



Intraregional variability of exotic and native zooplankton in Basque coast estuaries (inner Bay of Biscay): effect of secondary dispersion, estuary features and regional environmental gradients

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Abstract The zooplankton of five estuaries of the Basque coast (southeast Bay of Biscay) was surveyed in summer 2019 to check the intraregional spread of exotic species and analyse the cross-estuary differences in brackish and neritic communities related to the arrival of exotic species and the summertime geographic gradient of water temperature. Results revealed that all the studied estuaries have been colonized by *Oithona davisae* but not by *Acartia tonsa* and *Pseudodiaptomus marinus*. Environmental constraints due to estuarine hydrological features partly

explained such differences, and the role of secondary spread opportunities remained less clear. Cross-estuary differences in the brackish community mainly reflected changes in the dominance of the native species *Acartia bifilosa* relative to different exotic copepod species. The neritic holoplankton differed in the contribution of thermophilic species in agreement with the thermal west–east increase of surface water temperature in the inner Bay of Biscay in summer. The detection of large numbers of larvae of the exotic crab *Rhithropanopeus harrisi* only in one of the estuaries highlighted the role of exotic benthic species in enhancing intraregional differences in estuarine meroplankton. Differences in the dominance of neritic or brackish species as a function of salinity and distance from coastline were also explored.

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Introduction

Estuaries are complex ecosystems where freshwater, marine and terrestrial environments meet, showing strong intra- and inter-system environmental variability, mainly due to their differences in basin size, morphology, the relative magnitude of freshwater inflow and tidal exchange and dominance of the different water mixing forces (McLusky and Elliot, 2004). These morphological and hydrological differences

together with the geographic location, which may entail climate differences, account for the strong variability between estuaries in the productivity and structure of biotic communities (Kench, 1999).

The abiotic and biotic differences and their drivers can be different at different spatial and temporal scales. For a proper coverage of spatial scales, ideally, the cross-regional, the cross-estuary and within estuary scales should be considered. For some biological communities, such as those of fish, there is ample literature describing differences at those three spatial scales, but usually addressing differences at a single scale in each work. However, studies that cover the three spatial scales are much less abundant (Valesini et al., 2014). In this large body of studies with fish, for example, it is generally perceived that broader-scale regional differences act as the primary influence on the fish assemblages (i.e. given the potential for shifts in biogeography and/or climate) and that, within regions, estuary-scale differences such as estuarine morphology and bar type exert a major influence (Valesini et al., 2014). In other estuarine biological communities, such as those of zooplankton, we have much less information, particularly at cross-estuary and cross-regional scales.

Additionally, the human impacts, such as physical alteration, nutrient and organic matter enrichment, toxin discharges, overfishing and the introduction of exotic species (Day et al., 1989), also differ greatly between estuaries and, consequently, affect in different ways and magnitudes the original productivity, biodiversity and integrity of biological communities (Vasconcelos et al., 2007). In this context, estuaries have also the distinctive feature of being highly sensitive to the establishment of exotic species, which may cause measurable changes in the estuarine biota and services. These changes can result in damaging, beneficial or neutral impacts to local species and communities (Davis et al., 2011). The risk of introduction and settlement of exotic species in estuaries is enhanced by several factors. Their suitability, as sheltered coastal areas, for the development of port facilities for commercial shipping is one of the key factors (Nehring, 2006). Indeed, the main way of dispersion for planktonic invaders involves shipping, particularly through ballast water exchanges (Geburzi and McCarthy, 2018; Dexter et al., 2020). Ports promote transport of biota within and across regions (and oceans) and shipping may also enhance secondary spreads

of exotic biota. Similarly, an important factor is the ability of brackish species to tolerate strong environmental variations (Gollasch et al., 2000). The ability for introducing and settling in a new area is perceived to be particularly high in species with a broad environmental tolerance to both salinity (euryhaline) and temperature (eurythermal). Thus, euryhaline species or estuary dependent species may successfully settle at locations outside their native range if they are transported to estuaries with a significant variability in salinity (González-Ortegón and Moreno-Andres, 2021). Ecological niches in brackish waters of estuaries are naturally unsaturated and this is also a crucial factor favouring brackish exotic species settlements in estuaries (Nehring, 2006). Estuarine pollution level is also important, as it is a threat to the native populations and may allow a favourable selection of the non-native species in the host estuaries/sites (Gao et al., 2019). A good knowledge of the factors that can affect the colonization success of exotic species is crucial for the implementation of management plans by regional and intergovernmental decision-makers involved in the conservation and recovery of coastal ecosystems' integrity, function and services.

Zooplankton are pivotal in the functioning of aquatic systems. In estuarine environments, the distribution of zooplankton populations exhibits structured patterns determined by hydrological connectivity (Dexter et al., 2020). Some species disperse over long distances through stress-resistant eggs transported by floodwaters, birds, or air currents (Frisch et al., 2007), but anthropogenic activities, specifically the discharge of ballast water from commercial shipping vessels (Bradie et al., 2023) greatly facilitate the long-range transport. Furthermore, following their initial establishment, newly arrived estuarine species have the potential to undergo secondary spread within the estuary and to other nearby systems. However, the mechanisms and patterns of this secondary spread are not well understood, due to limited monitoring of zooplankton in estuaries (Dexter et al., 2020).

Regarding, the factors driving cross-estuary differences in zooplankton communities, Dexter et al. (2020) suggested that geographic distribution of exotic species is strongly constrained by geomorphic characteristics that define the salinity and mixing regimes of estuaries, reflecting the strong role that physical forces play in structuring estuarine zooplankton. However, comparisons of exotic zooplankton

across estuaries are really scarce in the literature. This insufficient knowledge hinders our understanding of invasion processes at regional scales. This is mostly because regular monitoring of zooplankton is carried out at only a small number of estuaries, making it difficult to understand the zooplankton community processes at cross-regional and cross-estuary scales. In European estuaries this may partly be due to the lack of encouragement by the European Union to monitor zooplankton as an indicator of environmental change in estuaries, since unlike the European Marine Strategy Framework Directive (EU-MSFD) (Ndah et al., 2022), the European Water Framework Directive (EU-WFD), which is applicable to coastal and estuarine areas, does not take into account the zooplankton.

We have been studying the zooplankton communities of the estuaries of Bilbao and Urdaibai (southeastern Bay of Biscay) since the 1980s, taking into account the environmental drivers that could affect the structures of the communities. From 2002 to 2003 onwards, in the estuary of Bilbao, which is in a rehabilitation process from highly polluted conditions in the past, exotic copepod species as *Acartia* (*Acanthacartia*) *tonsa* Dana, 1849, *Oithona davisae* Ferrari F.D. and Orsi, 1984 and *Pseudodiaptomus marinus* Sato, 1913, as well as copepods previously found in other estuaries of the Basque coast, i.e. the brackish species *Acartia* (*Acanthacartia*) *bifilosa* (Giesbrecht, 1881) and *Calanipeda aquaedulcis* Krichagin, 1873, arrived progressively. Some of these newcomers became dominant in brackish habitats and have had a marked effect on the abundance, structure and diversity of the zooplankton (Barroeta et al., 2020; 2022a). The exotic copepod species found in the estuary of Bilbao have also been observed in the estuary of Urdaibai; however, they have shown differences in colonization success, abundance and ecological niche (Barroeta et al., 2020, 2022b), suggesting that the morphological and hydrological features of estuaries, as well as their physical modification and pollution and rehabilitation processes are key factors in the colonization success of the newcomers. However, there is no information available on whether these exotic species have also colonized other estuaries along the Basque coast region.

Therefore, the main aim of the present study was to check whether the exotic copepod species identified in the estuaries of Bilbao and Urdaibai have also

colonized other estuaries of the Basque coast and to identify the environmental constraints that may have hindered a successful colonization in those estuaries from which they are absent. A secondary aim was to determine the differences in the native zooplankton, in both the brackish and the neritic communities, in relation to the inherent features of the estuaries and their location in the intraregional environmental gradient. Overall, this work can contribute to a better understanding of intraregional variability in estuarine zooplankton.

Materials and methods

Study area

The five systems we surveyed were, from west to east, the estuaries of Bilbao (43° 23' N, 03° 07' W), Plentzia (43° 25' N, 2° 57' W), Urdaibai (43° 22' N, 02° 43' W), Oria (43° 17' N, 2° 07' W) and Bidasoa (43° 23' N, 1° 46' W). They are close to each other, located within 176 km of the Basque coast, under the influence of waters from the southeastern Bay of Biscay (Fig. 1), and share the temperate-oceanic climate characterized by moderate winters and warm summers. They are all relatively short and shallow meso-macrotidal estuaries, showing semidiurnal tides. Nevertheless, differences in morphological and hydrodynamical features, as well as in water quality and anthropogenic impact are noticeable (Table 1).

The estuary of Bilbao is the largest and deepest one (from 0.5 m at the inner part to 32 m at the outer coastline site), and it also receives the highest freshwater inflows from the two main tributaries (Ibaizabal and Nerbioi) at the head of the estuary and from other smaller ones along the estuary. Its tidal flushing is relatively low (Valencia et al., 2004), the water column varies from partially mixed in the outer part to strongly stratified in the inner estuary and has a two-layered circulation, showing much lower residence time in above halocline layers of the channelized inner and middle reaches than in the below halocline layer of the outer zone (Abra harbour) (Uriarte et al., 2014). This is a result of its extensive morphological change due to land claiming on intertidal areas for urban and industrial developments and channelization and dredging to favour navigation. In the mid-twentieth century, the estuary of Bilbao was one of the most

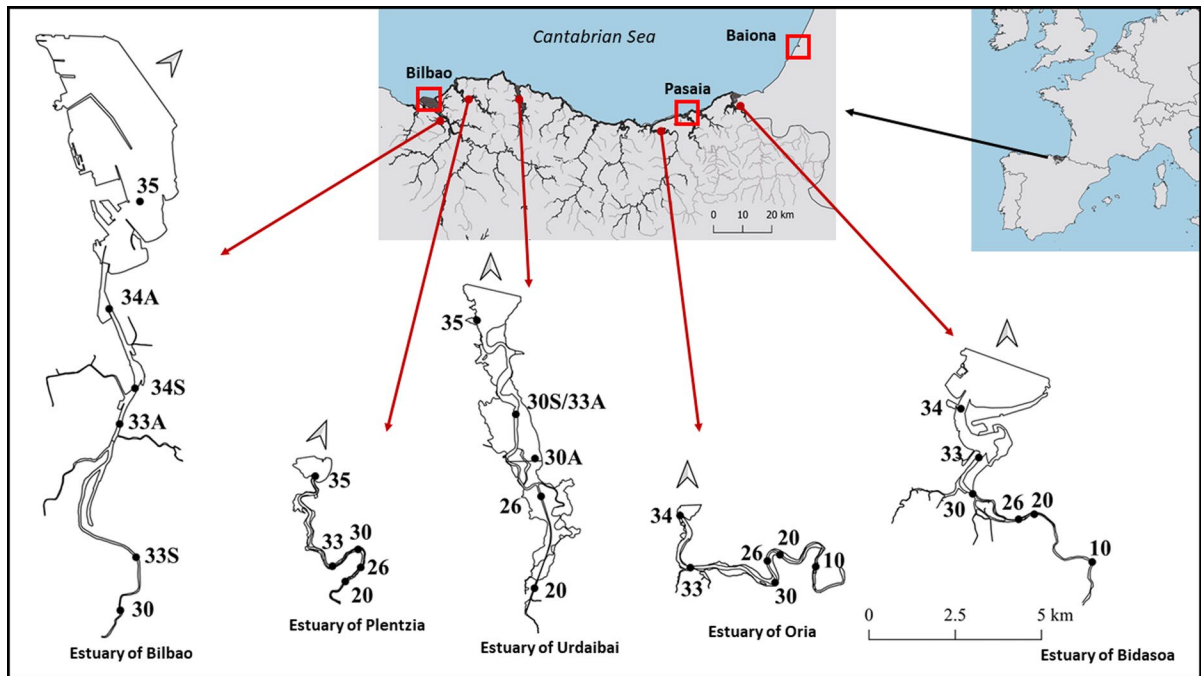


Fig. 1 Maps of the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa showing the salinity sites (35, 34, 33, 30, 26, 20 and 10) in the surveys of August (A) and September (S),

as well as their location in the Basque coast. The coastline is drawn. The locations (red squares) of the main commercial ports are also shown

Table 1 Main morphological and hydrological characteristics of the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa

Estuary	Length (Km)	² Area (Km ²)	⁴ Basin area (Km ²)	⁴ Mean volume (V) ($\times 10^6$ m ³)	¹ Mean river flow (m ³ /s)	² Average depth (m)	² Mean tidal prism (Ω) (m ³)	² Ω/V	⁴ Water column mixing
Bilbao	22.30	³ 1.50	1798.77	402.10	35.56	⁴ 9.50	⁴ 77,000,000	1.09	Highly stratified
Plentzia	9.21	0.38	172.22	2.20	4.73	3.07	833,500	1.20	Partially mixed
Urdaibai	13.70	1.89	183.21	12.87	3.60	2.59	4,858,300	1.47	Partially mixed
Oria	11.51	0.85	881.99	3.13	25.66	3.62	2,039,800	0.97	Partially mixed
Bidasoa	12.83	2.30	700.00	45.80	27.19	4.33	5,941,900	0.84	Highly stratified

Information from ¹Monge-Ganuzas et al. (2019), ²Villate et al. (1989), ³Cearreta et al. (2014), ⁴Borja et al. (2004)

polluted estuaries in Europe due to the industries located on its banks (Cearreta et al., 2000), but since 1980 it has undergone a rehabilitation process, with marked improvements in the water quality (Borja et al., 2006; Fdez-Ortiz de Vallejuelo et al., 2010; Villate et al., 2013). Presently the port facilities located in the outer part of the estuary (Abra harbour) are one of the most important marine transport and logistics centres in the European Atlantic Arc, receiving cargo

ships, and consequently ballast water, from worldwide (Bilbao Port, <https://www.bilbaoport.eus/en/the-port/>).

The estuary of Plentzia with a meandering shape is the smallest one, and it ends in a small, semi-enclosed embayment. The main tributary is the Butroi river, which shows perceptible pollution due to waste effluents from some industries settled in its basin (Orbea et al., 2002). During the first half of the twentieth

century, the estuary was in part channelized and artificial dykes were built for urban occupation and agriculture, but during the second half of the century natural regeneration of the impacted intertidal flats and marshes has been observed, as a consequence of the decline in agricultural activity (Cearreta et al., 2002) and currently, around 80% of the estuarine surface area is exposed at low tide.

The estuary of Urdaibai is the shallowest on average and also receives the lowest river flow from two small streams (Oka and Golako) at the head and another one (Mape) in the middle reaches. The system shows high tidal flushing and seawater dominance at high tide, the outer part of the estuary being well mixed and the inner part partially stratified (Villate et al., 2017). It is the central axis of the Urdaibai Biosphere Reserve, with extensive intertidal flats and sandy beaches in the outer reaches and salt marshes and reed beds in the middle and inner reaches. An artificial channel joins the main tributaries at the head of the estuary with the natural channel in the mid estuary, and a not very effective small wastewater treatment plant located in the upper artificial channel and still functional during the study period was the cause of occasional nutrient and chemical pollution (Solaun et al., 2018).

The also meandering estuary of Oria has a lower extension of intertidal and supratidal areas than the former two ones due in part to channelization and land claim. The Oria is the main river, but other small tributaries also flow into it. Once a highly polluted system, nowadays the water quality in the whole basin has improved considerably (Provincial Council of Gipuzkoa, 2020), due to the sanitation works that have been carried out, which included building a wastewater treatment plant. The middle-inner part is surrounded mainly by cattle raising and agricultural land, while the outer zone shows greater channelization and urbanization.

The estuary of Bidasoa takes the same name as the main river flowing into it. Though it has been channelized and modified in the inner-middle and the outer parts, it maintains its original isles, channels, and salt marshes in the middle-inner part and the bay-shaped basin (Txingudi Bay) of the outer reaches. The system shows a good ecological, chemical and global status (Solaun et al., 2018) and the best water quality of all estuaries of the western part of the Cantabrian Sea (Provincial Council of Gipuzkoa, 2020).

In all these estuaries there are small recreational ports (EKP, <https://www.ekpsa.eus/en/nuestros-puertos/>), and in the estuaries of Bilbao, Oria and Bidasoa there are also ports with commercial fisheries activity (Gobierno Vasco, https://www.euskadi.eus/web01a2arraku/es/contenidos/informacion/cofradias_pesca_dores_euskadi/es_dapa/index.shtml) but a commercial port with national and international ship traffic can only be found in Bilbao.

Data collection

For the purposes of the present study, daily samplings were carried out in the estuaries of Bilbao (on August 22 and September 23), Urdaibai (on August 23 and September 24), Oria (on September 5), Bidasoa (on September 6) and Plentzia (on September 12). The choice of sampling in late August–September was based on previous information obtained from the zooplankton time series in the estuaries of Bilbao and Urdaibai that points out that this is the time of the year most likely to find the three exotic copepod species *A. tonsa*, *O. davisae* and *P. marinus* in high abundance in estuaries of the Basque coast (Barroeta et al., 2020).

Because of the different extent of water masses of different salinity at each estuary, sampling sites within each estuary (Fig. 1) were the ones with water masses below the halocline of salinity around 35, 34, 33 and 30 in the estuary of Bilbao both in August and September, 35, 33, 30 and 26 in the estuary of Urdaibai on September, 35, 33, 30, 26 and 20 in the estuary of Plentzia and in the estuary of Urdaibai in August, and 34, 33, 30, 26, 20 and 10 in the estuaries of Oria and Bidasoa. In this work, we will refer to the sampling site as salinity site. All the samplings were conducted at high tide during neap tides. At each site, vertical profiles of salinity (Sal), water temperature (WT), and percentage saturation of dissolved oxygen (DOS) were obtained every 0.5 m using a multi-parameter water quality meter (YSI ProDSS). The Secchi disc depth (SDD) was also measured. Additionally, the average river flow ($\text{m}^3 \text{s}^{-1}$) (Rfl) during the 15 days prior to sampling was obtained from the Provincial Councils of Bizkaia (estuaries of Bilbao, Plentzia and Urdaibai) and Gipuzkoa (estuaries of Oria and Bidasoa).

Zooplankton samples were also collected below the halocline by 2–3 min horizontal tows using a

200 µm mesh size net equipped with a mechanical flow meter (Hydro-Bios). Zooplankton samples were preserved in 4% buffered formaldehyde. The qualitative and quantitative analyses of zooplankton samples were performed by diluting the sample to a known volume (10–1000 ml), to obtain a suitable density of individuals, and by extracting enough aliquots for the identification and counting of individuals until at least 100 individuals of the most abundant taxon and 30 individuals of the second and third most abundant taxa could be counted under an inverted stereomicroscope (Olympus IX70). Identification was made to the lowest possible taxonomic level, which was the species level for most copepods and main holoplankton groups, and a routine coarse category for most meroplankton groups, but distinguishing the easily recognizable zoea larvae of the crab *Rhithropanopeus harrisi* (Gould, 1841).

Data treatment

Both longitudinal and vertical distributions of salinity, percentage of dissolved oxygen and water temperature in each estuary were plotted using Surfer® 10 (Golden Software, LLC). For every estuary, at each sampling site, the salinity stratification index (Str; calculated as the maximum difference in salinity between consecutive depths (Villate et al., 2013), was plotted against the relative distance (RD; calculated as the percentage of the total length of the estuary, being 0 m the outermost point of the estuary i.e. the coastline).

For the statistical analysis of communities only the taxonomic categories showing a relative abundance > 0.1% were considered. However, related species showing a relative abundance < 0.1% were grouped in higher taxonomic categories in order to be included in the analyses. Thus, the cladocerans *Evadne spinifera* P.E. Müller, 1867, *Pseudevadne tergestina* Claus, 1877 and *Podon* sp. Lilljeborg, 1853 were grouped in the category of Podonidae, *E. spinifera* and *P. tergestina* being the most and second most abundant species, respectively. In addition, the adults of the copepod species *Paracalanus parvus* (Claus, 1863) and undistinguished juvenile stages that may also belong to the genus *Clausocalanus* Giesbrecht, 1888, *Pseudocalanus* Boeck, 1872 and *Ctenocalanus* Giesbrecht, 1888 were grouped into the PCPC-calanus category, although almost all of them can be

attributed to *P. parvus*. Moreover, the different taxa were classified into the categories of brackish exotic holoplankton (BEH), brackish native holoplankton (BNH), neritic holoplankton (NH), meroplankton (M) and thychoplankton (T).

In order to identify the main patterns of zooplankton variability between and within estuaries, the main taxa responsible for such patterns of variability and their relationships with the environmental variables, two canonical redundancy analyses (RDA) were performed by means of the Canoco 5 software with the data from all five estuaries jointly. In the first RDA, the environmental variables (WT, Sal, DOS, Str, SDD, depth, RD and Rfl) were added as explanatory variables and estuaries as supplementary variables. In the second RDA, the same procedure was carried out, but the salinity site was assigned as a categorical covariable, thereby removing the effect of salinity to look for other patterns of differences between estuaries. Both RDAs were performed by Monte Carlo permutation tests with 9999 iterations to assess the significance of the relationship between the environmental variables and the community composition. The resulting p-values were used to determine whether the observed patterns were statistically significant (p-value < 0.05) or occurred due to random chance. By employing the Monte Carlo test in our RDAs, we ensured robust statistical inference and increased the reliability of our findings.

Results

Environmental scenario

The estuary of Bilbao showed the highest vertical variations of salinity, since euhaline (salinity > 30) water masses reached the upper estuary below a thin layer of lower salinity water that expanded until the mid-zone (Fig. 2). In the estuaries of Oria and Bidasoa, the strongest vertical and longitudinal gradients of salinity were located in the middle sections of the estuaries, while in the estuaries of Plentzia and Urdaibai they were located nearer the outer end of the estuaries. The percentage of dissolved oxygen was high in most water masses across systems (Fig. 2). Overall, the values showed normoxia (80–110%) or mild hyperoxia (110–130%), except in the inner sections of the estuaries of Urdaibai and Bilbao. In these

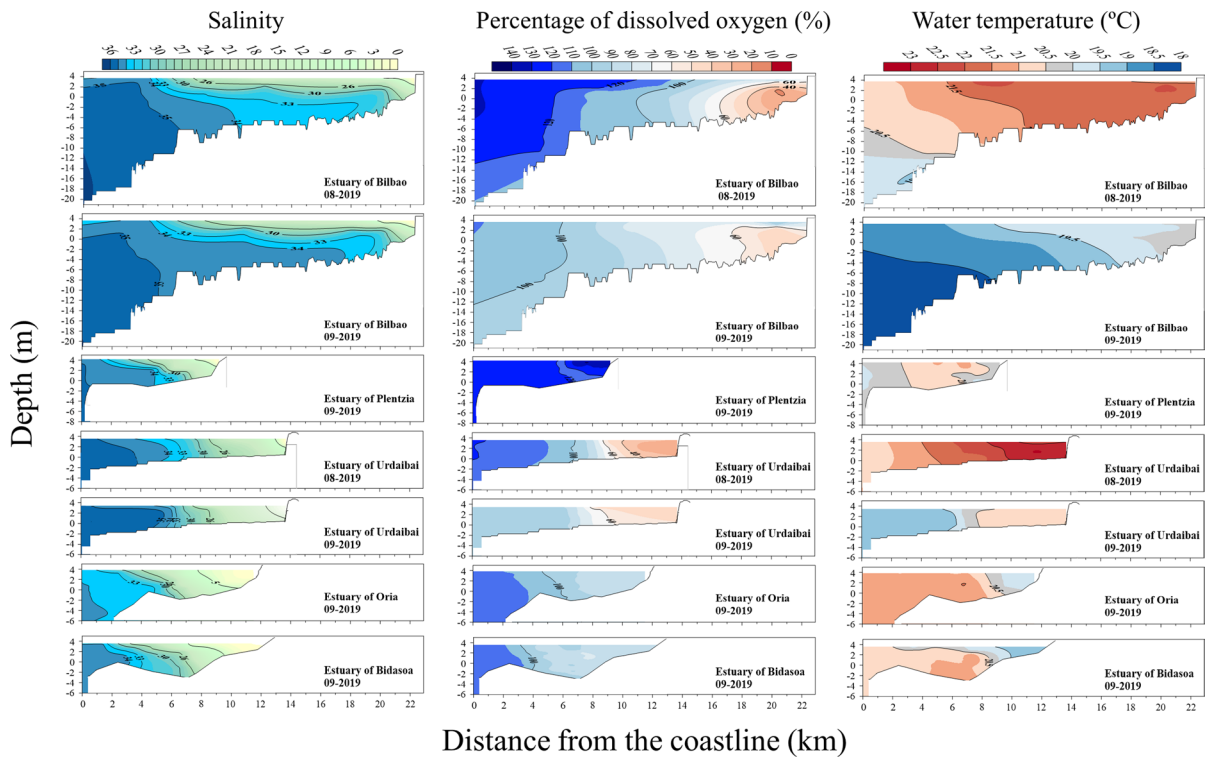


Fig. 2 Longitudinal and vertical distribution of salinity, percentage of dissolved oxygen (%) and water temperature (°C) in the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa in the surveys carried out during August and September 2019

latter systems, mild hypoxia (40–60%) and/or hypoxia conditions (15–40%) were registered, according to the dissolved oxygen criteria for water (Saroglia et al., 2010 and Hale et al., 2016 combined criteria). Water temperature (Fig. 2) ranged from 18 to 19 °C in the outer sections of the estuaries of Bilbao and Urdaibai during the late September surveys, to 22–24 °C in the inner estuary of Urdaibai during the late August survey. These temperature values exhibited different spatial patterns depending on the estuary and the sampling date. Overall, the longitudinal gradients were highest in the estuary of Urdaibai during both months and in the estuary of Bilbao in September, with a temperature increase from the outer to the inner reaches. In contrast, temperature increased from the inner to the outer reaches in the estuaries of Oria and Bidasoa. The strongest vertical gradients of temperature were found in the latter estuary, with decreasing values from surface to bottom in the middle part. The highest salinity stratification index was found in the estuary of Bilbao, whereas the lowest salinity stratification index was found in the estuary of Oria (Fig. 3).

In the estuary of Bilbao, the highest salinity stratification index was found in the inner part of the estuary, in the estuaries of Bidasoa and Oria in the middle part of the estuary and in the estuaries of Plentzia and Urdaibai in the outer part of the estuaries.

Exotic and native brackish copepod species

As shown in Table 2, *A. tonsa* was observed in high densities in the estuaries of Bilbao, Plentzia and Urdaibai, with mean values ranging from 628 to 5575 ind. m⁻³ and a maximum of > 20,000 ind. m⁻³ in the survey of August in the estuary of Urdaibai. However, *A. tonsa* was not found in the estuaries of Oria and Bidasoa. In contrast, *O. davisae*, was found in all estuaries, but it was significantly more abundant in the estuaries of Bilbao and Bidasoa (maxima > 600 ind. m⁻³) than in the others. The highest difference was observed when compared to the value measured in Urdaibai in September (with the highest density being < 1 ind. m⁻³). *P. marinus* was only observed in the estuary of Bilbao at

Fig. 3 Variation of the salinity stratification index in relation to the relative distance (in %) from the coastline of the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa during the surveys carried out in August and September 2019

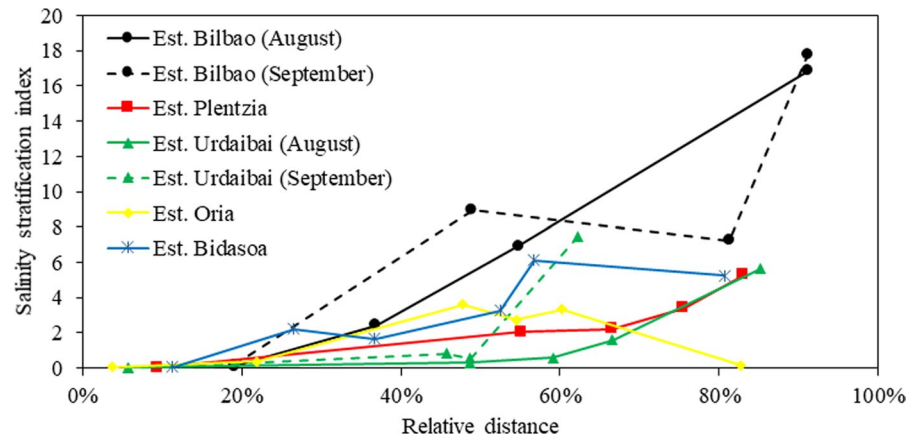


Table 2 Mean, minimum and maximum density (ind. m^{-3}) of the brackish native and exotic copepod species in the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa in the surveys carried out during August and September 2019

Estuary	Date	Metric	<i>Acartia tonsa</i>	<i>Oithona davisae</i>	<i>Pseudodiaptomus marinus</i>	<i>Acartia bifilosa</i>	<i>Calanipeda aquaedulcis</i>
Bilbao	22/08/2019	Mean	1579.3	243.6	41.0	1.7	0.9
		Minimum	0.0	0.0	0.0	0.0	0.0
		Maximum	3818.6	634.1	110.2	6.8	3.4
Bilbao	24/09/2019	Mean	1740.5	548.6	36.3	0.0	0.0
		Minimum	22.7	39.8	11.4	0.0	0.0
		Maximum	2571.9	1414.0	67.5	0.0	0.0
Plentzia	12/09/2019	Mean	1389.6	9.4	0.0	181.4	2.7
		Minimum	0.0	0.0	0.0	0.0	0.0
		Maximum	3996.3	40.8	0.0	675.9	13.6
Urdaibai	23/08/2019	Mean	5575.2	3.8	0.0	282.1	0.0
		Minimum	0.0	0.0	0.0	0.0	0.0
		Maximum	22,168.0	18.9	0.0	1307.3	0.0
Urdaibai	23/09/2019	Mean	627.5	0.2	0.0	87.7	0.0
		Minimum	5.6	0.0	0.0	0.0	0.0
		Maximum	2460.2	0.7	0.0	276.7	0.0
Oria	05/09/2019	Mean	0.0	24.0	0.0	751.2	0.4
		Minimum	0.0	0.0	0.0	0.0	0.0
		Maximum	0.0	96.0	0.0	2645.2	2.6
Bidasoa	06/09/2019	Mean	0.0	582.9	0.0	874.1	0.0
		Minimum	0.0	0.0	0.0	0.0	0.0
		Maximum	0.0	1299.1	0.0	1808.4	0.0

low densities, with a maximum of <110 ind. m^{-3} . Regarding native species, *A. bifilosa* appeared in all the estuaries, but with much lower densities in the estuary of Bilbao (maximum value of <10 ind. m^{-3}) than in the others. The highest densities were

observed in the estuaries of Oria and Bidasoa, with maxima of 2645 and 1808 ind. m^{-3} , respectively. Finally, *C. aquaedulcis* was only recorded in the estuaries of Bilbao, Plentzia and Oria in very low densities (maxima <14 ind. m^{-3}).

Zooplankton communities

As shown in Table 3, the brackish species *A. tonsa* in the estuaries of Bilbao, Plentzia and Urdaibai, and *A. bifilosa* in the estuaries of Oria and Bidasoa, reached the highest densities among all the zooplankton taxa considered, while Cirripedia larvae were the second most abundant taxon in the zooplankton of all estuaries. However, the dominance of *A. tonsa* was higher in the estuaries of Plentzia and Urdaibai (70 and 75%, respectively) than in the estuary of Bilbao (45%), and the dominance of *A. bifilosa* was higher in the estuary of Oria (47%) than in that of Bidasoa (24%). This decrease in the contribution of the dominant brackish species to the total zooplankton was mainly due to the high contribution (> 10%) of taxa such as Cirripedia larvae and *O. davisae* in the estuaries of Bidasoa and Bilbao, and also of the PCPC-calanus species group in the former estuary. PCPC-calanus was the most abundant taxon of the neritic assemblage in all estuaries, except in the estuary of Bilbao, where the appendicularian *Oikopleura* spp. Mertens, 1830 showed slightly higher density. Among neritic taxa, following PCPC-calanus and *Oikopleura* spp., copepod species such as *Acartia (Acartiura) clausi* Giesbrecht, 1889 and *Oithona nana* Giesbrecht, 1893, along with chaetognaths, were also abundant in all estuaries. However, cladocerans such as Podonidae (mainly *E. spinifera*) and *Penilia avirostris* Dana, 1849 were clearly more abundant in the estuaries of Oria and Bidasoa than in the other estuaries. Regarding meroplankton taxa, after Cirripedia larvae, the most abundant were Gastropoda larvae in the estuaries of Bilbao, Urdaibai and Bidasoa, Polychaeta larvae in the estuary of Plentzia and unidentified Brachyura larvae in the estuary of Oria. Additionally, in the estuary of Bidasoa the zoea larvae of the exotic brachyuran *R. harrisii* were found in large densities (142.2 and 59.1 ind. m⁻³ at the salinity sites of 20 and 10, respectively). As for tycho plankton forms, in general, density and contribution to total zooplankton were much lower in the estuary of Bilbao than in the other ones.

Spatial segregation of brackish and neritic assemblages

The distribution of the most abundant brackish and neritic species in relation to salinity, as well as to the relative position along the estuary length from the

coastline (Fig. 4) showed between-estuary differences in the position along the longitudinal salinity gradient at which brackish taxa replaced neritic taxa as dominant group in the zooplankton community. The replacement occurred at the highest salinity, between 34 and 35, in the estuary of Bilbao, since *A. tonsa* remained clearly dominant at the salinity of 34, while it occurred between 34 and 33 in the estuary of Plentzia and between 33 and 30 in the estuaries of Oria and Bidasoa. In the estuary of Urdaibai, the replacement was found at a salinity higher than 33 in August, but at a salinity lower than 31 in September.

However, differences between estuaries were not so clear in the relative distance at which the replacement occurred, since it took place within the second quarter of the estuary length, between 30 and 50% of the total length from the coastline in all cases.

Intra- and inter-estuary variability of zooplankton and their relationship to environmental variables

The first RDA analysis (Monte Carlo test, pseudo $F=4.9$ $P=0.0001$) showed that the first component (Axis 1), explaining 60.9% of the total variability explained by the environmental factors considered, primarily differentiated brackish native species (*A. bifilosa*), exotic species (*A. tonsa*, *O. davisae*), and tycho plankton (such as prawn of Gnathiidae, benthic ostracods, and harpacticoids) from the bulk of neritic holoplankton. The former brackish taxa were associated with greater relative distance from the coastline, higher stratification, and water temperature. In contrast, the bulk of neritic holoplankton taxa exhibited associations with higher water transparency, salinity, percentage of dissolved oxygen, and water column depth (Figs. 5a and 6). Regarding meroplankton groups, their scores along the first axis reflected the inward skewed distribution of polychaete larvae and the outward skewed distribution of medusae and larvae of gastropods, caridean decapods and bivalves. The second component (Axis 2; 21.6% of the total variability explained by the environmental factors considered) differentiated the zooplankton more associated with the estuary of Bilbao, with higher stratification, depth and salinity, from the zooplankton associated with the other estuaries, especially from the estuaries of Oria and Bidasoa. The brackish exotic species *P. marinus*, *A. tonsa* and *O. davisae*, the larvae of Cirripedia, Bivalvia and caridean Decapoda and the appendicularians of the genus *Oikopleura* were

Table 3 List of the main zooplankton taxa identified in the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa in the surveys carried out during August and September 2019. Abbreviation of the taxa as used in the RDAs. Classification into the categories of brackish exotic holoplankton (BEH), brackish native holoplankton (BNH), neritic holoplankton (NH), meroplankton (M) and tychoplankton (T). Mean density (ind. m⁻³) and contribution to total zooplankton (%) of each taxa in every estuary are also shown

Zooplankton taxa	Abbreviation	Category	Bilbao		Plentzia		Urdaibai		Oria		Bidasoa	
			ind.m ⁻³	%	ind.m ⁻³	%	ind.m ⁻³	%	ind.m ⁻³	%	ind.m ⁻³	%
<i>Acartia tonsa</i>	Ato	BEH	1660	45.16	1390	69.64	3376	75.41	0	0.00	0	0.00
<i>Oithona davisae</i>	Oda	BEH	396	10.77	9	0.45	2	0.04	24	1.51	583	16.15
<i>Pseudodiaptomus marinus</i>	Pma	BEH	39	1.06	0	0.00	0	0.00	0	0.00	0	0.00
<i>Acartia biflora</i>	Abi	BNH	1	0.03	181	9.07	196	4.38	751	47.14	874	24.20
<i>Acartia discaudata</i>	Adi	BNH	0	0.00	0	0.00	0	0.00	0	0.00	40	1.11
<i>Paracartia grani</i>	Pgr	BNH	0	0.00	0	0.00	6	0.13	0	0.00	13	0.36
<i>Acartia clausi</i>	Acl	NH	33	0.90	10	0.50	55	1.23	22	1.38	79	2.19
PCPC-calanus	PPc	NH	185	5.03	54	2.71	236	5.27	121	7.60	500	13.85
<i>Centropages typicus</i>	Cty	NH	47	1.28	7	0.35	22	0.49	3	0.19	4	0.11
<i>Temora stylifera</i>	Tst	NH	0	0.00	0	0.00	5	0.11	2	0.13	4	0.11
<i>Oithona nana</i>	Ona	NH	43	1.17	39	1.95	22	0.49	10	0.63	28	0.78
<i>Oithona similis</i>	Osi	NH	4	0.11	2	0.10	5	0.11	2	0.13	0	0.00
<i>Oncaea media</i>	Ome	NH	45	1.22	0	0.00	8	0.18	2	0.13	6	0.17
<i>Ditrichocorycaeus anglicus</i>	Dan	NH	10	0.27	3	0.15	1	0.02	3	0.19	5	0.14
<i>Euterpina acutifrons</i>	Eac	NH	19	0.52	30	1.50	5	0.11	6	0.38	36	1.00
Podonidae	Pod	NH	4	0.11	0	0.00	2	0.04	16	1.00	12	0.33
<i>Penilia avirostris</i>	Pav	NH	0	0.00	0	0.00	2	0.04	1	0.06	66	1.83
<i>Muggiaea</i> sp.	Mug	NH	26	0.71	2	0.10	2	0.04	3	0.19	4	0.11
<i>Chaetognatha</i>	Cha	NH	49	1.33	9	0.45	37	0.83	10	0.63	56	1.55
<i>Oikopleura</i> sp.	Oik	NH	273	7.43	52	2.61	18	0.40	120	7.53	190	5.26
<i>Fritillaria</i> sp.	Fri	NH	1	0.03	0	0.00	0	0.00	8	0.50	0	0.00
Medusae	Med	M	7	0.19	2	0.10	10	0.22	5	0.31	12	0.33
Gastropoda larvae	Gas	M	34	0.92	9	0.45	23	0.51	21	1.32	147	4.07
Bivalvia larvae	Biv	M	31	0.84	6	0.30	1	0.02	3	0.19	9	0.25
Polychaeta larvae	Pol	M	5	0.14	18	0.90	17	0.38	9	0.56	8	0.22
Cirripedia larvae	Cir	M	715	19.45	153	7.67	390	8.71	371	23.29	864	23.93
<i>Rhithropanopeus harrisi</i> larvae	Rha	M	0	0.00	0	0.00	0	0.00	0	0.00	34	0.94
Brachyura decapod larvae (others)	Bra	M	25	0.68	2	0.10	15	0.34	31	1.95	16	0.44
Caridea decapod larvae	Car	M	17	0.46	0	0.00	3	0.07	1	0.06	6	0.17

Table 3 (continued)

Zooplankton taxa	Abbreviation	Category	Bilbao		Plentzia		Urdaibai		Oría		Bidasoa	
			ind.m ⁻³	%	ind.m ⁻³	%	ind.m ⁻³	%	ind.m ⁻³	%	ind.m ⁻³	%
Ascidia larvae	Asc	M	5	0.14	1	0.05	2	0.04	0	0.00	3	0.08
Praniza of Gnathiidae	Pra	T	1	0.03	15	0.75	8	0.18	19	1.19	10	0.28
Ostracoda	Ost	T	0	0.00	0	0.00	3	0.07	29	1.82	0	0.00
Benthic harpacticoids	Bha	T	1	0.03	0	0.10	5	0.11	0	0.00	2	0.06

the taxa more strongly associated with the estuary of Bilbao, in opposition to the brackish native species *A. biflosa*, the thychoplankton (praniza of Gnathiidae and benthic ostracods), the larvae of Polychaeta, the larvae of the exotic crab *R. harrisi* and the neritic cladoceran *P. avirostris*, which characterized the communities of the other estuaries.

In the second RDA analysis (Monte Carlo test, pseudo F=2.8 P=0.0001), where the effect of the salinity gradient within estuaries was removed by using salinity site as a covariate (Figs. 5b and 7), the first component (Axis 1; 55.2% of the total variability explained by the environmental factors considered) showed an opposition between the assemblage of the brackish exotic species *A. tonsa*, *P. marinus* and *O. davisae* and the broad assemblage of neritic holoplankton taxa, within which the Podonidae (mainly *E. spinifera*) and *P. avirostris* cladocerans showed the highest opposition. This pattern of zooplankton variation was still linked to distance from the coastline, the stratification index, water transparency and dissolved oxygen but it illustrated between-estuary differences associated with a geographic pattern of variation from the estuary of Bilbao to the estuaries of Oría and Bidasoa rather than a pattern of variation along the longitudinal salinity gradient within estuaries (Figs. 1, 5b and 7). The second component (Axis 2; 22.0% of the total variability explained) showed the opposition mainly of the brackish exotic species *O. davisae* and *P. marinus*, the neritic appendicularians *Oikopleura* sp., and to a less extent also the larvae of bivalve and caridean decapod, the larvae of the exotic crab *R. harrisi* and *Acartia (Acartiura) discaudata* (Giesbrecht, 1881), all of them plotted at the positive end of the axis, from the tycho planktonic taxa and the larvae of polychaete, plotted in the negative side. In this case, the taxa plotted at the positive end of the axis characterized the estuaries of Bilbao and Bidasoa and those plotted at the negative end characterized the estuaries of Urdaibai and Plentzia, and they were mainly linked to high and low values of salinity stratification, river flow and depth, respectively.

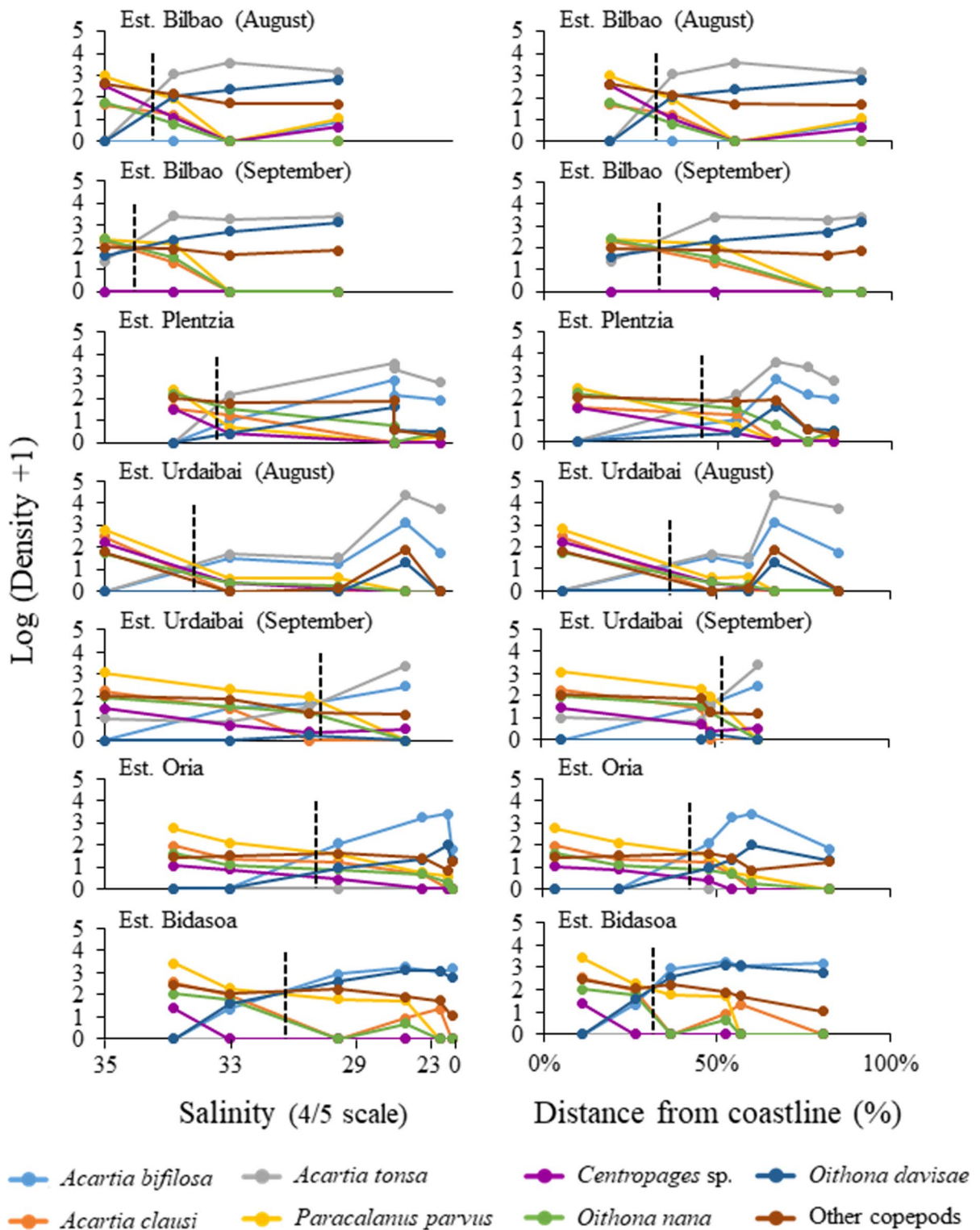


Fig. 4 Distribution of the brackish and neritic species of copepods in relation to salinity and relative distance (%) from the coastline in the estuaries of Bilbao, Plentzia, Urdaibai, Oria

and Bidasoa in the surveys carried out during August and September 2019. Vertical dotted line indicates the replacement of neritic species by brackish ones

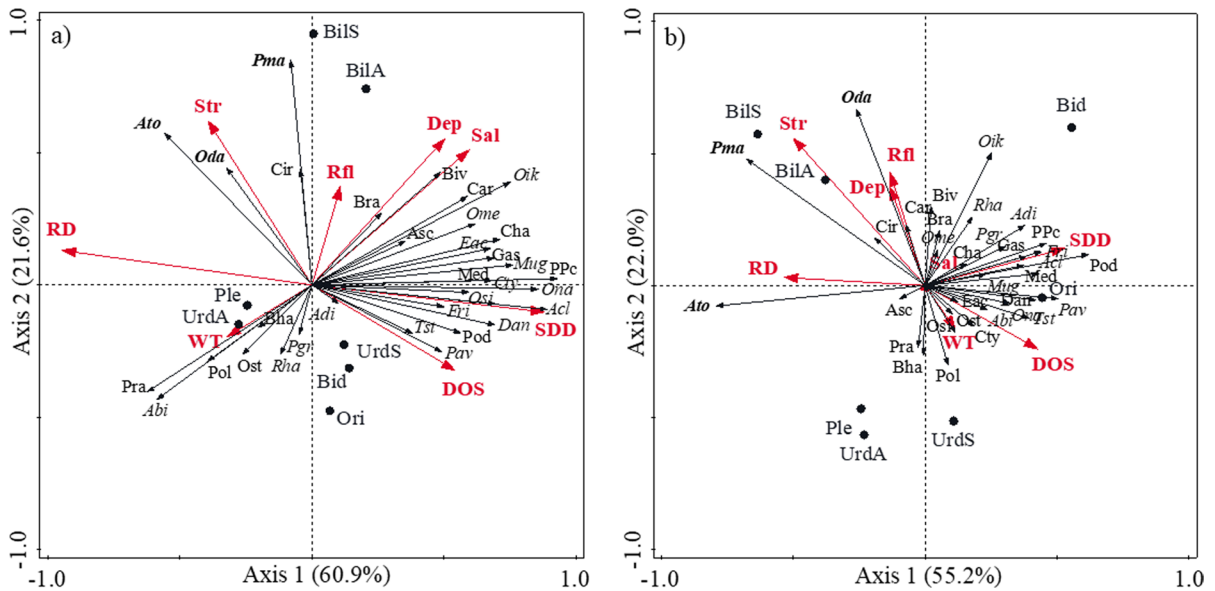


Fig. 5 **a** RDA triplot of zooplankton taxa, explanatory variables and estuaries as supplementary material and **b** RDA triplot of zooplankton taxa, explanatory variables and estuaries as supplementary material using salinity site as covariable. Taxa abbreviations as in Table 3. (*Sal* salinity, *WT* water temperature, *DOS* dissolved oxygen saturation, *SDD* Secchi

disc depth, *Dep* water column depth, *RD* relative distance from the coastline, *Str* salinity stratification index, *Rfl* river flow, *Bil* estuary of Bilbao, *Ple* estuary of Plentzia, *Urd* estuary of Urdaibai, *Ori* estuary of Orio, *Bid* estuary of Bidasoa, *A* August, *S* September)

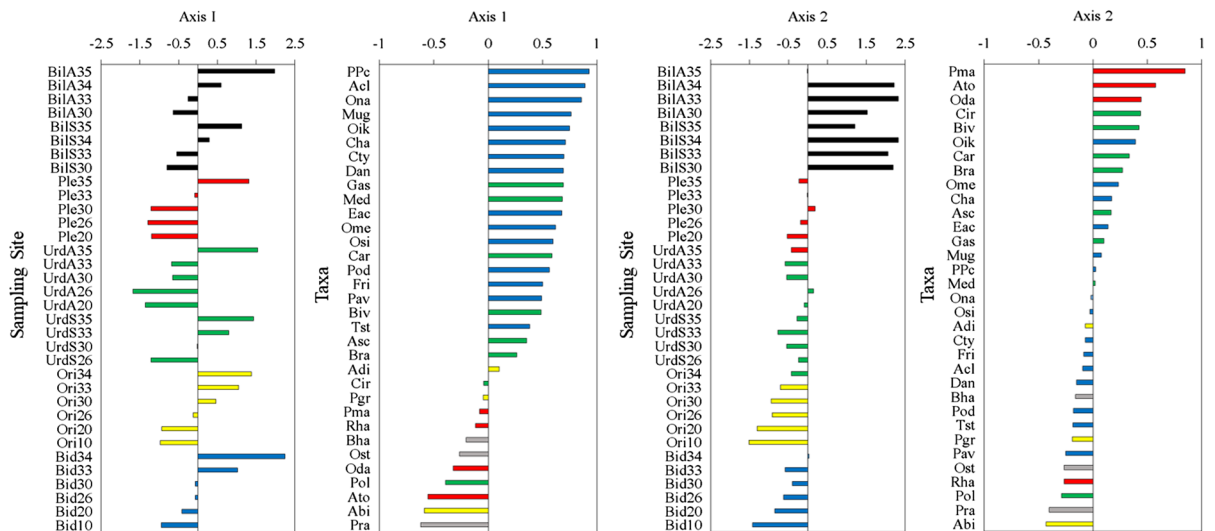


Fig. 6 Scores of salinity sites of the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa and zooplankton taxa along Axis 1 and Axis 2 of the RDA of zooplankton variability. Sampling sites were abbreviated and each estuary coloured (Bil for the estuary of Bilbao in black, Ple for the estuary of Plentzia in red, Urd for the estuary of Urdaibai in green, Ori for the

estuary of Oria in yellow and Bid for the estuary of Bidasoa in blue, *A* August, *S* September). Taxa abbreviations as in Table 3. Colours represent the different taxa categories (blue for neritic holoplankton, yellow for brackish native holoplankton, red for brackish exotic holoplankton, green for meroplankton and grey for tychoplankton)

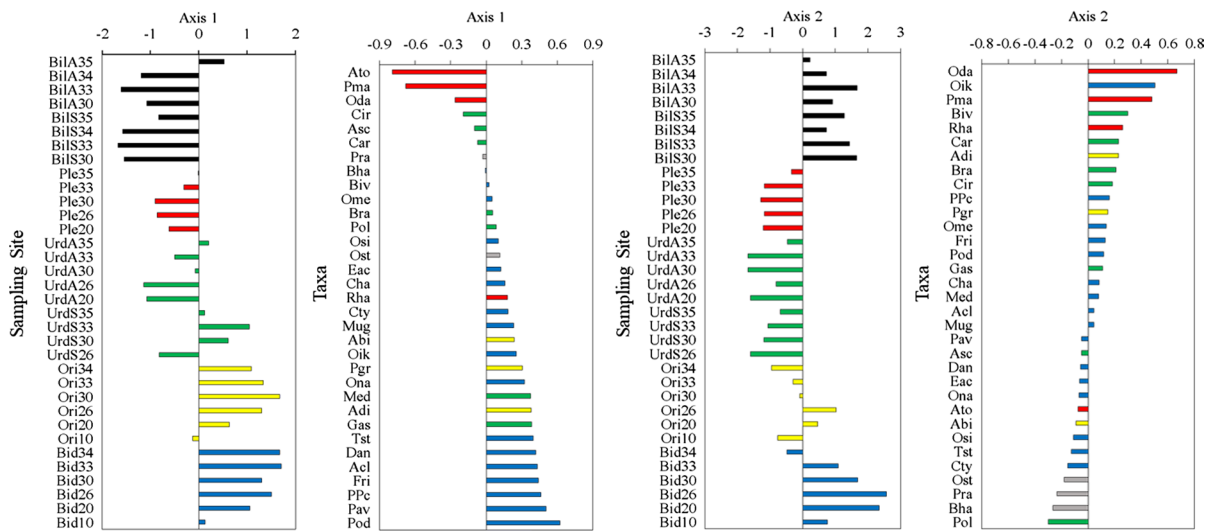


Fig. 7 Scores of salinity sites of the estuaries of Bilbao, Plentzia, Urdaibai, Oria and Bidasoa and zooplankton taxa along Axis 1 and Axis 2 of the RDA of zooplankton variability using salinity site as covariable. Sampling sites were abbreviated and each estuary coloured (Bil for the estuary of Bilbao in black, Ple for the estuary of Plentzia in red, Urd for the estuary of

Urdaibai in green, Ori for the estuary of Oria in yellow and Bid for the estuary of Bidasoa in blue, A: August, S: September). Taxa abbreviations as in Table 3. Colours represent the different taxa categories (blue for neritic holoplankton, yellow for brackish native holoplankton, red for brackish exotic holoplankton, green for meroplankton and grey for tycho plankton)

Discussion

Variations in the spread and abundance of the exotic species

O. davisae was the only exotic zooplankton species found in the brackish habitats of all five estuaries. An extensive literature on European coastal waters confirms its capability to colonize diverse brackish environments, including estuaries, lagoons, and open coastal waters (e.g. Altukhov et al., 2014; Cornils & Wend-Heckmann, 2015; Isinibilir et al., 2016; Zagami et al., 2018; Vidjack et al., 2019). The rapid expansion of *O. davisae* in the early twenty-first century has also been documented in Northeast Pacific estuaries (Dexter & Bollens, 2020). Notably, in the estuaries studied in the present work, *O. davisae* exhibited significantly higher densities in the more stratified and deeper estuaries of Bilbao and Bidasoa, while lower densities were observed in the predominantly mixed, shallow, and well-flushed estuaries of Plentzia and Urdaibai. These results are in agreement with findings of the requirement of stable water column conditions to ensure the year-round reproduction (Zagami et al., 2018) and to favour feeding activity

(Saiz et al., 2003) of *O. davisae* and also with those of previous studies on the differences in exotic copepods between the estuaries of Bilbao and Urdaibai (Barroeta et al., 2020, 2022b).

In contrast, *A. tonsa*, an earlier colonizer of European waters compared to *O. davisae* (Brylinski, 1981), is a well-established species in European brackish habitats (e.g. Azeiteiro et al., 2005; Chaalali et al., 2013; Camatti et al., 2019). However, it was not found in the easternmost estuaries of Bidasoa and Oria while it was abundant in the three estuaries on the western side, despite the contrasting physical and hydrological characteristics between the estuary of Bilbao and those of Plentzia and Urdaibai. This raises questions regarding the differential spread and successful colonization of *O. davisae* and *A. tonsa* in Basque estuaries. While both species are typical species of coastal and estuarine habitats with the ability to colonize various brackish environments due to their osmoregulatory abilities (Svetlichny and Hubareva, 2014), *A. tonsa* is expected to have higher colonization potential in small estuaries compared to *O. davisae*. This can be attributed to *O. davisae* being more susceptible to turbulence in the water column (Saiz et al., 2003) and lacking the ability to produce resting

eggs (Uye and Sano, 1995). Additionally, *A. tonsa* shows remarkable adaptability to different environmental conditions, including waters impacted by pollutants and trophic loadings, as observed in the Venice lagoon, (Camatti et al., 2019), the Gironde estuary (David et al., 2007) and the harbour of Dunkirk (Brylinski, 1981), both in France. Furthermore, it can thrive in habitats with low native zooplankton populations (Azeitero et al., 2005). Therefore, other factors influencing colonization patterns should be explored. In this sense, and without disregarding other non-human effective means of zooplankton dispersal, such as tides and coastal currents (Christy and Stancyk, 1982), the successful establishment of exotic brackish planktonic species in new estuaries or estuarine sites relies primarily on their arrival via commercial shipping routes (Gubanova, 2000) and recreational boating (Geburzi and McCarthy, 2018) between estuarine ports.

It is noteworthy that only the estuary of Bilbao includes a commercial port. This port receives cargo ships from around the world, and it is likely a significant hub for the introduction of exotic species. However, all five estuaries in our study have marinas for recreational or non-commercial fishing crafts, which could potentially play an important role in the secondary spread of species between estuaries (Wasson et al., 2001). Hence, the concurrent absence of *A. tonsa* and presence of *O. davisae* in the estuaries of Oria and Bidasoa pose a compelling challenge for future studies, that can be addressed through a comprehensive examination of the current zooplankton community in nearby ecosystems, coupled with molecular analyses that can track the spreading routes of these species based on the phylogeographic distribution of population's haplotypes (Albaina et al., 2016).

P. marinus has undergone a rapid spread from the Mediterranean Sea to the North Sea in recent years (Uttieri et al., 2020), but in the present study, it was only detected in the estuary of Bilbao in low density. Since *P. marinus* is also found offshore and exhibits epibenthic behaviour (Sabia et al., 2015), the absence of this species in other estuaries along the Basque coast may be attributed to its probable non-resident status. However, depending on the specific estuary, it is possible that *P. marinus* enters these estuaries periodically or occasionally. Long-term observations in the estuaries of Bilbao and Urdaibai support

this hypothesis, showing a continuous presence from year-to-year, although with modest abundance in the former estuary and very occasional appearances in very low numbers in the latter (Barroeta et al., 2020). The successful colonization of estuaries by *Pseudodiaptomus* species has been associated with the extent of marine intrusions (Cordell and Morrison, 1996) and the geomorphic characteristics that influence salinity and mixing patterns within estuaries (Dexter et al., 2020). Indeed, *Pseudodiaptomus* is typically abundant in plankton samples in transitional areas where high salinities meet low salinities (Sabia et al., 2015). Similarly, the seasonal occurrence of modest densities of *P. marinus* in the estuary of Bilbao, following its initial arrival, has been associated with the high inward penetration and maintenance of high salinity water masses during the dry season (Barroeta et al., 2020). These favourable conditions promote the advection and persistence of the *P. marinus* population in Bilbao (Barroeta et al., 2020). The study of Deschutter et al. (2018) concluded that nutrients and chlorophyll *a* were found to be of less importance, possibly due to the complex interactions between environmental variables. In contrast, the middle section of the estuary of Oria is shallower than the inner section, this limiting the penetration of the marine front landwards (San Vicente et al., 1988). Similarly, the estuary of Urdaibai undergoes large fluctuations in salinity between months (Villate, 1997), indicating frequent short-lived events of freshwater discharge. Those events likely flush out individuals of *P. marinus*, diminishing their chance of establishing dense populations. Conversely, the absence or sporadic occurrence of this species in the other shallower estuaries along the Basque coast seems to be attributable to the lack of a persistent salt wedge and stable water masses.

Another notable case of variation in the spread and colonization success of exotic species in the estuaries of the Basque coast involves the crab *R. harrisi*. A large abundance of zoea larvae of this decapod was only found in the plankton of the inner zone of the Bidasoa estuary, while these larvae were not observed in the other estuaries. *R. harrisi* was previously documented in this estuary in 1994–1995 (d'Elbée, 1998), but it was not included among the benthic exotic species reported in the estuary of Bilbao from 1989 to 2008 (Zorita et al., 2013). Likewise, it has never been detected in the zooplankton monitoring

conducted since 1998 in the estuaries of Bilbao and Urdaibai. This crab species native to the west coast of North America has spread extensively throughout continental Europe since 1870, likely facilitated by ballast water discharges or clinging to vessel hulls (Eno et al., 1997). It typically inhabits shallow environments with low salinity (Eno et al., 1997). In this regard, the estuary of Bidasoa, characterized by natural channels, salt marshes and high freshwater retention in the inner reaches, appears to provide a suitable habitat for this species.

The scarcity or absence of exotic species in systems that have the potential to receive individuals or propagules from nearby or adjacent systems suggest limitations imposed by local environments (Havel and Medley, 2006). In agreement with findings for the Northwest American estuaries by Dexter et al. (2020), we have also found that the geomorphologic features that affect hydrological conditions are the most likely factors constraining the geographic distribution of zooplankton in Basque coast estuaries. This appears to be the case for the very low abundance of *O. davisae* in the estuaries of Plentzia and Urdaibai, and the very rare presence or absence of *P. marinus* in all the shallow estuaries along the Basque coast, driven by the level of penetration of high salinity tidal waters, the flushing rate or the stability of the water column. The estuary of Bilbao, however, stands as an exception due to the significant anthropogenic morphological and hydrological alterations it has undergone (Cearreta et al., 2014), making it more favourable for the successful establishment of a wider range of colonizing species. Nonetheless, the gaps observed in the presence of *A. tonsa* in Basque coast estuaries may also be attributed to arrival opportunities or other unidentified factors. Understanding the dispersal routes and methods of exotic species is crucial for managing their introductions (Saul et al., 2017). The precautionary approach suggests focusing on managing invasion pathways to effectively address this issue and minimize their ecological impacts (Lodge et al., 2016).

Differences in the structure of the brackish assemblage and its distribution within estuaries

The spread and colonization success of exotic copepod species in the studied estuaries resulted in significant compositional differences in the summer

brackish community. In the estuary of Bilbao, the exotic species *A. tonsa* and *O. davisae* were dominant (Barroeta et al., 2020), while the native species *A. bifilosa* played a significant role in other estuaries. The estuaries of Urdaibai and Plentzia had similar brackish communities, with *A. tonsa* as the dominant species and *A. bifilosa* as the second most abundant. Prior to the first observation of *A. tonsa* in Basque estuaries in 2001, *A. bifilosa* was the dominant brackish copepod in these estuaries during summer (Villate et al., 2004). Instances where a newly introduced exotic species displaces a previously established and highly abundant species highlight the significance of competitive interactions in brackish ecosystems (Camatti et al., 2019; Dexter et al., 2020). Unlike the estuaries of Urdaibai and Plentzia, the estuaries of Oria and Bidasoa showed *A. bifilosa* as the primary contributor to the summer brackish community, with higher dominance in the estuary of Oria. *A. bifilosa* contribution in the estuary of Bidasoa was similar to that of *O. davisae*. Accordingly, the summer brackish community in the estuary of Oria showed the lowest diversity and the lowest impact from exotic copepod species. In contrast, the higher diversity and species richness observed in the brackish community of the estuary of Bidasoa can be attributed to the similar densities of the two main species and the significantly high abundance of *A. discaudata* and *Paracartia grani* Sars G.O., 1904, which is unprecedented according to previous studies (Villate et al., 2004). Present results suggest that the semiconfined bay-shaped morphology of the outer part of the estuary of Bidasoa may determine the higher suitability of this system for these species. Exotic species are widely recognized for their negative impacts on ecosystems at various levels (Gallardo et al., 2016). However, it is important to acknowledge that they can also have positive effects on certain ecosystems (Davis et al., 2011). Overall, the arrival of exotic copepods has had a positive impact on brackish copepod communities in the surveyed estuaries, resulting in increased brackish copepod density, diversity, and species richness. The estuary of Bilbao, which previously lacked a well-established brackish copepod community, has now developed one due to the presence of exotic species (Barroeta et al., 2022a). In the other estuaries, native brackish species populations are present, although the dominance of *A. bifilosa* has diminished. These observations are in agreement with findings

that non-native aquatic species often outperform native species and are favoured under environmental change (Sorte et al., 2013).

Additionally, our findings have shown that relative distance from the coastline exerts a greater influence than salinity in separating brackish copepod communities from neritic ones and this is in accordance with observations in other transitional systems, where spatial differences in zooplankton communities were also associated with distance from the estuary mouth (Champalbert et al., 2007; Elliot and Kaufmann, 2007). In the estuary of Urdaibai, brackish zooplankton populations were observed to shift from the inner zone during high tide to the outer zone during low tide, covering distances exceeding one-third of the estuary's length during spring tides (Villate, 1997). This suggests that the dominance of brackish populations occurs in water masses that remain within the estuary without being flushed out. In the estuary of Bilbao, the replacement of brackish populations with neritic populations takes place at higher salinities (between 34 and 35), thus preventing the brackish populations from being flushed out of the estuary.

Other cross-estuary differences in the zooplankton communities

The RDA analysis of the pooled abundance of all taxa from all the studied estuaries revealed important contributions from meroplankton, tychoplankton, and neritic holoplankton to differences between estuaries. These differences can be attributed to differences in environmental factors among estuaries or to their geographic location along the Basque coast. Among meroplankton taxa, Cirripedia larvae and Bivalvia larvae appeared in opposition to Polychaeta larvae. The observed variations in meroplankton contributions among estuaries are related to differences in the surface area of suitable benthic habitats for each taxonomic group, in agreement with observations elsewhere (Bae et al., 2022). Estuaries like Bilbao and Bidasoa, with extensive natural and artificial hard substrates, provide suitable habitats for sessile forms such as cirripedes. On the other hand, the estuaries of Plentzia and Urdaibai, with larger surface areas of soft sediment, support a more abundant polychaete community. Gnathiidae isopods, mainly juveniles of *Paragnathia formica* (Hesse, 1864), played a significant role in differentiating the other estuaries from the

estuary of Bilbao. These isopods are hematophagous ectoparasites of fishes (Manship et al., 2012) and are commonly found in relatively high abundance in shallow European estuaries (e.g. Marques et al., 2007). The presence of these isopods and other temporary zooplankton in the water column is favoured by the combined effect of shallow depths and tidal influences (Villate, 1997).

The dominant west-to-east pattern of zooplankton variation, as identified by the RDA analysis after removing the effect related to the longitudinal salinity gradients, involved not only brackish taxa but also neritic holoplankton species such as Podonidae cladocerans (primarily *E. spinifera*, *P. tergestina*, and *P. avirostris*), as well as *Temora stylifera* (Dana, 1849), although the latter one to a lesser extent. These findings agree with the higher abundance of these taxa in the zooplankton communities of the eastern Basque coast estuaries. These species belong to a coastal thermophilic assemblage (Siokou-Frangou et al., 1998) that exhibits peak abundance during the warmest period in the waters of the inner Bay of Biscay (Fanjul et al., 2017). In our study, these species showed spatial patterns of variation that correspond to the intraregional gradient of surface sea temperature in the inner Bay of Biscay during summer. On average, the eastern part of the Basque coast shows temperatures that are at least 0.5 °C higher than those in the western part (Costoya et al., 2015).

Conclusions

This study has highlighted the need for increasing cross-estuary comparisons, which are very scant in the literature, to better unravel the complexity of drivers of the presence and abundance of exotic zooplankton species in estuaries. Our results on the factors that have affected the colonization of Basque coast estuaries by exotic zooplankton are in agreement with others obtained from cross-estuary comparisons in the following:

1. The presence of exotic species depend on the chances of arrival of individuals or propagules. In this sense, and assuming vessel navigation is the most likely way of arrival to Basque coast estuaries, we hypothesize that, given its intense international maritime traffic, the Bilbao port has

been the main entrance point for *A. tonsa* in the Basque coast, and that it has reached the western estuaries of Plentzia and Urdaibai through secondary spread from the estuary of Bilbao. Similarly, a lower vessel traffic between the eastern and western estuaries could be the reason for the absence of this species in the eastern estuaries. However, the presence of *A. tonsa* in ports/estuaries to the northeast of the Bidasoa estuary which have a higher contact through maritime traffic with this estuary should be investigated. Molecular studies that can track the spreading routes of these species based on the phylogeographic distribution of population's haplotypes would also be very helpful.

- Physical factors related to hydro-morphological features of estuaries are key drivers, although the main constraining factor can be species specific. For example water column stability seems very influential for *O. davisae*, whereas a persistent salt wedge and stable water masses appear as important factors in the case of *P. marinus*. This also supports the idea that inter-estuarine comparisons should cover a wider variety of exotic zooplankton.

Additionally, our data have shown that the history of anthropogenic impacts (damaging and rehabilitation activities) in estuaries is also a prominent factor. The estuary of Bilbao is now in a rehabilitation phase from intense past pollution conditions in which the inner estuary was almost devoid of zooplankton. When conditions improved for zooplankton in brackish waters the presence of ecological niches not fully saturated by brackish zooplankton, is the most likely reason for the higher abundance of exotic brackish copepods in this estuary than in other Basque coast estuaries. Some exotic brackish species such as *A. tonsa* seem well adapted to take advantage of the transitional conditions from highly polluted to lower pollution.

Understanding the dispersal routes and factors favouring/constraining the colonization of specific exotic species is crucial for managing their introductions and minimizing their potential ecological impacts.

Cross-estuary differences in the zooplankton communities also involve non-exotic taxa. In Basque coast estuaries, meroplankton groups' abundances

were influenced by the estuarine sediment type (soft or rocky) and some holoplankton abundances by the west-to-east gradient of water temperature.

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Data availability Data will be made available at a reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare.

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