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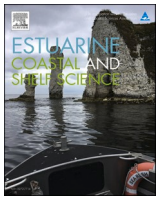
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Response of native and non-indigenous zooplankton to inherent system features and management in two Basque estuaries: A niche decomposition approach

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ABSTRACT

The realized ecological niches of native zooplankton and non-indigenous species (NIS) were analysed in the contrasting estuaries of Bilbao and Urdaibai from 1998 to 2015 in order to study their responses to the particular features of each estuary and the biotic and abiotic changes along the study period. The marginality and tolerance of zooplankton taxa was estimated for the two estuaries together using the OMI analysis, whilst, by means of the WitOMI analysis, niches were differentiated into spatial subniches to assess the differences between estuaries and into temporal subniches to determine the variations in time within each estuary. The changes in the niche overlap of the main native and NIS species were calculated with the D-metric. Results showed that the combined effect of salinity gradients and seasonal variations, linked to temperature, defined the main spatio-temporal niches of zooplankton taxa in the overall environmental scenario of both estuaries. Thus, those factors separated winter-spring neritic, summer-autumn neritic, warm water affinity brackish and limnetic species. Secondarily, river discharge and hydrological stability accounted for the higher presence of freshwater and some brackish species in the estuary of Bilbao and tychoplanktonic organisms in the estuary of Urdaibai. At the regional scale the marginality and tolerance of zooplankton taxa reflected a combination of spatial and seasonal niche breadth and difference of abundance between estuaries. The number of taxa with niches not clearly explained by the environmental variables studied was higher in the estuary of Urdaibai, whilst the new arriving species occupied realized niches well defined by the environmental variables and overall zooplankton taxa showed a better discrimination from generalist to specialist behaviours in the estuary of Bilbao. This was related to inherent abiotic and biotic features of each estuary, which make the estuary of Urdaibai more refractory to the settlement of NIS species. Moreover, in the estuary of Bilbao, several neritic and brackish species experienced temporal changes in niche breadth and overlap that was attributed both to the environmental improvement related to rehabilitation plans in the system and the impact of the species that arrived during the study period. In the estuary of Urdaibai only small changes attributable to the limited impact of NIS were inferred, since no meaningful changes in environmental conditions were perceived.

1. Introduction

Estuaries are complex coastal systems that may differ largely between and within them in geomorphology, hydrodynamics (i.e. influence of seawater and freshwater flows, distribution of salinity, circulation and stratification/mixing patterns), sediments, as well as in functional features related to the geographic location, energetics and human impacts (e.g. Pritchard, 1952; Odum et al., 1974) and have been traditionally classified in a great variety of revisable types (Whitfield and Elliott, 2011). As a consequence, estuaries also evidence marked

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differences in habitat availability and opportunities for the establishment of species with different ecological niches.

Zooplankton species and assemblages found in estuaries with a well-established salinity gradient increasing from the river to the sea are roughly classified in the classical freshwater (limnetic), brackish (estuarine) and marine categories of the Remane model for the distribution of species in estuaries (Whitfield et al., 2012). Furthermore, the following classification of holoplanktonic copepods according to the position/-segregation along the axial gradient: true estuarine, marine-estuarine, euryhaline marine and stenohaline marine (Jeffries, 1967) is also used. However, the relevance of each category may be highly variable between and within estuaries since it depends on the inherent environmental features of the system, which determine the number and extent of environmental niches available to zooplankton species. While estuaries located at the end of large rivers are characterized by high presence of freshwater species and dominance of a species-poor brackish assemblage composed mainly by copepods of the genera *Eurytemora* and *Acartia* in the northern hemisphere (Castel, 1981; Ambler et al., 1985; Taylor, 1987; Baretta and Malschaert, 1988; Roman et al., 2001), marine-dominated estuaries are characterized by the penetration of a species-rich neritic assemblage landwards and the dominance of a marine-estuarine assemblage mainly composed of *Acartia* congeneric species in brackish habitats (Castel and Courties, 1982; Alcaraz, 1983; Seo et al., 2021). This is the case for most of the small estuaries located along the Basque coast in the inner Bay of Biscay, which are mostly shallow sea-dominated tidal systems (Borja et al., 2004) mainly inhabited by neritic zooplankton seawards and a brackish assemblage dominated by *Acartia* species landwards, but lacking or with a scarce presence of typically mesohaline-oligohaline (i.e. *Eurytemora* spp.) and limnetic zooplankton (Villate et al., 2004). However, the two estuaries that we chose to study have contrasting histories of human impact, which strongly modified the estuarine basin, the hydrological and sedimentary processes and the environmental quality in the case of the estuary of Bilbao, and allowed the maintenance of a greater morphological, environmental and biological integrity in the case of the estuary of Urdaibai.

Human perturbations, e.g. system uses, physical changes and pollution, determine and limit the occupation of estuaries by zooplankton in different ways. Among the estuaries of the Basque coast, this was most evident in the estuary of Bilbao, where no brackish zooplankton but a neritic community restricted to the lower part was reported in the early 1980s, due to the high level of pollution along most of the estuarine system (Villate, 1991). Significant differences in the composition and abundance distribution of zooplankton taxa between this estuary and the estuary of Urdaibai attributable to pollution were still observed in the late 1990s (Uriarte and Villate, 2004, 2005), after the Regional Water Authority implemented in 1979 a management plan for the recovery of environmental quality. The gradual improvement of environmental conditions in the estuary of Bilbao allowed the progressive recolonization of the mid and inner estuary by zooplankton populations that gradually occupied the empty niches available after the most detrimental conditions were no longer in place. Neritic species were the first to occupy those niches and later new brackish species arrived (Uriarte et al., 2016). In addition, this estuary homes a major commercial port area that undoubtedly favours the arrival of non-indigenous species (NIS) and biological invasions (Geburzi and McCarthy, 2018; Dexter et al., 2020), as it was evidenced by the fact of the initial colonization of the inner estuary by brackish NIS copepods instead of native brackish copepods present in nearby estuaries which have been less affected by man-driven environmental degradation (Barroeta et al., 2020). The colonization success of NIS, however, varies depending on their ability to face the biotic and abiotic constraints within the recipient ecosystem (Chan and Briski, 2017). Competition, exploitation and other biotic interactions may promote (or hinder) the spread and establishment of the NIS in the new environment (e.g. Freestone et al., 2013; Alofs and Jackson, 2014; Gallardo et al., 2015). Although the possibility of "niche shift" has been widely debated, i.e., changes in the realized niche of a species in relation to the centroid of the niche, the margins, and/or frequency of occupied environmental conditions (Guisan et al., 2014), it is commonly accepted that species do occupy the same niche in their new range as in their native range (Laeseke et al., 2020).

Recent improvements in mathematical computing allowed relating the species response to the environmental conditions within an ecological niche approach (Guisan et al., 2017). Based on observational data, it is possible to describe the realized ecological niche theorized by Hutchinson (Colwell and Rangel, 2009). Guisan et al. (2014) classified niche studies in two approaches: ordination and ecological niche models. While the first approach is more robust to detect niche changes and quantify niche overlap (Broennimann et al., 2012), the second one tends to provide a better mathematically formalized niche representation. The so called outlying mean index (OMI, Dolédec et al., 2000) and within outlying mean index (WitOMI, Karasiewicz et al., 2017) analyses belong to the ordination framework. They make up for the drawbacks of old indexes based on resource availability (Levins, 1968; Hulbert, 1978; Smith and Zaret, 1982), densities (Morisita, 1959) or relative abundances (Macarthur and Levins, 1967; Pianka, 1973; Hulbert, 1978), giving a greater evenness to the significance of all the sampling units, even in the case of rare species. These methods show more adequately the response to the environmental variations, allowing a better description of the changes in the niches. Thus, it can be a useful tool in cases of environmental alterations, such as the case of the introduction of NIS (Broennimann et al., 2012; Guisan et al., 2014). In addition, those methods are useful for calculating niche metrics such as centroid (i.e. marginality) or niche breadth (i.e. tolerance) (Dolédec et al., 2000; Karasiewicz et al., 2017). By niche decomposition, WitOMI analysis provides new insights to studying ecological niche at finer scale: the so-called subniche (Karasiewicz et al., 2017). Furthermore, to understand the realization of species ecological niche it may be important to take in account both abiotic and biotic components. Niche overlap between species is a parameter commonly used to assess biological interactions. Among the set of different measurements of niche overlap, the D-metric is one of the most widespread methods because of its simplicity (Warren et al., 2008).

The aim of the present study was to determine and compare the realized ecological niche of zooplankton taxa in the contrasting estuaries of Bilbao and Urdaibai from 1998 to 2015, a period in which new species of copepods arrived at both systems (Barroeta et al., 2020) and the estuary of Bilbao experienced a marked environmental improvement in water conditions associated to a clean-up plan (e.g. Villate et al., 2013; Cajaraville et al., 2016; Irabien et al., 2018), while no interventions were implemented in the less perturbed estuary of Urdaibai. The specific aims were (i) to estimate the ecological niches of both native zooplankton and new arriving species within the regional environmental framework of the two estuarine systems, (ii) to determine the spatial subniches in each system in order to highlight the main discrepancies on the realized subniches between estuaries, and (iii) to differentiate the data series into temporal subniches in order to assess the temporal responses of zooplankton communities to biotic and abiotic stressors in each estuary during the study period. The starting hypothesis was that zooplankton communities, including the addition of NIS, in estuaries are primarily determined by the inherent environmental features of the system and they vary with time depending on local human activities, including management actions, and therefore meaningful differences in the realized niche of native and non-native components should be found both between estuaries and with time.

2. Method

2.1. Study area

The nearby estuaries of Bilbao (43° 23′N, 03° 07′W) and Urdaibai (43° 22′N, 02° 43′W) are located on the south-eastern Bay of Biscay

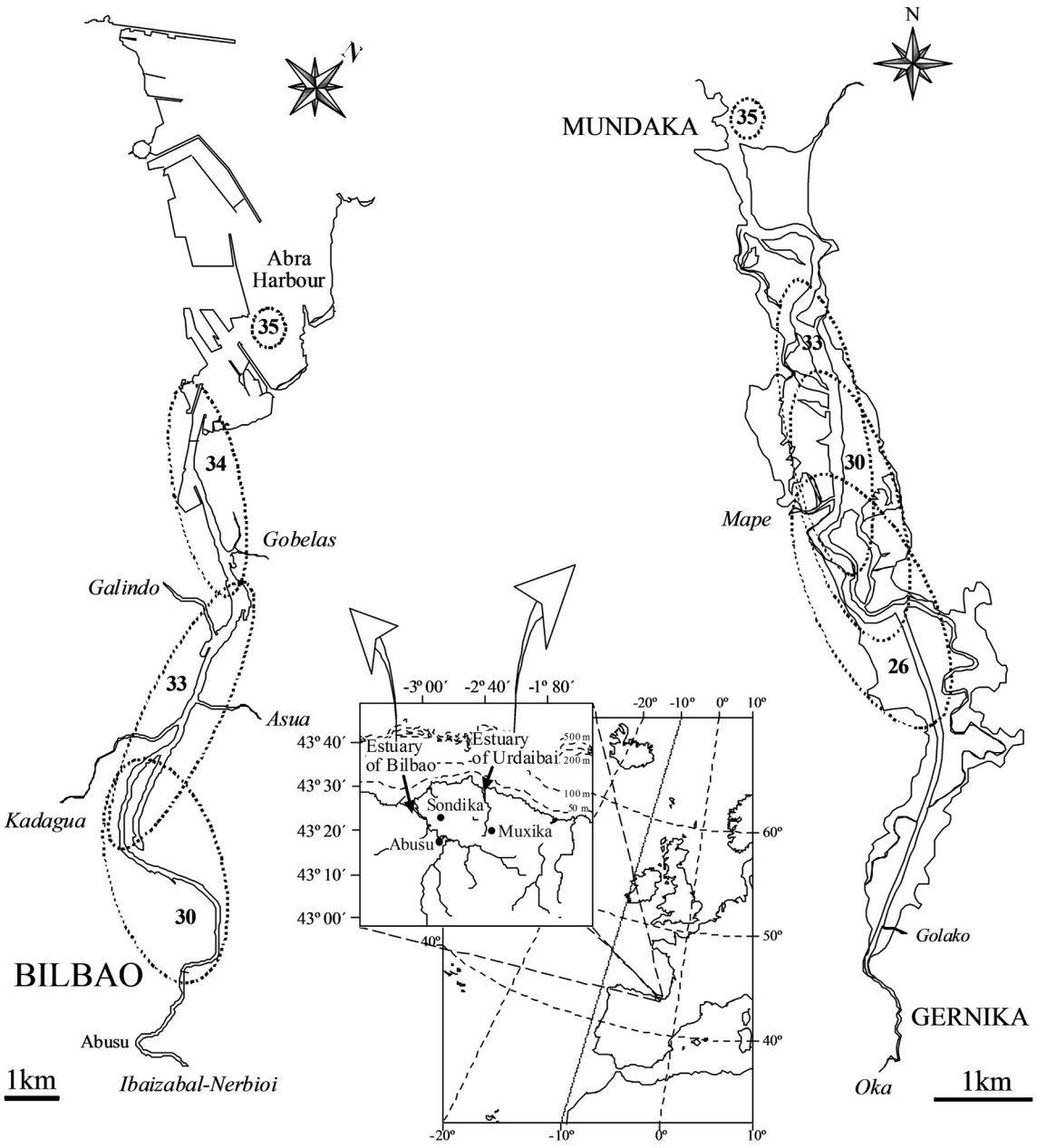


Fig. 1. Maps of the estuaries of Bilbao and Urdaibai showing the spatial range of the salinity zones (26, 30, 33, 34 and 35) sampled.

(Fig. 1). Therefore, they are under the same temperate-oceanic climate with moderate winters and warm summers. Nevertheless, they have large differences with respect to morphology, hydrodynamic conditions and water quality.

The meso-macrotidal estuary of Bilbao is a relatively short (~23 km long) and shallow (0.5 m deep at the inner part and 32 m deep at the outer coastline site) system, where euhaline water masses span along most of its length (Villate et al., 2013). It is characterized by strong stratification in the inner part but it is partially mixed in the outer part (Intxausti et al., 2012). Water residence time is lower in the channelized upper and middle reaches than in the outer Abra embayment, and it is also much lower in above halocline layers (0.1–1.6 days) than in below halocline layers (0.3–11.6 days) for most of the estuary length (Uriarte et al., 2014). The main rivers, Ibaizabal and Nerbioi, enter at the estuary head, but other smaller streams drain into the middle reaches. The high industrialization along its banks and raw sewage discharges turned the estuary into one of the most polluted systems in Europe during the mid-20th century (Irabien et al., 2018). Nevertheless, since the late 1970s, a comprehensive plan for the sanitation of the metropolitan area of Bilbao started and noticeable improvements in the ecological status have been observed in the last two decades, e.g. decrease in heavy metal pollution (Fdez-Ortiz de Vallejuelo et al., 2010), increase in dissolved oxygen levels (Villate et al., 2013) and an enhancement of biological diversity (Borja et al., 2006). Currently, this estuary is a strongly man-modified system, very channelized and intensely dredged (Cearreta et al., 2004) in order to facilitate intense international maritime traffic. 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.

The estuary of Urdaibai is a meso-macrotidal system of ~12.5 km long and ~3 m depth on average characterized by a strong horizontal salinity gradient. The outer part is dominated by high tidal flushing and well-mixed marine water at high tide, whilst the middle-inner part is partially stratified (Villate et al., 2017). The flow rate of the main river (Oka) that flows into the head of the estuary is very low, and seawater entering with the tide dominates within the estuary at high tide (Iriarte

et al., 2015). In comparison to the estuary of Bilbao, this system and its watershed bear low human pressure and they constitute the Biosphere Reserve of Urdaibai. The system is characterized by extensive intertidal flats and sandy beaches in the lower reaches and salt marshes and reed beds in the middle and upper reaches. Nowadays, it has a low to moderate anthropogenic impact. A small sewage treatment plant located in the inner estuary is the main source of pollution (Cotano and Villate, 2006) and shipyard activities in the middle zone were responsible for periodic dredging in the outer half of the estuary until 2003. Since then the stop of dredging activities caused a gradual reduction in depth in the system (Monge-Ganuzas et al., 2013).

2.2. Data acquisition

Since 1998 zooplankton community was monitored monthly at high tide at different salinity zones of the estuary of Bilbao (30, 33, 34 and 35)

Table 1
Average (±standard deviation) density of zooplankton taxa used in the OMI analysis at each salinity zone of the estuaries of Bilbao (30, 33, 34 and 35 salinities) and Urdaibai (26, 30, 33 and 35 salinities) during the study period (1998–2015). The PCPC-calanus assemblage gathered *Paracalanus* sp., *Clausocalanus* sp., *Pseudocalanus* sp. and *Ctenocalanus* sp. Taxa abbreviations (Abb) are also included. In bold density maxima recorded for each taxon at each salinity zone in each estuary. In bold values from the salinity zone where density was highest for each taxon in each estuary.

Taxa	Abb	Estuary of Bilbao				Estuary of Urdaibai			
		30	33	34	35	26	30	33	35
Copepods									
Acartia bifilosa	Abi	**56.6 ± 341.1**	7.5 ± 87.3	9.0 ± 125.8	0.1 ± 1.43	1426.0 ± 3442.4	**1436.7 ± 5345.8**	395.5 ± 1335.9	3.6 ± 18.1
Acartia clausi	Acl	43.9 ± 132.7	191.1 ± 484.1	605.3 ± 1568.8	**969.8 ± 1873.6**	22.4 ± 70.0	58.0 ± 165.4	165.2 ± 661.2	**1068.0 ± 3997.6**
Acartia tonsa	Ato	**881.8 ± 2090.2**	749.2 ± 1904.7	156.6 ± 473.5	31.9 ± 412.0	**1041.7 ± 7361.6**	221.1 ± 895.3	45.9 ± 196.5	0.3 ± 3.3
Acartia discaudata	Adi	0.4 ± 2.6	2.3 ± 9.7	**8.6 ± 31.6**	1.6 ± 6.6	0.0 ± 0.4	0.1 ± 0.8	**0.6 ± 6.0**	0.3 ± 1.9
Paracartia grani	Pgr	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 12.4	6.2 ± 35.6	**14.1 ± 134.2**	0.1 ± 0.5
Acartia margalefi	Ama	4.8 ± 18.6	**8.5 ± 69.2**	4.9 ± 16.5	0.7 ± 5.2	0.1 ± 1.7	0.2 ± 1.4	**2.4 ± 13.7**	0.1 ± 0.8
Calanus sp.	Cala	0.7 ± 4.1	4.3 ± 22.5	5.6 ± 19.7	**17.4 ± 81.6**	0.1 ± 1.4	0.5 ± 1.7	1.9 ± 6.7	**3.9 ± 11.4**
Calocalanus sp.	Calo	0.1 ± 1.8	0.2 ± 1.6	1.3 ± 6.5	**1.9 ± 7.0**	0.3 ± 1.5	1.1 ± 6.4	1.3 ± 6.6	**2.0 ± 7.2**
PCPC-calanus	PCPC	25.9 ± 59.2	105.7 ± 189.4	216.0 ± 339.4	**543.0 ± 762.4**	27.5 ± 67.4	76.2 ± 172.2	150.5 ± 232.0	**526.0 ± 1206.4**
Centropages sp.	Cen	0.8 ± 4.3	4.6 ± 15.9	16.2 ± 42.7	**51.5 ± 148.2**	2.6 ± 8.8	8.0 ± 26.6	16.1 ± 50.6	**36.3 ± 133.5**
Temora longicornis	Tlo	0.8 ± 6.8	1.5 ± 5.3	4.4 ± 15.5	**15.3 ± 64.7**	1.6 ± 9.7	4.3 ± 43.9	8.2 ± 100.7	**11.1 ± 56.3**
Temora stylifera	Tsty	1.0 ± 4.3	5.5 ± 23.9	15.9 ± 58.2	**52.8 ± 187.8**	1.8 ± 13.7	4.9 ± 29.8	10.5 ± 34.9	**24.6 ± 93.9**
Pseudodiaptomus marinus	Pma	**9.0 ± 79.4**	5.4 ± 37.3	1.4 ± 7.1	0.0 ± 0.0	0.0 ± 0.0	**0.0 ± 0.2**	0.0 ± 0.1	0.0 ± 0.0
Calanipeda aquaedulcis	Caq	**293.7 ± 1435.7**	6.2 ± 27.5	0.8 ± 4.5	0.0 ± 0.1	**0.4 ± 2.0**	0.2 ± 0.8	0.1 ± 0.6	0.0 ± 0.1
Candacia sp.	Cand	0.0 ± 0.4	0.0 ± 0.1	0.0 ± 0.2	**0.3 ± 1.6**	0.0 ± 0.1	**1.2 ± 13.6**	0.0 ± 0.3	0.2 ± 1.3
Oithona plumifera	Opl	0.5 ± 3.4	2.3 ± 11.9	5.5 ± 24.0	**13.2 ± 49.1**	0.4 ± 2.3	1.3 ± 5.23	4.4 ± 29.7	**11.6 ± 35.4**
Oithona nana	Ona	6.4 ± 17.5	20.7 ± 59.4	60.4 ± 237.3	**98.5 ± 289.4**	8.9 ± 31.7	15.9 ± 47.3	28.0 ± 71.5	**56.5 ± 174.8**
Oithona similis	Osi	7.0 ± 16.8	29.1 ± 67.4	52.6 ± 91.3	**74.2 ± 115.9**	26.7 ± 66.8	81.0 ± 230.6	93.7 ± 252.5	**111.6 ± 212.5**
Oithona davisae	Oda	**295.1 ± 1149.6**	103.7 ± 483.1	74.6 ± 638.5	2.3 ± 30.6	0.9 ± 4.6	**5.2 ± 66.0**	0.4 ± 2.9	0.0 ± 0.0
Fresh water copepods	Fwcop	**37.5 ± 284.5**	16.3 ± 138.1	2.9 ± 21.1	0.4 ± 2.5	**7.0 ± 61.2**	3.5 ± 17.6	1.7 ± 5.5	0.4 ± 2.1
Oncaea sp.	Onc	1.1 ± 3.3	3.6 ± 10.8	13.3 ± 50.4	**46.9 ± 174.6**	6.0 ± 27.4	12.7 ± 40.6	30.0 ± 86.7	**77.2 ± 246.3**
Ditrichocorycaeus anglicus	Dan	0.2 ± 0.8	0.5 ± 1.8	2.0 ± 8.4	**5.6 ± 19.5**	0.4 ± 2.0	1.4 ± 7.1	3.1 ± 7.0	**6.9 ± 17.1**
Euterpina acutifrons	Eac	2.7 ± 11.3	8.4 ± 18.3	26.3 ± 58.9	**45.0 ± 101.0**	8.0 ± 28.1	26.2 ± 176.2	41.5 ± 138.0	**56.8 ± 162.9**
Microsetella sp.	Micr	0.1 ± 0.6	0.0 ± 0.2	0.1 ± 0.7	**0.2 ± 1.3**	0.0 ± 0.3	0.2 ± 1.0	0.5 ± 4.2	**1.4 ± 4.8**
Harpacticoids	Harp	0.9 ± 3.2	0.9 ± 2.8	1.6 ± 4.4	**2.1 ± 7.2**	42.7 ± 152.4	**56.4 ± 399.8**	33.4 ± 240.5	7.4 ± 21.5
Non-copepod groups									
Medusae	Med	4.7 ± 28.0	2.9 ± 6.1	8.9 ± 18.6	**16.8 ± 38.5**	5.1 ± 38.0	7.1 ± 41.4	**8.9 ± 20.3**	**8.8 ± 23.2**
Siphonophores	Sip	1.2 ± 4.0	10.7 ± 39.1	25.2 ± 80.2	**49.4 ± 89.9**	1.4 ± 5.5	4.8 ± 20.0	8.9 ± 29.9	**15.1 ± 46.3**
Cyphonauta larvae	Cyp	0.2 ± 1.8	0.2 ± 1.1	1.7 ± 7.9	**2.9 ± 7.4**	0.3 ± 1.5	1.3 ± 5.2	2.5 ± 9.4	**4.2 ± 11.7**
Gastropod larvae	Gas	20.4 ± 48.7	27.4 ± 51.7	47.0 ± 81.4	**76.7 ± 157.0**	**637.6 ± 2840.9**	309.1 ± 1228.0	177.0 ± 360.4	245.7 ± 754.6
Bivalve larvae	Biv	**284.6 ± 2210.8**	50.2 ± 202.8	59.1 ± 269.9	52.9 ± 117.1	4.6 ± 16.4	12.1 ± 38.8	22.3 ± 52.2	**54.6 ± 143.1**
Polychaeta larvae	Poly	16.1 ± 34.1	**30.9 ± 72.7**	14.8 ± 32.8	7.2 ± 16.2	**45.8 ± 107.9**	36.3 ± 114.3	33.3 ± 168.2	10.2 ± 20.1
Chaetognaths	Cha	1.9 ± 6.4	7.8 ± 40.7	10.5 ± 33.4	**22.2 ± 59.9**	1.8 ± 5.6	3.2 ± 9.9	4.5 ± 14.0	**8.9 ± 28.2**
Cladocera	Cla	15.8 ± 89.5	55.3 ± 194.0	134.2 ± 348.7	**270.7 ± 555.7**	8.3 ± 33.7	19.6 ± 74.7	40.9 ± 122.8	**106.9 ± 289.6**
Ostracods	Ost	0.1 ± 0.6	**0.2 ± 1.1**	0.1 ± 0.4	0.0 ± 0.1	6.4 ± 11.4	**8.8 ± 19.8**	6.6 ± 21.9	1.6 ± 6.1
Cirripede larvae	Cir	244.8 ± 629.9	930.3 ± 2437.9	**1023.0 ± 2334.1**	959.9 ± 1736.8	**476.6 ± 1138.7**	336.5 ± 748.0	250.8 ± 760.1	273.4 ± 825.2
Isopods	Iso	0.5 ± 1.6	0.3 ± 1.2	**0.7 ± 2.1**	0.7 ± 2.9	**16.6 ± 59.8**	15.0 ± 26.8	14.5 ± 29.2	±7.5
Mysids	Mys	**0.9 ± 10.3**	0.0 ± 0.2	0.1 ± 1.6	0.0 ± 0.1	**0.9 ± 9.2**	0.1 ± 0.5	0.2 ± 1.3	0.0 ± 0.1
Decapod larvae	Dec	1.1 ± 4.6	11.7 ± 38.1	18.4 ± 48.9	**28.2 ± 69.3**	5.4 ± 21.7	**12.4 ± 55.1**	12.1 ± 28.4	8.4 ± 22.1
Echinoderm larvae	Ech	0.0 ± 0.6	0.5 ± 3.2	2.1 ± 7.7	**4.6 ± 16.5**	0.1 ± 1.0	0.6 ± 4.1	0.6 ± 3.9	**1.5 ± 6.3**
Appendicularians	App	47.2 ± 146.6	103.5 ± 180.3	155.3 ± 298.8	**184.4 ± 316.3**	7.9 ± 27.6	31.6 ± 133.0	39.9 ± 83.2	**71.5 ± 160.6**
Doliolids	Dol	0.2 ± 1.6	0.9 ± 5.7	9.8 ± 51.2	**44.6 ± 184.8**	0.3 ± 3.4	0.2 ± 1.2	1.4 ± 7.7	**15.6 ± 69.8**
Ascidian larvae	Asc	0.1 ± 0.6	0.4 ± 2.1	0.3 ± 2.2	**0.8 ± 4.8**	0.0 ± 0.1	**0.2 ± 2.9**	0.2 ± 0.9	0.1 ± 0.9
Ichthyoplankton	Ict	1.0 ± 4.3	2.4 ± 7.7	7.4 ± 20.9	**15.1 ± 50.9**	0.7 ± 3.3	1.0 ± 4.3	1.3 ± 4.2	**2.5 ± 7.6**

and Urdaibai (26, 30, 33 and 35) (Fig. 1). At each salinity zone, salinity, temperature and dissolved oxygen vertical profiles were recorded every 0.5 m using a multi-parameter water quality meter (YSI 556MPS). Secchi disk depth was also measured. Water was collected at mid-depth below the halocline with a Niskin bottle and filtered (Whatman GF/C) for the spectrophotometric chlorophyll *a* determination, according to Lorenzen (1967). Mesozooplankton samples were collected below the halocline by 2–3 min horizontal tows using a 200-μm mesh size net with 25 cm of diameter at the mouth, equipped with a mechanical flow meter. These samples were preserved in 4% buffered formaldehide, and individuals from an aliquot were identified and counted (until at least 100 individuals of the most abundant species and 30 individuals of the second and third most abundant species were recorded) to the lowest possible taxonomic level using optical inverted microscopes. River flow of the main rivers and precipitation data were obtained from the Provincial Council of Bizkaia.

2.3. Data pre-treatment

In the present study, we used the biological and environmental data obtained from 1998 to 2015 in the ongoing monitoring program of hydrography and plankton in the estuaries of Bilbao and Urdaibai. Sporadic missing values (less than 11% for the variable with most gaps) of both environmental and zooplankton variables in the time series were filled with the mean of the preceding and following month values. However, in the data series of the estuary of Urdaibai more than two consecutive months/year were missing for U26 chlorophyll *a* concentration between 1998 and 2000; therefore, in that case only data from 2001 to 2015 was used for the analyses. Likewise, rare zooplankton species were excluded based on an index mixing species abundances and frequencies using the abundance sorting method adapted from Ibanez and Dauvin (1998), in our case retaining only taxa with a presence >0.01% over the study period in any of the estuaries (Table 1). Copepods were studied at species level in most cases or grouped to genus level or species assemblages with common habitat features, whilst the rest of zooplankton were grouped into major taxonomic categories. Salinity stratification index was calculated as the maximum difference in salinity between consecutive depths (Villate et al., 2013).

2.4. Data analysis

To investigate the ecological niche of the zooplankton taxa, outlying mean index (OMI) and within outlying mean index (WitOMI) analyses were performed (Dolédec et al., 2000; Karasiewicz et al., 2017). Both multivariate explorative methods allow studying the species (i.e. matrix site-taxon) and environment (i.e. matrix site-environmental variables) relationships, much like the commonly used canonical correspondence (CCA) and redundancy analyses (RDA). However, OMI and WitOMI analyses can detect and represent both unimodal and linear responses of every species along the environmental gradient, while CCA (unimodal response) and RDA (linear response) can only detect one of them (Dolédec et al., 2000; Karasiewicz et al., 2017). OMI analysis introduced marginality, tolerance and residual tolerance indexes for every species j in its habitat in absolute and relative values (Dolédec et al., 2000). Marginality index (OMI_j) is the distance between the average species habitat (centroid) to the average of the sampling area (origin). Tolerance index (Tol_j) is a measurement of the niche breadth of the species. Residual tolerance ($Rtol_j$) represents the unexplained variance by the analysis. The scores sum of the previous indexes gives the inertia of the explanation of the environmental variables for each species (Dolédec et al., 2000). On the other hand, WitOMI analysis enables to study species' niche at a temporal and/or spatial finer scale, as a consequence of the niche decomposition into subsets to create subniches (Karasiewicz et al., 2017). For this purpose, WitOMI analysis combines the OMI properties with the K-select analysis species marginality decomposition (Calenge et al., 2005). For every subset k (specific environmental conditions) and species j, marginality ($WitOMIG_{kj}$), tolerance (Tol_{kj}) and residual tolerance ($Rtol_{kj}$) can be calculated in reference to the subset conditions (i.e. the so-called WitOMIGk analysis) or to the overall conditions (i.e. the so-called WitOMIG analysis). In the present study only the $WitOMIG_k$ analysis was performed. Zooplankton niches were studied as follows: (i) first, using data pooled for the realized niche of the selected 43 taxa (Table 1) obtained during the study period (1998–2015) at both estuaries jointly by an OMI analysis based on a PCA of the eight monitored environmental variables. The marginality significance ($p < 0.05$) for each taxon was assessed by means of a Monte Carlo permutation test (1000 permutations), under the null hypothesis that each taxon is non-dependent on the set of environmental factors under study (Dolédec et al., 2000). (ii) Then, the realized subniches for the same taxa as in (i) were analysed from the output of the previous OMI analysis by means of a WitOMI analysis. This analysis was carried out under two subsets corresponding to each of the two estuaries. The statistical significance ($p < 0.05$) of the marginality for every taxon was assessed in relation to the average environmental conditions of each subset ($WitOMIG_k$ analysis) through a Monte Carlo permutation test (1000 permutations). Finally, (iii) in order to disentangle the temporal changes induced by the arrival of new copepod species during the study period in each estuary, a WitOMI analysis as in (ii) was performed for the 3 periods that were established according to the sequential arrival of such species (Barroeta et al., 2020). Period 1 was prior to the establishment of *Acartia tonsa* and *Oithona davisae* populations and spanned from 1998 to 2002; period 2 covered from 2003 to 2009 with *A. tonsa* and *O. davisae* present in both estuaries, but before the occurrence of *Pseudodiaptomus marinus*, and finally, period 3, from 2010 to 2015 was initiated with the first occurrences of *P. marinus* and the marked increase of the colonising species *Calanipeda aquaedulcis* in the estuary of Bilbao. In total six subsets were selected: B.1, B.2, B.3, U.1, U.2, U.3, corresponding to each estuary (B: Bilbao, U: Urdaibai) and time period. Again, the statistical significance ($p < 0.05$) of the marginality for each taxon in relation to the average environmental conditions of each subset were tested through a Monte Carlo permutation test (1000 permutations).

In addition, the niche overlap between NIS copepods and native copepods that appeared at least five times in the same period subset (Broennimann et al., 2012) were calculated. For this purpose, the ecological niche of every species was estimated. The subsets of environmental conditions represented by the first two axes of the OMI analysis were gridded into a 100 × 100 grid. Then, based on the species occurrence in every grid in the subset, the species niches were estimated using a kernel density estimation (see Figure 2 in Hernandez Fariñas et al., 2015). Finally, the D-metric was used to quantify the niche overlap between each pair of species (Warren et al., 2008; Schoener, 2013):

$$D_{1,2} = 1 - \frac{1}{2}\sum_{ij}\left|p_{1ij} - p_{2ij}\right|$$

Where p_{1ij} and p_{2ij} are respectively the occupancy of the species p_1 and p_2 in the *ij* grid. The D-metric ranges between 1 (full overlap between both species) and 0 (no overlap).

All numerical analyses were performed using the R Studio software (Team, 2013), with the packages *ade4* (Bougeard and Dray, 2018) for OMI and WitOMI analyses and *ecospat* for the niche overlaps calculations (Broennimann et al., 2020).

3. Results

3.1. Distribution of zooplankton community taxa with salinity in each estuary

As shown in Table 1, most of the 43 zooplankton taxa recorded in the estuaries of Bilbao and Urdaibai were most abundant in the highest salinity zone of 35, i.e. 16 and 14 out of the 25 species (assemblages) of copepods, respectively, and 12 and 10 taxa out of the 18 non-copepod

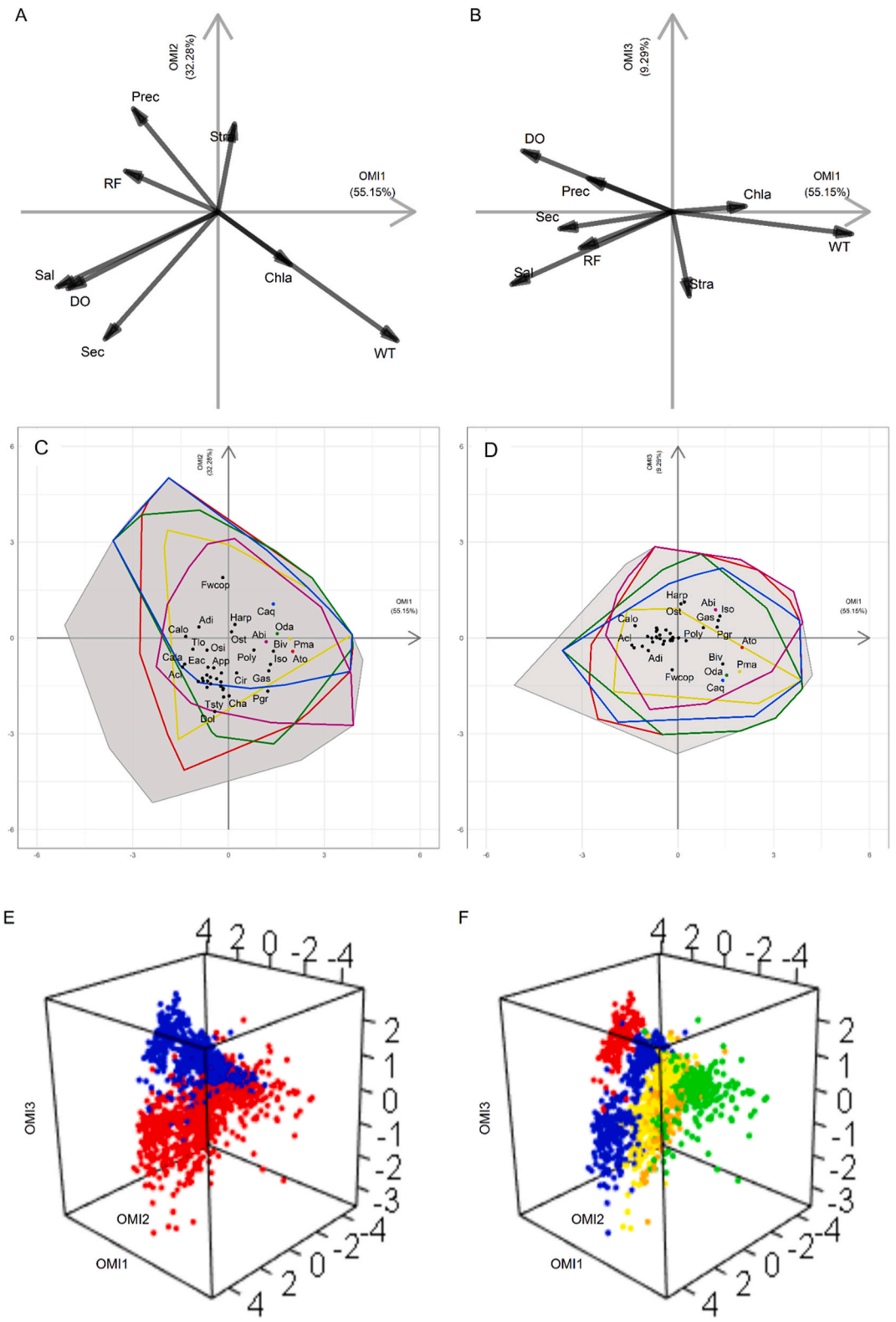


Fig. 2. Joint OMI analysis of the zooplankton community of the estuaries of Bilbao and Urdaibai. Top panels: Canonical weights of environmental variables on (A) the first two factorial axes (OMI1 and OMI2) and (B) the first and third factorial axes (OMI1 and OMI3). Mid panels: realized niche centroids of the statistically significant zooplankton taxa on (C) OMI1 and OMI2 and (D) OMI1 and OMI3. Coloured points represent NIS and *A. bifilosa* and *C. aquaedulcis* copepods. The light grey shaded convex polygon represents the environmental conditions constrained at the regional scale (pooled data for the two estuaries). Bottom panels: plots of all sampling points in the first three factorial axes of the OMI analysis, distinguishing between (E) estuaries (red dots for the estuary of Bilbao, and blue dots for the estuary of Urdaibai) and (F) salinity zones (red dots for the 26 salinity, blue ones for the 30 salinity, yellow ones for the 33 salinity, orange ones for the 34 salinity and green ones for the 35 salinity zone). Taxa abbreviations as in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

groups, respectively. The dominant copepod species that peaked at 35 salinity in both estuaries were the calanoids *Acartia clausi* and the assemblage PCPC-calanus (mostly *Paracalanus parvus*) and the cyclopoids *Oithona similis* and *Oithona nana.* Among non-copepod groups, the most abundant holoplankton groups that peaked at 35 salinity in both estuaries were cladocerans and appendicularians. The copepods that reached highest densities at the lowest salinity zones of both estuaries were the NIS *Acartia tonsa*, *Calanipeda aquaedulcis* and the assemblage of freshwater cyclopoid species. *Acartia bifilosa* and the NIS *Pseudodiaptomus marinus* and *Oithona davisae* peaked in both estuaries at the salinity zone of 30. The assemblage of benthic harpacticoid species also peaked at this salinity zone in the estuary of Urdaibai. *Acartia discaudata* and *Acartia margalefi* peaked at the intermediate salinities of 33–34 in both estuaries, and so did *Paracartia grani* in the estuary of Urdaibai. Among the most abundant non-copepod groups of the meroplankton, cirripede larvae and gastropod larvae peaked at the lowest salinity zone (26) of the estuary of Urdaibai but at the high salinity zones of 34 and 35, respectively, in the estuary of Bilbao. In contrast, bivalve larvae peaked at the lowest salinity in this estuary but at the highest salinity in that of Urdaibai.

According to the Monte Carlo test, of the 43 available taxa from the general inventory, only four taxa (*Acartia margalefi, Candacia* sp., *Microsetella* sp. and Mysids) showed no detectable influence of the environmental variables under study (Table 2), and as a consequence, they were not included in the subsequent analyses.

3.2. Zooplankton community niches in the environmental space of both estuaries jointly

The three first axis of the joint OMI analysis for the zooplankton community of both estuaries explained 96.72% of the variability (Fig. 2). The first component (OMI1, 55.15% of the variability) underscored seasonal and spatial segregation of zooplankton taxa mainly explained by the opposite effects of water temperature and closely

Table 3
Environmental variables (precipitation, river flow, water temperature, salinity, stratification, dissolved oxygen, *Secchi* disk and chlorophyll *a*) scores for each of the main three axis of the OMI analysis. In bold the highest scores at each axis.

Environmental variables	Axis 1	Axis 2	Axis 3
Precipitation	−0.250	**0.398**	0.240
River flow	−0.274	0.157	−0.260
Water temperature	**0.530**	**−0.497**	−0.159
Salinity	**−0.476**	−0.290	**−0.522**
Salt stratification	0.049	**0.340**	**−0.606**
Dissolved oxygen	**−0.443**	−0.297	**0.440**
Sechhi disk	−0.334	**−0.491**	−0.121
Chlorophyll *a*	0.215	−0.205	0.040

Table 2
Niche parameters of the zooplankton taxa selected by Monte Carlo permutation tests. The inertia (I), marginality (OMI), tolerance index (Tol) and residual tolerance index (Rtol) were computed for every taxon at a regional scale (both estuaries jointly) and inertia (I_K), marginality ($WitOMIG_K$), tolerance (Tol_K) and residual tolerance ($Rtol_K$) were calculated for every taxon at a local scale (the estuary of Bilbao and the estuary of Urdaibai). Significant p-values ($p < 0.05$) of Monte Carlo permutations (1000) are in bold. Taxa abbreviations as in Table 1.

	Both estuaries jointly					Estuary of Bilbao					Estuary of Urdaibai				
Taxa	I	OMI	Tol	Rtol	P	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	P	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	P
Abi	7.87	2.26	0.99	4.62	**0.001**	6.46	2.77	1.14	2.55	**0.002**	6.18	0.76	1.43	4.00	0.085
Acl	11.32	3.17	3.15	5.00	**0.001**	11.52	3.23	3.43	4.86	**0.001**	6.90	1.95	0.87	4.07	**0.001**
Ato	9.71	4.33	1.61	3.77	**0.001**	8.05	3.18	1.36	3.51	**0.003**	9.67	7.26	1.00	1.41	**0.001**
Adi	5.59	1.45	0.91	3.23	**0.001**	4.86	0.79	0.76	3.31	0.127	4.15	2.03	0.76	1.36	**0.007**
Pgr	6.75	4.63	0.44	1.68	**0.039**	—	—	—	—	—	5.95	3.83	0.43	1.69	0.093
Cala	14.24	3.06	3.47	7.72	**0.001**	14.28	2.79	3.37	8.12	**0.010**	6.42	1.53	0.92	3.97	**0.027**
Calo	9.24	2.03	1.23	5.98	**0.001**	10.15	2.54	2.06	5.55	**0.001**	6.07	2.59	0.45	3.03	**0.002**
PCPC	9.25	2.30	2.17	4.79	**0.001**	10.41	3.13	2.46	4.82	**0.001**	6.07	1.33	1.54	3.19	**0.001**
Cen	8.74	2.98	1.62	4.14	**0.001**	10.19	3.89	1.86	4.44	**0.001**	5.44	2.12	1.16	2.17	**0.002**
Tlo	9.07	1.75	1.39	5.93	**0.016**	10.48	4.11	2.82	3.55	**0.004**	8.31	4.37	1.74	2.20	**0.004**
Tsty	7.96	3.66	1.23	3.07	**0.001**	9.12	5.11	1.10	2.91	**0.001**	5.64	1.90	1.07	2.67	**0.002**
Pma	10.59	4.93	2.46	3.20	**0.013**	10.86	5.21	2.39	3.26	**0.033**	2.68	1.35	0.14	1.19	0.448
Caq	8.89	5.40	0.37	3.12	**0.001**	8.42	4.95	0.42	3.05	**0.023**	4.75	1.19	1.28	2.28	0.417
Opl	7.02	2.07	1.38	3.56	**0.001**	7.64	3.01	1.55	3.08	**0.001**	6.06	1.60	0.81	3.65	**0.006**
Ona	8.50	2.64	1.86	4.00	**0.001**	9.80	4.03	1.81	3.97	**0.001**	5.69	1.04	1.54	3.11	**0.004**
Osi	7.37	0.73	1.30	5.34	**0.001**	8.99	1.59	2.42	4.99	**0.001**	4.82	0.46	0.82	3.54	**0.002**
Oda	9.57	3.86	2.14	3.57	**0.001**	9.57	3.88	1.92	3.76	**0.002**	1.78	0.35	0.17	1.27	0.600
Fwcop	11.82	5.92	2.62	3.29	**0.002**	10.25	7.14	0.90	2.22	**0.007**	6.35	1.39	2.44	2.52	0.260
Onc	7.11	2.11	1.56	3.44	**0.001**	9.34	5.08	1.43	2.84	**0.001**	4.53	0.71	0.95	2.87	**0.029**
Dan	7.69	2.56	1.47	3.65	**0.001**	8.88	4.11	1.76	3.00	**0.001**	6.13	1.53	1.13	3.47	**0.001**
Eac	7.66	1.47	2.14	4.05	**0.001**	10.14	3.63	2.86	3.66	**0.001**	4.75	0.64	0.99	3.12	**0.012**
Harp	5.98	1.73	0.70	3.55	**0.014**	8.38	0.41	3.99	3.98	0.625	4.48	0.59	0.69	3.21	0.458
Med	7.62	1.28	1.77	4.58	**0.001**	8.36	1.75	2.21	4.41	**0.001**	5.46	0.87	1.02	3.58	**0.001**
Sip	8.45	2.28	2.02	4.15	**0.001**	8.98	2.93	2.09	3.96	**0.001**	5.00	0.51	0.80	3.69	**0.012**
Cyp	7.09	1.90	1.66	3.53	**0.001**	8.52	3.22	1.95	3.35	**0.001**	4.67	0.91	1.08	2.68	**0.003**
Gas	9.79	2.98	2.07	4.74	**0.001**	9.01	1.51	3.31	4.19	**0.007**	8.27	2.56	1.74	3.96	**0.005**
Biv	9.07	2.88	2.25	3.95	**0.018**	9.67	3.87	1.97	3.83	**0.027**	5.46	0.99	1.02	3.45	0.264
Poly	8.83	0.99	2.89	4.94	**0.001**	7.56	0.14	2.06	5.36	0.287	7.57	1.67	2.44	3.46	**0.001**
Cha	10.83	3.36	1.74	5.73	**0.001**	12.03	4.56	1.89	5.58	**0.001**	6.49	1.92	0.79	3.79	**0.001**
Cla	8.91	2.54	2.32	4.05	**0.001**	9.31	2.99	2.43	3.90	**0.001**	5.94	1.02	1.04	3.89	**0.002**
Ost	6.30	1.40	0.56	4.34	**0.001**	6.41	1.48	0.82	4.12	**0.001**	5.07	0.26	1.82	3.00	0.229
Cir	8.20	1.32	1.32	5.56	**0.001**	8.20	1.76	1.73	4.71	**0.001**	7.34	1.91	1.90	3.54	**0.001**
Iso	8.44	2.93	1.84	3.67	**0.001**	8.71	0.68	3.08	4.94	**0.013**	6.93	1.77	2.24	2.92	**0.002**
Dec	9.44	2.71	2.37	4.35	**0.001**	10.74	4.09	2.74	3.91	**0.001**	5.46	1.22	1.25	2.99	**0.001**
Ech	9.52	2.81	2.67	4.03	**0.001**	10.08	3.86	2.39	3.82	**0.001**	6.28	1.71	1.03	3.54	**0.005**
App	7.73	1.15	2.10	4.48	**0.001**	7.99	1.32	2.56	4.11	**0.001**	5.55	0.75	1.16	3.64	**0.001**
Dol	9.01	5.69	0.79	2.53	**0.001**	9.68	6.49	0.66	2.53	**0.001**	5.95	2.43	1.34	2.18	**0.018**
Asc	7.42	2.22	2.35	2.86	**0.002**	8.42	3.40	2.34	2.68	**0.008**	3.28	0.20	0.21	2.87	0.914
Ict	8.25	2.43	1.89	3.93	**0.001**	8.52	3.05	1.92	3.55	**0.001**	6.38	0.23	0.75	5.40	0.432

related variables like salinity and dissolved oxygen (Fig. 2A and B, and Table 3). Warm and brackish water affinity taxa such as *A. tonsa, P. marinus*, *O. davisae, C. aquaedulcis A. bifilosa, P. grani*, and bivalve and gastropod larvae were plotted towards the positive end of this axis, whilst late winter-early spring taxa of neritic origin, such as *A. clausi*, *Calanus* sp., *Temora longicornis* and *Calocalanus* sp. were plotted in the most negative positions (Fig. 2C and D, and Fig. 3). The second axis (OMI2, 32.28% of the variability) also reflected seasonal and longitudinal variations, but mainly conditions of high temperature and water transparency as opposed to conditions of high precipitation and water column salt stratification (Figs. 2A and 3 and Table 3). This factor mainly illustrated the opposition of a zooplankton assemblage mostly associated to winter-spring stratified conditions, i.e. freshwater copepods and *C. aquaedulcis*, on the positive end and a neritic assemblage of warm water affinity taxa represented by doliolids, *Temora stylifera* and chaetognaths on the negative one (Figs. 2C and 3). The third component (OMI3, 9.29% of the variability) mainly separated the lowest salinity zones of the two estuaries (Fig. 2B, E and 2F), in relation to the higher water column stratification and salinity, and the lower dissolved oxygen concentration in the estuary of Bilbao (Fig. 2B and Table 3). *C. aquaedulcis*, *O. davisae*, *P. marinus* and freshwater copepods were the best related to the high stratification conditions that characterized the inner estuary of Bilbao, in opposition to *A. bifilosa*, which together with some tychoplanktonic forms (benthic harpacticoids and ostracods) were the most distinctive taxa of the inner estuary of Urdaibai (Fig. 2D, E and 2F).

Tolerance and marginality values of zooplankton taxa are shown in Fig. 4 and Table 2. *Calanus* sp. and *A. clausi* showed highest tolerance and relatively high marginality. *C. aquaedulcis*, doliolids and *P. grani*, differed from most of the other zooplankton components due to their high marginality and low tolerance. The three NIS (*A. tonsa*, *P. marinus* and *O. davisae*), also differed from the other taxa due to their high marginality but had intermediate values of tolerance. Among neritic copepod species, the highest marginality was for *T. stylifera*. *A. discaudata* and benthic harpacticoids and ostracods showed both low marginality and tolerance. *A. bifilosa* also showed low tolerance, but its marginality was similar to that of most taxa. The highest marginality, with relatively high tolerance, was obtained for freshwater copepods,

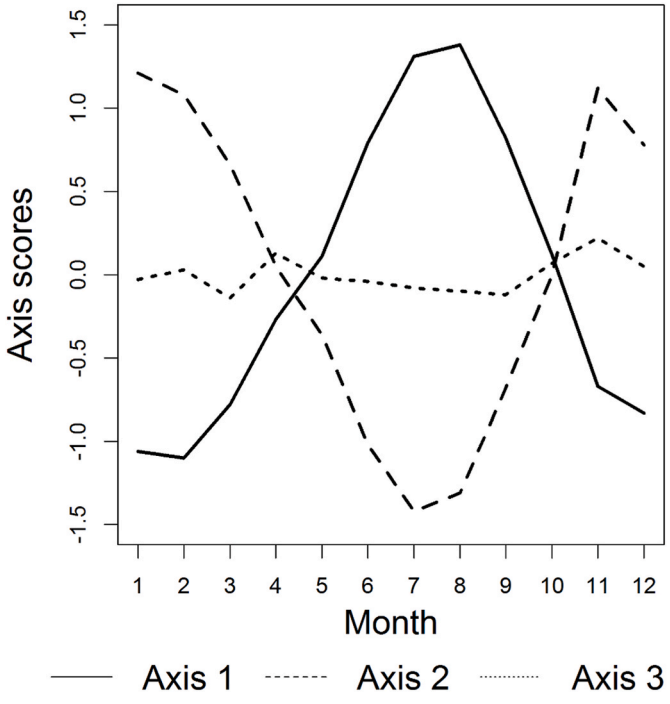


Fig. 3. Month to month variations of the three main axes of the OMI analyses.

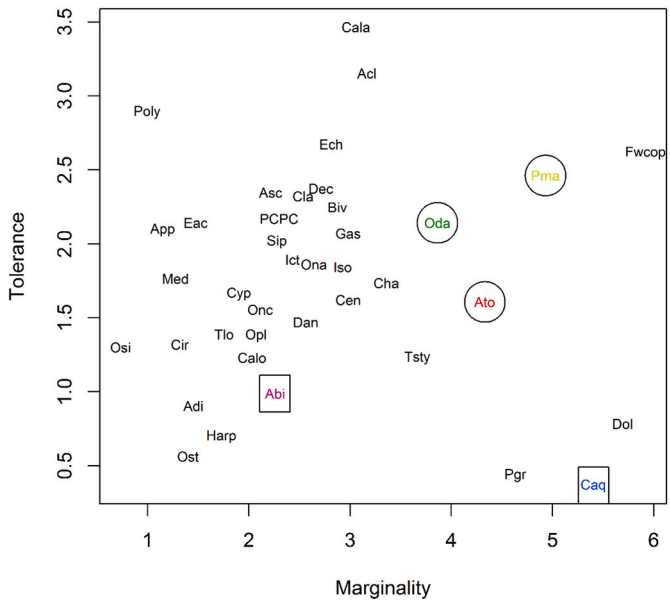


Fig. 4. Bivariate plot of all zooplankton taxa according to their marginality and tolerance. NIS and *A. bifilosa* and *C. aquaedulcis* copepods are shown inside circles and squares, respectively. Copepod taxa colours and abbreviations as in Fig. 2 and Table 1, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

whilst the lowest marginality, with relatively low tolerance, for *O. similis*. Polychaete larvae differed by their low marginality and high tolerance.

3.3. Between-estuary differences in zooplankton niches

On the 39 selected taxa of both estuaries, the Monte Carlo permutation tests were significant for 26 taxa in the subsets of both estuaries, 3 were not significant in the subset of the estuary of Bilbao and 11 were not significant in the subset of the estuary of Urdaibai (Table 2). The tests for benthic harpacticoids were not statistically significant in any estuary; for polychaete larvae and the copepod *A. discaudata* they were not statistically significant in the estuary of Bilbao, whilst for several groups (ascidian and bivalve larvae, ostracods, ichthyoplankton and freshwater copepods), the native copepods *A. bifilosa*, *P. grani* and *C. aquaedulcis*, and the NIS *O. davisae* and *P. marinus* they were not statistically significant in the estuary of Urdaibai.

The tolerance or the marginality of most copepod species and zooplankton groups were higher in the estuary of Bilbao than in the estuary of Urdaibai (Fig. 5A and D). The most noticeable exception was the higher marginality of *A. tonsa* in the estuary of Urdaibai. In addition, tolerance and marginality values were correlated negatively for both copepod species and zooplankton groups in the estuary of Bilbao ($p = 0.002$ and $p = 0.007$, respectively) but not correlated in the estuary of Urdaibai ($p = 0.165$ and $p = 0.604$, respectively). Regarding copepod species, comparatively, summer-autumn neritic species of warm water affinity like *O. nana, T. stylifera* and *Oncaea* sp. showed higher tolerance and lower marginality in the estuary of Urdaibai, whilst winter-spring neritic species like *T. longicornis* and *Calocalanus* sp. showed higher tolerance and lower marginality in the estuary of Bilbao (Fig. 5B and C, and Table 2). Among *Acartia* species, the neritic species *A. clausi* showed much higher tolerance in the estuary of Bilbao and similar marginality in both estuaries, whilst the NIS *A. tonsa* reached higher tolerance in the estuary of Bilbao but showed much higher marginality in the estuary of Urdaibai. Regarding non-copepod groups (Fig. 5E and F), doliolids were the only taxa that had higher tolerance in the estuary of Urdaibai and higher marginality in the estuary of Bilbao, and gastropod larvae and isopods those that showed higher marginality in the estuary of Urdaibai

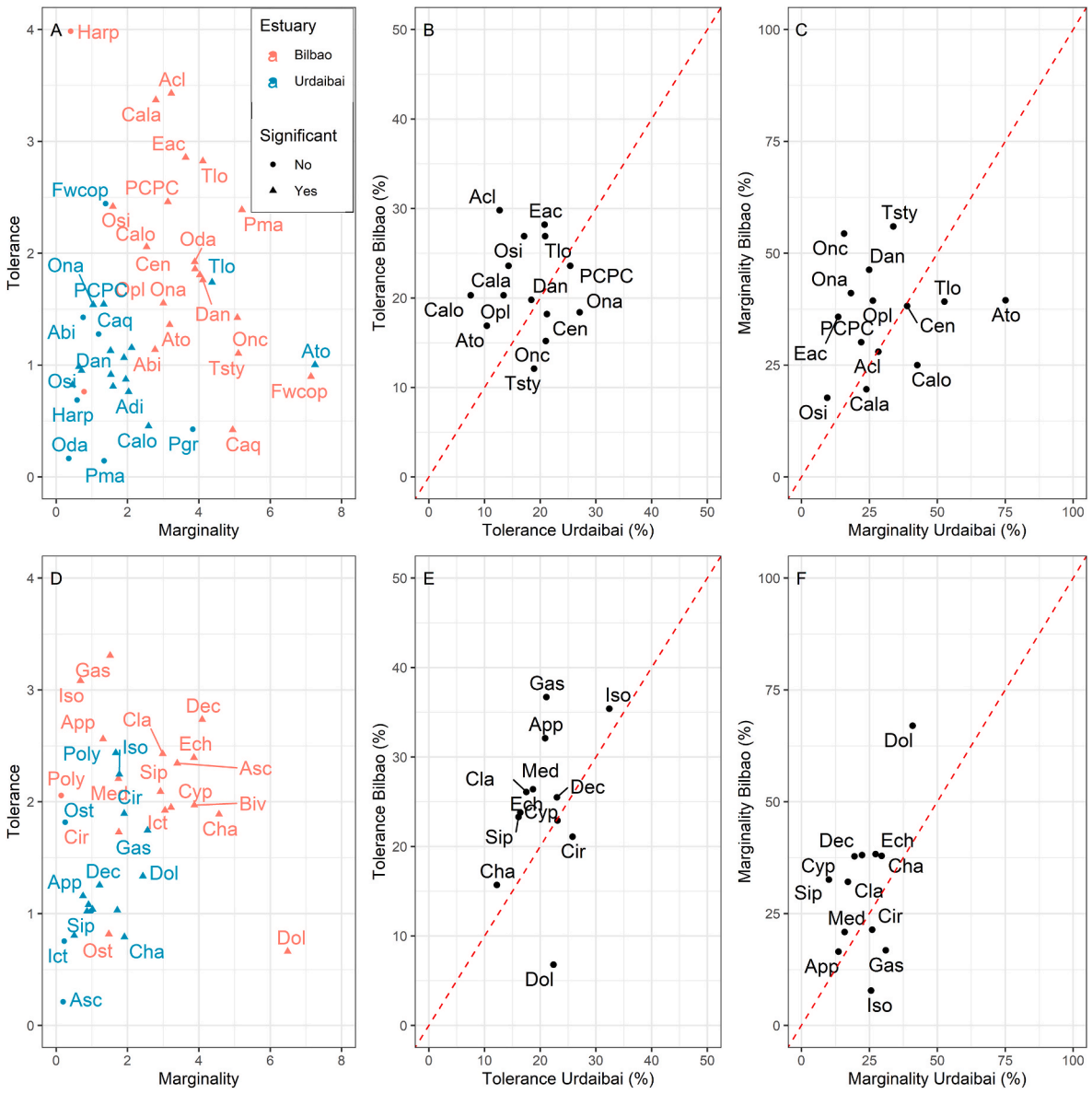


Fig. 5. (A and D) Plots of tolerance (Tol_k) and marginality ($WitOMIG_k$) from the suborigin of each subset, i.e. each estuary. Taxa with statistically significant realized subniches are represented by triangles. (B and E) Plots of taxa according to their tolerances (Tol_k, in percentage) in the estuaries of Bilbao and Urdaibai. (C and F) Plots of taxa according to their marginalities ($WitOMIG_k$, in percentage) in the estuaries of Bilbao and Urdaibai. Top (A, B and C) and bottom (D, E and F) panels show copepod species and non-copepod taxa, respectively. Taxa abbreviations as in Table 1.

and higher tolerance in the estuary of Bilbao.

3.4. Temporal changes of zooplankton niches in each estuary

According to the Monte Carlo permutation test only four taxa (cirripede larvae, *A. clausi*, decapod larvae and appendicularians) showed significant values of marginality in the six subsets and for the 35 remaining taxa, 25 were significant at least in two periods in the same estuary (Table 4). However, the number of taxa with statistically significant values was clearly higher in the estuary of Bilbao than in the estuary of Urdaibai in each of the three periods. In both estuaries, the mean environmental conditions of the three periods were close to the origin of the OMI components (Figs. 6A and 7A), but in the estuary of Bilbao a gradual displacement of the centroid over time along the first OMI component was observed, whilst in the estuary of Urdaibai the three centroids overlapped. The niche decomposition into temporal subsets highlighted niche shifts along the two main OMI components over time for most of the neritic copepods, including the congeneric species of the NIS, in the estuary of Bilbao (Fig. 6B and C). For the non-copepod groups niche shifts were also evident in some cases in this estuary (Fig. 6D and E). In the estuary of Urdaibai, no niche shift was observed, or was clearly lower than in the estuary of Bilbao, but shifts did not occur in the same direction for the few neritic copepods for which significant values were obtained (Fig. 7C). The exception was the remarkable shift of the congeneric *A. clausi* along the second axis from period 1 to period 2 (Fig. 7B). As for non-copepods, no shift, or weak shifts in different directions were observed in the estuary of Urdaibai (Fig. 7D and E).

The temporal change of the niche overlap between NIS and the other copepod species differed between estuaries (Fig. 8). In the estuary of Bilbao, the niche overlap of *A. tonsa* and *O. davisae* with most of the neritic and congeneric species increased from period 1 to period 2, and decreased in period 3, with the main exception of the overlap of *A. tonsa* with *A. bifilosa*, which clearly increased from period 2 to period 3. In the estuary of Urdaibai, however, *A. tonsa*'s niche overlap with most of taxa increased from period 2 to period 3, except with *Calanus* sp. and *P. grani*.

Table 4
Niche parameters of the zooplankton taxa. In the spatio-temporal subsets, inertia (I_K), marginality ($WitOMIG_K$), tolerance (Tol_K) and residual tolerance ($Rtol_K$) were estimated at the local scale ($WitOMIG_K$ analysis, see result section iii). Significant p-values ($p < 0.05$) of Monte Carlo permutations (1000) are in bold. Taxa abbreviations as in Table 1.

Taxa	Estuary of Bilbao												
	Period 1					Period 2					Period 3		
	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	p	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	p	I_k	$WitOMI_k$	Tol_k
Abi	—	—	—	—	**—**	7.80	6.78	0.52	0.49	**0.001**	6.15	2.47	1.00
Acl	7.05	1.85	1.35	3.85	**0.015**	5.73	1.05	0.94	3.75	**0.005**	18.69	9.87	4.05
Ato	6.16	2.42	2.06	1.68	0.060	8.98	3.74	1.53	3.71	**0.008**	7.65	3.42	1.17
Adi	4.42	0.85	0.45	3.12	0.513	4.97	1.32	0.97	2.69	0.183	5.33	1.78	0.77
Pgr	—	—	—	—	**—**	—	—	—	—	**—**	—	—	—
Cala	6.66	3.48	1.00	2.19	0.055	5.46	0.83	1.05	3.59	0.343	25.31	9.10	4.92
Calo	4.29	1.84	0.42	2.03	0.141	7.53	2.63	2.07	2.83	**0.013**	17.83	6.05	3.51
PCPC	8.56	1.68	1.67	5.21	**0.008**	7.08	2.25	1.67	3.16	**0.001**	13.28	5.50	3.16
Cen	8.99	3.19	1.31	4.50	**0.019**	6.40	2.25	1.24	2.91	**0.008**	11.94	6.17	1.82
Tlo	5.39	1.16	1.43	2.80	0.441	5.05	1.55	0.89	2.62	0.231	13.45	7.33	2.29
Tsty	6.14	3.20	0.57	2.37	**0.036**	8.59	4.79	1.07	2.72	**0.001**	11.87	9.52	1.20
Pma	—	—	—	—	**—**	—	—	—	—	**—**	12.34	6.69	2.45
Caq	9.20	8.41	0.02	0.78	**0.044**	8.79	8.37	0.25	0.17	**0.019**	9.12	5.65	0.57
Opl	6.91	1.99	1.45	3.46	0.075	7.35	3.23	1.42	2.70	**0.008**	13.87	11.29	1.21
Ona	8.20	1.28	1.18	5.74	0.070	7.45	2.95	1.67	2.83	**0.001**	11.47	7.16	1.73
Osi	6.24	1.24	1.38	3.63	**0.018**	5.79	0.91	1.05	3.83	**0.005**	14.34	4.24	4.29
Oda	5.01	1.18	1.29	2.54	0.358	12.38	6.96	2.44	2.98	**0.002**	8.69	4.14	1.11
Fwcop	7.80	1.96	1.99	3.85	0.308	9.81	7.57	0.53	1.72	**0.012**	13.83	5.75	2.06
Onc	7.47	3.24	0.96	3.27	**0.007**	7.31	3.91	0.96	2.45	**0.001**	11.03	7.39	1.55
Dan	8.05	2.81	1.06	4.19	**0.019**	7.70	2.67	1.68	3.35	**0.002**	9.61	5.89	1.83
Eac	6.77	1.29	1.39	4.09	0.055	7.32	1.79	1.73	3.81	**0.005**	11.85	5.85	2.96
Harp	7.80	0.24	1.54	6.01	0.860	8.19	0.22	2.61	5.36	0.864	8.44	1.66	3.83
Med	7.99	1.21	1.50	5.28	**0.031**	7.14	1.86	1.82	3.45	**0.002**	9.79	2.49	2.66
Sip	6.40	1.48	1.11	3.80	**0.027**	8.09	2.25	1.50	4.34	**0.001**	12.25	7.34	1.77
Cyp	6.96	2.19	1.26	3.52	**0.024**	6.22	2.41	0.81	3.00	**0.003**	12.12	5.80	3.01
Gas	7.35	1.48	1.71	4.15	0.154	7.02	1.15	2.48	3.38	0.151	10.49	1.93	4.36
Biv	13.84	3.42	5.33	5.09	0.077	9.58	3.13	2.48	3.97	0.089	10.73	5.61	1.87
Poly	7.04	0.52	1.73	4.79	0.190	7.10	0.16	1.31	5.64	0.509	7.77	0.06	1.21
Cha	6.49	1.94	1.10	3.44	**0.023**	12.48	5.47	2.79	4.22	**0.001**	14.62	9.47	2.67
Cla	6.11	2.08	0.98	3.06	**0.010**	6.96	1.88	2.00	3.08	**0.001**	14.98	8.18	3.27
Ost	7.28	1.32	1.60	4.37	0.062	7.61	1.46	1.53	4.62	**0.012**	4.92	2.19	0.25
Cir	10.23	1.61	2.08	6.54	**0.004**	6.00	1.60	1.42	2.98	**0.001**	9.91	2.41	2.38
Iso	5.57	0.19	0.78	4.60	0.733	6.94	1.22	1.88	3.84	**0.018**	11.73	1.20	3.01
Dec	8.71	3.12	1.51	4.08	**0.002**	7.05	1.67	2.13	3.25	**0.002**	12.52	5.89	2.74
Ech	5.61	1.95	1.02	2.64	0.111	7.36	2.89	1.51	2.96	**0.008**	17.33	12.86	2.82
App	7.20	1.23	1.61	4.36	**0.004**	6.27	0.80	2.18	3.29	**0.001**	10.12	2.31	3.02
Dol	9.26	6.46	0.85	1.96	**0.027**	9.25	6.26	0.56	2.42	**0.009**	10.86	8.54	0.78
Asc	3.34	1.42	0.17	1.76	0.487	7.04	2.56	1.63	2.85	0.109	9.07	4.61	2.30
Ict	5.55	3.06	0.61	1.88	**0.022**	6.45	2.53	1.28	2.64	**0.008**	12.40	4.81	3.45

Similarly, *O. davisae*'s niche overlap increased from period 2 to period 3 with congeneric species but varied with the neritic taxa. As *P. marinus* did not appear more than 5 times in most of the temporal subsets, it was not possible to assess changes in its overlap with other copepod species. The species that showed the highest overlap with the NIS *A. tonsa* and *O. davisae* did not change from period 2 to period 3 in any estuary. In the estuary of Bilbao, both NIS showed higher overlap in periods 1 and 2 with PCPC-calanus than with any of the respective congeneric species. In the estuary of Urdaibai, however, both showed the highest overlap in periods 1 and 2 with their congeneric species *A. bifilosa* and *O. nana*, respectively.

4. Discussion

4.1. Zooplankton taxa in the overall environmental scenario of the two estuaries

Results revealed that a blend of spatial and temporal patterns of water physical and chemical features drove the overall environmental segregation of the major zooplankton assemblages that inhabited the estuaries of Bilbao and Urdaibai. As observed before in other coastal transitional environments, salinity and temperature showed the highest contribution to model the studied zooplankton communities (Marques et al., 2008; Albaina et al., 2009; Zervoudaki et al., 2009; Oda et al., 2018). The OMI highlighted the typical differentiation (in terms of salinity habitat) of estuarine zooplankton in freshwater, brackish and marine neritic assemblages. Moreover, a clear seasonal differentiation of a neritic winter-spring assemblage and a neritic summer-autumn assemblage was clearly observed. Those neritic assemblages were constituted by taxa that rotate in the dominance or occurrence between the first and the second half of the annual cycle in the biogeographic region where our estuaries are located (Fanjul et al., 2018). The components of the brackish assemblage, which included all the NIS (*A. tonsa*, *O. davisae* and *P. marinus*) and *C. aquaedulcis*, showed weaker temporal segregation than those of the neritic assemblage. The three NIS, however, had higher warm water affinity than *C. aquaedulcis*, as it is corroborated by the occurrence of the annual peaks of *A. tonsa*, *O. davisae* and *P. marinus* in summer-early autumn and that of *C. aquaedulcis* in late spring-early summer (Barroeta et al., 2020). The OMI also evidenced between-estuary differences in the zooplankton assemblages of the inner part, due mainly to the higher presence of freshwater copepods in the estuary of Bilbao and tychoplanktonic forms (benthic harpacticoids and ostracods) in the estuary of Urdaibai, but also due to the higher abundance of the species *A. bifilosa* in the estuary of

Estuary of Bilbao		Estuary of Urdaibai														
Period 3		Period 1					Period 2					Period 3				
$Rtol_k$	p	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	p	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	P	I_k	$WitOMI_k$	Tol_k	$Rtol_k$	P
2.69	0.064	4.97	2.27	0.83	1.86	0.064	8.04	0.91	1.38	5.75	0.215	5.10	0.48	1.57	3.04	0.537
4.77	**0.001**	7.95	4.36	2.23	1.36	**0.001**	6.74	3.18	1.13	2.43	**0.001**	5.95	2.11	1.45	2.39	**0.002**
3.06	**0.029**	—	—	—	—	—	10.66	8.09	1.19	1.38	**0.001**	7.43	5.90	0.60	0.94	**0.007**
2.78	0.206	3.82	2.45	0.03	1.34	0.155	3.81	2.59	0.55	0.68	**0.036**	5.52	1.83	1.80	1.90	0.169
—	—	1.95	1.95	0.00	0.00	0.527	6.13	4.13	0.29	1.70	0.131	4.13	2.27	0.86	1.00	0.423
11.29	**0.002**	6.22	1.27	2.24	2.71	0.306	6.31	1.91	1.23	3.17	0.100	6.84	2.59	0.34	3.91	0.051
8.27	**0.008**	3.02	2.02	0.31	0.69	0.165	8.39	3.28	0.62	4.49	**0.008**	8.33	3.76	0.78	3.79	**0.014**
4.61	**0.001**	4.65	0.36	0.73	3.56	0.195	6.54	1.25	1.34	3.95	**0.007**	6.61	2.92	1.81	1.89	**0.003**
3.95	**0.003**	4.05	0.50	0.89	2.67	0.510	5.94	3.36	0.92	1.66	**0.006**	5.14	1.31	1.15	2.68	0.095
3.84	**0.022**	8.87	6.88	1.14	0.84	**0.019**	7.04	4.26	1.33	1.45	**0.029**	3.26	0.62	0.44	2.20	0.638
1.14	**0.002**	5.95	2.17	1.49	2.30	0.085	5.38	1.96	0.55	2.87	**0.035**	4.29	1.29	1.09	1.90	0.183
3.21	0.109	—	—	—	—	—	—	—	—	—	—	2.71	1.38	0.10	1.23	0.660
2.90	0.135	4.92	1.81	0.58	2.53	0.537	4.83	1.70	2.23	0.90	0.472	4.52	0.89	0.98	2.66	0.791
1.37	**0.002**	4.36	0.70	0.93	2.73	0.486	6.36	1.76	0.77	3.83	0.052	5.88	5.35	0.00	0.52	**0.006**
2.58	**0.001**	5.12	0.24	0.80	4.08	0.757	5.11	0.74	1.21	3.16	0.119	6.14	2.05	1.53	2.57	**0.016**
5.81	**0.001**	3.54	0.60	0.53	2.42	0.092	5.13	0.54	1.01	3.58	**0.039**	4.82	0.83	0.68	3.32	**0.023**
3.44	**0.045**	2.87	2.87	0.00	0.00	0.080	1.38	0.58	0.13	0.66	0.624	4.21	1.24	0.66	2.31	0.320
6.02	0.088	2.90	0.33	0.34	2.24	0.866	3.98	0.27	0.59	3.13	0.907	9.03	5.32	1.86	1.85	**0.036**
2.08	**0.001**	3.48	0.42	0.63	2.43	0.532	4.75	1.02	0.74	3.00	0.066	5.46	0.94	1.80	2.73	0.124
1.90	**0.003**	5.92	1.37	0.91	3.64	0.102	6.03	1.70	1.04	3.30	**0.019**	6.31	2.33	1.75	2.23	**0.018**
3.04	**0.002**	6.25	1.21	2.42	2.62	0.056	4.98	1.26	0.89	2.82	**0.026**	3.70	0.66	0.51	2.52	0.156
2.95	0.291	5.59	0.65	1.51	3.44	0.530	4.64	0.90	0.58	3.16	0.415	3.62	0.47	0.43	2.71	0.617
4.64	**0.005**	4.57	0.39	0.74	3.44	0.349	6.32	0.92	0.92	4.48	**0.015**	4.84	1.63	0.92	2.30	**0.005**
3.14	**0.001**	4.82	0.51	1.29	3.02	0.343	5.23	0.61	0.79	3.83	0.078	4.11	1.23	0.64	2.24	**0.025**
3.32	**0.001**	4.66	1.15	1.08	2.43	0.211	4.45	1.04	0.92	2.48	0.060	5.19	1.69	1.12	2.38	**0.041**
4.21	0.113	4.65	2.63	0.71	1.32	**0.043**	10.53	3.01	2.99	4.53	**0.008**	5.66	2.46	1.20	2.00	**0.035**
3.25	0.052	5.23	0.53	1.51	3.19	0.547	5.42	1.37	0.87	3.18	0.223	5.58	1.21	1.60	2.78	0.250
6.50	0.918	6.11	1.53	1.54	3.04	**0.035**	8.45	2.22	2.77	3.46	**0.001**	5.31	0.53	1.52	3.26	0.185
2.49	**0.001**	6.51	1.05	1.23	4.24	0.137	6.77	1.90	0.74	4.13	**0.003**	5.98	2.75	0.53	2.70	**0.003**
3.53	**0.001**	6.82	1.44	1.25	4.13	**0.032**	5.87	2.78	1.28	1.81	**0.001**	4.28	0.52	0.42	3.35	0.220
2.48	**0.021**	5.36	0.48	0.98	3.90	0.410	4.65	0.36	1.90	2.39	0.357	5.40	0.42	2.00	2.99	0.360
5.12	**0.001**	8.59	1.22	2.29	5.08	**0.012**	7.88	1.94	1.96	3.99	**0.001**	5.55	2.36	1.36	1.83	**0.001**
7.52	0.066	5.10	1.04	1.49	2.57	0.124	8.42	2.81	2.58	3.03	**0.004**	4.91	0.75	1.49	2.67	0.148
3.89	**0.001**	5.15	1.41	1.08	2.66	**0.039**	6.03	1.21	1.46	3.37	**0.007**	5.36	1.92	0.94	2.50	**0.006**
1.64	**0.001**	5.13	1.80	0.62	2.71	0.145	5.78	1.47	1.01	3.30	0.089	7.61	3.34	0.88	3.39	**0.016**
4.80	**0.001**	6.61	1.57	2.28	2.76	**0.006**	5.10	1.22	1.41	2.48	**0.001**	5.48	1.00	1.23	3.25	**0.004**
1.54	**0.013**	4.14	2.50	0.06	1.58	0.176	7.97	4.69	1.78	1.51	**0.017**	3.71	1.15	0.18	2.37	0.441
2.16	**0.066**	7.61	7.61	0.00	0.00	**0.031**	2.15	0.82	0.52	0.81	0.665	6.55	2.92	1.15	2.48	0.138
4.14	**0.003**	3.60	0.28	0.73	2.59	0.766	6.27	0.77	0.78	4.71	0.175	7.52	0.74	1.22	5.56	0.262

Urdaibai and *C. aquaedulcis* and *O. davisae* in the estuary of Bilbao. These reveal different environmental scenarios due to additional factors that modulate the predominant effect of spatial salinity gradients and seasonal variations of temperature in shaping zooplankton communities in estuaries. Thus, the higher inputs of freshwater that transport limnetic organisms into the estuary of Bilbao seem responsible for the higher presence of freshwater copepods in this system. The shallower depth and higher extent of intertidal areas promote the incorporation of benthic organisms to the water column by turbulence and tidal washing in the estuary of Urdaibai (Villate, 1997). Additionally, the higher stratification and stability of the water column in the estuary of Bilbao, as a result of its channelization and deepening (Uriarte et al., 2014), is considered too.

OMI results revealed differences in tolerance and marginality values between the species of the community at the regional scale (both estuaries). *Calanus* sp. and *A. clausi* showed the highest tolerance. *A. clausi* was also found among the taxa that presented the highest tolerance values in the Lagoon of Venice (Camatti et al., 2019). This indicates high niche breadths, which is coherent with their wide range of tolerance to salinity and temperature (Pedersen and Tande, 1992; Gaudy et al., 2000; Møller et al., 2012) and their similar abundance in both estuaries. However, clear environmental preferences were also brought to light by their marked salinity preferences and well-defined seasonal cycles in both estuaries (Uriarte and Villate, 2005; Fanjul et al., 2017). The low tolerance and marginality of taxa with low abundances such as benthic ostracods and harpacticoids and the copepod *A. discaudata* reflect narrow but not well-defined saline and thermal niches, suggesting that they may be more influenced by environmental factors not considered in the present study. This is corroborated by their marked difference in abundance between estuaries. The presence of benthic taxa in plankton samples is much higher in the estuary of Urdaibai due to the stronger hydrodynamics and shallowness of this system (Uriarte and Villate, 2004), but their occurrence is expected to be rather irregular due to the temporal asynchrony of winds, river discharges and tides which are the main drivers of benthic organisms' resuspension into the water column (Madariaga et al., 1992; Villate, 1997). Regarding *A. discaudata*, its seasonal pattern was little consistent over time in the estuary of Bilbao (Villate et al., 2018). Although a low marginality may also be due to a niche location close to the mean of environmental condition none of these taxa show optima at intermediate values of the two main factors, i.e. temperature and salinity, since *A. discaudata* is associated with low temperature and relatively high salinity in the estuary of Bilbao (Villate et al., 2018) and benthic harpacticoids and ostracods occur mainly at low salinity in the estuary of Urdaibai (Villate, 1982, 1991).

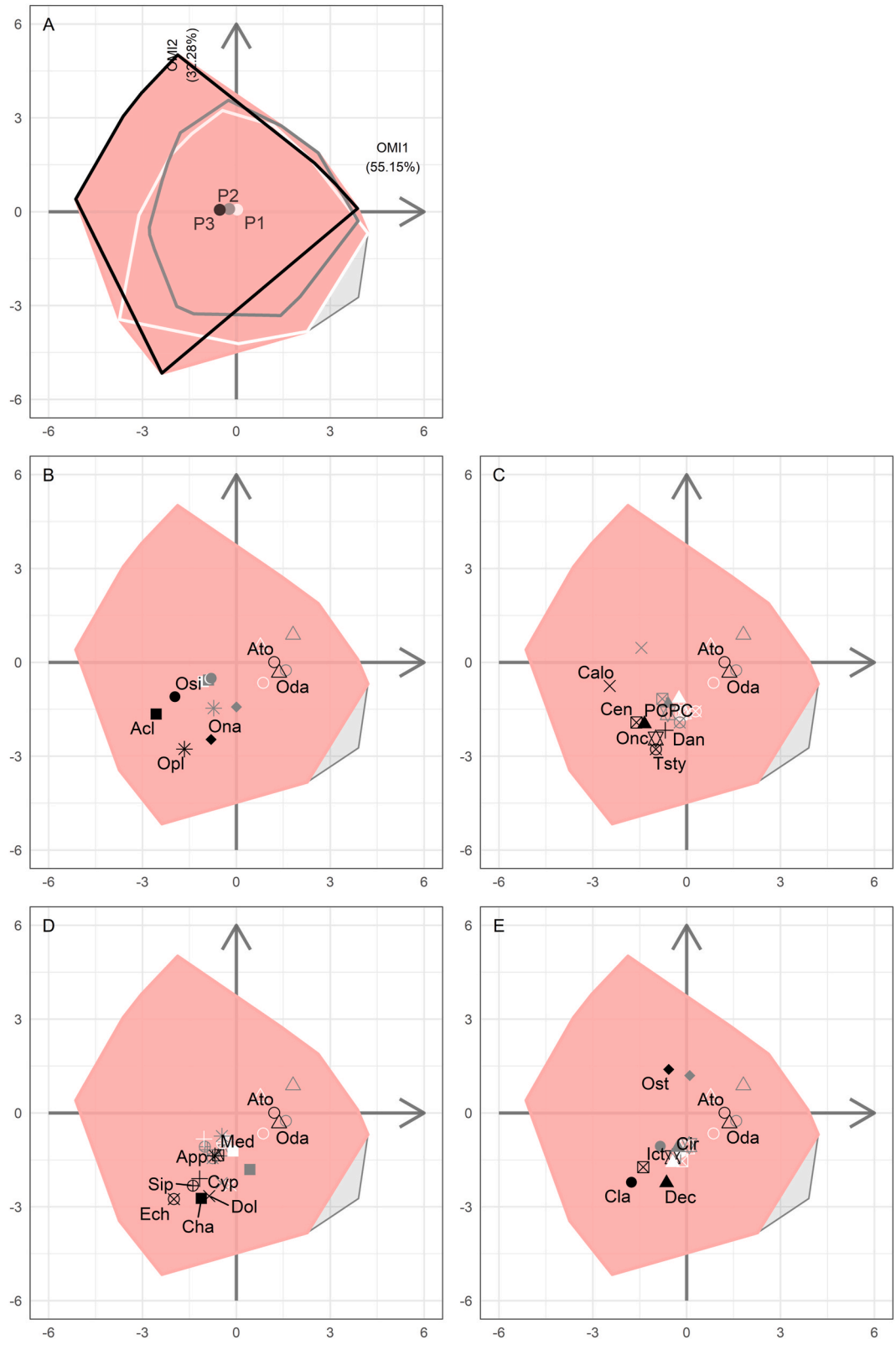


Fig. 6. Plot of the results of the WitOMI analysis of the spatio-temporal subsets in the two first OMI axes. The light grey shaded convex polygons represent the overall environmental conditions constrained at the regional scale (data pooled for the two estuaries under study). The red shaded convex polygons represent the habitat conditions of the estuary of Bilbao. (A) Representation of the environmenal conditions and the suborigins for each period (Period 1: white polygon and symbol; Period 2: grey polygon and symbol; Period 3: black polygon and symbol). The rest of panels show the centroids of the NIS together with those of (B) significant congenerics, (C) neritic copepod taxa, (D) neritic non-copepod taxa and (E) estuarine non-copepod taxa in the estuary of Bilbao, represented in the first two OMI axes. Each symbol stands for a species (see labels in the plot) and the colour of the symbol shows the time period (white for period 1, grey for period 2, and black for period 3). Taxa abbreviations as in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

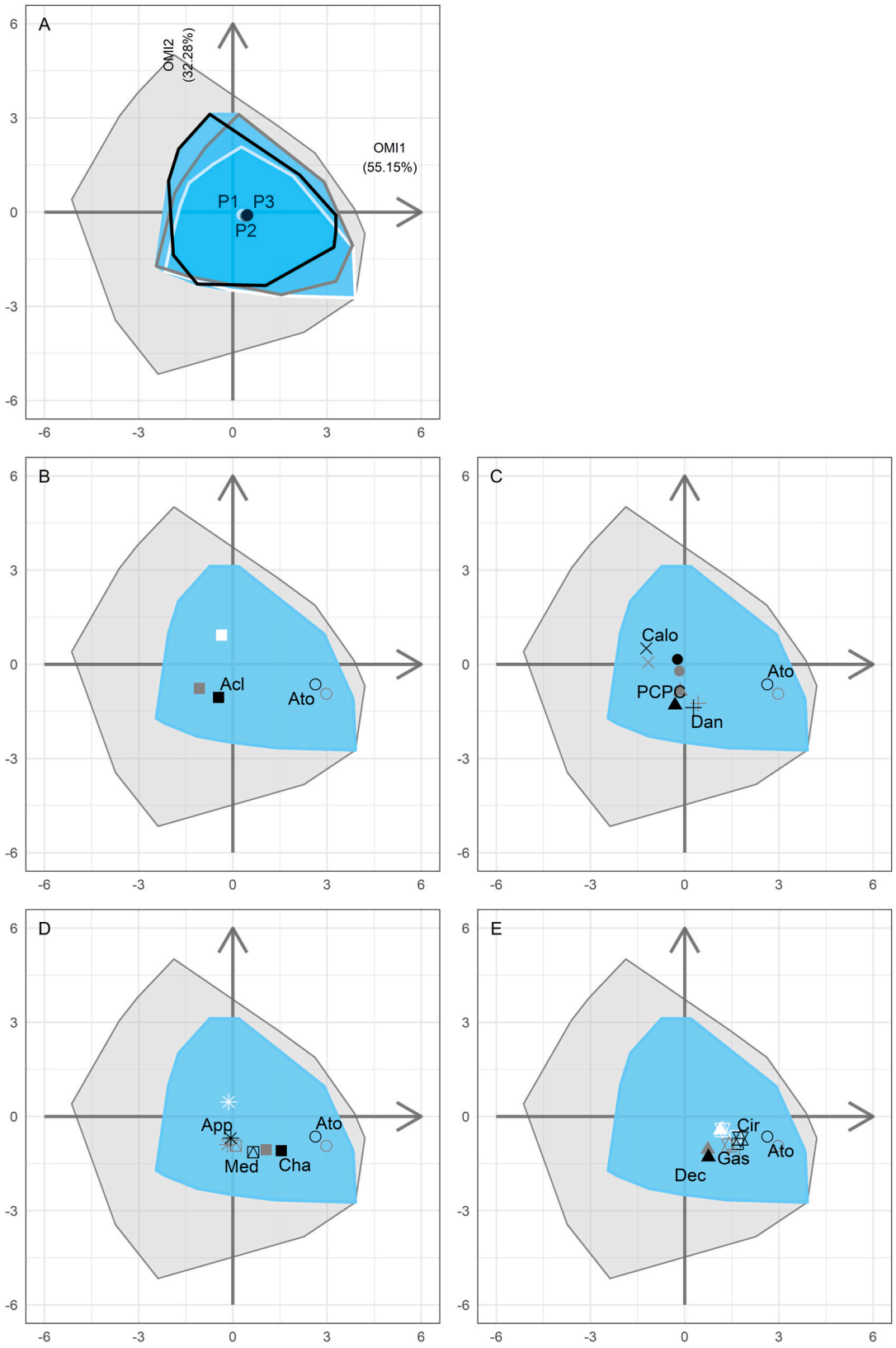


Fig. 7. As in Fig. 6, but for the estuary of Urdaibai, using blue shaded polygons to show the habitat conditions in this estuary. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

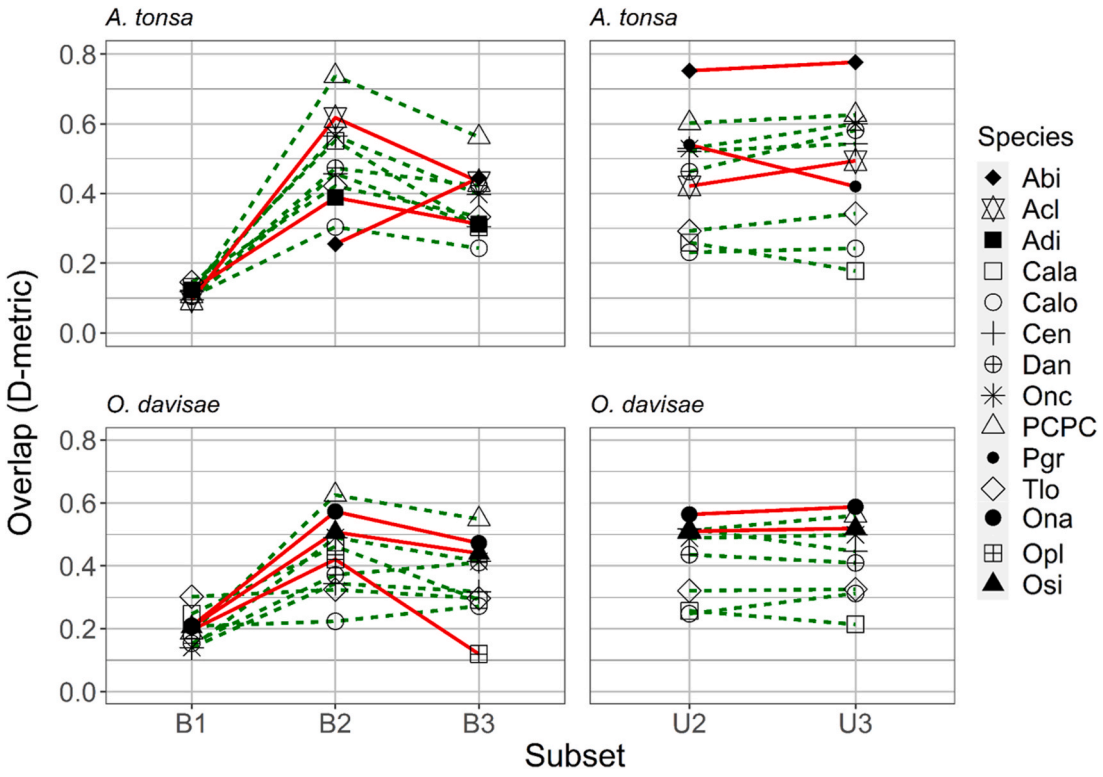


Fig. 8. Bivariate plots of niche overlap changes over time of *A. tonsa* and *O. davisae* with neritic copepod (in dashed green lines) and congeneric taxa (in solid red lines) in the estuary of Bilbao (left panels) and the estuary of Urdaibai (right panels). Symbols correspond to the species whose niche overlaps with that of *A. tonsa* (top panels) or *O. davisae* (bottom panels). Taxa abbreviations as in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Paracartia grani, doliolids, and *C. aquadulcis* also showed very low tolerance but, in contrast with the former taxa, very high marginality. This denoted that they were the taxa with smallest niche breadth and clearest habitat restriction, in agreement with their marked differences in abundance between estuaries and their highly constrained spatial and or temporal distributions. *P. grani* has only been found (in low numbers) in the estuary of Urdaibai, spatially constrained to intermediate salinity habitats between those of the neritic species and the dominant brackish species and with a conspicuous seasonal peak in the warm period (Villate, 1982; Villate et al., 2018). Doliolid abundance decreased dramatically with decreasing salinity and showed an irregular presence over the studied years with short-lived occurrences in both estuaries (Fanjul et al., 2017). This agrees with their tendency to bloom only under conditions of high temperature and stratification (Pinchuk et al., 2021). *C. aquaedulcis* was much more abundant in the estuary of Bilbao after experiencing a sudden increase in this system in 2010 (Barroeta et al., 2020), but it was the colonizer with the most restricted spatial distribution within the upper reaches, likely because oligohaline waters are the optimal for their reproduction (Svetlichny et al., 2012). The high marginality of the NIS *A. tonsa, P. marinus* and *O. davisae*, at the regional scale was also related to their noticeable differences in abundance between estuaries and well-defined seasonal patterns of abundance (Barroeta et al., 2020), but their relatively high tolerance is in agreement with their wider spatial distributions along the salinity gradient as compared to *C. aquaedulcis*. Although laboratory experiments have addressed that those NIS can tolerate large ranges of temperature and salinity (Lance, 1963; Castro-Longoria and Williams, 1999; Chaalali et al., 2013b; Sabia et al., 2015; Zagami et al., 2018), in these estuaries of the Bay of Biscay they seem more constrained seasonally than spatially, likely being due to a greater effect of the seasonal variations in river discharge, gravitational circulation and residence time, than by spatial variations in salinity.

4.2. Importance of local conditions in realized niches

WitOMI analysis allowed an evaluation at a finer scale of zooplankton community of each estuary, highlighting differences in the realized niche of taxa between the estuaries of Bilbao and Urdaibai. The number of taxa with no significant marginality was markedly higher in the estuary of Urdaibai (>25% of the taxa) than in the estuary of Bilbao (<10% of the taxa). No significant marginality may imply that species are not influenced by the environmental conditions studied (Karasiewicz et al., 2017) due to the ubiquity of the species within the studied subset or to insufficient data available to evaluate the ecological niche of the species in the subset. In this study, only the case of *A. bifilosa* in the estuary of Urdaibai could be related to ubiquity, since it is a dominant species found along the entire salinity range studied and throughout the entire annual cycle and its spatial maxima were found to move from 26 to 30 salinity zones and seasonal maxima from spring to summer (Villate et al., 2018; Barroeta et al., 2020). The high adaptability of this species to estuarine environments is corroborated by the displacement of its optimal salinity niche towards lower salinity and the different seasonal patterns observed in other estuaries (Baretta and Malschaert, 1988; Soetaert and Van Rijswijk, 1993; David et al., 2007), as well as by its capacity to grow successfully in systems with contrasting trophic environments (Burdloff et al., 2002). *A. biflosa* is also found to be able to recover rapidly from banks of resting eggs in sediments of the Baltic Sea (Katajisto et al., 1998) or when populations are washed from the water column by river floods in small systems like the estuary of Urdaibai (Uriarte et al., 1998; Uriarte and Villate, 2006). Most of the cases of copepod species with no significant marginality in this latter estuary (e.g. *P. marinus*, *C. aquaedulcis*, *O. davisae* and *P. grani*) can be, however, attributed to the scarcity of observations of the species in the estuary. The scarcity of such species in the estuary of Urdaibai seems to be mainly due to abiotic constraints, such as hydrological instability and the lack of

marine water intrusion in depth (Barroeta et al., 2020). Salinity intrusions have been associated to the establishment of invasive *Pseudodiaptomus* species in estuaries (Cordell and Morrison, 1996) and *O. davisae* seems to require low water exchange to prosper (Zagami et al., 2018).

Overall, the highest values of both tolerance and marginality were found in the estuary of Bilbao and, in addition, tolerance and marginality were inversely related in this estuary but not in the estuary of Urdaibai. This evidenced differences in the realized niche of zooplankton taxa attributable to inherent environmental differences between estuaries. Low marginality and high tolerance are distinctive of generalists occupying the average conditions of the study area whilst high marginality and low tolerance characterize the most specialized taxa, restricted to particular habitats and environmental conditions (Sillero, 2011; Le Coz et al., 2018). Accordingly, the taxa of the estuary of Bilbao depicted a clear progression of taxa from the most generalists (e.g. benthic harpacticoids, *A. clausi* and *Calanus* sp., among the copepods, and isopods, gastropod larvae and appendicularians, among non-copepod groups) to the most specialized (e.g. freshwater species, *C. aquaedulcis* and *T. stylifera,* among copepods, and doliolids among non-copepod groups), whilst such pattern of niche differentiation from clear generalists to clear specialists was not observed in the estuary of Urdaibai. This suggests that the estuary of Bilbao has the conditions to allow zooplankton taxa to adjust their realized niche within the range of the studied environmental variables according to their capacities, while the estuary of Urdaibai seems to show inherent constraints that hinder a well-defined realized niche in the environmental frame of studied environmental variables. According to Karasiewicz et al. (2017), the reason for the realized subniche change in marginality and tolerance can be explained by the difference between both subsets average habitat conditions and subset habitat condition constraints which will affect the species' realized niche.

Although the estuary of Urdaibai offers a greater salinity range to zooplankton, the estuary of Bilbao shows greater range of dissolved oxygen, suspended organic material concentration and stratification (Uriarte and Villate, 2004; Iriarte et al., 2010; Villate et al., 2017). In this sense, a higher heterogeneity of certain environmental factors across sites potentially increases the effect of environmental filtering on the zooplankton community structuring (Le Coz et al., 2018). The tolerance and marginality of zooplankton taxa were not correlated at the regional scale (both estuaries together) and the negative correlation obtained in the estuary of Bilbao was a result of the noticeable changes in marginality and tolerance of several taxa from the regional to the local system scale. This suggests that differences between estuaries may lead to the distortion of the generalist or specialist behavior of species regardless of their tolerance ranges. Analyzing the regional occupancy of stream diatoms, Heino and Soininen (2006) also found no correlation between the tolerance and the marginality and indicate that generalist species may occur in atypical environmental conditions and may also be able to occur more widely. Similarly, Hernandez Fariñas et al. (2015), assessing phytoplankton realized niches along a coastal area, showed that taxa with low marginality could be both tolerant and non-tolerant.

Between-estuary comparisons by taxa evidenced that warm affinity neritic taxa like *O. nana*, *T. stylifera*, *Oncaea* sp. and doliolids, which peak from August to October (Fanjul et al., 2017), had a greater specialist behaviour (lower tolerance and higher marginality) and cold-affinity neritic taxa like *T. longicornis*, *Calocalanus* sp., *Calanus* sp. and *A. clausi*, which peak from March to June (Fanjul et al., 2017), had a greater generalist behaviour (higher tolerance and lower or similar marginality) in the estuary of Bilbao than in the estuary of Urdaibai. These facts seem to be due to differences in the seasonal niche breadth related to the differences in the magnitude and seasonality of food availability in the neritic zone of each estuary. The outer estuary of Bilbao is fertilized by the estuarine plume and shows mesotrophic conditions and a seasonally extended period of high phytoplankton biomass with annual maxima in summer. However, the outer estuary of Urdaibai shows lower trophic status derived from the decline of phytoplankton biomass in summer after the early spring peak (Iriarte et al., 2015). This accounts for the higher density and most conspicuous seasonal patterns of abundance of summer-autumn species around the summer maxima of phytoplankton biomass in the estuary of Bilbao, as well as for the seasonal spread towards summer of the species peaking in spring in this estuary, whilst their abundances drop in summer in the estuary of Urdaibai (Fanjul et al., 2017; Villate et al., 2018).

Concerning brackish species, the much higher marginality of the NIS *A. tonsa,* in the estuary of Urdaibai could be better explained by biotic than by abiotic constraints, as may occur in biological invasions (Guisan et al., 2014). Brackish waters show high level and similar seasonal pattern of chlorophyll *a* in both estuaries (Iriarte et al., 2016), essential for *A. tonsa* development in coastal systems (Paffenhöfer and Stearns, 1986), but the presence of potential competitors at the arrival of this species differed largely between estuaries. The estuary of Bilbao was lacking brackish copepod species, whilst the congeneric brackish species *A. bifilosa* dominated the zooplankton community of the inner estuary of Urdaibai, thus preventing the complete occupation of the brackish niche by *A. tonsa* in this system (Villate et al., 2018; Barroeta et al., 2020). The biotic resistance exerted by native species has also been hypothesized to influence the realized ecological niche of this species in other estuaries (Chaalali et al., 2013a).

4.3. Different response of zooplankton communities in time

Results highlighted sequential environmental changes and niche shifts of the zooplankton communities throughout time only in the estuary of Bilbao. The clear seaward shifts in the realized niche of the native neritic species *A. clausi, O. similis, O. nana and O. plumifera* (congeneric species of the two main NIS) and *Calocalanus* sp., *Centrophages* sp., *Oncaea* sp. and PCPC-calanus (among others) in this estuary could be attributed either to changes in environmental conditions or to changes in biotic interactions within the estuary (Pearman et al., 2008). In our case, the combined effect of environmental changes and competitive pressure by the new colonizers seems the most plausible explanation. The observed progressive changes in environmental conditions were consistent with the improvement in water quality, including the increase of dissolved oxygen concentration, during the study period as a result of the rehabilitation plan carried out in this estuary (Villate et al., 2013; Iriarte et al., 2016; Cajaraville et al., 2016). However, water quality improvement in the estuary of Bilbao involved the recolonization of the inner part of the estuary by copepods (Uriarte et al., 2016), as in other systems (Mialet et al., 2011). Indeed the study by Aravena et al. (2009) revealed that the colonization by *A. tonsa* caused the seaward displacement of *A. clausi* by competitive pressure in relation to oxygenic conditions. Niche shifts of congeneric species after the establishment of *O. davisae* have also been reported in other coastal environments (Isinibilir et al., 2016; Zagami et al., 2018). Biotic interactions between the studied NIS and their congeneric neritic species can lead to a high spatial segregation in estuaries (Azeiteiro et al., 2005) due to the fact that *A. tonsa*, *O. davisae* or *A. bifilosa* are found to be competitively superior in brackish environments (Gunter, 1956; Lance, 1963; Calliari et al., 2006), but physiologically limited to compete with their congeneric neritic species in high salinity waters (Dahl, 1956; Hedgpeth, 1957; Gaudy et al., 2000).

In this estuary, the niche overlap of *A. tonsa* and *O. davisae* with most congeneric and non-congeneric copepod species was higher in the period following their successful colonization (period 2003–2009) than after a longer time of cohabitation (period 2010–2015). This observation suggests that the time of coexistence led to optimal spatial segregation. The increase in the niche overlap of *A. tonsa* with *A. bifilosa* in the last period was due to the fact that *A. bifilosa* arrived to the estuary of Bilbao in 2007, when *A. tonsa* was already well established, and experienced an expanding phase during the last years of the study (Barroeta et al., 2020). In fact, both species were able to share the ecological niche

initially only occupied by *A. clausi* in the estuary of Bilbao (Aravena et al., 2009). The addition of new data to the zooplankton series in the future might allow to better understand the competition between these two species by using a case study in which the order of arrival of competing species to the system is the opposite to the expected one in natural conditions.

The lack of such niche shifts or changes in niche overlap through time in the estuary of Urdaibai seems to be due to the fact that this estuary, compared to the estuary of Bilbao, did not seem to experience major changes of environmental conditions. Moreover, the NIS that arrived had a negligible impact in the case of *O. davisae* and *P. marinus*, and a much more moderate impact in the case of *A. tonsa,* as compared to the impacts in the estuary of Bilbao (Barroeta et al., 2020). In addition, neritic and estuarine zooplankton assemblages were abundant and well-differentiated before the arrival of NIS, with the native brackish species *A. bifilosa* dominating the estuarine community and maintaining neritic species restricted to the high salinity waters of the outer reach (Villate et al., 2018). The most affected species by the NIS in the estuary of Urdaibai was *A. bifilosa*, whose annual abundance peak occurred around two months earlier after the establishment of *A. tonsa* (Barroeta et al., 2020).

In summary, the use of OMI and WitOMI analyses allowed us to describe the structure of the zooplankton community in the environmental context of two Basque estuaries and determine the influence of local environmental factors and biotic interactions in the realized niches of the species under study in each estuary. In that sense, the inherent environmental features of each estuary allowed a better definition of generalist or specialist behaviours of zooplankton taxa in the estuary of Bilbao than in the estuary of Urdaibai. Finally, these analyses also allowed the identification of interannual changes in the realized niches of the community components that occurred as a result of the impact of the species that arrived to the estuaries, but also to the changes in the environmental conditions. Such changes were evident in the estuary of Bilbao but almost negligible in the estuary of Urdaibai.

CRediT authorship contribution statement

Ziortza Barroeta: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Théo Garcia:** Writing – review & editing, Methodology, Writing – original draft. **Ibon Uriarte:** Writing – review & editing, Supervision, Funding acquisition. **Arantza Iriarte:** Funding acquisition, Supervision, Writing – review & editing. **Fernando Villate:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Fernando Villate reports financial support was provided by University of the Basque Country. Ziortza Barroeta reports financial support was provided by University of the Basque Country. Fernando Villate reports a relationship with University of the Basque Country that includes: employment. Arantza Iriarte reports a relationship with University of the Basque Country that includes: employment. Ibon Uriarte reports a relationship with University of the Basque Country that includes: employment. Ziortza Barroeta reports a relationship with University of the Basque Country that includes: funding grants.

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