 2016. Zooplankton recolonization of the inner estuary of Bilbao: influence of pollution abatement, climate and non-indigenous species. J. Plankton Res. 38, 718–731.

Usov, N., Kutcheva, I., Primakov, I., Martynova, D., 2013. Every species is good in its season: Do the shifts in the annual temperature dynamics affect the phenology of the zooplankton species in the White Sea? Hydrobiologia 706, 11–33. http://dx.doi.org/10.1007/s10750-012-1435-z.

Verity, P.G., Borkman, D.G., 2010. A decade of change in the Skidaway river estuary. III. Plankton. Estuar. Coasts 33, 513–540. http://dx.doi.org/10.1007/s12237-009-9208-2.

Vieira, L.R., Guilhermino, L., Morgado, F., 2015. Zooplankton structure and dynamics in two estuaries from the Atlantic coast in relation to multi-stressors exposure. Estuar. Coast. Shelf Sci. 167, 347–367. http://dx.doi.org/10.1016/j.ecss.2015.10.012.

Villate, F., 1989-1990. Zooplanktoni buruzko aurreikerketa koantitatiboa Gernika-Mundakako itsasadarrean (A preliminary quantitative study of the zooplankton in the estuary of Gernika-Mundaka). Munibe 41, 3–30 (in Basque with English abstract).

Villate, F., 1991. Zooplankton assemblages in the shallow tidal estuary of Mundaka (Bay of Biscay). Cah. Biol. Mar. 32, 105–119.

Villate, F., Orive, E., 1981. Abundancia y distribución de las poblaciones de Cladoceros en el estuario de Plencia. Investig. Pesq. 45, 327–336.

Villate, F., Aravena, G., Iriarte, A., Uriarte, I., 2008. Axial variability in the relationship of chlorophyll *a* with climatic factors and the North Atlantic Oscillation in a Basque coast estuary, Bay of Biscay (1997–2006). J. Plankton Res. 30, 1041–1049. http://dx.doi.org/10.1093/plankt/fbn056.

Villate, F., Iriarte, A., Uriarte, I., Intxausti, L., de la Sota, A., 2013. Dissolved oxygen in the rehabilitation phase of an estuary: Influence of sewage pollution abatement and hydro-climatic factors. Mar. Pollut. Bull. 70, 234–246.

Whitfield, A.K., 2005. Fishes and freshwater in southern African estuaries – a review. Aquat. Living Resour. 18, 275–289. http://dx.doi.org/10.1051/alr:2005032.