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1 Seasonal variation in biochemical and energy content of size-fractionated zooplankton
2 (North-Western Mediterranean Sea)

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17 **Highlights**

- 18 • Proteins are the dominant compound of zooplankton
- 19 • Biochemical and energy content differ between zooplankton groups and size
20 classes
- 21 • Copepods, decapod larvae and chaetognaths represent the most energy-rich
22 groups
- 23 • Biochemical and energy content vary seasonally with environmental
24 parameters
- 25 • Zooplankton energy peaks in 200-1000 µm size classes in all seasons

Abstract

Zooplankton plays a prominent role in marine pelagic food webs, but its contribution to organic matter transfer from phytoplankton to upper level consumers is modulated by size and specific composition. The biochemical composition (protein, carbohydrate and lipid concentrations) and energy content of size- and group-fractionated zooplankton were analyzed in the NW Mediterranean Sea (Marseille) over 18 months. Proteins were the most abundant biochemical compounds in all size classes and zooplankton groups, and provided the largest part of the energy content. The medium (200-1000 μm) size classes, mainly composed of copepods, crustacean larvae and eggs, presented a higher protein and energy content than both the smallest (80-200 μm) size class, mainly composed of phytoplankton, and the largest (>1000 μm) size classes, composed of siphonophores, salps and chaetognaths. Strong and similar seasonal variations in zooplankton biochemical composition were observed in all size classes, with higher energy content associated with cold oxygen- and nutrient-rich waters in spring 2017 and winter 2018. This study evidenced the importance of size- and group-fractionating methods to achieve a better insight into energy and nutrient transfer up into pelagic food webs, and understanding of zooplanktivorous fish feeding preferences, depending on the variable quality of zooplankton prey.

Keywords

Zooplankton; Size classes; Taxonomic groups; Biochemical composition; Energy content; Seasonal variations

1. Introduction

Zooplankton is considered as an essential compartment of aquatic ecosystems because of its pivotal role in food webs (Banse, 1995). Due to their intermediate trophic position, zooplankton organisms not only transfer the organic matter (OM) and energy from primary producers to higher trophic level consumers (Frederiksen et al., 2006; Saiz et al., 2007), but they also influence the energy density of the food available to zooplanktivores (Harmelin-Vivien et al., 2019). During the last decade, a decreasing trend was observed in biomass, landings, and body condition of commercially important sardines and anchovies in the Mediterranean Sea, particularly in the Gulf of Lion, while the abundance of non-marketable sprats was increasing (Saraux et al., 2019; Van Beveren et al., 2014). Previous studies have hypothesized that the decline of these small zooplanktivorous pelagic fishes could be due to bottom-up control linked to changes in the availability or quality of their food, which mainly consists of mesozooplankton from 200 μm to 1000 μm in size (Costalago et al., 2012; Le Bourg et al., 2015). This hypothesis thus highlighted the interest of studying the biochemical composition of size-fractionated zooplankton in order to better estimate the amount of energy provided by different size classes to upper-level consumers in the pelagic food webs of the NW Mediterranean Sea.

In zooplankton, proteins and lipids are the two most important biochemical substances (Mauchline, 1998; Postel et al., 2000; Ventura, 2006), in contrast to carbohydrates which represent a major component of phytoplankton (15 to 35% of phytoplankton biomass) (Biddanda and Benner, 1997). Proteins are the main constituents of muscle tissues and metabolic enzymes and serve as carriers of genetic information, while lipids serve as energy reserve, membrane components, and antioxidants (Kattner et al., 2007). During the past decades, our knowledge of various

aspects of plankton chemistry has developed, and more studies have dealt with the organic and calorific content of marine zooplankton species (Båmstedt, 1986; Barroeta et al., 2017; Omori and Ikeda, 1992). The protein and carbohydrate composition of zooplankton organisms is known to be species- and size-specific (Guisande, 2006; Ventura, 2006). Similarly, the lipid content of marine zooplankton differs among species, and can range from a few percent to more than 60% of individual dry weight (Lee et al., 2006). Works on organic fraction analysis have often been restricted to variations in only certain biochemical compounds (lipids separately from proteins, or nothing about carbohydrates) (Norrbin and Båmstedt, 1984), but few have recorded the three compound types simultaneously (Ventura, 2006). However, quantifying the protein, carbohydrate and lipid content of organisms enables determination of their energy content, which provides information on their nutritional quality as prey for upper-level predators (Harmelin-Vivien et al., 2019; Spitz et al., 2010). Despite the complex structure and functions of zooplankton (Steinberg and Landry, 2017), previous biochemical studies considered zooplankton as a homogenous compartment (Goswami et al., 1981; Kumari et al., 1993), or investigated only a few plankton groups, especially large organisms (Choe et al., 2003; Harmelin-Vivien et al., 2012). However, body size determines potential predator-prey interactions (Emmerson and Raffaelli, 2004), rates of production (Banse and Mosher, 1980), energy requirements and metabolism (Brown et al., 2004), and mortality rates (Hirst and Kiorboe, 2002). A size-based approach has thus been proposed as a useful means to obtain a better insight into the structure and functioning of marine food webs, and used in plankton studies (Carlotti et al., 2008; Rolff, 2000; Saiz et al., 2007).

While many biochemical studies on zooplankton have been performed worldwide (Ara, 2001; Färber-Lorda et al., 2009; Jo et al., 2017), little work has been done on the Mediterranean Sea. Morris et al. (1984) and Yilmaz and Besiktepe (2010)

described the biochemical composition of Mediterranean plankton, but only for small (pico-, nano- and microplankton) and large (>2000 μm) zooplankton groups (salps), respectively, while Barroeta et al. (2017) investigated only the energy content of roughly divided micro- and mesozooplankton. A high variability in feeding strategy in each size class has been highlighted in Mediterranean zooplankton communities (Bănară et al., 2014; Espinasse et al., 2014), but no study has yet been performed exploring their biochemical composition with a size-fractionated approach, or considering different zooplankton groups. Furthermore, as the taxonomic composition of zooplankton varies between seasons (Calbet et al., 2001), its biochemical composition and energy content may also vary accordingly, and may be linked to seasonal variations of environmental factors, which influence zooplankton metabolic processes (Gaudy et al., 2003; Lee et al., 2017). We thus hypothesized that zooplankton size classes and taxonomic groups may differ in their biochemical composition and energy content, and vary with the seasonal variations of environmental factors and organism metabolism.

The main aims of this study were thus (1) to determine the biochemical composition of different zooplankton size-fractions (80-200 μm , 200-300 μm , 300-500 μm , 500-1000 μm , 1000-2000 μm and >2000 μm size classes) and of some important zooplankton taxonomic groups (copepods, decapod larvae, chaetognaths, salps, siphonophores, teleost eggs) in the NW Mediterranean Sea, by measuring their concentrations in organic compounds (proteins, carbohydrates and lipids) and their energy content in Joules; (2) to explore the seasonal variations of biomass and biochemical composition of the four dominant zooplankton size classes (from 80 μm to 1000 μm); and (3) to analyze the influence of environmental factors on their biochemical composition. The ultimate objective was to provide more comprehensive

information on the food quality and energy content supplied by mesozooplankton to upper level zooplanktivorous consumers in the NW Mediterranean Sea.

2. Material and methods

2.1. Study site

Zooplankton sampling was performed at the Solemio (43.24°N; 5.29°E) site, located in the Bay of Marseille in the south of France (NW Mediterranean Sea) (Fig. 1), on board the RV ANTEDON II of the OSU Pytheas Institute. Solemio is a site of the long-term national programme for littoral observation, SOMLIT (<http://somlit.epoc.u-bordeaux1.fr>), where phytoplankton and a wide range of environmental parameters, including temperature (T, °C), salinity (S, PSU), oxygen (O₂, ml L⁻¹), nitrates (NO₃, µmol L⁻¹), phosphates (PO₄, µmol L⁻¹), chlorophyll *a* (Chl *a*, µg L⁻¹), particulate organic carbon (POC, µg L⁻¹), particulate organic nitrogen (PON, µg L⁻¹) and suspended particulate matter (SPM in mg L⁻¹), have been recorded every two weeks since 1994. The Bay of Marseille, located in the eastern part of the Gulf of Lion, is influenced by intrusions of the Northern Current on the shelf (Millot, 1987; Petrenko et al., 2005), and occasionally by intrusions of the Rhone river plume and the coastal Huveaune River (Fig. 1), depending on winds and rain events (Cresson et al., 2012; Fraysse et al., 2014). This bay is also subjected to anthropogenic and terrestrial inputs from the Marseille sewage treatment plant at Cortiou (Fig. 1) (Millet et al., 2018). Thus, three main potential sources of particulate OM (seawater, river runoff and sewage water particles), with different biochemical characteristics, are available to zooplankton in the Bay of Marseille (Bănaru et al., 2014).

2.2. Sampling and sample treatment

For biochemical analyses, zooplankton was sampled monthly from October 2016 to

March 2018 during the SOMLIT programme in order to benefit from the environmental data corresponding to our samples. Zooplankton was sampled vertically from 50 m depth (close to bottom) to the surface with a 80 μm mesh net (50 cm mouth diameter) towed at 1 m s^{-1} . Five vertical tows were performed to collect enough material for the analyses. Four samples were dedicated to biochemical analyses and one to quantification of plankton biomass. Samples were filtered through six different sieves ranged in a column (2000, 1000, 500, 300, 200 and 80 μm mesh size), allowing the separation of six size classes of zooplankton (80-200 μm , 200-300 μm , 300-500 μm , 500-1000 μm , 1000-2000 μm and >2000 μm).

The identification of the main taxonomic groups in each size class was done under a binocular microscope, and the relative importance of the different groups was estimated. The main copepod groups were identified following the website 'Marine Planktonic Copepods' (<https://copepodes.obs-banyuls.fr/en/loc.php?loc=14>) (Razouls et al., 2005). At all dates, a sufficient amount of bulk matter was collected to perform all biochemical analyses for the four smaller size classes (from 80-200 μm to 500-1000 μm), but only occasionally for the two largest size classes (1000-2000 μm and >2000 μm), which were often collected in small quantity. Therefore, when possible, organisms of these two large size classes were sorted under a microscope into six broad zooplankton groups: copepods, decapod larvae, chaetognaths, salps, siphonophores and eggs (mostly teleost eggs), and the individuals of all samples were gathered to obtain enough material for analyses. For smaller size classes, sorting was difficult due to the small size of organisms and the quantity necessary for biochemical analyses. Thus, the seasonal variations in the biochemical composition of zooplankton could be analyzed only on four zooplankton size classes, from 80-200 μm to 500-1000 μm . Samples for biochemical analyses were then frozen at -22°C , freeze-dried and ground into a homogenized fine powder prior to analyses.

To quantify zooplankton biomass, the material of each size class was filtered on pre-weighed GFF filters, and dried in an oven at 60°C for 72 hours. Then, filters were weighed again and zooplankton biomass of each size class was expressed as mg m⁻³ dry weight (DW) per volume of filtered water.

2.3. Biochemical analyses

Protein, lipid and carbohydrate concentrations were assessed by three different spectrophotometric methods. Total proteins were determined according to (Lowry et al., 1951) and were expressed as bovine sero-albumin equivalent. For the determination of total proteins, 0.3 mg of ground dry matter was used in each replicate. Carbohydrates were determined after the Dubois method (Dubois et al., 1956), and expressed as glucose equivalents. Lipids were determined following the method of Bligh and Dyer (1959), and expressed as tripalmitic acid equivalent. For the determination of carbohydrates and lipids, 0.6 mg of ground dry matter was used for each replicate. For each type of analysis, triplicates were run on well-homogenized material and concentrations were expressed in µg mg⁻¹ DW. Ashes and the residual organic compounds not recovered by these standard biochemical assays (chitin for example) were noted as 'ash content' and considered as the residual content in each milligram. To minimize the high variation of data from one sampling date to the other, and to take into account the time of component integration into the different zooplankton size classes, the seasonal variations in biochemical composition of the four size classes considered (from 80-200 µm to 500-1000 µm) were analyzed by aggregating data into three-month periods according to Mediterranean hydrological seasons (Seguin, 1981): winter (January to March), spring (April to June), summer (July to September) and autumn (October to December).

Energy content estimation

The energy value (E_i) of each zooplankton size class and group was estimated in Joules per milligram DW using the following equation:

$$E_i = [(C_{Pro} \times 21.4) + (C_{Carb} \times 17.2) + (C_{Lip} \times 35.6)] \text{ J mg}^{-1} \text{ DW},$$

where C_{Pro} = concentration of proteins in the size class or group i in $\mu\text{g mg}^{-1}$ DW,
 C_{Carb} = concentration of carbohydrates in the size class or group i in $\mu\text{g mg}^{-1}$ DW,
 C_{Lip} = concentration of lipids in the size class or group i in $\mu\text{g mg}^{-1}$ DW. The coefficients 21.4 for proteins, 17.2 for carbohydrates and 35.6 for lipids are the conversion factors in Joules recommended for zooplankton by (Postel et al., 2000).

The total amount of energy provided by zooplankton per cubic meter per season ($E_T \text{ J m}^{-3}$) in the 0-50 m water column was calculated as the sum, for the four size classes considered, of the biomass (B_i in mg DW m^{-3}) of each size class at a given season multiplied by its energy value E_i (J mg^{-1} DW): $E_T = \sum(B_i \times E_i)$.

Biomasses (mg DW m^{-3}) of the four zooplankton size classes (80-1000 μm) analyzed per season were used to calculate the total amount of energy provided by zooplankton at the Solemio site, as the two largest size classes were not collected in sufficient amount and represented each only a low percentage of the biomass sampled with this kind of plankton net.

2.4. Statistical analyzes

The effect of size and group on the biochemical composition of zooplankton was tested by means of one-way ANOVA or non-parametric Kruskal-Wallis tests after testing for normality and homogeneity of variances, followed by appropriate paired comparison tests, using the software R (R Core Team, 2017). A redundancy analysis (RDA) was used to relate the biochemical characteristics of zooplankton (biochemical concentrations and energy content) to environmental and zooplankton factors (size, season, T, S, O_2 , NO_3 , PO_4 , Chl a , POC, PON, and SPM). RDA is a constrained

ordination analysis that enables the representation of multivariate data in a reduced number of axes of the greatest variability (Legendre and Legendre, 2012). As variables were expressed in different units ($\mu\text{g mg}^{-1}$ DW, J mg^{-1} DW, $\mu\text{mol L}^{-1}$, $\mu\text{g L}^{-1}$, etc.), the analysis was done on transformed reduced centered data. *F* tests based on 999 permutations were used to test the significance of RDA axes, the overall effect of the environment (all variables taken into account) and the effect of each environmental variable. A forward selection of RDA was then performed for determining the minimum number of explanatory factors that could explain a significant proportion of variation in the biochemical data. The RDA analysis was done using the software R (package ‘vegan’).

3. Results

3.1. Biochemical composition and energy value of size classes and groups

Proteins were the dominant biochemical component in all zooplankton size classes analyzed, representing a mean percentage of $x \pm \text{SD} = 26.8 \pm 15.1\%$ DW, $n = 270$, followed by lipids ($10.5 \pm 8.9\%$ DW, $n = 270$). Carbohydrates ($6.7 \pm 3.4\%$ DW, $n = 270$) presented a much lower percentage. Ashes and non-recovered organic compounds represented a mean percentage of $56.1 \pm 18.6\%$ DW, $n = 270$). All size classes contained a high percentage of ash ($>48.5\%$), with the highest ash content (84.3%) recorded in the largest size class ($>2000 \mu\text{m}$). The biochemical composition of zooplankton significantly differed among size classes (Table I). The highest protein content was recorded in the $300\text{-}500 \mu\text{m}$ size class, and was not significantly different from that of the $200\text{-}300 \mu\text{m}$ and $500\text{-}1000 \mu\text{m}$ size classes. In contrast, the lowest values for protein content were recorded in the largest size class $>2000 \mu\text{m}$. Carbohydrate content peaked in the $1000\text{-}2000 \mu\text{m}$ size class, while their minimum value was recorded in the $>2000 \mu\text{m}$ size class. The highest lipid content was observed

in the 500-1000 μm size class, but did not significantly differ from that of the smaller size classes (below 500 μm), while the lowest lipid values were recorded in two larger size classes (1000-2000 μm and >2000 μm). The highest energy content was obtained in the 500-1000 μm , 300-500 μm and 200-300 μm size classes in decreasing order of importance, while the lowest energy content was observed in the largest size class (>2000 μm). Energy was mainly linked to the proportion of proteins, which provided around 53.3% (38.1%-60.5% depending on the size class) of the total energy content, while lower energy content was provided by lipids (33.3%, from 24.3% to 42.3%) and carbohydrates (13.4%, from 8.3% to 20.6%).

Size classes differed in their taxonomic composition (Table II), which in turn may influence their biochemical composition. The smallest size class (80-200 μm) was generally composed of a mixture of phytoplankton (diatoms such as *Chaetoceros* spp., dinobionts such as *Gymnodinium* spp., *Heterocapsa* spp.), detritus and microzooplankton (mostly bivalves and gastropod larvae, and young copepod stages such as eggs, nauplii and copepodites), in decreasing order of importance. The 200-300 μm size class was dominated by small copepods (copepodites of Paracalanidae, Oithonidae, Oncaeidae, *Microsetella* spp. and Corycaeidae), the 300-500 and 500-1000 μm size classes were dominated by larger copepods (Paracalanidae, *Acartia* spp., Centropagidae, *Candacia* spp., *Calanus* spp. and *Paraeuchaeta* spp.), which differed in size and developmental stages in the different size classes. These size classes were also composed of different crustaceans, such as decapod larvae (including zoea, and megalops), cladocerans (*Evadne* spp., *Pleopis* spp., *Penilia* spp.) and euphausiids. The 500-1000 and 1000-2000 μm size classes also contained eggs of teleosts and crustaceans, with a larger proportion of teleost eggs. The two largest size classes (> 1000 μm) were dominated by gelatinous zooplankton (salps such as blastozoid or oozoid generations of Thaliacea, calycophoran siphonophores such as

Eudoxoides spp., *Lensia* spp., *Muggiaea* spp., and chaetognaths such as Sagittidae), and also contained some pteropods (Creseidae and Cavoliniidae), amphipods *Hyperia* spp., and different species of euphausiids.

The different groups analyzed (copepods, decapod larvae, chaetognaths, salps, siphonophores, and teleost eggs) differed in their biochemical composition (Table III). All groups contained a high percentage of ash (>47%), the highest proportions being found in siphonophores and salps (89% and 83% respectively). Decapod larvae and copepods presented the highest protein (>410 $\mu\text{g mg}^{-1}$ DW) and energy content (>11 J mg^{-1} DW), but did not differ significantly from those of chaetognaths and eggs. Conversely, siphonophores and salps presented significantly lower protein (<220 $\mu\text{g mg}^{-1}$ DW) and energy content (<7 J mg^{-1} DW). The highest carbohydrate content (>42 $\mu\text{g mg}^{-1}$ DW) was observed in salps, and the lowest (<13 $\mu\text{g mg}^{-1}$ DW) in chaetognaths. The highest lipid content (>61 $\mu\text{g mg}^{-1}$ DW) was observed in decapod larvae, and the lowest (<24 $\mu\text{g mg}^{-1}$ DW) in salps. Thus, the energy-rich copepods, decapod larvae, chaetognaths and eggs contrasted with the energy-poor salps and siphonophores. The energy content of these organisms was mainly issued from proteins (73.4%), then lipids (21.5%) and carbohydrates (5.1%). The relative importance of proteins in energy peaked in chaetognaths, copepods, and decapod larvae (77-79%), in eggs for lipids (30.8%) and in salps for carbohydrates (18.5%).

3.2. Seasonal variation of zooplankton biomass and community composition

The total biomass represented by the 80-1000 μm zooplankton varied with season, with higher values (>6.0 mg DW m^{-3}) in spring and summer 2017, and lower values (<4.4 mg DW m^{-3}) in autumn 2016 and 2017, and winter 2017 and 2018 (Table IV). The mean biomass of the two smaller size classes (80-200 μm and 200-300 μm) peaked in summer 2017, while those of the two largest ones (300-500 μm and

500-1000 μm) peaked in spring 2017, although these differences were not significant. Whatever the size class, the biomass of zooplankton decreased from autumn 2016 to winter 2017, increased abruptly in spring and summer 2017, and finally decreased again in autumn 2017 and winter 2018. However, the biomass of the three largest size classes was higher in autumn 2017 and winter 2018 than in autumn 2016 and winter 2017, resulting in higher total biomass of zooplankton in those seasons the second year of study, even if the difference was not statistically significant.

Broadly, during the sampling period, the zooplankton community collected in spring was mostly dominated by copepod nauplii and copepod species belonging to the Centropagidae, followed by Clauso/Paracalanidae, Oithonidae and Oncaeidae, two harpacticoid copepods (*Microsetella* spp. and *Euterpina acutifrons*), Corycaeidae (groups present at all seasons) and fine particle filter-feeding cladocerans (*P. avirostris*, *Podon* and especially *Evadne* spp.). In summer, decapod larvae (brachyurans and macrurids) dominated the zooplanktonic community in the largest size class analyzed, and the dominant copepods belonged to Oithonidae and Acartidae. Other zooplankton, such as mollusc larvae and chaetognaths, also occurred, but in much lower abundance. In autumn, zooplankton were numerically dominated by *Microsetella* spp., *E. acutifrons*, and Oithonidae, followed in lower abundance by *Evadne* spp. and other groups such as pteropods (*Creseis* spp., Caviolinidae), appendicularians and chaetognaths. Finally, in winter, the zooplankton community was once again dominated by calanoid copepods, mostly Clauso/Paracalanidae, along with some Calanidae, Centropagidae, Acartidae, *Microsetella* spp., *E. acutifrons*, and Oithonidae, and mollusc larvae. In general, calanoid copepods dominated in abundance during spring and winter, whereas cyclopoid copepods were more abundant in summer and autumn.

334 3.3. Seasonal variation of biochemical composition and energy content

335 The biochemical composition of zooplankton from 80 μm to 1000 μm differed
336 significantly between seasons (Table V). The highest percentages of ash were observed
337 in autumn 2016 and winter 2017 (70.6% and 62.2% respectively), and the lowest
338 percentage in winter 2018 (32.5%). Protein content peaked in winter 2018, while lipid
339 content peaked in spring 2017, and these peaks were observed in all size classes
340 (Supplementary Table S1). This resulted in a significantly higher energy content of
341 zooplankton in these two seasons ($>13 \text{ J mg}^{-1} \text{ DW}$), followed by summer and autumn
342 2017 (around $10 \text{ J mg}^{-1} \text{ DW}$). By contrast, the significantly lower concentrations of all
343 biochemical components in autumn 2016 led to the lowest energy value of zooplankton
344 in that season ($<7 \text{ J mg}^{-1} \text{ DW}$). Differences in biochemical concentrations and energy
345 content were also observed between years for the same season. Protein and lipid
346 concentrations were significantly lower in autumn 2016 and winter 2017 than in
347 autumn 2017 and winter 2018, resulting in a significantly lower energy content of
348 zooplankton during the first year of the study ($P = 0.002$).

349 The total energy (E_T) provided by mesozooplankton per cubic meter to upper-level
350 consumers at Solemio site in the 0-50 m water column varied with season and years
351 (Fig. 2). In each season, the highest proportions of E_T were provided by the 300-500
352 μm (36.5%) and 500-1000 μm (33.3%) size classes, while the 200-300 μm size class
353 contributed 24.6% to the E_T and the smallest size class (80-200 μm) contributed the
354 least, with only 5.5%. Low E_T values were recorded in autumn 2016 and winter 2017
355 ($<30 \text{ J m}^{-3}$), followed by a peak in total energy content of zooplankton in spring 2017
356 ($>100 \text{ J m}^{-3}$). Then E_T decreased in summer and autumn 2017 (from 64 to 46 J m^{-3}),
357 before increasing again in winter 2018 (75 J m^{-3}). Higher E_T values were recorded in
358 autumn 2017 (x 1.7) and winter 2018 (x 2.9) compared to those observed in autumn
359 2016 and winter 2017. This higher E_T content was linked to an increase in both

biomass and biochemical concentrations of zooplankton during the second year of study (Tables IV and V).

3.4. Influence of environmental factors

The mean values of environmental parameters by season used to run the RDA were presented in Table VI. The RDA axes overall explained 57.6% of the inertia of the biochemical composition of zooplankton at Solemio (permutations F test, $p < 0.001$). The forward selection procedure of the RDA retained eight variables (three size classes: 80-200 μm , 200-300 μm and 300-500 μm , salinity, temperature, oxygen, nitrate and phosphate concentrations) that explained significantly the variations in the biochemical composition of zooplankton (Fig. 3). The RDA indicated that salinity was the most important factor, followed by size (200-300 μm) and nitrate concentration, in explaining the biochemical composition of zooplankton on axis RDA 1 (28.1% of the total variance), while oxygen, temperature, phosphate concentration and size (80-200 μm and 300-500 μm) were associated with axis RDA 2 (21.6% of the total variance). PON, POC, chlorophyll a , and SPM concentrations were not significant to explain the variation in biochemical composition of zooplankton. Proteins, lipids, and energy were all positively correlated with the medium size classes (200-300 μm and 300-500 μm), and negatively with the smallest size class (80-200 μm). Higher lipid, energy and protein content was positively associated with higher salinity, oxygen, nitrate and phosphate concentrations, and lower sea temperature. Two groups of seasons can be distinguished, since most points of autumn 2016 and 2017, summer 2017 and winter 2017 were positioned on the positive part of axis RDA 2, while those of spring 2017 and winter 2018 were positioned on the negative part. Zooplankton of the first group, characterized by low protein, lipid and energy content, corresponded to an environment characterized by

high temperature, low oxygen and low nutrient concentrations. The second group of zooplankton presented high biochemical and energy concentrations, and corresponded to low temperature, but high oxygen and nutrient concentrations.

4. Discussion

The biochemical composition of marine organisms is an important parameter to apprehend their nutritional quality as prey for upper-level consumers (Spitz et al., 2010). The present study is the first to explore the quality, quantified by the biochemical composition and energy content, of different zooplankton size classes and taxonomic groups in the NW Mediterranean Sea. Our results indicated that zooplankton quality was strongly influenced by size class, taxonomic composition and season. The comparison of our data with those of the literature is often difficult because of different plankton sampling methods (net mesh size), measuring methods, units in which results are expressed (wet weigh, dry weight, ash-free dry weight, and carbon weight), and the conversion factors used to calculate the energy content (Postel et al., 2000; Yebra et al., 2017), as discussed by Harmelin-Vivien et al. (2019). To facilitate comparisons, concentration values of biochemical components in the literature were transformed and expressed as percentages of total organic components when values of proteins, carbohydrates and lipids were available.

4.1. Biochemical composition of zooplankton

In this study, proteins were always identified as the dominant organic compounds in zooplankton communities, representing a mean percentage of 58.3% of organic components in the Mediterranean Sea, which reflected their prominent role as structural components for growth and metabolic reserve (Carlotti et al., 1993). Lipids (21.4% in the present study) formed the second most important component, while

carbohydrates represented generally lower concentrations (but 20.3% here). These results were consistent with those recorded for zooplankton in tropical (Goswami et al., 1981; Kumar et al., 2013; Kumari et al., 1993), temperate (Chen and Chen, 1992; Jo et al., 2017; Kuroshima et al., 1987; Mayzaud and Martin, 1975; Raymont et al., 1971) and polar zones (Percy and Fife, 1981; Torres et al., 1994; Yun et al., 2015), although there are regional differences. Harmelin-Vivien et al. (2019) found 63.1% of proteins and 27.8% of lipids in zooplankton from subantarctic Kerguelen waters in springtime, while Goswami et al. (1981) recorded lower proportions of proteins (49.7%) and lipids (11.4%) in the tropical Andaman Sea. The high lipid content of zooplankton organisms in polar regions, particularly before entering in cold seasons, is a well-documented pattern, since lipids form the most energy-rich reserves under extreme cold conditions (Donnelly et al., 1994; Lee et al., 2006). In contrast, the low protein and lipid content of zooplankton in tropical regions is related to low food concentrations available year round to zooplankton in warm waters (Lee et al., 2006). The generally low carbohydrate content of zooplankton suggests that they might not contribute substantially towards body reserves (Kumar et al., 2013).

4.2. Effect of size and group composition

We found that both size and taxonomic composition influence the biochemical composition of zooplankton in the Bay of Marseille. Considering the size, the highest concentrations of biochemical components and energy content were recorded in the medium size classes of zooplankton (200-1000 μm), with a decrease of concentrations (particularly proteins and lipids) in the largest size classes (>1000 μm). A similar size-related profile of energy content is also reported by Dessier et al. (2018) in the NE Atlantic zooplankton, and is related to the high proportion of protein- and energy-rich copepods and decapod larvae in the medium size classes. Generally both

protein and lipid concentrations of organisms increased with increasing size of individuals (Båmstedt, 1988; Guisande, 2006), particularly in the size range 200-1000 μm for many mesozooplanktonic organisms, such as copepods, in temperate regions. The low concentrations of biochemical components in the largest size classes ($>1000 \mu\text{m}$) observed in the Bay of Marseille can be attributed to the dominance of protein- and energy-poor gelatinous organisms, such as salps and siphonophores. These results contrast with those observed in cold high-latitude oceans, where an increase in all component concentrations with zooplankton size classes is generally recorded (Guise, 2006; Hagen and Auel, 2001; Harmelin-Vivien et al., 2019), and is related to the high abundance of euphausiids and large-sized copepods and amphipods in the $>1000 \mu\text{m}$ size classes, many species having their adult stage far above this size.

Concentrations in biochemical composition have been observed to differ widely between taxonomic groups and species of zooplankton (Donnelly et al., 1994; Harmelin-Vivien et al., 2019; Kumar et al., 2013; Lee et al., 2006; Mayzaud and Martin, 1975; Nakai, 1942; Percy and Fife, 1981). In our study, zooplankton species composition in the Bay of Marseille differed between size classes, but the dominant groups were similar to those previously recorded in the Gulf of Lion (Bănar, 2014; Champalbert, 1996; Espinasse et al., 2014). Detritus, micro-phytoplankton and small copepod stages dominated the smallest size class (80-200 μm). Detritus could be related to the proximity of the large city of Marseille, the constant inputs of Cortiou sewage wastewaters and sporadic river water runoff (Bănar, 2014; Millet et al., 2018). Copepods dominated the 200-300 μm , 300-500 μm , and 500-1000 μm size classes, while the 1000-2000 μm and $>2000 \mu\text{m}$ size classes were highly diversified and dominated by gelatinous organisms, as previously observed in the Mediterranean Sea (Bănar, 2014; Champalbert, 1996; Razouls and Kouwenberg, 1993; Saiz et al., 2007). Copepods were rich in proteins and lipids

(82.7% and 11.4%), as observed in many previous studies (Harmelin-Vivien et al., 2019; Mauchline, 1998; Ventura, 2006). Most copepods convert part of their food to lipids, stored as reserves (Lee et al., 2006), depending on their metabolism and body size (Vogedes et al., 2010; Zarubin et al., 2014). Calanoid copepod species may have higher lipid and thus energy content than cyclopoid copepods (Ventura, 2006). The high contribution of energy from lipid in eggs (30.8%) compared to other groups reflects the importance of reserve investment for offspring development (Guisande and Harris, 1995). In contrast, gelatinous zooplankton, such as salps and siphonophores, contain a high percentage of water, less protein but higher proportions of carbohydrates, as previously observed in other regions (Harmelin-Vivien et al., 2019; Lucas, 2009). However, among gelatinous organisms, chaetognaths are characterized by a high protein content, which may be linked to their predatory behavior, particularly upon copepods (Terazaki, 1995). The higher percentage of chaetognaths in the 1000-2000 μm size class in Marseille may explain the higher protein and lipid content of this size class, compared to the >2000 μm size class dominated by salps and siphonophores.

4.3. Seasonal variability of zooplankton biochemical composition

Wide seasonal variations of zooplankton biomass and energy content were observed in the Bay of Marseille, both presenting their maximum values in spring. The contrast between high zooplankton biomass in spring and low biomass in winter observed in the present study is a classical pattern observed in most western Mediterranean regions and is related to an increase in primary production in spring (Gaudy, 1985; Gaudy et al., 2003). Seasonal variations of zooplankton biomass often express the variations of copepod density, which displays considerable fluctuations with several peaks of abundance and five to seven generations during the year

(Champalbert, 1996; Seguin, 1981). However, two groups of seasons with contrasted biochemical composition of zooplankton were distinguished in the Bay of Marseille (Fig. 4), independently of biomass value. Spring 2017 and winter 2018 were characterized by energy-rich zooplankton ($>13 \text{ J mg}^{-1} \text{ DW}$), and were related with low temperature and high salinity, oxygen and nutrients. In contrast, zooplankton presented a lower energy density ($<11 \text{ J mg}^{-1} \text{ DW}$) in all other seasons, which was related with higher water temperature and lower nutrient concentrations.

García-Comas et al. (2011) demonstrated that low temperature and high salinity correlate with high zooplankton populations in the Mediterranean Sea, due to a higher phytoplankton production favored by an increase in nutrients induced by winter water mixing and convection. Similarly, Donoso et al. (2017) observed an increase in zooplankton biomass in spring after a winter convection episode. High nutrient inputs in the Bay of Marseille favor particularly the development of picophytoplankton and nanophytoplankton (Grégori et al., 2001), which have recently been recognized as the main zooplankton food resources in the Mediterranean Sea (Hunt et al., 2017). The amount of food available would in turn increase the organic matter and energy content of zooplankton organisms, as observed for *Calanus finmarchicus* by Comita et al. (1966). These authors indicated that if a spring increase in *Calanus* calorific value is observed in all years, an additional peak in winter may occur when environmental and feeding conditions are favorable, as observed here during the second winter studied.

Thus, the relationships between environmental factors and the biochemical composition of mesozooplankton observed in the Bay of Marseille according to both seasons and years, are probably in fact mediated by the development of phytoplankton and microheterotroph blooms, as well as by the various developmental stages of copepods.

However the two peaks of zooplankton energy differed in their biochemical

composition and the underlying mechanisms. In spring 2017, the peak of energy was due to a conspicuous increase of lipid concentrations, while the high energy content in winter 2018 was related to very high protein concentrations, along with rather high lipid content. While in both seasons, the main part of the mesozooplankton biomass (72%) was provided by the 300-500 and 500-1000 μm size classes, with a high abundance of energy-rich copepods, their size and composition differed. In spring 2017, there was a huge abundance of small-sized species (Oncaeidae, Oithonidae) and nauplii stages, linked to rapid reproduction processes of copepods in that season (Gaudy, 1985), along with numerous lipid-rich crustacean and teleost eggs. Eggs provided a large part of the lipids quantified, but there was also probably an increase in structural polar lipids, due to the high individual abundance. This hypothesis was supported by the fact that lipids increased in all size classes, and not only in larger ones, in which eggs were more abundant. Conversely, in winter 2018, zooplankton abundance was twice lower than in spring 2017, with much larger individuals (data not shown), particularly of large-sized protein-rich Calanidae and bivalve larvae. Båmstedt (1988) demonstrated that protein content in copepods is better correlated with individual length than lipids, which display a higher individual variability. The protein/lipid ratio increases then with the size of copepods (Båmstedt, 1988). Thus, the high protein concentrations recorded in winter 2018 could be linked to the larger size of copepod individuals and the presence of protein-rich groups such as mollusk larvae.

4.4. Influence of zooplankton energy content on food web functioning

The mean energy content of zooplankton in the Bay of Marseille ($10.7 \text{ J mg}^{-1} \text{ DW}$) appears to be within the range of values recorded in other regions (while differences could be due to different size ranges analyzed and different methods): from 7.5 J mg^{-1}

DW to 12.5 J mg^{-1} DW in the Andaman Sea (Kumar et al., 2013), 9.4 J mg^{-1} DW in Kerguelen waters (Harmelin-Vivien et al., 2019), 9.6 J mg^{-1} DW in the Chukchi Sea (Yun et al., 2015), and 13.4 J mg^{-1} DW in the Japan Sea (Jo et al., 2017). Our results indicated that the energy density of zooplankton peaked in the medium size classes (200-1000 μm) and varied widely with seasons and years depending on environmental factor variations, among which temperature, salinity and nutrient inputs seemed to prevail. Many studies highlight the feeding selectivity of predators, which generally target specific prey sizes and species to fulfill their metabolic requirements, as observed in the Gulf of Lion (Mellon-Duval et al., 2017), but prey quality is also of crucial importance for consumers (Albo-Puigserver et al., 2017; Spitz et al., 2010). Stomach content analyses have revealed the importance of mesozooplankton (mostly 200-1000 μm) as the main prey of small planktivorous fish species, such as sardine and anchovy (Le Bourg et al., 2015), and the quality of their food has been questioned to be a possible cause of their decline in body condition (Brosset et al., 2016; Saraux et al., 2019). We demonstrated in the present paper that these fishes indeed select the most energy-rich zooplankton size classes (200-1000 μm) and taxonomic groups (copepods), but that the quality of these prey may vary widely over time (and probably also in space, as demonstrated in the Bay of Biscay by Dessier et al. (2018)). Thus, the metabolism of sardine and anchovy in the NW Mediterranean Sea could have been constrained by the low quality of their prey in previous years, resulting in the observed poor body condition. A better understanding, on the basis of further studies combining stable isotope analyses with a modelling approach, of how environmental factors influence the blooms of the different phytoplankton size classes and microheterotrophs, and their trophic pathways up to the different zooplankton size classes (Hunt et al., 2017), and of the resulting effects on their biochemical and energy content, would be necessary to fully understand the small pelagic fishery crisis

(Saraux et al., 2019; Van Beveren et al., 2014). It would also provide a basis for anticipating the possible impacts of global climate change on these fishery resources in the Mediterranean Sea, where changes in water convection and river inputs could drastically alter the inputs of nutrients at the base of the food web functioning.

5. Conclusion

The biochemical composition of size-fractionated zooplankton was analyzed seasonally at the Solemio site in the Bay of Marseille, NW Mediterranean Sea. The biochemical composition of zooplankton differed with size classes and groups, and varied between seasons and years according to environmental factor variations. We observed that (1) the highest energy content was recorded in the 300-500 μm and 500-1000 μm mesozooplankton size classes, which were dominated by copepods, the most energy-rich group of organisms analyzed; (2) the largest zooplankton size class (>2000 μm) presented the lowest energy content, due to the high importance of energy-poor salps and siphonophores; and (3) high seasonal variations of the biochemical composition occurred, with higher energy content in all zooplankton size classes in low temperature - high oxygen and nutrient-rich waters (generally spring and winter). Our results, obtained over 18 months, clearly suggested that community composition strongly determined the biochemical content of each zooplankton size class. The inter-annual variability obtained for two successive autumn-winter periods appeals for repeating this study over multiyear period. In the Bay of Marseille, the high energy level of mesozooplankton may result for certain years from nutrient enrichment by winter convection events and/or from terrestrial inputs linked to precipitations during the cold seasons. Therefore, decadal observations of the atmospheric and anthropic forcing conditions on the planktonic food web functioning and in fine on the quantity and quality of the zooplankton are required. Such further

studies should combine biochemical and stable isotope analyses with a modelling approach in order to better understand the varying energy budget of trophic compartments in pelagic food webs and their impacts on fish dynamics.

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