JOURNAL OF PLANKTON RESEARCH | VOLUME 33 | NUMBER 7 | PAGES 1104–1118 | 2011

Does the study of microzooplankton community size structure effectively define their dynamics? Investigation in the Bay of Biscay (France)

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Received September 13, 2010; accepted in principle January 7, 2011; accepted for publication January 16, 2011

Corresponding editor: John Dolan

Studies of microzooplankton dynamics based on the analysis of community taxonomic composition are very time-consuming and manpower demanding. Recent developments in automatic plankton counting technology offer a new way to consider plankton dynamics with straightforward analysis of community size structures. The present study aimed to determine if cell size is a good descriptor of microzooplankton dynamics. The dynamics of the microzooplankton community of the Bay of Biscay (France) at three sites and during four sampling periods was analyzed using three different classification criteria: taxonomy, body size (ng C $cell^{-1}$) and equivalent spherical diameter (ESD, μm). A Mantel test revealed that there was no difference in the characteristics of microzooplankton dynamics when studying either community size structure or taxonomic composition. Moreover, a BIO-ENV analysis confirmed that the biotic and abiotic factors selected for affecting microzooplankton community dynamics were the same among the three classification types. Considering these results, it is argued that microplankton dynamics is well defined by the study of the community size structure. While focusing on microzooplankton size structure seems promising, homogenizing the size descriptor used among studies would be needed in order to make worldwide data comparisons and ESD/biovolume should be favored.

KEYWORDS: microzooplankton; size structure; dynamics; taxonomy; Bay of Biscay

INTRODUCTION

Open ocean photosynthesis is dominated by pico-nanophytoplanktonic production (0.2–20 μm diameter, Sieburth *et al.*, 1978), with most of the active primary producers smaller than 3 μm (Waterbury *et al.*, 1979; Fenchel, 1988). A complex assemblage of viruses, bacterioplankton and protozoans coexist with and support these small oceanic primary producers through their role in nutrient regeneration (Azam *et al.*, 1983; Ducklow and Carlson, 1992; Sherr and Sherr, 2000). Larger phytoplankton such as diatoms and dinoflagellates show greater variability than pico-nanophytoplankton and so do their predators, i.e. larger protists and metazoans (Fenchel, 1988). Primary production is transferred to higher levels through two main

doi:10.1093/plankt/fbr009, available online at www.plankt.oxfordjournals.org. Advance Access publication February 21, 2011

pathways: the classical food chain and the microbial food web, according to the size of the main primary producers (Azam *et al.*, 1983; Sherr *et al.*, 1986; Sommaruga, 1995; Thingstad and Rassoulzadegan, 1999). Therefore, depending on which pathway is dominating the planktonic food web, the amount of energy lost (mainly through respiration), matter recycled and sedimented, and organic carbon available to planktonic predators (mainly fish) will vary. For these reasons, our understanding of plankton dynamics and plankton food web functioning is intimately related to our appreciation of global biogeochemical fluxes. Despite the need for information about these issues when studying global warming concerns, current knowledge of the identity and the functional role of the major plankton groups as well as their dynamics prevent us from evolving from the current simple plankton models towards more detailed and adaptative ecosystem modeling (Ducklow, 2003).

Since the paper of Azam *et al.* (Azam *et al.*, 1983), a large number of studies have focused on the dynamics and the role of nano-microzooplankton in oceanic, coastal and halieutic environments. Nano-microzooplankton are trophic intermediaries in pelagic food webs, permitting the transfer of carbon from pico- and nanoplankton to metazoans (Sherr *et al.*, 1986; Pierce and Turner, 1992). They occupy an essential trophic node in microbial food webs and their dynamics may be either controlled by predation or by resource availability as well as hydrography (e.g. Sanders, 1987; Cowlishaw, 2004). Despite such an essential role in aquatic ecosystems, their taxonomic diversity is high and not well known. In the early nineties, Sleigh (Sleigh, 1991) discussed the taxonomy of heterotrophic protists, which are the main components of the nano-microzooplankton community, and stated "many species, and possibly phyla, remain to be described" (Sleigh, 1991). Today, almost 20 years later, and despite progress in molecular biology techniques, protozoan taxonomy is still subject to study, discussion and controversy (e.g. Finlay, 2004; Adl *et al.*, 2005).

Traditional studies based on taxonomic composition of plankton communities are difficult to implement efficiently due to the limitation of resources, time and manpower to process samples (Culverhouse *et al.*, 2006). However, size is another possible way to classify plankton organisms, and the terminology of Sieburth (Sieburth *et al.*, 1978) is useful and has become widely accepted. Recent developments in metabolic theory (Brown *et al.*, 2004) confirm older studies (e.g. Moloney and Field, 1989) in suggesting that size determines many biological properties of organisms such as respiration, nutrient uptake, production, ontogenetic growth, etc. (e.g. Zeuthen, 1970; Gillooly *et al.*, 2001; West *et al.*, 2003; Lopez-Urrutia *et al.*, 2006). Moreover, in aquatic ecosystems, size usually determines predator–prey interactions (e.g. Frost, 1972; Peters and Downing, 1984; Caparroy *et al.*, 2000). As such, size distribution of plankton may be used as an indicator of ecosystem and trophic status as described in Gaedke's studies on Lake Constance (e.g. Gaedke, 1993). Additionally, recent studies have shown that one of the aquatic ecological responses to direct and indirect impacts of global warming is the reduction of body size (Daufresne *et al.*, 2009; Moran *et al.*, 2010). Regarding all these issues, studying planktonic and especially nano-microzooplanktonic dynamics based on the community size structure seems to be an interesting and promising research focus.

New technologies for automatic plankton counting are now available to plankton ecologists. Such instruments, e.g. the FlowCAM technology (Sieracki *et al.*, 1998), are succeeding in size classification and identification of planktonic cells. Therefore, they would be very useful in studying the dynamics of planktonic community size structure. However, before commencing such studies, it is essential to ensure that size is a sufficient plankton community descriptor on its own. In other words, it is essential to understand whether the dynamics resulting from those size-based analyses show the same pattern and the same spatio-temporal differences as the dynamics resulting from the taxonomy-based analysis.

This study aims to investigate whether the seasonal and spatial dynamics of the nano-microzooplankton may be revealed by looking at the size structure of the community. Extensive data on planktonic communities collected in 2004 in the Bay of Biscay were used to answer this question. Size-abundance spectra (SAS) are used to understand the overall changes in the nano-microzooplankton composition and a comparison between taxon-based and size-based classifications helps evaluate the relevance of the size structure analysis.

METHOD

Study site and hydrography

The continental shelf of the Bay of Biscay (Fig. 1) is up to 200 km wide with a surface area of 223 000 km^2. Its hydrological structure is principally influenced by the seasonal dynamics of the Loire and the Gironde river plumes (Lazure and Jegou, 1998) and the shelf ecology shows a strong variability in time related to temperate zone climatic fluctuations (Koutsikopoulos *et al.*, 1998). At four periods in 2004 (08–10 February, 23–25 April, 09–11 June and 30 September–02 October), three stations were sampled, located on the continental shelf,

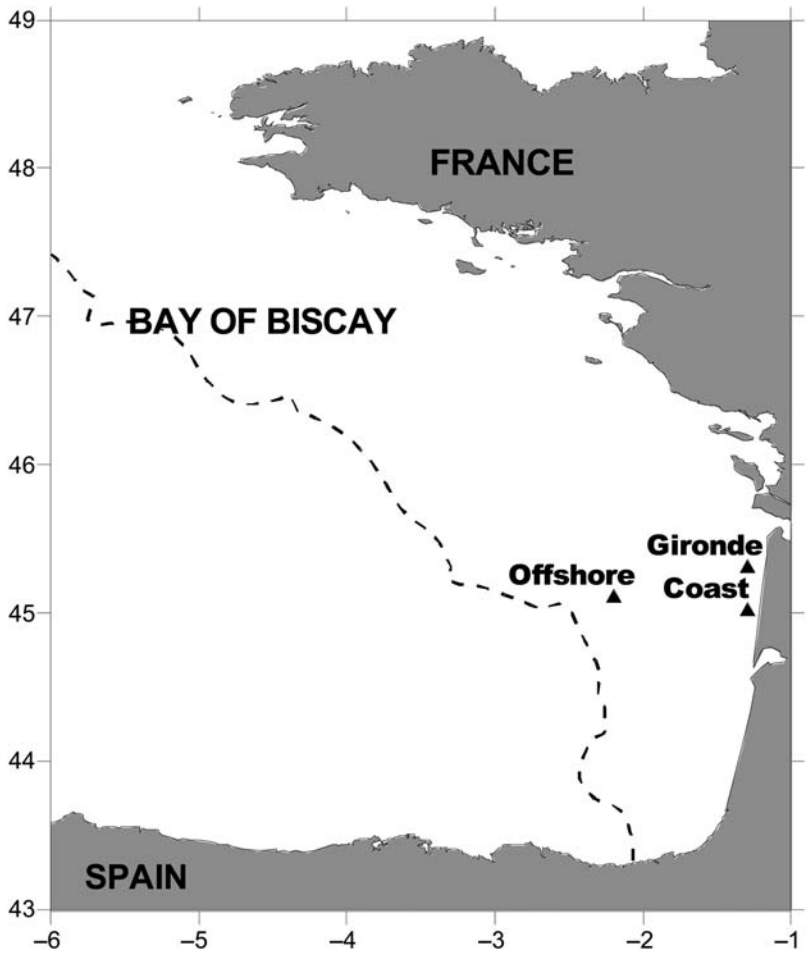

Fig. 1. Map of the Bay of Biscay with the location of the three study sites. Dashed line is a schematic representation of the continental shelf limits.

on an estuary–coast–offshore triangle: "Gironde" (01°30′W, 45°30′N), "Coast" (01°30′W, 45°01′N) and "Offshore" (2°20′W, 45°10′N) (Fig. 1). Salinity and temperature profiles were measured using a conductivity–temperature–density (CTD) probe (Sea-Bird SBE 9). Concurrently, irradiance and fluorescence profiles of the water column were measured using, respectively, a photosynthetically active radiation sensor and a fluorometer attached to the CTD. The depths of the photic zone and maximum fluorescence were then evaluated in order to adapt the plankton sampling accordingly.

Water and plankton sampling and analysis

At each station and sampling period, we collected water samples with 12-L Niskin bottles at three different depths: subsurface, maximum fluorescence and bottom of the photic zone.

Samples for dissolved inorganic nutrients analyses: nitrate (NO_3), nitrite (NO_2), silicate ($Si(OH)_4$) and phosphate (PO_4) were immediately filtered through Whatman GF/F filters so that the filtrate could be stored at −20°C until analysis in the laboratory with a Skalar autoanalyzer (Strickland and Parsons, 1972).

Samples for bacteria and picophytoplankton counting were fixed with formaldehyde (final concentration 2%), frozen in liquid nitrogen and enumerated using a FACSCan flow cytometer (Bd-Bioscience) (Marie *et al.*, 2000). Nanoflagellates were fixed with buffered paraformaldehyde (final concentration 1%) then stained with DAPI and counted on 0.8 μm black polycarbonate filters by epifluorescence microscopy (Sherr *et al.*, 1994). Heterotrophic nanoflagellates (HNF) were distinguished from pigmented (autotrophic) nanoflagellates (ANF) by the absence of chlorophyll fluorescence. Microphytoplankton (diatoms and dinoflagellates) were fixed with formaldehyde (final concentration 1%) plus alkaline Lugol (final concentration 1%), enumerated and measured by inverted microscopy (Utermöhl, 1958). Heterotrophic and mixotrophic dinoflagellates (HDF) were determined from

Table I: Factors and formulae with their reference used to convert biovolume to carbon mass of each plankton organism

Plankton organisms	Conversion factor or formulae	References
Bacteria	0.016 pg C $cell^{-1}$	Labry *et al.* (2002)
Cyanobacteria	0.104 pg C $cell^{-1}$	Blanchot and Rodier (1996)
Eucaryotic picophytoplankton	0.22 pg C $cell^{-1}$	Shinada *et al.* (2005)
Nanoflagellates	0.125 pg C μm^{-3} = 3.14 pg C $cell^{-1}$ (with mean biovolume of 25.2 μm^3)	Pelegri *et al.* (1999) and our data of biovolumes
Dinoflagellates	Log_{10} C (in pg C $cell^{-1}$) = −0.353 + 0.864 * log_{10} V	Menden-Deuer and Lessard (2000)
Diatoms	Log_{10} C (in pg C $cell^{-1}$) = −0.541 + 0.811 * log_{10} V	<3000 μm^3
	Log_{10} C (in pg C $cell^{-1}$) = −0.933 + 0.881 * log_{10} V	>3000 μm^3; Menden-Deuer and Lessard (2000)
Naked Ciliates	0.19 pg C μm^{-3}	Putt and Stoecker (1989)
Ciliate Tintinnids	C (in pg C $cell^{-1}$) = 444.5 + 0.053 * LV	Verity and Langdon (1984)
Copepod nauplii	pg C $ind.^{-3}$ = 0.08 × V	Gowing *et al.* (2003)

V: biovolume (in μm^3); LV: Lorica volume (in μm^3).

morphologic species recognition and relevant literature (e.g. Lessard and Swift, 1986). Ciliates were stained with alkaline Lugol (1% final concentration), counted and measured by inverted fluorescence microscopy. Ciliate samples from surface and bottom of the photic zone collected in February at the Gironde station were not analyzed due to poor preservation. Diatom, dinoflagellate and ciliate biovolumes were calculated by transforming the cell's shapes into geometric figures. Samples of metazoan microplankton were obtained by gently filtering 10 L of collected seawater through a 63 μm size mesh. The organisms retained were then diluted in filtered (<63 μm) seawater and preserved in buffered formaldehyde (final concentration 2%). They were counted under a binocular microscope. All the conversion factors and equations used to convert the abundance of pico-, nano- and microplankton into biomass were obtained from the literature (Table I). The equivalent spherical diameter (ESD) of each cell was obtained by calculating the diameter (μm) of a hypothetical sphere of equivalent volume. The two conversions from biovolume to biomass and from biovolume to ESD resulted in exponential relationships between the body mass and ESD of nano-microzooplankton cells in this study (Fig. 2).

Mesozooplankton was collected from vertical tows through the entire photic zone using a 200 μm mesh WP2 net, preserved in buffered formaldehyde (final concentration 2%) and counted under a binocular microscope. A second replicate was used to measure the mesozooplankton dry weight. Mesozooplankton carbon biomass was determined by multiplying dry weights of each sample by a factor of 0.38 (Bode *et al.*, 1998).

Data analysis

Phytoplankton and nano-microzooplankton abundances ($cells.L^{-1}$) and cell volumes (μm^3) were used to create the SAS. The construction of biovolume classes on an octave (log_2) scale resulted in a maximum number of 26 size classes, ranging from 0.09 to 5.87×10^6 μm^3. Linear relationships in the SAS were obtained by plotting the log_{10} of total abundance by size class against the log_{10} of the lower limit of the corresponding octave size class (e.g. Huete-Ortega *et al.*, 2010). SAS slopes were estimated from a linear regression analysis of each spectrum.

The microzooplankton community was then classified under three different classification criteria (cf. Table II). HNF were not included in the analysis due to a lack of detail on their size and taxonomic structure.

(1) Taxa: 10 classes based on Class level for metazoans (Copepod nauplii) and Order level for unicellulars (Ciliate Haptorida; Ciliate Halteriida; Ciliate Chaerotrichida; Ciliate Cyclotrichida; Ciliate Strombiida; Ciliate Peniculida; Dinoflagellate Peridiniales; Dinoflagellate Dichtyochales; Dinoflagellate Gymnodiniales).
(2) Body sizes: 10 classes based on an Octave scale from 112 to 114,688 ng C $cell^{-1}$.
(3) ESD: 10 classes based on a 4.4 μm interval from 28 to 72 μm.

All data were normalized prior to statistical analyses; using a double-root transformation on the abundance and biomass data and a log-transformation on the environmental data (Legendre and Legendre, 1998). Two types of analyses were applied to the data sets: Mantel test (Legendre and Legendre, 1998) to analyze whether the different ways of classifying nano-microzooplankton lead to different description of its spatial/temporal dynamics, and BIO-ENV procedure, to analyze the biotic (i.e. trophic) and abiotic (i.e. environmental) factors impacting nano-microzooplankton

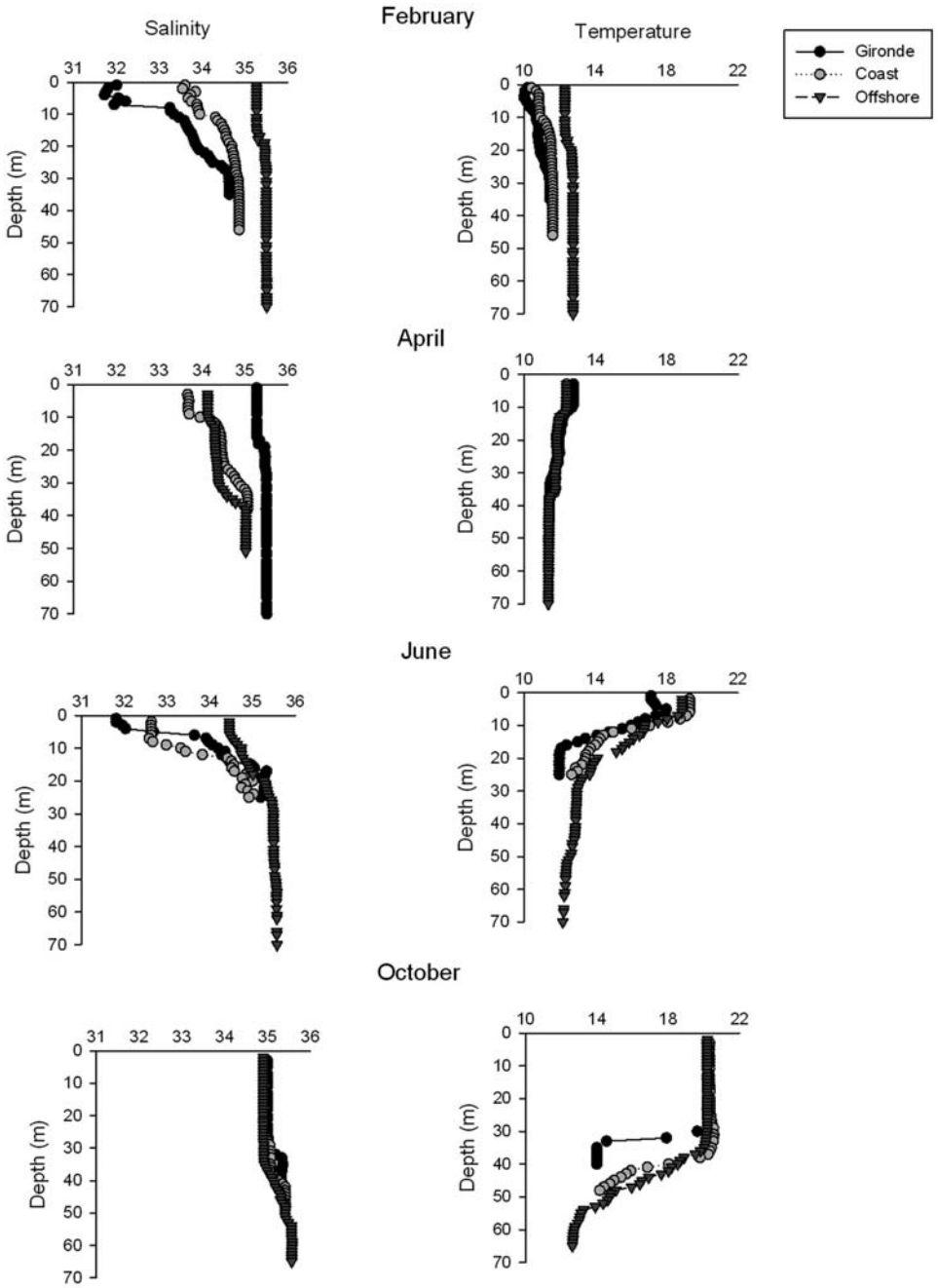

Fig. 2. Temperature (°C) and salinity (PSU) profiles of the water column at the three stations and the four sampling periods.

community dynamics and to compare the factors selected for each of the classification types.

First, we constructed three matrices using nano-microzooplankton abundances of the three classification types (taxa, body sizes and ESD). We used the average abundance values throughout the water column (Table II). The 3 matrices have 12 rows (3 sites and 4 sampling periods) and 7 columns (7 different classes, for each classification). These matrices were used to calculate dissimilarity matrices in order to model the resemblance between the sampled sites/months by the mean values of community composition for each of the three nano-microzooplankton classification types. Bray and Curtis distance measure was used as it is particularly suitable for quantitative data (Legendre and Legendre, 1998). We then applied the Mantel test to see if there was a correlation between the different distance matrices (Legendre and Legendre, 1998). In a simple

Table II: Seasonal abundances (cells L^{-1}) with their standard deviations, of the different nano-microzooplankton classes from the three classification types (taxa, ESD and body size) on average over the photic zone at the three stations

		Gironde				Coast				Offshore			
		February	April	June	October	February	April	June	October	February	April	June	October
Taxons	Haptorida	0.0	53.2	228.0	0.0	30.4	34.2	585.2	0.0	17.1	21.7	34.2	0.0
	Halteriida	0.0	38.0	15.2	0.0	49.4	169.1	36.1	5.7	49.4	275.9	3.8	22.8
	Chaerotrichida	7.6	824.6	361.0	57.0	731.4	805.6	1428.8	167.2	1263.5	622.4	201.4	158.0
	Cyclotrichida	0.0	1086.8	182.4	3.8	274.1	3927.3	304.0	17.1	205.2	1222.1	3.8	19.0
	Strombidiida	53.2	2576.4	2228.7	573.8	701.6	4368.1	1941.8	313.5	1052.6	1584.6	220.4	235.3
	Peniculida	0.0	3.8	3.8	0.0	11.4	0.0	7.6	1.9	11.4	14.8	0.0	15.2
	Peridiniales	277.3	3050.7	7395.6	66.2	0.0	1456.0	1670.9	69.3	69.3	665.6	485.3	28.9
	Dichtyochales	208.0	0.0	92.4	34.7	69.3	0.0	0.0	365.4	450.7	0.0	69.3	0.0
	Gymnodiniales	1040.0	16432.0	5500.4	332.0	277.3	11162.7	7846.2	1061.9	7522.7	9692.8	5148.0	2617.3
	Nauplii	43.1	44.3	31.2	47.6	20.4	39.6	17.5	24.3	30.2	9.9	12.1	2.5
Body size (ng C $cell^{-1}$)	112–224	208.0	10558.7	849.2	239.6	346.7	7094.0	4576.3	1081.2	6850.9	5961.4	2809.0	1489.0
	224–448	208.0	167.2	19.0	0.0	146.8	237.5	91.6	5.7	407.5	310.1	281.1	51.3
	448–896	0.0	4094.7	1419.0	3.8	232.3	4062.0	1509.8	34.4	491.1	1429.5	333.1	124.5
	896–1792	746.5	5531.7	5329.8	483.9	805.9	4959.2	4878.9	599.3	2273.4	4697.0	2344.1	1261.8
	1792–3584	76.9	3434.8	8042.8	245.1	466.5	3460.6	2598.8	262.4	458.4	1023.7	229.9	146.7
	3584–7168	138.7	179.5	41.8	3.8	22.3	1031.5	68.4	0.0	103.5	412.6	138.7	3.8
	7168–14338	208.0	68.4	279.3	81.7	14.3	379.0	5.7	15.2	19.0	118.6	7.6	43.9
	14338–28672	0.0	19.0	13.3	9.5	60.8	38.0	83.6	0.0	34.2	119.7	0.0	0.0
	28672–57344	0.0	95.0	24.7	0.0	49.4	788.5	19.0	3.8	3.8	151.6	26.6	14.1
	57344–114688	43.1	44.3	31.2	47.6	20.4	45.3	17.5	24.3	30.2	9.9	12.1	2.5
ESD (μm)	28–32.4	208.0	425.6	568.1	19.0	277.4	461.7	2018.2	140.6	1026.9	334.0	417.0	85.0
	32.4–36.8	208.0	10152.1	281.1	220.6	69.3	6632.3	2613.6	940.6	5893.3	5627.4	2669.3	1404.0
	36.8–41.2	0.0	984.2	190.0	3.8	356.4	3298.4	353.4	22.8	482.6	1406.8	7.6	100.7
	41.2–45.8	530.9	6176.5	6156.1	358.8	367.6	5663.0	5255.6	535.9	2125.7	4158.4	2378.7	1251.8
	45.8–50	431.2	4320.1	8231.5	332.2	868.1	3150.1	2839.1	304.9	581.4	1726.9	520.7	160.7
	50–54.4	0.0	483.5	41.8	7.6	44.2	684.8	68.4	0.0	36.1	201.8	3.8	7.6
	54.4–58.8	208.0	1489.3	512.3	115.9	51.8	1333.6	581.3	53.4	454.0	497.5	146.3	111.2
	58.8–63.2	0.0	15.2	5.7	3.8	60.8	38.0	79.8	0.0	36.1	119.7	0.0	0.0
	63.2–67.6	0.0	95.0	24.7	0.0	49.4	788.5	19.0	3.8	5.7	151.6	26.6	14.1
	67.6–72	43.1	51.9	38.8	53.3	20.4	45.3	21.3	24.3	30.2	9.9	12.1	2.5

Mantel test, two matrices were compared. First a Pearson correlation (r) was calculated between the matrices and after this, the second matrix was shuffled 10 000 times and the correlation recalculated. This permutation procedure was done in order to construct a law of distribution of correlation coefficients, in the absence of statistically significant relation between the two matrices. The original coefficient was then compared with this distribution in order to determine its statistical significance (P-value). The Mantel statistics were calculated for the three pairs formed by the different nano-microzooplankton classifications: taxa/body sizes, taxa/ESD, body sizes/ESD.

The second analysis was the BIO-ENV routine (Clarke and Warwick, 2001) done using the software package PRIMER 6 (PRIMER-E, Plymouth, UK). The BIO-ENV analysis selected trophic and environmental variables that best explain patterns in the nano-microzooplankton assemblage. The test was conducted by maximizing a Spearman rank correlation between the resemblance matrices of environmental and trophic variables (Euclidean distance) and community abundances (Bray–Curtis distance). The significance of these results was tested using permutation tests. The environmental parameters analysed were: temperature (°C), salinity (PSU), NO_2 concentration (μmol L^{-1}), $N0_3$ concentration (μmol L^{-1}), PO_4 concentration (μmol L^{-1}) and N/P ratio. The trophic parameters tested were the biomass (μg C L^{-1}) of the possible nano-microzooplankton prey as well as their possible predators: heterotrophic bacteria, cyanobacteria, picoeukaryotes, autotrophic nanoflagellates, autotrophic dinoflagellates <20 μm, autotrophic dinoflagellates >20 μm, diatoms <20 μm, diatoms >20 μm and mesozooplankton.

RESULTS

Seasonal and spatial dynamics of environmental conditions

At the Gironde and Coast sites (Fig. 2), the water column varied from salinity stratification in winter and spring to thermal stratification in summer. In June at these two

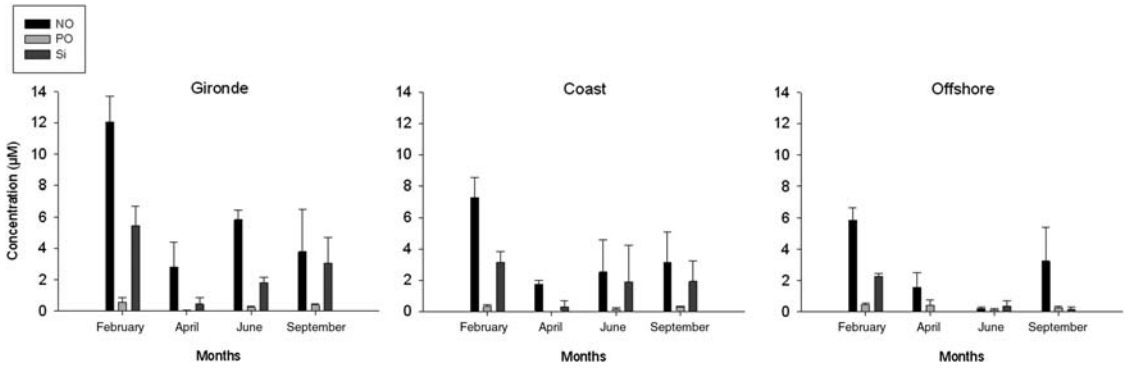

Fig. 3. Seasonal variations of nutrient concentration averages (µM) over the photic zone of the three stations: nitrites and nitrates (N tot), phosphates (PO_4) and silicates (Si).

coastal sites, the water column showed both types of stratification (Fig. 2). For example, at the Gironde site, changes in salinity and temperature between the surface to a depth of 10 m were 31.8 to 34.3 PSU, and 17.2 to 14.7°C; at the Offshore site, the water column showed a moderate salinity stratification in June with only a surface salinity of 34.5 PSU (Fig. 2). The water column at the Offshore site was not stratified in winter and early spring (February and April, Fig. 2) but became progressively thermally stratified from June to October; in late summer, the water temperature stays around 20.1°C from the surface to a depth of 30 m subsequently dropping lower than 18°C below a depth of 40 m (Fig. 2).

At the three sites, the highest average concentrations of total NO_2+NO_3 within the photic zone were observed in February with, for example, 12.1 ± 1.6 µmol L^{-1} at the Gironde site (Fig. 3). In April, June and September, NO_2+NO_3 average concentrations varied from 5.8 ± 0.6 to 0.2 ± 0.1 µmol L^{-1} within the photic zones of the three sites studied (Fig. 3). Considering all three sites and all four study periods, the average $Si(OH)_4$ concentrations within the photic zone were always lower than 6 µmol L^{-1} and the average PO_4 concentrations within the photic zone never exceeded 1 µmol L^{-1} (Fig. 3). The N/P ratio was highest in April at the two coastal sites (77 at Gironde and 173 at Coast) and lowest in September (below the Redfield ratio with 10 at Gironde and 11 at Coast). The highest N/P ratio at the Offshore site was found in September but did not exceed 13.

Seasonal and spatial variations in phytoplankton biomass and composition

The average autotrophic biomass within the photic zone decreased from February to October at the Gironde site (376.6 ± 459.3 to 10.9 ± 2.4 µg C L^{-1}, Fig. 4). At the Coast and Offshore sites, the average photic zone autotrophic biomass first increased between February and April, reaching, respectively, 116.2 ± 15.6 and 155.8 ± 40.1 µg C L^{-1} (Fig. 4a) and then decreased in June and October. Large diatoms were responsible for more than 70% of the total biomass in February, April and June at the Gironde site and in February and April at both Coast and Offshore sites (Fig. 4b). In February, diatoms were dominated by large chain forming cells (e.g. *Thalassiosira* sp.) at the two coastal sites. Smaller diatoms (i.e. diatoms <20 µm) such as *Leptocylindrus minumus* became more abundant in April at the Coast site (Fig. 4b). The biomass of smaller autotrophs (ANF, cyanobacteria and picoeukaryotes) proportionally increased from April to October at all three stations and dominated in October (>60% of average biomass, Fig. 4b).

Seasonal and spatial variations of nano-microzooplankton biomass and taxonomic composition

At the three sites, average biomass within the photic zone of nano-microzooplankton peaked in April at Gironde, Coast and Offshore with 27.9 ± 13.2, 57.1 ± 32.8 and 27.2 ± 13.6 µg C L^{-1}, respectively (Fig. 5a). The average biomass was still relatively high in June at the Gironde and Coast sites (>19.5 µg C L^{-1}, Fig. 5a) but never exceeded 8.2 µg C L^{-1} in February and October. The Offshore site always had the lowest average biomass of the three sites (Fig. 5a).

Ciliates, especially naked ciliates, accounted for a large part (>70%, Fig. 5b) of the total average nano-microzooplankton biomass at Coast site in February and April as well as in April at the Offshore site. The relative proportion of ciliates in the average nano-microzooplankton biomass was always the highest in

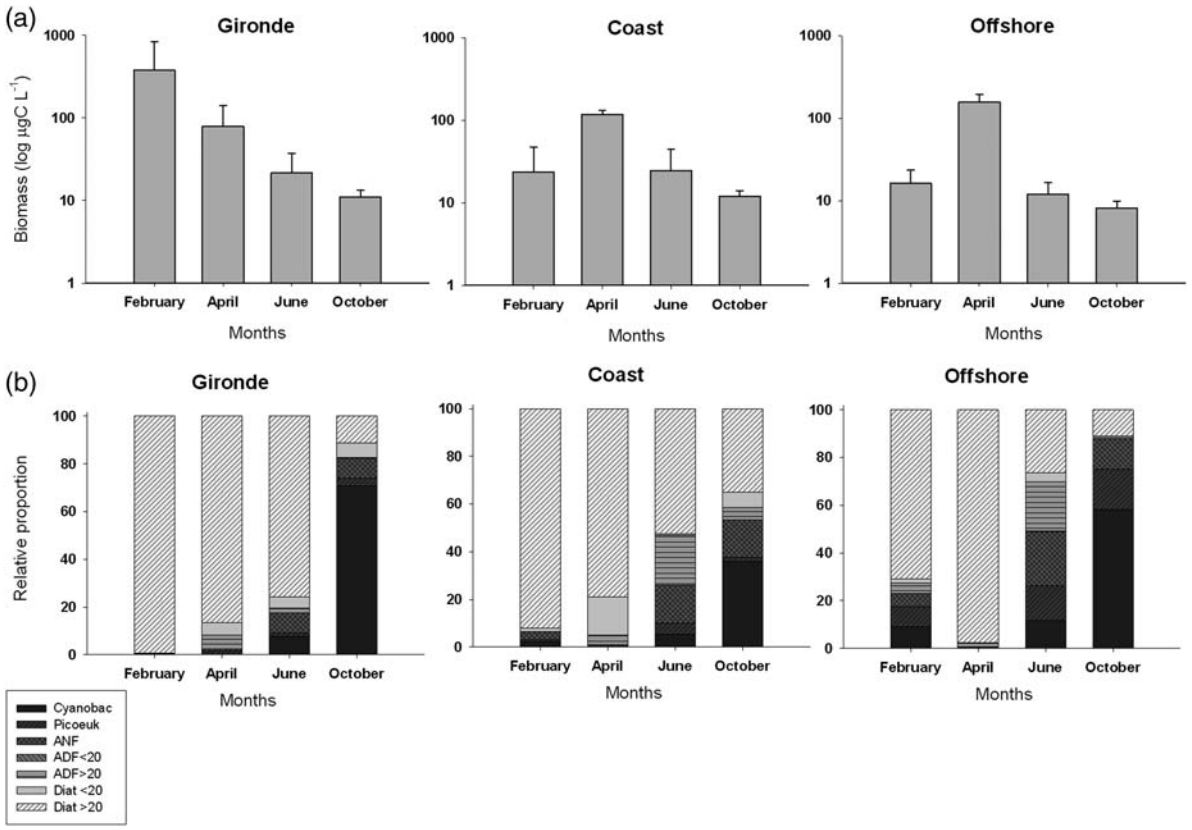

Fig. 4. Seasonal variations of the average autotrophic biomass (**a**) and its relative composition (**b**) over the photic zone at the three stations. Diat > 20 for diatoms >20 µm, diat < 20 for diatoms <20 µm, ADF > 20 for autotrophic dinoflagellates >20 µm, ADF < 20 for autotrophic dinoflagellates <20 µm, ANF for autotrophic nanoflagellates, Picoeuk for eukaryotic picophytoplankton, Cyanobac for cyanobacteria.

April at the three study sites (Fig 5b). The relative proportion of nauplii biomass was higher in February and October compared to April and June at all sites (Fig. 5b) despite the small range of variation in their absolute abundance. The rest of the nano-microzooplankton biomass was largely due to unarmoured dinoflagellates (up to 61.8% of the total biomass, in June at Offshore site, Fig. 5b). Even though the HNF had very high abundances, their contribution to the total biomass of the nano-microzooplankton was very low, overall less than 8% (Fig. 5b).

Seasonal and spatial dynamics of phytoplankton and nano-microzooplankton size structure

Analysis of the SAS showed significant inverse linear relationships at each site and every sampling periods with determination coefficients (r^2) higher than 0.5 and regression coefficients ranged from 0.5 and 1 (Figs 6 and 7).

For any size class common to both, phytoplankton had higher abundances than nano-microzooplankton at Gironde and Coast sites in February (Fig. 6). However, as observed at the Offshore site in October, abundances of medium size classes of microzooplankton were sometimes higher than the abundances of phytoplankton in similar size classes (Fig. 6). In February, phytoplankton SAS showed a well-defined plateau for large size classes at the three study sites. This plateau could still be noticed in April and June but with highest variability in the phytoplankton abundance.

Linear regression slopes of the combined phyto-zooplankton and phytoplankton-only SAS showed similar patterns. The slope at the Offshore site showed the highest temporal variations with a slope becoming first flatter from February to April (-0.86 to -0.51 for combined SAS and -0.80 to -0.43 for phytoplankton SAS) and then steeper between April and October (-1.01 for combined SAS and -1.02 for phytoplankton SAS) (Fig. 7a and b). Both combined and phytoplankton-only SAS slopes at the Gironde and Coast sites became steeper from February to October (Fig. 7a and b). The combined SAS slopes of the three sampling sites were the steepest and the most significant in October ($b = -0.97$, -0.90 and -1.01 and $r^2 = 0.94$, 0.88 and 0.95, respectively, at Gironde, Coast and

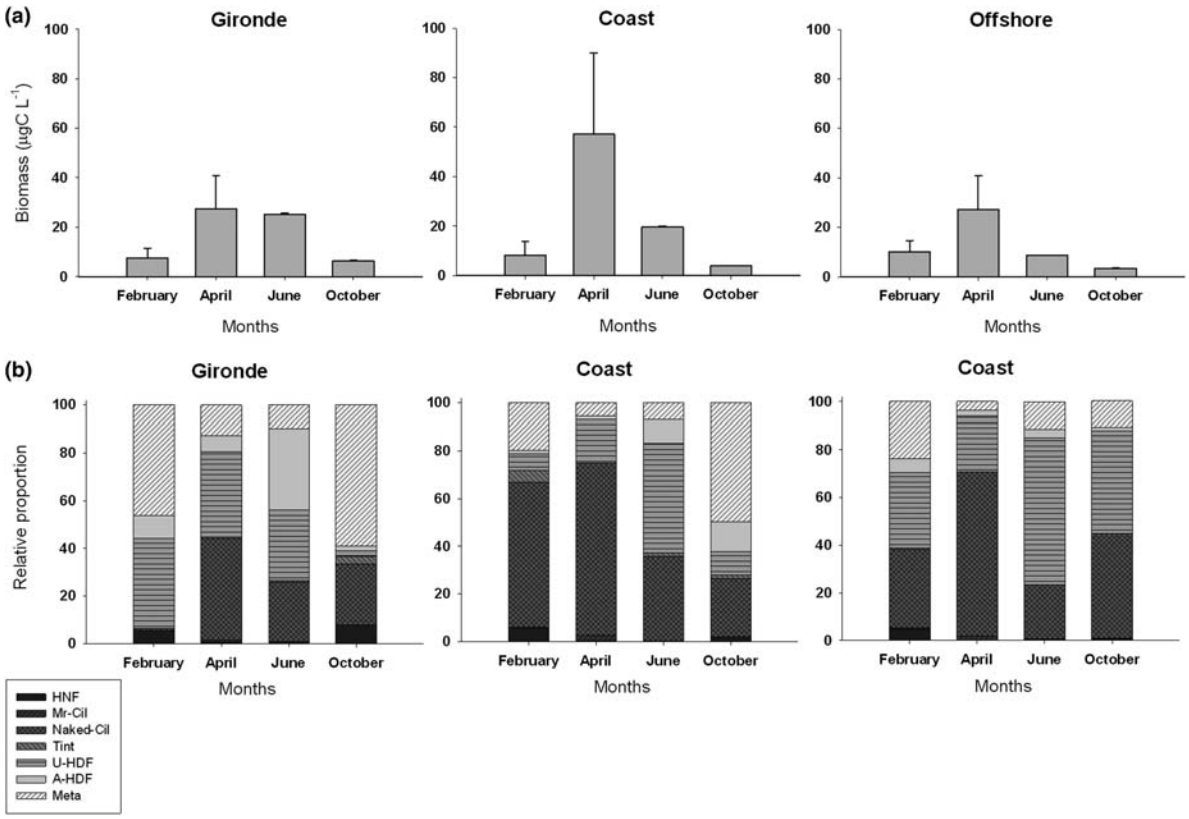

Fig. 5. Seasonal variations of average biomass of nano-microzooplankton (**a**) and its relative composition (**b**) over the photic zone at the three stations. HNF for heteronanoflagellates; U-HDF for unarmoured hetero-dinoflagellates; A-HDF for armoured hetero-dinoflagellates; N-Cil for naked ciliates; Tint for tintinnid ciliates; Mr-Cil for Myrionecta rubra ciliates; Meta for metazoans.

Offshore sites; Fig. 7a). Both nano-microzooplankton SAS slopes at the Offshore and Coast sites became flatter from February to April and then steeper from April to October (Fig. 7c). At the Gironde site, the SAS slope varied slightly during the first three sampling periods, but it became steeper in October and became very similar to the slopes of the two other sites (-0.81, -0.81 and -0.77, respectively, at Gironde, Coast and Offshore sites; Fig. 7c).

Comparing the three classification types

The Mantel test revealed a positive and significant correlation between the three microzooplankton classification types (Table III). The level of similarities between each of the 12 data sets (3 sites and 4 sampling periods) in terms of nano-microzooplankton composition was comparable with every classification we used ($r > 0.8$ and $P < 0.0001$). In other words, there was no difference in the characteristics of microzooplankton dynamics when studying either community size structure or taxonomic composition.

Linking microzooplankton dynamics and environmental conditions

Rank correlation of physical variables to the microzooplankton abundances indicated that the best physical variables driving community patterns were salinity and NO_3 concentration whatever classification types were used (Table IV). BIO-ENV analysis also showed that the microzooplankton dynamics were associated with diatoms >20 µm, bacteria, HNF and mesozooplankton dynamics with every classification type used (Table IV). It was finally noted that the correlation coefficient obtained with the BIO-ENV analyses, while significant, were not in any case very high (<0.3, Table IV).

DISCUSSION

In temperate marine environments, seasonal variations in physico-chemical parameters of the water column have a strong influence on the structure of phytoplankton communities (Margalef, 1958; Fenchel, 1988; Breton *et al.*, 2000; Chang *et al.*, 2003). In summer,

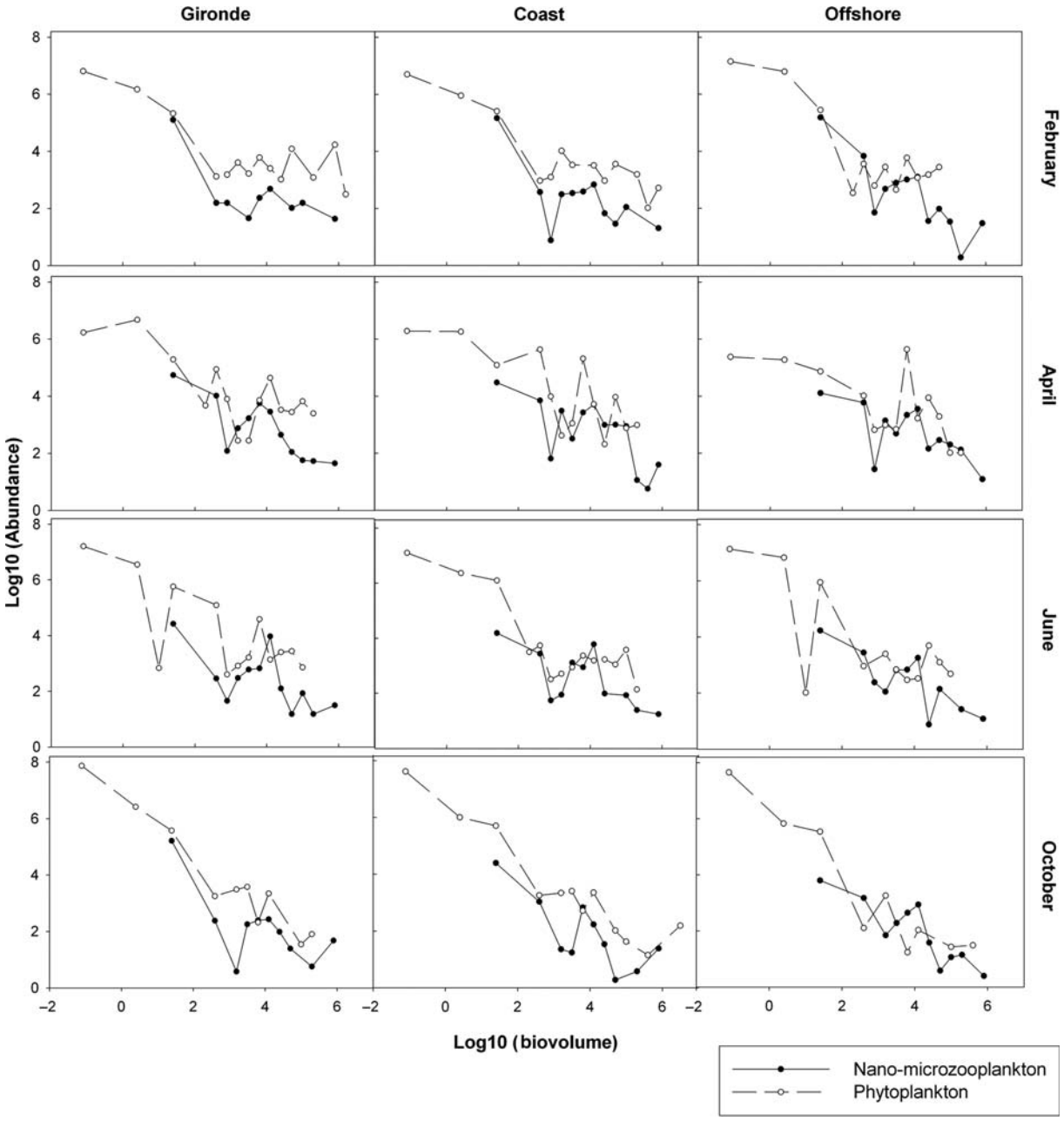

Fig. 6. Size-abundance spectra of phytoplankton (pico- to microphytoplankton) and nano-microzooplankton at the three sites and for the four sampling periods. Size is represented by cell biovolumes (μm^3).

vertical stratification is generally associated with nutrient limitation and regenerated production based on the uptake of ammonium by small-celled phytoplankton populations (Margalef, 1958; Valiela, 1995; Chang *et al.*, 2003). On the other hand, winter/spring vertical mixing is usually associated with nitrate availability and new production by large-celled phytoplankton populations (Margalef, 1958; Valiela, 1995; Bury *et al.*, 2001; Irigoien *et al.*, 2005). In addition, in coastal areas receiving river, the latter may be increased due to the accumulation of terrestrial nutrients in late winter/spring (Domingues *et al.*, 2005; Alvarez *et al.*, 2009). Although the Bay of Biscay is no exception with highest thermal water column stratification in late summer at the three study sites and high concentrations of nitrate in late winter at the site closest to the Gironde estuary, areas under the influence of the Gironde runoff showed high salinity stratification as early as February. As a

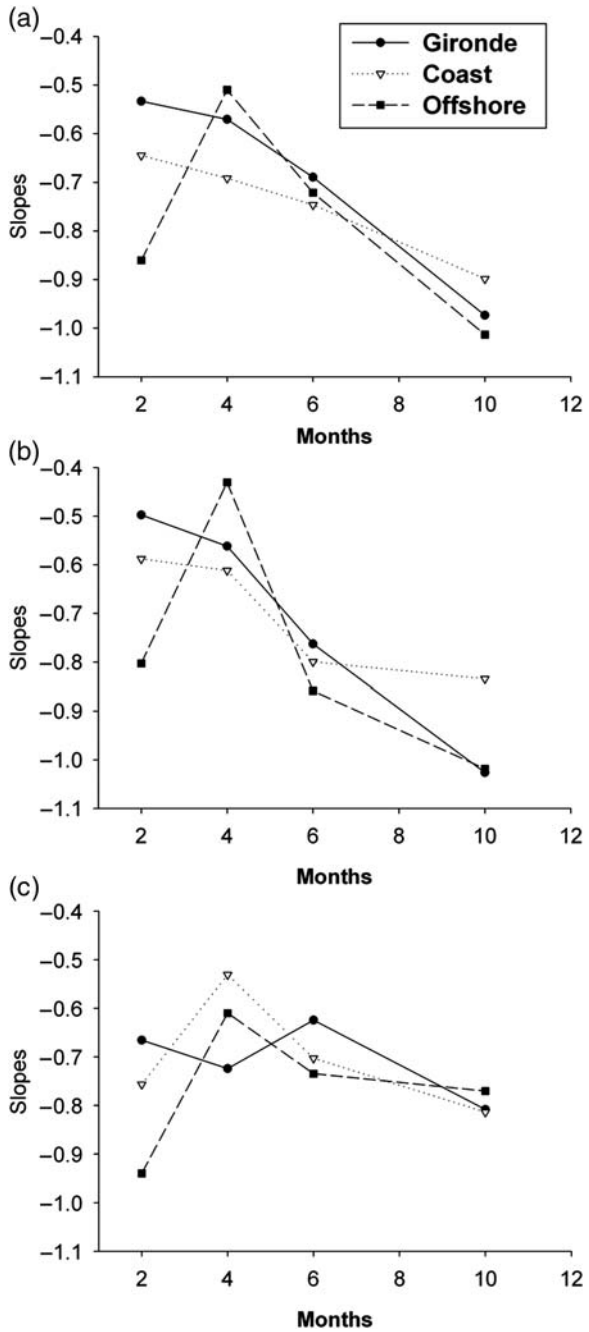

Fig. 7. Temporal and spatial variations of the linear regression slope ($r^2 > 0.5$) of the size-abundance spectra built with (**a**) combined phytoplankton (pico- to microphytoplankton) and nano-microzooplankton data, (**b**) phytoplankton data only and (**c**) nano-microzooplankton data only.

consequence, phytoplankton biomass and composition show strong spatio-temporal variations with large diatoms dominating the community in winter/spring and small autotrophic cells dominating in late summer. Microzooplankton are direct predators on phytoplankton cells (Capriulo, 1991; Capriulo *et al.*, 1991; Calbet and Landry, 2004). As a bottom-up effect, we can assume that changes in the microzooplankton community will follow changes in the phytoplankton community structure. Indeed, heterotrophic protist abundances have been described as a function of food availability in many previous studies (e.g. Dolan and Coats, 1990). Following this argument, the lack of suitable resources in September (dominance of cyanobacteria) and February (large diatoms) might be the principal factor responsible for the low summer and winter microzooplankton biomass in the Bay of Biscay. Verity (Verity, 1986) and Lynn and Montagnes (Lynn and Montagnes, 1991) show that the distribution of ciliates generally shows a close association with nanophytoplankton in temperate coastal ecosystems. In this study, ciliate biomass seems to be more closely related to microphytoplankton than nanoplankton, but this might be because those kinds of biomass composition snapshots are not adequate to show the real level of biomass of highly grazed prey that is produced. However, the BIO-ENV analysis revealed that HNF biomass was one of the main factors explaining the changes in the microzooplankton taxonomic structure. In late summer, the proportion of nauplii in the microzooplankton community increases along the coast of the Bay of Biscay since most of the reproduction of adult copepods occurs during summer (Sautour and Castel, 1998).

Most of the trophic relationships within planktonic food webs are based on size, i.e. the larger consuming the smaller (Platt and Denman, 1977; Moloney and Field, 1991; Caparroy *et al.*, 2000; Stock *et al.*, 2008). Therefore, the size structure of nano-microzooplankton should reflect the phytoplankton composition with large grazers more abundant when large diatoms are the main primary producers. When the community is described with SAS, this situation would then correspond to flatter slopes, i.e. lower absolute values (Platt and Denman,

Table III: Statistical details of the Mantel tests run to compare similarities between the dynamics of nano-microzooplankton community using three different classification types: taxa, body size and ESD

	Mantel statistics	
Explanatory datasets	*r*	*P*-value
Taxons versus body size	0.861	<0.0001
Taxons versus ESD	0.932	<0.0001
Body size versus ESD	0.922	<0.0001

r refers to Pearson correlation coefficient. Statistical significance (*P*) was estimated using 10 000 permutations.

Table IV: Statistical details of the BIO-ENV analysis run to link nano-microzooplankton dynamics and environmental conditions (biological and physical conditions)

		Correlation coefficient *r*		
Environmental conditions	Best parameters	Taxon classification	ESD classification	Body size classification
Physical	Salinity and NO_3 concentration	0.229	0.208	0.202
Biological	Diatoms >20, bacteria, HNF, mesozooplankton	0.172	0.188	0.240

r refers to Spearman rank correlation coefficient. Statistical significance (*P*) was estimated with permutations and is <0.01.

1977; Rodriguez and Mullin, 1986). This is what was observed with this data set. The three study sites show temporal differences in the phytoplankton dynamics with a diatom bloom occurring as soon as February closer to the coast but only in April offshore. This time difference is also visible when analyzing the changes in nano-microzooplankton SAS slope at the three sampling sites. The Gironde site has a flatter slope in February due to the presence of larger cells in the nano-microzooplankton community, showing the earlier occurrence of the diatom bloom in the estuarine waters. The nano-microzooplankton SAS slope at Offshore and Coast sites became flatter in April showing an increase of large cells in the grazer community after the peak of the diatom bloom. In October, the nano-microzooplankton communities at the three study sites have similar size distributions, which can be related to similar size distributions of the primary producers. The slopes of the linear regressions, when fitted to the three types of SAS, are steepest (i.e. highest absolute value) in October when bacteria are very abundant, picophytoplankton is the principal primary producer and grazers are small.

In the literature, most of the studies on plankton size structure have focused on phytoplankton (e.g. Cermeno and Figueiras, 2008; Huete-Ortega *et al.*, 2010) or the modeling of the whole planktonic community (e.g. Baird and Suthers, 2007). However, with the increasing interest in the nano-microzooplankton community as a major player in the planktonic food web (Azam *et al.*, 1983), numerous studies have described nano-microzooplankton dynamics in very different environments (e.g. Shinada *et al.*, 2003; Gaedke and Wickham, 2004; Fileman and Leakey, 2005). Identification of microzooplankton cells using classical microscopic methods is highly time-consuming and new technologies such as automatic plankton counters are quickly extending their attraction among ecologists (see review in Benfield *et al.*, 2007). However, these technologies, despite being very efficient at counting cells, do not have the same capabilities in cell identification as an expert human eye. A good understanding of the community dynamics depends on a good description of its composition and structure. Size parameters of nano-microzooplankton cells are easy to compile, but the question is whether they are a sufficient descriptor on their own.

The first problem occurs when choosing the best size parameter to use. Each study corresponds to a different parameter: length, diameter, ESD, biovolume, cell carbon content, etc. (e.g. Kimmel *et al.*, 2006; Cermeno and Figueiras, 2008; Basedow *et al.*, 2010). Despite aiming to describe the same characteristic of a cell and its size, this variety may be an obstacle to those who would like to compare data from different studies. This study considered the three most widely used parameters used, biovolume, ESD and body mass. Since ESD and biovolume are directly related to one another, one can be used to compare the structure of the other without any difficulty. However, depending on the conversion factor used, body mass may not be directly proportional to the first two parameters. In this case, the general relationship between ESD and body mass matches an exponential slope. Although ESD does not represent the trophic value of the nano-microzooplanktonic cell, it is an easier parameter to measure or estimate than the body mass. Moreover, since the definition of ESD is the same for cells of every size and every sea, it would be more reliable as a comparable and universal parameter. Therefore, we recommend using ESD as a proxy of size when studying nano-microzooplanktonic size structure.

The second problem, and without doubt the most important, concerns the efficiency of size descriptors to define microzooplankton dynamics as clearly as the taxonomy. Before addressing this problem, the definition of "dynamics" of a community needs to be agreed upon. As proposed in Lindeman's paper on tropho-dynamics (1942), the dynamics of a community is the pattern of its changes in size and composition over time and space resulting from the interactions between the living members of the community and reactions of those members to the non-living environment. Following this definition, the microzooplankton dynamics portrayed with size descriptors should show the same pattern of changes and of differences between seasons and sites as the dynamics portrayed with taxonomy. The Mantel test used in this study focused on comparing the patterns of

the spatio-temporal differences observed in the structure and composition of the microzooplankton community with three different descriptors (taxonomy and two size parameters). Statistics did not reveal any differences between the three dynamics. Therefore, we can argue that using the size structure to characterize the dynamics of nano-microplankton leads to revealing the same spatio-temporal patterns as when using a taxonomic structure.

Finally, the ultimate goal of community dynamics studies is the understanding of the relationships of a community with its environment and more precisely with its trophic environment (i.e. Lindeman, 1942). Knowledge of community dynamics is necessary in food web and ecosystem process studies as it provides a way to analyze the transfer rates of energy and matter and the impact of environmental disturbances (de Ruiter *et al.*, 2005). The historical way to examine community dynamics has been by changes in taxonomic structure and the trophic relationships between taxonomic groups (e.g. Andersen and Sorensen, 1986; Uitto *et al.*, 1997). We have shown in this study that size descriptors efficiently define microzooplankton dynamics, but are they also good in revealing biotic and abiotic factors controlling that dynamics? Although correlations do not necessarily mean direct causal relationships such as trophic links, they might give information on the physical and biological parameters influencing microzooplankton dynamics (Legendre and Legendre, 1998). In our study, the interest of such an analysis was to compare the results obtained with the three different types of classification. The results of the BIO-ENV analyses were the same for the three types of classification compared with very low but similar Spearman rank correlation coefficients.

In conclusion, we argue that nano-microplankton dynamics are well defined by the study of their size structure. The use of automatic plankton counters such as the FlowCAM technology (Sieracki *et al.*, 1998) is therefore relevant despite the lack of precise taxonomic identification. However, homogenizing the size descriptor used among studies is needed in order to make worldwide data comparisons and ESD/biovolume should be favored. We also recommend further investigations on size-based trophic relationships and size structure dynamics in order to confirm the results found in the Bay of Biscay.

ACKNOWLEDGEMENTS

The authors wish to thank the two anonymous reviewers who helped improving the manuscript. In addition, the authors thank captains and crews of R/V Thalia for support activities, C. Courties (Observatoire Océanologique de Banyuls, France) for the flowcytometry analyses, F. Mornet (IFREMER, FREDD, France) and V. Huet (LIENSs, France) for nutrient and chlorophyll measurements, P. Malterre and M-J Capdeville (students of University of La Rochelle, France) for metazoan counts.

FUNDING

This study was supported by the French "Programme National Environment Côtier"—Bay of Biscay working site—and Ifremer.

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