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► To cite this version:

Théo Garcia, François Carlotti, Gilles Lepoint, Loïc Guilloux, Javier Angel Tesán-Onrubia, et al.. The decadal variability of elemental, isotopic, and biochemical compositions of coastal Mediterranean zooplankton responds to environmental changes. *Marine Biology*, 2024, 171 (5), pp.119. <10.1007/s00227-024-04430-5>. <hal-04702949>

HAL Id: hal-04702949

<https://hal.science/hal-04702949v1>

Submitted on 19 Sep 2024

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The decadal variability of elemental, isotopic, and biochemical compositions of coastal Mediterranean zooplankton responds to environmental changes

Théo Garcia¹ · François Carlotti¹ · Gilles Lepoint^{2,3} · Loïc Guilloux¹ · Javier Angel Tesán-Onrubia¹ · Baptiste Grassi¹ · Victor Russias¹ · Daniela Bănaru¹

Abstract

The functioning of Mediterranean coastal waters is deeply affected not only by global environmental changes (e.g., global warming, climate change) but also by improvements in sewage treatment as required by the European Water Framework Directive. These changes affect nutrient concentrations and cycling, and primary production. The present paper presents the time series analysis (2005–2020) of stable isotope ratios (hereafter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), elemental (C and N), and biochemical compositions (proteins, lipids, carbohydrates) within the 300–500 μm zooplankton size fraction from bimonthly monitoring samples (0–55 m) collected in the Bay of Marseille (NW-Mediterranean Sea). The zooplankton seasonality was mostly related with the dynamic of proxies of phytoplankton biomass and nutrients in the system. In spring, protein content and energy increased and $\delta^{15}\text{N}$ of zooplankton decreased; in late spring, lipid and carbohydrate content peaked; and in late summer, $\delta^{13}\text{C}$ increased. At the interannual scale, major changes in the zooplankton biochemical and isotopic composition have occurred since 2009. Lipid content decreased while protein and stable isotope ratios increased. These shifts were concomitant with changes in the nutrient concentrations, nutrient stoichiometry, and particulate organic matter concentrations. The changes in biochemical composition led to an increase of energy content from 2011, but the total quantity of energy available to higher trophic positions remained stable because of a reduction in biomass. The variations of the zooplankton stable isotopes and biochemical compositions can be indicative of changes in individual biochemistry (energy allocation) and/or in the zooplankton community (changes in relative abundance of lipid-rich vs protein-rich species).

Keywords Gulf of Lion · Zooplankton time series · SOMLIT monitoring · Coastal waters · Regime shift · Nutrient decrease · Bay of marseille

Introduction

The Mediterranean Sea is an oligotrophic Sea; nonetheless, more productive areas that support important fishery activities have been identified in the Northwestern Mediterranean Sea (e.g., Gulf of Lion, Catalan Sea) (Durrieu de Madron et al. 2011 and references therein). These productive areas are influenced by multiple forcing such as continental inputs, upwellings, deep convections, and eddies (MEDOC GROUP, 1970; Millot 1999; Diaz et al. 2008). Time series analyses over the last two decades showed that the French Mediterranean coast underwent a decline in nutrient concentrations (in particular phosphate, nitrate, nitrite, and ammonium), and an increase in temperature and salinity (Goberville et al. 2010; Lheureux et al. 2021). Due to improved sewage treatment, resulting notably from implementation of

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the Water Framework Directive (WFD), a strong decrease of nutrient input from the Rhone (average flow 1242 m³/s, data on <https://www.hydro.eaufrance.fr/>) (Saraux et al. 2019) into the Gulf of Lion has been observed (Feuilloley et al. 2020; Raimbault et al. 2021). In addition, the Mediterranean Sea has also experienced the effects of global change, with an increase in temperature and salinity and long-term effects on water circulation, and, potentially, on natural advection of nutrients from deep water to coastal waters (Margirier et al. 2020). It has been suggested that all these changes have affected small pelagic fish, through zooplankton bottom-up control, in the Gulf of Lion (Le Bourg et al. 2015; Brosset et al. 2016; Saraux et al. 2019). The hypothesis of bottom-up control postulates that zooplankton nutritional quality and quantity have changed in response to primary production change, linked to nutrient load and cycling changes (Saraux et al. 2019). However, no quantitative data on temporal variations in zooplankton nutritional quality in the Gulf of Lion have yet been acquired and used to support this hypothesis.

Zooplankton organisms play a pivotal role in the pelagic food web. They transfer energy and carbon from primary producers to organisms in upper trophic positions such as fish, sea birds, and marine mammals (Banse 1995; Bănarău et al. 2013). The study of zooplankton stable isotope ratio and biochemical composition has been central in the last decades, notably to identify and to quantify the energy pathway in the pelagic ecosystem (Eddy et al. 2021). Various methods have been developed aiming to specify zooplankton biochemical quality, from bulk biomass, size fractionated biomass, and up to individual organisms, including differentiation in species, stage, and sex, using carbon and nitrogen stoichiometry, calorimetric bomb, or proximate composition (i.e., quantify the concentration of molecules, e.g., lipids, proteins, and carbohydrates) (Bamstedt 1986; Ventura 2006; Schaafsma et al. 2018; and references therein). Proteins, lipids, and carbohydrates have different functions in the biology of aquatic organisms (see Postel et al. 2000; Ventura 2006 and references therein). Proteins have structural roles in the body of an organism, for example in muscle tissues. Lipids are mainly allocated for energy reserves, reproduction, and cell membranes. Finally, carbohydrates supply energy (Ventura 2006; Lee et al. 2006; Thomas et al. 2022). The proximate composition approach by determining the energy values of the macronutrients provides information on zooplankton nutritional quality and physiological functions. Although some discrepancies may emerge from different analytical methods, zooplankton organisms are globally characterized by high protein, medium lipid, and low carbohydrate content (Ventura 2006). In addition, carbon and nitrogen stoichiometry (C:N ratio) are commonly used for the proxy lipid: protein ratio. The biochemical and energy content of zooplankton vary according to inherent (e.g., sex, life stage, size) and/or external (e.g., environment, habitat,

prey seasonal variation) factors (Morris and Hopkins 1983; Ventura 2006; Cass et al. 2014; Barroeta et al. 2017; Chen et al. 2019; Harmelin-Vivien et al. 2019).

In addition to providing detailed protocols for the study of zooplankton biochemistry, Yebra et al. (2017) advocated the need to apply them during zooplankton monitoring. Analyses of stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are commonly used to study zooplankton trophic ecology (Minagawa and Wada 1984; Rolff 2000; Post 2002). $\delta^{13}\text{C}$ provides information on the origin of the organic food source. $\delta^{15}\text{N}$ in each size fraction is indicative of organic food sources, and also their trophic position and the values estimated for the successive size fractions highlight the vertical dimension of the trophic web. To explore decadal variations in the zooplankton biochemistry, elemental composition and stable isotope ratios may provide a new basis for understanding the functioning and the structure of the plankton community. However, time series analyses of zooplankton stable isotope ratios and biochemical content over multiple years or decades remain scarce (Mackas et al. 2012; Ohman et al. 2012; Espinasse et al. 2022; Brito-Lolaia et al. 2022). Although numerous studies in the NW-Mediterranean focused on zooplankton biochemistry (Champalbert and Kerambrun 1973; Gaudy and Champalbert 1998; Barroeta et al. 2017; Chen et al. 2019; Yebra et al. 2020; Tesán-Onrubia et al. 2023), none based on a long-term monitoring survey has been published. Since 2005, zooplankton has been monitored bimonthly in the Bay of Marseille (NW-Mediterranean Sea), in parallel with the national survey (RNO SOMLIT) of environmental parameters (Goberville et al. 2010; Lheureux et al. 2021). The 200–1000 μm zooplankton community over this period was mainly dominated by copepods (Garcia et al. 2023) in agreement with previous studies in the Bay of Marseille (Bănarău et al. 2014; Chen et al. 2019). Nevertheless, Garcia et al. (2023) showed a decrease between 2005 and 2020 in the zooplankton size fractions biomass and total abundance, changes in groups and species relative abundance (i.e., decrease of the dominance of calanoids and oithonoids copepods), and modification of the zooplankton community phenology.

The aim of the present work was to answer the following question: how does the biochemical, elemental, and stable isotopic composition of the zooplankton 300–500 μm size fraction respond to environmental forcing over the period 2005–2020 in the context of climate change and the implementation of the Water Framework Directive? For this purpose, we first analyzed the temporal variations in elemental, isotopic, and biochemical composition of this size fraction for sixteen years (2005–2020) in the Bay of Marseille (NW-Mediterranean Sea), by investigating their seasonal and interannual variations. Then, the detected zooplankton variations were analyzed relative to the biotic and abiotic environment of the Bay of Marseille. In particular, the trophic

relationship between zooplankton and particulate organic matter, mainly composed of phytoplankton (Liénart et al. 2017; Tesán-Onrubia et al. 2023), was studied using stable isotopic ratios.

Materials and methods

Zooplankton sampling

From 2005 to 2020, mesozooplankton has been sampled bimonthly by a vertical haul at the Frioul monitoring Station (43.24N, 5.29E, 60 m depth, see Fig. 1), from 55 m to the surface, using a WP2 200 μm mesh-size plankton net. The volume of filtered sea water was estimated by multiplying the surface area of the mouth of the net by the sampled depth. The cod end content was separated in the laboratory on a sieve column into 6 size fractions: 80–200 μm , 200–300 μm , 300–500 μm , 500–1000 μm , 1000–2000 μm , and > 2000 μm . The time lag between sampling and size separation in the laboratory was much longer than common gut clearance of zooplankton. The zooplankton organisms from each size fraction were placed on pre-combusted (450 $^{\circ}\text{C}$, 2 h), pre-weighed Whatmann GF/F filters and dried 48 h at 60 $^{\circ}\text{C}$ for dry weight (DW) measurements, using a microscale ($d = 0.0001$ g). During the 2005–2020 period, at least one sample per month (with a few missing values for some months) of the 300–500 μm size fraction was used for biochemical, stable isotope, and elemental analyses.

Biochemical analysis

One mg of zooplankton was weighed using a microscale. Proteins, carbohydrates, and lipids were extracted following the methods of, respectively, Lowry et al. (1951) (i.e., in Folin phenol solution), Dubois et al. (1956) (i.e., in phenol–sulfuric acid solution), and Bligh and Dyer (1959) (i.e., in monophasic methanol–dichloromethane solution). The absorbance was measured using a spectrophotometer (Shimadzu, UV-1280). Different wavelengths were used for measuring proteins, carbohydrates, and lipids: 700 nm, 490 nm, and 360 nm respectively. The absorbance was converted to mass units for the different biochemical compounds by using an external calibration curve. The concentrations values were expressed in $\mu\text{g}\cdot\text{mg}^{-1}$ DW. Applying the Postel et al. (2000) constants, the concentrations of the biochemical compounds of the 300–500 μm size fraction were converted into an energy metric (21.4 $\text{kJ}\cdot\text{g}^{-1}$ for proteins, 17.2 $\text{kJ}\cdot\text{g}^{-1}$ for carbohydrates, and 35.6 $\text{kJ}\cdot\text{g}^{-1}$ for lipids). The sum of the obtained values for the three compounds enabled us to determine the energy concentration (i.e., energy density) of the sample per unit of DW (E_{con} , in $\text{kJ}\cdot\text{g}^{-1}$ DW). The energy concentration issued from this size fraction and available for their consumers (E_{ava} , in $\text{kJ}\cdot\text{m}^{-3}$) was calculated by multiplying E_{con} by the biomass ($\text{mg}\cdot\text{m}^{-3}$) of the 300–500 μm size fraction in the water column.

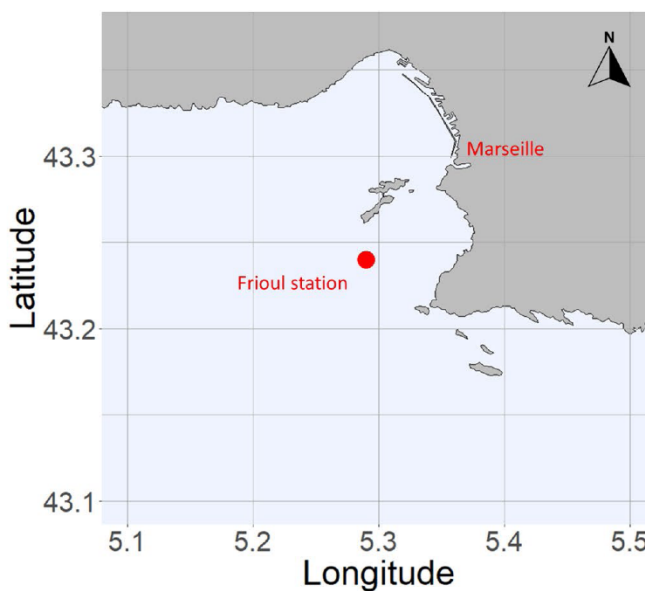
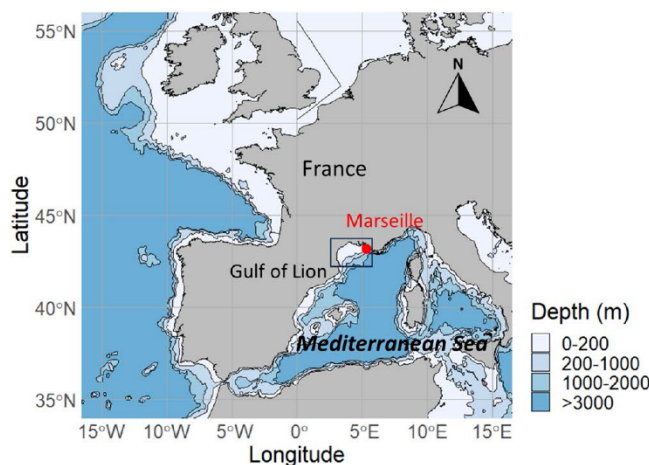


Fig. 1 Localization of the Bay of Marseille at Basin (NW Mediterranean) and Gulf of Lion scale (left panel). Localization of the Frioul sampling station in the Bay of Marseille (right panel). Color gradient

refers to the bathymetry on the left panel. Maps were produced using Natural Earth and France-GeoJSON (Gregoire 2018) open access data

Stable isotope and elemental compositions

The 300–500 µm zooplankton fraction was retrieved with a scalpel from the surface of the filters. For carbon isotopes analysis, a weighed amount of sample (0.8 mg DW, i.e., optimum value for mass spectrometer) was placed in a silver cup. Then, acidification was done directly by adding HCl 1% solution to avoid possible bias related to inorganic carbonates (Pinnegar and Polunin 1999). No acidification was performed on samples used for nitrogen isotope analyses. The dried matter was placed in a tin cup and weighted with a microscale ($d = 0.00001$ g). Samples were desiccated in an oven for two hours at 50 °C before being enclosed in a cup. The isotopic compositions of C and N, as well as carbon and nitrogen concentration relative to dry weight (%DW) were measured in continuous flow using an elemental analyzer (vario MICRO Cube Elementar, Hanau Germany) coupled to an isotope-ratio mass spectrometer (PrecisION, Elementar UK, Cheadle, United Kingdom). Stable isotope ratios of carbon and nitrogen were expressed in δ notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively; Coplen 2011) and in ‰ relative to international references (Vienna Pee Belemnite for $\delta^{13}\text{C}$ and N_2 in atmospheric air for $\delta^{15}\text{N}$) according to the formula:

$$\delta X_{\text{sample}} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000,$$

where X is ^{13}C or ^{15}N , R is the isotope ratio between heavy and light isotopes ($^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$, for carbon and nitrogen, respectively).

Certified materials for stable isotopes from the International Atomic Energy Agency (IAEA, Vienna, Austria) were IAEA N-1 (ammonium sulfate; $\delta^{15}\text{N} = 0.4 \pm 0.2\text{‰}$) and IAEA C-6 (sucrose; $\delta^{13}\text{C} = -10.8 \pm 0.5\text{‰}$). Sulfanilic acid (Sigma-Aldrich, Overijse, Belgium; $\delta^{13}\text{C} = -25.6 \pm 0.4\text{‰}$; $\delta^{15}\text{N} = -0.1 \pm 0.4\text{‰}$; means \pm SD) was used as second quality control for stable isotopes and as elemental standard (N: 8.01%DW, C: 41.8%DW).

Anthropogenic emissions of CO_2 in the atmosphere are depleted in ^{13}C and, therefore, affect $\delta^{13}\text{C}$ measurements (known as Suess effect, Bacastow et al. 1996). In long-term studies, the Suess effect can affect the observed $\delta^{13}\text{C}$ values and bias interpretation (Quay et al. 1992). A correction of the decreasing trend induced by Suess effect at a rate of $-0.0282\text{‰}\cdot\text{year}^{-1}$ (Quay et al. 1992) in the $\delta^{13}\text{C}$ was applied on zooplankton and $\text{POM}_{\text{surface}}$ series of $\delta^{13}\text{C}$.

Stoichiometry of carbon and nitrogen (C:N ratio, w:w) of 300–500 µm zooplankton series was derived as proxy of lipids/proteins ratio.

Environmental data

Zooplankton samples were collected in parallel with a long-term national program (RNO SOMLIT, www.somlit.fr). Since 1994, this long-term monitoring program includes CTD casts and water sampling by means of Niskin bottles. The following parameters are measured bimonthly: temperature (T in °C), salinity (S), oxygen concentration (O_2 in $\text{mL}\cdot\text{L}^{-1}$), nutrient concentrations (i.e., ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) in $\mu\text{mol}\cdot\text{L}^{-1}$), concentration of suspended particulate matter (SPM) in $\text{mg}\cdot\text{L}^{-1}$, concentrations of particulate organic carbon (POC), particulate organic nitrogen (PON), and chlorophyll *a* (Chl *a*, in $\mu\text{g}\cdot\text{L}^{-1}$). The parameters are measured at subsurface, maximum fluorescence, and bottom for each sampling date. In this study, the values for the three depths were averaged to obtain an integrated value over the water column. From temperature and salinity profiles, mixed layer depth (MLD) was considered as the depth with a difference of $0.03 \text{ kg}\cdot\text{m}^{-3}$ from the density at -10 m (de Boyer Montégut et al. 2004).

Since 2008, analyses of stable isotopes of carbon ($\delta^{13}\text{C}\text{-POM}_{\text{surface}}$) and nitrogen ($\delta^{15}\text{N}\text{-POM}_{\text{surface}}$) are done routinely on water subsurface samples (-1 m, filtered on a 0.7 µm Whatmann GF/F filter to retain particulate organic matter) during SOMLIT surveys in the Bay of Marseille.

Cytometry analyses have been performed in subsurface sample since late 2009 to determine abundance and size of plankton groups that composes POM: high nucleic acid bacteria, low nucleic acid bacteria, total bacteria, *Cryptophytes*, *Prochlorococcus*, *Synechococcus*, pico- and nano- eucaryotes.

More detailed information on SOMLIT parameters and protocols can be found in www.somlit.fr.

Finally, precipitation and wind data were obtained from Météo France (<https://donneespubliques.meteofrance.fr/>) to represent the meteorological context that can affect zooplankton variability (i.e., terrestrial inputs from precipitation and water mixing during strong wind events). From wind raw data (i.e., speed and direction), the wind vector was projected on a NW–SE axis (wind stress) to capture the intensity of the two main wind regimes: the north-western (Mistral, positive values of wind stress index) and south-eastern winds (negative values of wind stress index). Large-scale atmospheric conditions were represented by means of North Atlantic Oscillations (NAO, <https://www.ncdc.noaa.gov/teleconnections/nao/>) and Western Mediterranean Oscillations (WeMO, <https://crudata.uea.ac.uk/cru/data/moi/>).

Statistical analyses

Data pretreatment and analyses were performed using the R software (R Core Team 2022). Statistical analyses were considered significant when p -value < 0.05 .

Time series pretreatment

Time series (Table 1, summary of the time series) were regularized (i.e., averaged when more than one sample was available within a month) and analyzed at a monthly scale.

Multiple Imputation with Bayesian PCA procedure (MIPCA, Audigier et al. 2015), using the R package ‘missMDA’, was implemented to impute missing values. This procedure enables robust allocation for missing values in a dataset up to 30%. In our case, the proportion of missing values is less than 7%.

Zooplankton time series analyses

To investigate collinearity between zooplankton indices (biochemical and energy content, stable isotope ratios, and elemental composition), Pearson correlations were performed between each pair of zooplankton variables, with the method recommended by (Pyper and Peterman 1998), with correction of degree of freedom for autocorrelations following the modified Chelton method.

Interannual trends and 95% confidence interval of zooplankton variables were estimated using local polynomial regressions (LOESS). Trends were considered significant when the 95% confidence interval of the LOESS regression could not contain a horizontal line.

On every time series, a breakpoint detection procedure was used to highlight structural change among univariate time series (“strucchange” package in R, Zeileis et al. 2002). A maximum of three breakpoints was investigated. The optimum number of breakpoints was selected according to the Bayesian Information Criterion.

Monthly seasonal patterns were assessed by fitting LOESS regression. As for interannual trends, the significance of the seasonal patterns was determined based on whether the horizontal lines fell within the 95% confidence interval.

Relationship between zooplankton elemental, isotopic, and biochemical composition, and environmental conditions

Partial least squares (PLS) analysis aims at explaining the relationship (linear regression) between two datasets of continuous variables. This analysis belongs to the family of dimension reduction methods between two data tables (along with, e.g., canonical correspondence analysis or redundancy analysis); however, in contrast to other methods, PLS maximizes the covariance between latent variables (i.e., principal component) rather than correlations (i.e., between variables). Furthermore, by construction in regression mode, PLS investigates the asymmetric roles of the two datasets. To quantify the relationship between variables of

two different datasets, an association score can be delivered by calculating the scalar product value between each pair of vectors of n length representing coordinates of the variables on the n dimensions of the PLS axes.

PLS was applied to disentangle the response of zooplankton elemental, isotopic, and biochemical composition in relation to abiotic and biotic environmental variables.

Two PLS were performed to study the relationships between:

- (i) variations of zooplankton elemental, isotopic, and biochemical composition (i.e., response dataset) and environmental data (i.e., explanatory dataset) over the period 2005–2020.
- (ii) variations of zooplankton elemental, isotopic, and biochemical compositions (i.e., response dataset), and environmental data including subsurface water measurements for cytometry and POM stable isotope ratios analyses (i.e., explanatory dataset) over the period 2010–2020 (i.e., when cytometry and stable isotope data are both available for the whole year).

Clustered image mapping (CIM) is a graphical method used to represent the association between variables of the two datasets. CIM is a heatmap which uses association scores among variables of two datasets. A hierarchical clustering is performed on row and column to better visualize groups of variables that present a similar association with variables of the other dataset. The R package ‘mixOmics’ (Cao and Welham 2021) was used for performing PLS.

Differences (Δ , ‰) between stable isotope ratios composition of zooplankton ($\delta^{13}\text{C}$ -zoo and $\delta^{15}\text{N}$ -zoo) and subsurface POM ($\delta^{13}\text{C}$ -POM_{surface} and $\delta^{15}\text{N}$ -POM_{surface}) were investigated by means of ANOVA to investigate trophic relationships between the two compartments. The temporal dynamics (seasonal and interannual) of stable isotopic composition of subsurface POM and their difference (Δ , ‰) relative to the stable isotope ratio of the zooplankton were used to assess temporal changes in the trophic relationship.

Results

Ranges of zooplankton elemental, isotopic and biochemical compositions in the time series

$\delta^{13}\text{C}$ -zoo and $\delta^{15}\text{N}$ -zoo mean values (respectively, -21.4 ‰ \pm SE 1.0, $n = 192$, and 4.4 ‰ \pm SE 1.0, $n = 192$) were higher than mean values of $\delta^{13}\text{C}$ -POM_{surface} and $\delta^{15}\text{N}$ -POM_{surface} (respectively, -22.3 ‰ \pm SE 1.1, $n = 156$, and 2.6 ‰ \pm SE 1.3, $n = 156$) over the whole period (Table 2 and Table S1). Values of mean difference (Δ , ‰) between 300 and 500 μm size-fraction zooplankton and POM_{surface}

Table 1 Summary of the variables used for statistical analyses in this study, the units, the period, and the sources

Variable (abbreviation)	Unit	Period	Source
Wind stress (wind_stress)	–	2005–2020	Météo France
Precipitations (prec)	mm	2005–2020	Météo France
Maximum Layer Depth (MLD)	m	2005–2020	SOMLIT
Western Mediterranean Oscillation (WeMO)	–	2005–2020	Martin-Vide and Lopez-Bustins 2006
Northern Atlantic Oscillation (NAO)	–	2005–2020	NOAA
Temperature (T)	°C	2005–2020	SOMLIT
Salinity (S)	–	2005–2020	SOMLIT
Oxygen (O)	mL.L ⁻¹	2005–2020	SOMLIT
Ammonium concentration (NH ₄)	µmol.L ⁻¹	2005–2020	SOMLIT
Nitrate concentration (NO ₃)	µmol.L ⁻¹	2005–2020	SOMLIT
Nitrite concentration (NO ₂)	µmol.L ⁻¹	2005–2020	SOMLIT
Phosphate concentration (PO ₄)	µmol.L ⁻¹	2005–2020	SOMLIT
Particulate organic carbon (POC)	µg.L ⁻¹	2005–2020	SOMLIT
Particulate organic nitrogen (PON)	µg.L ⁻¹	2005–2020	SOMLIT
Suspended particulate matter (SPM)	µg.L ⁻¹	2005–2020	SOMLIT
Chlorophyll a (CHLA)	µg.L ⁻¹	2005–2020	SOMLIT
High nucleic acid bacteria count	Cells.mL ⁻¹	2010–2020	SOMLIT
High nucleic acid bacteria size index	–	2010–2020	SOMLIT
Low nucleic acid bacteria counts	Cells.mL ⁻¹	2010–2020	SOMLIT
Low nucleic acid bacteria size index	–	2010–2020	SOMLIT
Total bacteria counts	Cells.mL ⁻¹	2010–2020	SOMLIT
Total Bacteria size index	–	2010–2020	SOMLIT
<i>Cryptophytes</i> counts	Cells.mL ⁻¹	2010–2020	SOMLIT
<i>Cryptophytes</i> size index	–	2010–2020	SOMLIT
<i>Synechococcus</i> counts	Cells.mL ⁻¹	2010–2020	SOMLIT
<i>Synechococcus</i> size index	–	2010–2020	SOMLIT
<i>Prochlorococcus</i> counts	Cells.mL ⁻¹	2010–2020	SOMLIT
<i>Prochlorococcus</i> size index	–	2010–2020	SOMLIT
Picoeukaryotes counts	Cells.mL ⁻¹	2010–2020	SOMLIT
Picoeukaryotes size index	–	2010–2020	SOMLIT
Nano-eukaryotes counts	Cells.mL ⁻¹	2010–2020	SOMLIT
Nano-eukaryotes size index	–	2010–2020	SOMLIT
Isotope ratios δ ¹³ C of surface particulate organic matter (δ ¹³ C-POM _{surface})	–	2008–2020	SOMLIT
Isotope ratios δ ¹⁵ N of surface particulate organic matter (δ ¹⁵ N-POM _{surface})	–	2008–2020	SOMLIT
Biomass of 300–500 µm fraction (Biomass)	mg DW.m ⁻³	2005–2020	Present study
Isotope ratios δ ¹³ C of 300–500 µm fraction (δ ¹³ C-zoo)	–	2005–2020	Present study
Isotope ratios δ ¹⁵ N of 300–500 µm fraction (δ ¹⁵ N-zoo)	–	2005–2020	Present study
Content of carbon	%C	2005–2020	Present study
Content of nitrogen	%N	2005–2020	Present study
Carbon: nitrogen ratio of 300–500 µm fraction (C:N)	–	2005–2020	Present study
Energy content of 300–500 µm fraction available for predators (E _{ava})	kJ.m ⁻³	2005–2020	Present study
Energy content of 300–500 µm fraction per unit of dry weight (E _{con})	kJ.g ⁻¹ DW	2005–2020	Present study
Concentration of proteins of 300–500 µm fraction (Prot)	kJ.g ⁻¹ DW	2005–2020	Present study
Concentration of carbohydrates of 300–500 µm fraction (Carb)	kJ.g ⁻¹ DW	2005–2020	Present study
Concentration of lipids of 300–500 µm fraction (Lipi)	kJ.g ⁻¹ DW	2005–2020	Present study

Table 2 Summary of the characteristics of the stable isotope ratios, biochemical and elemental composition time series of the 300–500 μm zooplankton size fraction

Variable	Unit	Mean (min/max)	Shift year	Mean (min/max) before shift	Mean (min/max) after shift
$\delta^{13}\text{C}$ -zoo	‰	-21.43 (-25.83/-19.19)	-	-	-
$\delta^{15}\text{N}$ -zoo	‰	4.37 (0.75/6.89)	2009	3.93 (1.00/5.22)	4.52 (0.75/6.89)
%C	%	47.53 (26.19/74.42)	2010	45.05 (26.19/58.38)	48.87 (31.49/74.42)
%N	%	11.04 (4.88/20.49)	2007	9.62 (5.41/12.99)	11.27 (4.88/20.49)
C:N	-	4.32 (2.98/6.20)	2007	4.62 (4.1/6.2)	4.27 (2.98/5.3)
E_{con}	kJ.g^{-1} DW	5.08 (3.51/8.78)	2011	4.72 (3.51/6.25)	5.29 (3.59/8.78)
Biomass	mg.m^{-3}	1.95 (0.16/10.87)	-	-	-
E_{ava}	kJ.m^{-3}	10.1 (0.71/67.70)	-	-	-
Proteins	$\mu\text{g.mg}^{-1}$ DW	155.67 (67.71/345.87)	2008	134.57 (70.09/246.26)	161.68 (67.76/345.79)
Proteins	kJ.g^{-1} DW	3.33 (1.45/7.40)	2008	2.88 (1.50/5.27)	3.46 (1.45/7.40)
Lipids	$\mu\text{g.mg}^{-1}$ DW	40.17 (8.71/171.35)	-	-	-
Lipids	kJ.g^{-1} DW	1.43 (0.31/6.10)	-	-	-
Carbohydrates	$\mu\text{g.mg}^{-1}$ DW	20.93 (2.33/43.02)	2013	22.67 (11.04/37.79)	19.19 (2.33/43.02)
Carbohydrates	kJ.g^{-1} DW	0.36 (0.04/0.74)	2013	0.39 (0.19/0.65)	0.33 (0.04/0.74)

E_{con} corresponds to energy content of 300–500 μm fraction per unit of dry weight. E_{ava} corresponds to the energy content of 300–500 μm fraction available for upper trophic positions

stable isotope ratios ranged between negative values (minimum of -2.9 and -2.6 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to positive (maximum of 3.9 and 4.9 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, Table S1). Over the study period, the average differences were 0.9 ‰ for carbon (\pm SE 1.4 $n = 156$) and 1.9 ‰ for nitrogen (\pm SE 1.3 $n = 156$). Differences were significant for both $\delta^{13}\text{C}$ values (ANOVA, $df = 1$, $F = 55.46$, p -value < 0.001) and $\delta^{15}\text{N}$ values (ANOVA, $df = 1$, $F = 146.2$, p -value < 0.001).

Among the biochemical components measured, proteins were the most concentrated (155.67 in $\mu\text{g.mg}^{-1}$ DW, \pm SE 38.97, $n = 192$) and represented the largest contribution to the dry weight (71.16% ash free dry weight, AFDW, \pm SE 8.19, $n = 192$), followed by lipids (40.26 in $\mu\text{g.mg}^{-1}$ DW, \pm SE 15.59, $n = 192$, 18.88% AFDW, \pm SE 6.81, $n = 192$) and carbohydrates (20.94 in $\mu\text{g.mg}^{-1}$ DW, \pm SE 5.82, $n = 192$, 9.96% AFDW, \pm SE 3.29, $n = 192$). On average, the carbon content represented 47.54% of the DW \pm SE 5.6, $n = 192$ (ranging from 26.19 to 59.46% DW) while nitrogen content represented 11.04% of the DW \pm SE 1.74, $n = 192$ (ranging from 4.88 to 14.60% DW). The C:N mean ratio was $4.32 \pm$ SE 0.34, $n = 192$ (values between 2.97 and 6.20).

Of the 36 correlations performed on zooplankton indices, 16 were not significant. An ordination of the correlogram (Fig. 2) enabled us to separate two groups of variables that were positively correlated. The first was composed of C:N ratio, lipids, and carbohydrates; the second was composed of $\delta^{13}\text{C}$ -zoo, E_{ava} , %C, %N, proteins, and E_{con} . Note that $\delta^{15}\text{N}$ -zoo present only one positive correlation with carbohydrates. In addition, although E_{con}

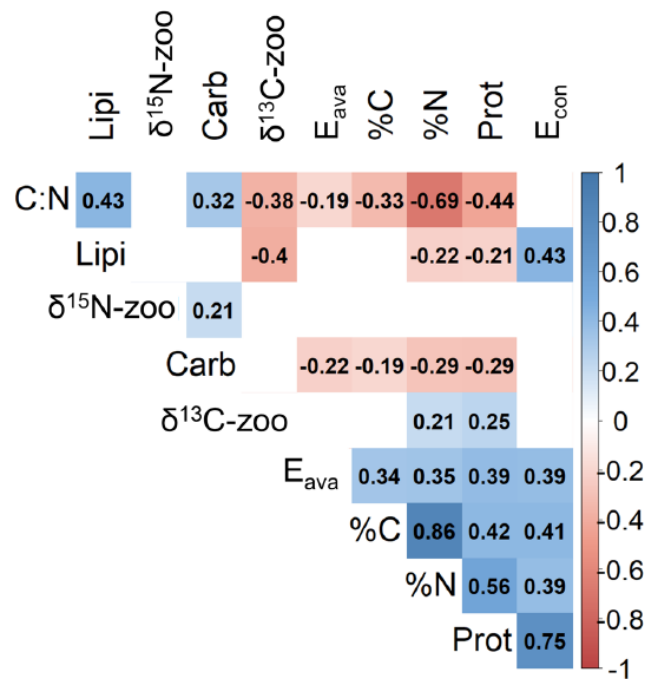


Fig. 2 Correlogram of zooplankton indices. When significant correlations were found (p -values < 0.05), correlation coefficient is indicated on the matrix

was present in the second group, this variable presented a significant positive correlation with lipid content. In addition, a significant correlation ($\rho = 0.45$, p -value < 0.001) was found between C:N ratio and lipids: proteins ratio (Figure S1).

Temporal variations of the elemental, isotopic and biochemical compositions

LOESS regressions of interannual stable isotopes variations (Fig. 3) highlighted an increase in the $\delta^{13}\text{C}$ -zoo ratio trend until 2009, and a stabilization thereafter. This pattern was quite similar for $\delta^{15}\text{N}$ -zoo, and the initial increase was associated with a breakpoint in 2009. C:N ratio and lipids showed a decrease until 2009, a breakpoint was found for C:N in 2007. Increase of proteins, %C, %N, and E_{con} induced breakpoints, respectively, in 2008, 2011, 2007, and 2010. Carbohydrates decreased over the period with a breakpoint in 2013. No breakpoint was detected for energy available for consumers of the 300–500 μm fraction, E_{ava} , and its biomass series. After a slight increase, biomass decreased since 2009. E_{ava} did not present significant variations.

Every series displayed significant seasonal patterns (Fig. 4). $\delta^{13}\text{C}$ -zoo reached its minimum values in April before increasing (maximum values in November). $\delta^{15}\text{N}$ -zoo reached minimum values in March and September, inversely %N, proteins, and E_{con} presented two periods of increase during spring and autumn. Lipids, C:N, and carbohydrates tended to peak in late spring–early summer. E_{ava} and biomass series peaked similarly in April.

Figure 5 shows the seasonal patterns of zooplankton indices before and after the main shift period (i.e., 2009).

Except for $\delta^{15}\text{N}$ -zoo, $\delta^{13}\text{C}$ -zoo, biomass, and E_{ava} , the variables did not show significant seasonal patterns for the years before 2009. In contrast, all zooplankton variables showed a significant seasonal pattern after 2009. Differences between the two periods before and after 2009 in $\delta^{15}\text{N}$ -zoo, C:N, %C, %N, proteins, and E_{con} could be observed for several months (i.e., considering the months where the confidence intervals do not overlap). For $\delta^{15}\text{N}$ -zoo, higher values were observed between January and July after 2009. For %C, %N, proteins, and E_{con} , the main differences occurred during the spring and autumn periods (except for %C with no significant differences in autumn). Finally, differences between the two periods were observed for the C:N ratio mainly in fall. For all variables, the confidence intervals of the pre-2009 regressions are much wider than those of the post-2009 regressions.

Environmental influence on zooplankton elemental, isotopic and biochemical compositions

The two first axes of the PLS analysis explained, respectively, 40.15% and 59.38% of the environmental dataset and zooplankton dataset (Fig. 6A). The first component is mostly explained by variations of POC, O_2 , and PON (positively) for environmental variables; and by C:N ratio (positively), $\delta^{13}\text{C}$ -zoo, and $\delta^{15}\text{N}$ -zoo (negatively) for

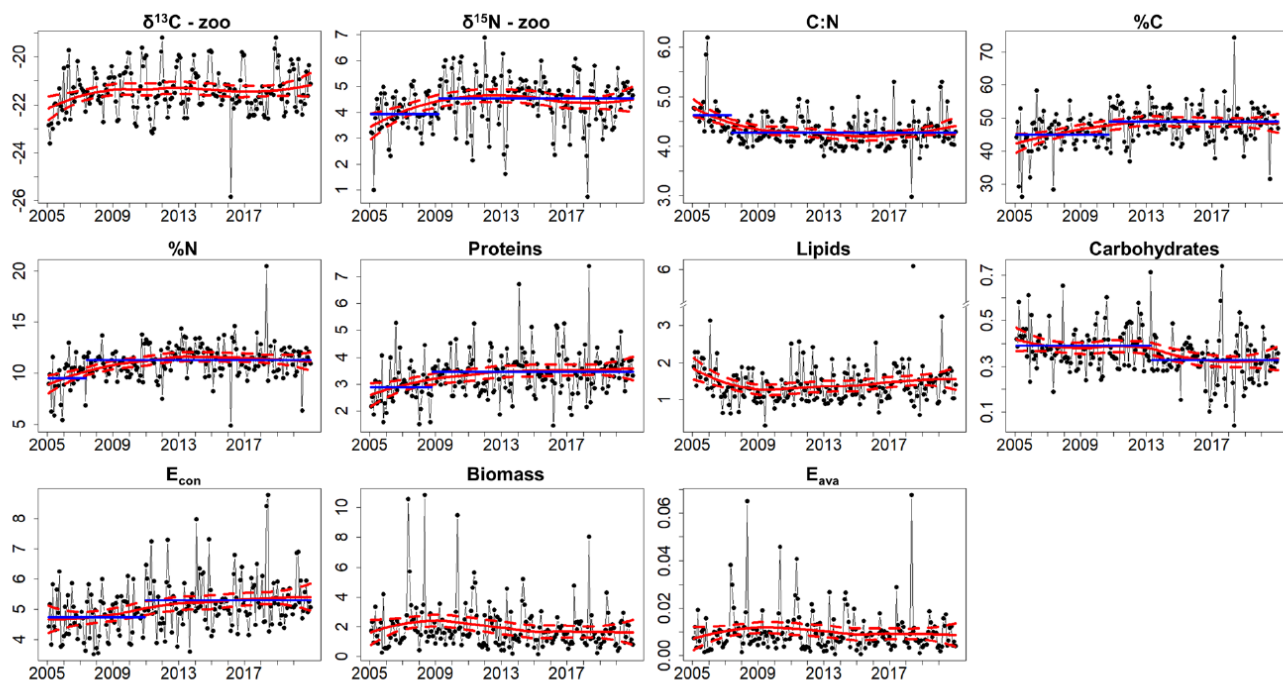


Fig. 3 Monthly interannual variations of the zooplankton time series (black points and lines). The continuous red lines correspond to the local regressions smoothing to describe the trends (dashed lines correspond to 95% confidence interval). The blue lines correspond to the mean values of the series before and after a breakpoint date. For

series without blue lines no breakpoints are detected. $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are expressed in ‰, C:N is unitless, E_{con} in $\text{kJ}\cdot\text{g}^{-1}$ DW, Biomass in $\text{mg}\cdot\text{m}^{-3}$, E_{ava} in $\text{kJ}\cdot\text{m}^{-3}$, Proteins, Carbohydrates and Lipids in $\text{kJ}\cdot\text{g}^{-1}$ of DW, %C and %N are expressed as % of DW

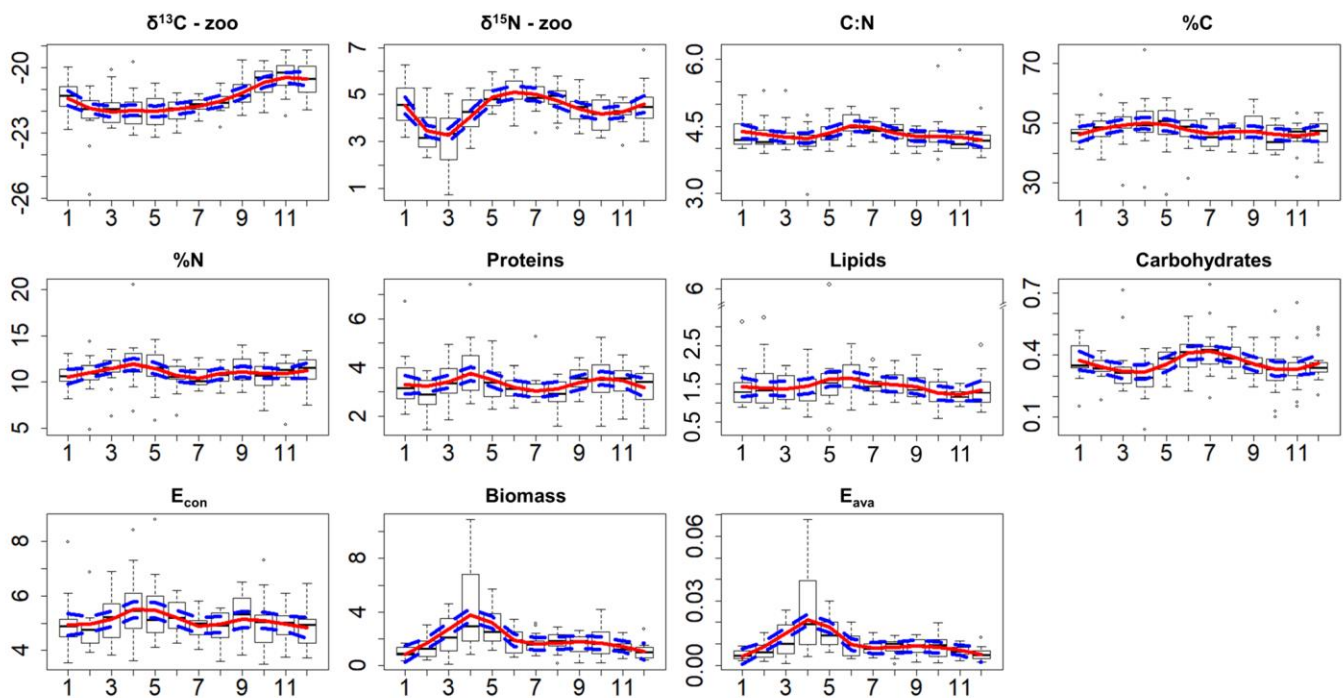


Fig. 4 Boxplot of the seasonal pattern of the zooplankton time series. When significant LOESS regression models were found the seasonal effect is represented by a continuous red line. The 95% confidence interval is represented by dashed blue lines. $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$

are expressed in ‰, C:N is unitless, E_{con} in kJ.g^{-1} DW, Biomass in mg.m^{-3} , E_{ava} in kJ.m^{-3} , Proteins, Carbohydrates and Lipids in kJ.g^{-1} of DW, %C and %N are expressed as % of DW

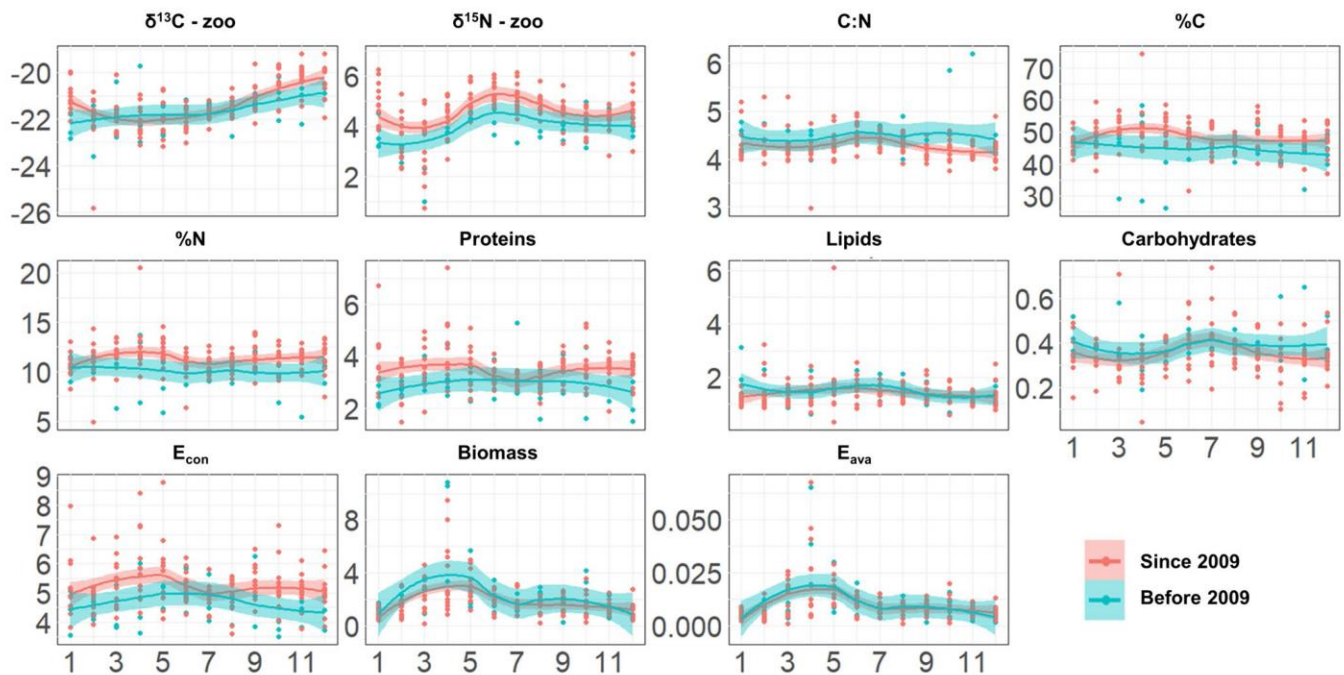


Fig. 5 Seasonal pattern of the zooplankton indices over two periods of the time series, before 2009 (in blue) and after 2009 (in red). Seasonal LOESS models were represented for both periods by a continuous line and the 95% confidence interval by shading. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

are expressed in ‰, C:N is unitless, E_{con} in kJ.g^{-1} DW, Biomass in mg.m^{-3} , E_{ava} in kJ.m^{-3} , Proteins, Carbohydrates and Lipids in kJ.g^{-1} of DW, %C and %N are expressed as % of DW

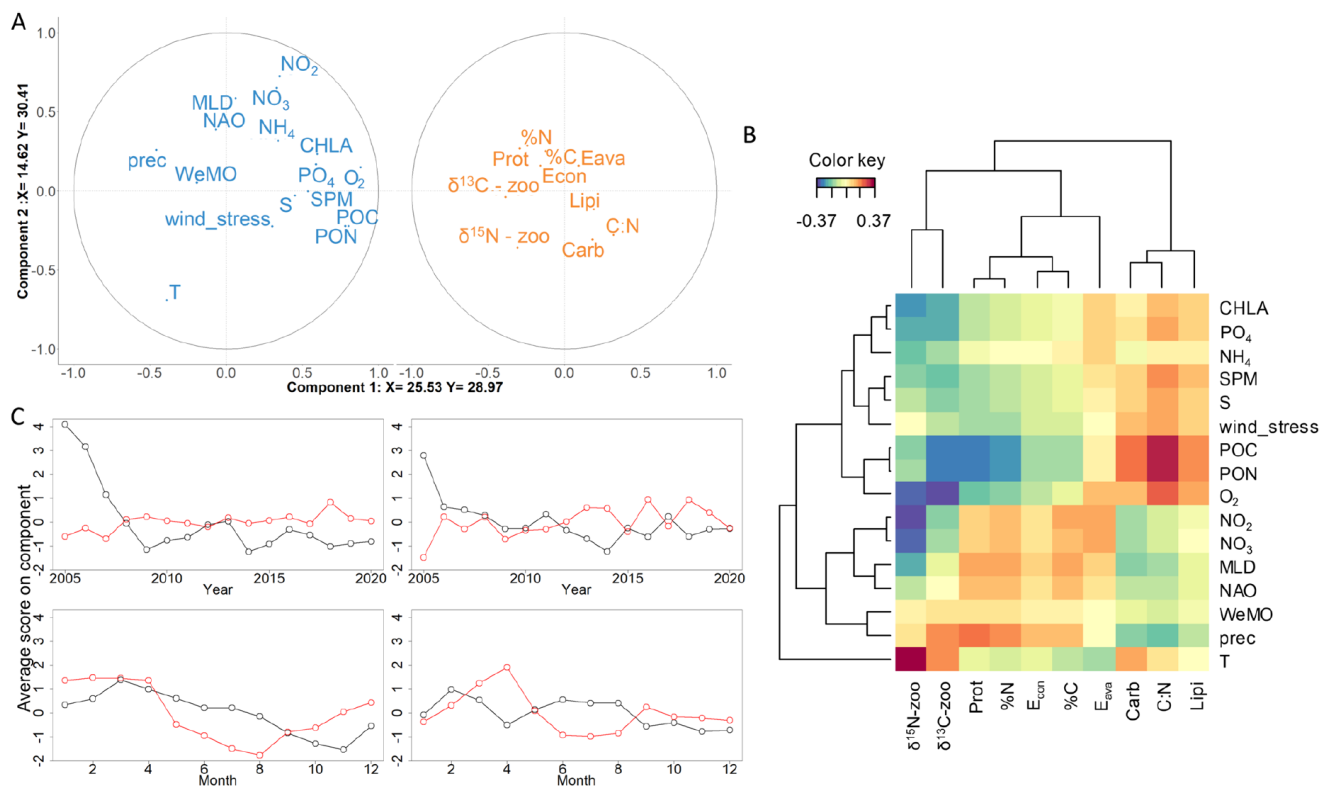


Fig. 6 Results of the Partial Least Squares analysis performed on environmental and zooplankton series from 2005 to 2020. **A** Correlation circle plot representing environmental variables (in blue) and zooplankton variables (i.e., isotope and biochemical compositions in orange). **B** Clustered image map representing the association between environmental variables (in column) and zooplankton variables (in

raw). **C** Top panels represent the year-to-year averaged scores of components 1 (in black) and 2 (in red) of the environmental variables (left) and for the zooplankton variables (right). The bottom panels represent the monthly averaged scores of components 1 (in black) and 2 (in red) of the environmental variables (left) and for the zooplankton variables (right)

zooplankton variables. The second component is mostly explained by variations of NO_3 , NO_2 , MLD (positively) and T (negatively) for environmental variables; and by %C, %N (positively), carbohydrates, and $\delta^{15}\text{N}$ -zoo (negatively). The clustered image mapping (CIM) (Fig. 6B) mainly showed positive association of $\delta^{15}\text{N}$ -zoo with temperature and negative association with MLD, NO_3 , O_2 and NO_2 . $\delta^{13}\text{C}$ -zoo, proteins, %N, %C, and E_{con} (to a lesser extent) were associated positively with precipitation (and T for $\delta^{13}\text{C}$ -zoo) and negatively with O_2 , POC, and PON. E_{ava} was related with MLD, NO_3 , and NO_2 . Carbohydrates, lipids, and C:N ratios were mainly positively associated with POC, PON, and O_2 . Yearly average of first component scores for both data sets (environmental and zooplankton) highlighted common decreasing trends (Fig. 6C). This revealed the common decreasing trend of POC, PON, lipids, carbohydrates, and C:N, and the increasing trend of proteins, %C, %N, $\delta^{13}\text{C}$ -zoo, and $\delta^{15}\text{N}$ -zoo. The slight interannual increase in average values of the second component of the environmental dataset may indicate a slight increase in MLD, NO_3 , and NO_2 values. No common interannual trend was observable for the second component

on the zooplankton dataset. Monthly average of first and second component scores for the environmental dataset displayed clear seasonal patterns. The second component of the zooplankton data (red curve) separated the variables that peaked in summer ($\delta^{15}\text{N}$ -zoo, carbohydrates, C:N, and lipids), from variables that peaked in spring and autumn (proteins, %N, %C, Econ, Eava).

Figure 7 shows the association matrix of a complementary PLS between variations of zooplankton stable isotope and biochemical compositions with the environment which included cytometry and stable isotope ratios from POM surface samples over the period 2010–2020. On one hand, this second PLS showed the positive relationship of some zooplankton variables (i.e., E_{ava} , proteins, %N, E_{con} , and %C) with phytoplankton variables (e.g., CHLA, counts of phytoplankton groups, POC, and PON), and the negative association of $\delta^{13}\text{C}$ -zoo with these plankton variables. On the other hand, $\delta^{15}\text{N}$ -zoo, carbohydrates, lipids, and C:N were mostly associated with the size of phytoplankton groups (e.g., bacteria size, nanoeucaryote size) and sub-surface POM stable isotope ratios.

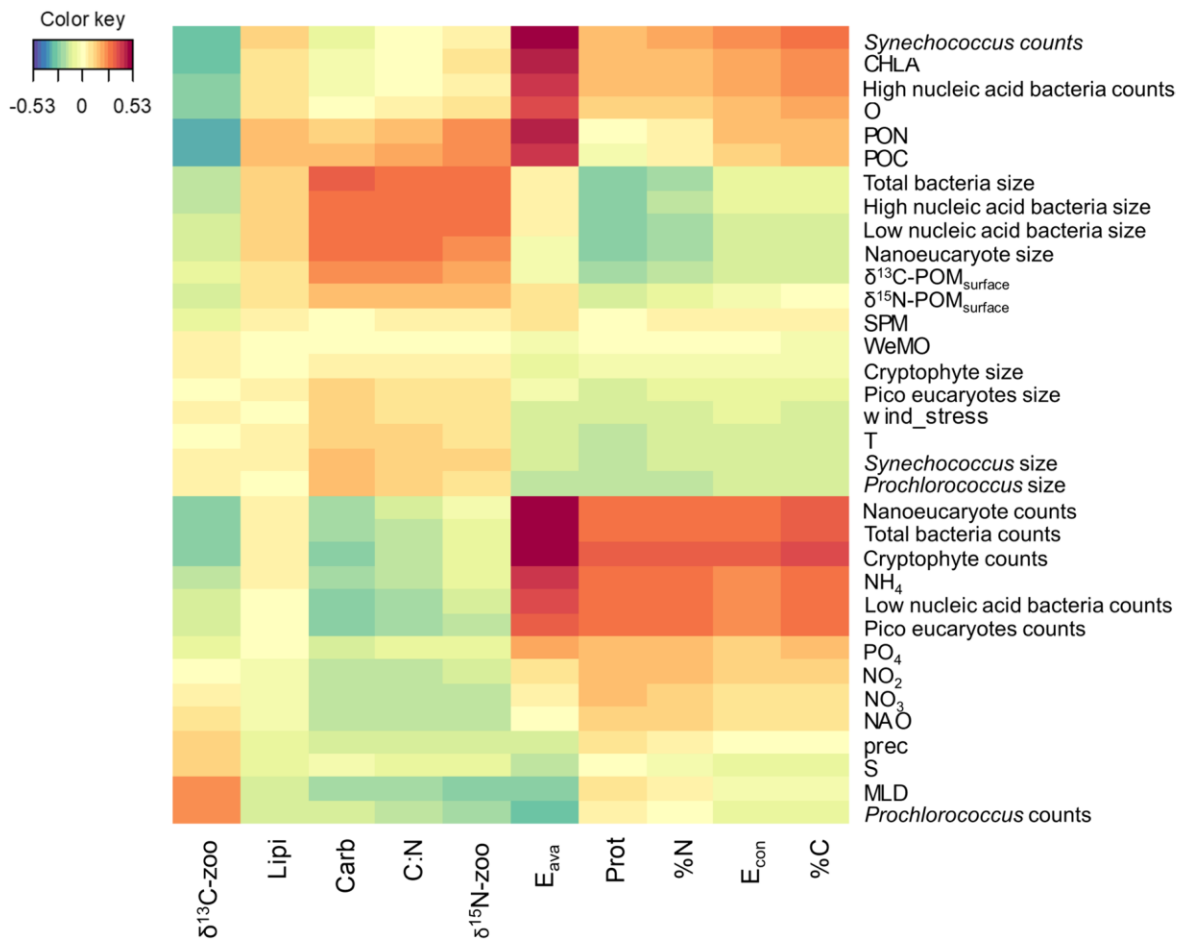


Fig. 7 Clustered image map of the Partial Least Squares analysis performed on environmental (including cytometry data and subsurface isotope ratio of the POM) and zooplankton series from 2010 to 2020.

The association between environmental variables (in column) and zooplankton variables (in row)

Discussion

Zooplankton elemental, isotopic, and biochemical compositions

The present study represents the first biochemistry, stable isotope ratio, and elemental analyses on zooplankton long time series (2005–2020) published for the Mediterranean Sea. Overall, the average values of biochemical compounds and stable isotope ratios acquired over the present study on the 300–500 μm size fraction zooplankton time series from the Bay of Marseille were within the range of some previous studies performed in shorter duration (some particular months/seasons or years) in the same Bay (Bănaru et al. 2014; Chen et al. 2019, 2023; Tesán-Onrubia et al. 2023) and in the eastern part of the Gulf of Lion (Espinasse et al. 2014) (Table 3). However, for the same periods, some differences between the present study and the previous ones may have resulted from the use of different mesh size nets with potential consequences for zooplankton composition (i.e. 60

μm size net in Tesán-Onrubia et al. 2023; 80 μm size net in Chen et al. 2023 vs 200 μm size net in the present study). The zooplankton carbon and nitrogen contents, and the stoichiometric C:N ratios found in our study were in line with values commonly observed for small- and medium-sized copepods in the North Western Mediterranean Sea (Champalbert and Kerambrun 1973; Gorsky et al. 1988; Gaudy and Champalbert 1998; Saiz et al. 2020).

The relative importance of the three biochemical components in terms of contribution to the energy density found in our study (proteins 64.66%, lipids 27.99%, and carbohydrates 7.35%) is in full agreement with zooplankton observations in the Mediterranean Sea and beyond (Raymont et al. 1967, 1969; Morris and Hopkins 1983; Ventura 2006; Harmelin-Vivien et al. 2019; Chen et al. 2019; Lobus et al. 2020; Chen et al. 2023; and references in Table 4). The absolute concentrations of biochemical components measured in our study were among the lowest values observed elsewhere (Table 4). Due to the wide diversity of analytical methods, the concentrations of biochemical components

Table 3 Comparison of the stable isotope ratios in the size fraction 300–500 obtained in the present study and in previous studies carried out within or nearby the bay of Marseille

Organisms/size fraction	Site	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Net device/mesh size net	References/period
300–500 μm	Marseille	4.37 (0.75/6.89)	-21.43 (-25.83/-19.19)	WP2/200 μm	Present study/2005–2020
300–500 μm	Marseille	3.93 (1.00/5.22)	-21.60 (-23.61/19.71)	WP2/200 μm	Present study/before 2009
300–500 μm	Marseille	4.52 (0.75/6.89)	-21.38(-25.83/-19.19)	WP2/200 μm	Present study/after 2009
300–500 μm	Marseille	2.52	-23.00	WP2/200 μm	Bănaru et al. (2014)/2009–2010
300–500 μm	Gulf of lion	2.75	-22.16	Bongo net/120 μm	Espinasse et al., (2014)/ January 2011
300–500 μm	Gulf of lion	3.99	-23.43	Bongo net/120 μm	Espinasse et al., (2014)/ May 2010
200–500 μm	Mediterranean Sea	3.2	-22.6	Hydrobios/60 μm	Tesán-Onrubia et al. (2023)/ Spring 2019
300–500 μm	Marseille	4.0	-22.0	WP2/80 μm	Chen et al. (2023)/2016–2018

Average values (and ranges between parentheses) are reported

may present wide differences in the literature (e.g., proteins measurements see Brey et al. 2010; Zaguri et al. 2021). In addition, some discrepancies of zooplankton biochemistry might result from different sampling gear. For example, the 80 μm mesh size net used in Chen et al. (2019), and a 200 μm mesh size net sample the zooplankton community differently (Pansera et al. 2014). Therefore, the absolute values should be compared with caution.

Seasonality of zooplankton elemental, isotopic and biochemical compositions, and relation to environmental conditions

The present analysis of the historical zooplankton time series of the Bay of Marseille (16 years) provided more robust results regarding seasonal patterns of the stable isotope ratios of nitrogen and carbon, and overall agree with previous observations on shorter durations (Wainright and Fry 1994; Bouillon et al. 2000; Kibirige et al. 2002; Fanelli et al. 2011; Bănaru et al. 2014).

The seasonal variations of the nitrogen and carbon stable isotope ratios could be related to terrestrial inputs and water mixing (correlated with temperature variations) that promote nutrients availability for plankton seasonal successions (in term of species and trophic regime) in the NW-Mediterranean Sea (García-Comas et al. 2011; Romagnan et al. 2015). A change in the food source of zooplankton 300–500 μm (i.e., influenced by variations in the proportion of carnivorous/omnivorous copepods within the size fraction that feed on other sources than POM, e.g., microzooplankton) might have been hypothesized, whether $\Delta^{15}\text{N}$ between POM and zooplankton increased or decreased. However, the values remained constant around 1.9‰ all year round, within the ranges of values cited in the literature (Tiselius and Fransson

2016). Therefore, the zooplankton $\delta^{15}\text{N}$ variations should be attributable rather to factors that directly or indirectly affect POM. The following mechanisms are likely to best explain $\delta^{15}\text{N}$ variations in the plankton food web:

- (i) the enrichment/depletion in ^{15}N of the residual nutrient pool and a food web relying on regenerated production (Savoie et al. 2003). Phytoplankton prefers to uptake ^{14}N over ^{15}N , showing generally low $\delta^{15}\text{N}$ values when blooms start. This leads to a residual nutrient pool progressively enriched in ^{15}N . The association between the isotopic enrichment of nutrients during depletion by primary production and, consequently, the progressive increase in plankton $\delta^{15}\text{N}$ values has already been documented in the literature (Wada and Hattori 1976; Goering et al. 1990; Savoie et al. 2003).
- (ii) a change in the pico-, nano-, and micro-plankton community that compose the POM which by cascade effect might have affected zooplankton 300–500 μm composition and resulted in an elongation of the plankton food web. For example, this might be linked to the appearance of ciliates within the POM during the most oligotrophic periods and, in the 300–500 μm zooplankton, the appearance of omnivorous copepods that may feed on heterotrophic plankton (Saiz et al. 2007). An indicator of changes in the POM composition might be the size. Our results showed that the variation of zooplankton $\delta^{15}\text{N}$ values was positively associated with the size of plankton organisms present in the POM. Increases in $\delta^{15}\text{N}$ values are commonly observed along size gradients of plankton organism (Bănaru et al. 2014; Espinasse et al. 2014; Figueiredo et al. 2020).

Table 4 Comparison of the biochemical content in the size fraction 300–500 or in targeted zooplankton organisms obtained in the present study and in previous studies

Organisms/size fraction	Site	%Prot DW	%Lipi DW	%Carb DW	%C DW	%N DW	C:N	Net device/ Mesh size net	Reference/ period
300–500 µm	Marseille	15.57 (6.77/34.59)	4.03 (0.87/17.14)	2.1 (0.3/4.3)	47.47 (26.2/74.5)	11.0 (4.9/20.7)	4.36 (2.3/10.5)	WP2/200 µm	Present study/2005–2020
300–500 µm	Marseille	13.66 (7.00–24.62)	4.14 (1.78–8.80)	2.23 (1.11–3.80)	44.80 (26.19/58.38)	10.12 (5.41/13.69)	4.48 (3.98/6.2)	WP2/200 µm	This study/ before 2009
300–500 µm	Marseille	16.20 (6.77–34.59)	3.99 (0.87–17.14)	2.05 (0.24–4.30)	48.45 (31.49/74.42)	11.35 (4.88–20.49)	4.28 (2.98/5.3)	WP2/200 µm	This study/after 2009
300–500 µm	Marseille	33.6	10.2	6.2	–	–	–	WP2/80 µm	Chen et al. (2019)/2016–2018
Copepods	Marseille	41.3	5.7	3.0	–	–	–	WP2/80 µm	Chen et al. (2019)/2016–2018
Copepods	Marseille	–	–	–	(32.8–57.4)	(7.5–12.6)	–	–	Champalbert et al. (1973)
> 200 µm	Marseille	–	–	–	–	–	4.57	WP2/200 µm	Gaudy and Champalbert (1998)/1992–1995
200–500 µm	Mediterranean Sea	28	4	8	–	–	–	Hydro-bios/60 µm	Tesán-Onrubia et al. (2023)/ spring 2019
Calanoid copepod	Marine review	38.74 (23.9–64.3)	25.0 (1.9–73)	2.8 (0.2–8.5)	50.7	9.5	–	–	Ventura (2006)
Copepods	Marine review	47.2 (13.1–82.6)	23.6 (0.5–74.5)	9.4 (0.7–29.7)	47.6 (21.4–67.5)	9.7 (4.6–15.8)	5.06 (2.9–12.74)	–	Brey et al. (2010)
200–500 µm	Kerguelen	18.6	7.1	3.3	37.3	6.8	–	Bongo/120 µm and 330 µm	Harmelin-Vivien et al. (2019)/spring 2011
> 500 µm	Kara Sea	43.4	26.7	4.9	47.5	7.3	6.6	Bongo/500 µm	Lobus et al. (2020)/July 2019
Copepod	Andaman Sea	61.2	14.4	4.4	21.4	7.3	2.9	Indian ocean standard net	Goswami et al. (1981)/1979–1980
Copepod	Southern Ocean	–	–	–	53.3	9.4	6.5	ORI C and ORI 100 net	Omori (1969)/1967–1968

Average values (and ranges between parentheses) are reported

In the Bay of Marseille, Liénart et al. (2018) highlighted that the POM composition differed seasonally according to local forcing dynamics (e.g., river flows). The association between precipitations and $\delta^{13}\text{C}$ shows the importance of terrestrial input during rains at the end of the year. The seasonal variations of $\delta^{13}\text{C}$ values are most likely explained by changes in the plankton composition present in the surface

POM, due to a cascade effect (see Figure S2). The complementary PLS performed over a smaller range of years (2010–2020), highlighted that plankton group composition (i.e., in POM) influenced zooplankton $\delta^{13}\text{C}$ values. Stable isotope analyses showed that pico- and nano-phytoplankton are the favorite prey of small and medium sized zooplankton in the NW-Mediterranean Sea (Hunt et al. 2017;

Tesán-Onrubia et al. 2023). Gearing et al. (1984) found that carbon stable isotope ratios of primary producers varied according to taxonomic groups. In the Bay of Marseille, the succession between cold water phytoplankton (i.e., *Cryptophytes*, pico- and nano-eucaryotes and total bacteria) and warm water phytoplankton (i.e., *Prochlorococcus*) might have resulted in variations of zooplankton $\delta^{13}\text{C}$ values (see also Figures S2, S3). Sampling of plankton (including microzooplankton) from the entire water column and/or at maximum chlorophyll depth should be considered for future monitoring. These analyses should in addition include analyses of the stable isotope ratio of other zooplankton size classes to better investigate the trophodynamic across the plankton food web (Tesán-Onrubia et al. 2023; Chen et al. 2023).

In addition, our study shows a negative correlation between proteins and lipids, often observed among zooplankton organisms (Raymont et al. 1969). The increase of lipid content in organisms in late spring-early summer, after the main zooplankton spring peak, might be the result of (i) energy storage allowed for reproduction, or (ii) by the appearance of lipid-rich species, i.e., herbivores (Lee et al. 2006). Reciprocally, protein, %N, and energy concentrations peak during the richest period in terms of nutrients during, i.e., spring and autumn.

Interannual dynamic of zooplankton elemental, isotopic and biochemical compositions

Our analysis suggested that 2009 has been a pivotal year for the 300–500 μm zooplankton fraction in the Bay of Marseille. The change in the 300–500 μm biochemistry coincided with the decrease in nutrient (NO_2 , NH_4^+ , NO_3^- , PO_4^{3-}) concentrations (Lheureux et al. 2021) and a notable change in the stoichiometry of nutrient concentrations (i.e., decrease of $\text{NH}_4^+:\text{NO}_3^-$ ratio, Figure S4) in the Bay of Marseille (Garcia et al. 2023). Variations of nitrogen sources may affect autotrophic processes and the nitrogen stable isotope composition, as pico- and nanoplankton groups present preferential uptake for ammonium (Probyn and Painting 1985) which is depleted in ^{15}N (i.e., lower $\delta^{15}\text{N}$ values). This has already been hypothesized in the NW-Mediterranean Sea (Hunt et al. 2017). The cyanobacteria decrease observed over the time series, which uptake atmospheric N_2 , may have also contributed to the increase in the smaller fraction $\delta^{15}\text{N}$ values.

In the Bay of Marseille, these changes seemed to have also affected the phytoplankton, as evidenced by the interannual variations of decreasing phytoplankton size, by the decreasing trend in POM concentration (Garcia et al. 2023 and see Figure S5) and in POM carbon stable isotope ratios (Figure S2). In the Bay of Marseille, the year 2008 marks the installation at the Cortiou sewage treatment plant of a new

microbiological treatment system designed to reduce particulate organic matter by 90–95% (Raimbault et al. 2021). The decrease in POM concentration is a general pattern in monitored French coastal stations. It has been related to the reduction of nutrient inputs to coastal areas in relation with European legislation and the implementation of the Water Framework Directive and global changes (Goberville et al. 2010; Lheureux et al. 2021).

The variations in stable isotope composition and biochemistry highlight changes in the zooplankton community in response to a change in their food. $\delta^{15}\text{N}$ values shifted toward higher values and the biochemical composition has markedly changed (i.e., lower concentrations of lipids and higher concentrations of proteins). The changes in the zooplankton 300–500 μm size fraction can be a response to (i) individual and/or (ii) community adaptations to oligotrophic conditions. (i) On one hand, the decrease in the quantity and the different type of food may have affected energy availability for zooplankton and, thus, their energy allocation (i.e., giving preference to structural form with proteins rather than reserves with lipids). In addition, the change of the trophic position of their prey might have forced zooplankton to feed on heterotrophic or mixotrophic prey rather than directly on autotroph plankton present in the POM. (ii) On the other hand, the changes in zooplankton biochemistry and stable isotope compositions may reveal different species assemblages. Such changes in POM composition might have favored the appearance of omnivorous/carnivorous zooplankton in comparison to strict herbivorous zooplankton (i.e., that present higher reserves in lipids, Lee et al. 2006). Moreover, since lipids may constitute important reserves for the copepod's reproduction (Lee et al. 2006; Thomas et al. 2022), their temporal decrease observed during the time series may have influenced copepods population dynamics, by reducing recruitment of copepod nauplii larvae, and then calanoids and oithonoids abundance, and *in fine* the observed biomass decrease in the successive mesozooplankton size classes (Garcia et al. 2023). Further analyses on copepod ecological and functional niches (i.e., giving trophic details on the community composition) based on species level taxonomic identification are needed to assess the hypothesis of change and/or adaptation of the zooplankton community to oligotrophic conditions.

One might expect that after the shift period in 2009, the seasonal patterns of zooplankton variables would have been affected. Before 2009, our results showed (i) lower $\delta^{15}\text{N}$ values from January to July, (ii) weaker seasonal pattern for most variables (C:N ratio, %C, %N, Proteins, Lipids, Carbohydrates, E_{con}) that may lead to differences in the values for these components during the spring and/or autumn periods (depending on the variables). However, these results seemed to be driven by the uneven number of observations between the two periods notably with fewer observation before 2009

(4 years vs 12 years). The results of the present analysis should therefore be interpreted with caution.

Our results suggest that water management in coastal areas combined with global changes may impact the elemental, isotopic, and biochemical composition of zooplankton through the reduction of nutrient concentrations and the change in phytoplankton dynamics. The 300–500 µm zooplankton fraction underwent a change in its biochemical composition with lesser amounts of lipids and carbohydrates and higher protein content. The changes in the concentration of the biochemical component have led into an increase in the energy concentration (with a shift in 2011). However, the decrease in the biomass of the 300–500 µm zooplankton size-fraction led finally to a stability of available energy in the water column. As the copepod dominated 300–500 µm size fraction corresponds to the preferred size range of planktivorous fish in the Bay of Marseille (Chen et al. 2019, 2021), one might expect that these changes would have affected planktivorous fish (as hypothesized by Saraux et al. 2019). The lower lipid content of their preferred prey may have contributed to the decrease of their relative body condition and given rise to the fisheries crisis observed after 2009 (Saraux et al. 2019; Chen et al. 2021, 2023). The present work highlights the relevance of performing stable isotope and biochemical analyses during zooplankton monitoring in the context of global change and human pressures and regulations.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00227-024-04430-5>.

Acknowledgements The authors acknowledge the contribution of all participants in the Bay of Marseille SOMLIT monitoring program and the crew of the R.V. Antedon II. We thank P. Raimbault, N. Garcia, and M. Lafont for making available the physical and biogeochemical data. We would like to thank all past PhD and MSc students for their contribution to the sampling effort of the zooplankton time series linked with the on-going SOMLIT monitoring program initiated by FC in 2002. This work contributes to the valorization of observational time series strategy and to the understanding of pelagic ecosystem functioning developed respectively by the national research organization ILICO and by previous research in the frame of the CNRS-INSU MISTRALS MERMEX program (subprograms IPP and MERITE). The biochemical and isotopic treatments have been supported by the INSU/EC2CO project ZOO-INDEX (Call 2021; PI D. Bănară & F. Carlotti) from the French National program CNRS-INSU-EC2CO (Ecosphère Continentale et Côtière). Thanks are also addressed to Michael Paul for English proofreading.

Author contributions Conception and design of the study: Théo Garcia, François Carlotti, Daniela Bănară. Acquisition of data: Gilles Lepoint, Loïc Guilloux, Baptiste Grassi, Victor Russias, François Carlotti, Daniela Bănară. Analysis of data: Théo Garcia. Drafting of the manuscript: Théo Garcia, François Carlotti, Daniela Bănară. Revising/ editing of the manuscript: all authors. Project administration and funding acquisition: François Carlotti, Daniela Bănară.

Funding The present project is supported by funding from Région Sud and the Parc National des Calanques in the form of a PhD grant to

T. Garcia, and by the *Comité Régional des Pêches Maritimes et des Elevages Marins PACA*. The present work is supported by funding of the project ZOO-INDEX (Call 2021; PI D. Bănară & F. Carlotti) from the French National program CNRS-INSU-EC2CO (Ecosphère Continentale et Côtière). D. Bănară benefited from leave within the ANR CONTAMPUMP project (no ANR-19-ce34-0001).

Data availability The datasets generated during and/or analyzed during the current study should be available shortly from a doi data repository soon to be published by the authors.

Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

Ethical approval All applicable international, national, and/or institutional guidelines for sampling and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

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