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

3

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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

26 **Abstract**

27 Zooplankton plays a prominent role in marine pelagic food webs, but its contribution
28 to organic matter transfer from phytoplankton to upper level consumers is modulated
29 by size and specific composition. The biochemical composition (protein, carbohydrate
30 and lipid concentrations) and energy content of size- and group-fractionated
31 zooplankton were analyzed in the NW Mediterranean Sea (Marseille) over 18 months.
32 Proteins were the most abundant biochemical compounds in all size classes and
33 zooplankton groups, and provided the largest part of the energy content. The medium
34 (200-1000 μm) size classes, mainly composed of copepods, crustacean larvae and
35 eggs, presented a higher protein and energy content than both the smallest (80-200 μm)
36 size class, mainly composed of phytoplankton, and the largest (>1000 μm) size
37 classes, composed of siphonophores, salps and chaetognaths. Strong and similar
38 seasonal variations in zooplankton biochemical composition were observed in all size
39 classes, with higher energy content associated with cold oxygen- and nutrient-rich
40 waters in spring 2017 and winter 2018. This study evidenced the importance of size-
41 and group-fractionating methods to achieve a better insight into energy and nutrient
42 transfer up into pelagic food webs, and understanding of zooplanktivorous fish
43 feeding preferences, depending on the variable quality of zooplankton prey.

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49 **Keywords**

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

52 **1. Introduction**

53 Zooplankton is considered as an essential compartment of aquatic ecosystems
54 because of its pivotal role in food webs (Banse, 1995). Due to their intermediate
55 trophic position, zooplankton organisms not only transfer the organic matter (OM)
56 and energy from primary producers to higher trophic level consumers (Frederiksen et
57 al., 2006; Saiz et al., 2007), but they also influence the energy density of the food
58 available to zooplanktivores (Harmelin-Vivien et al., 2019). During the last decade, a
59 decreasing trend was observed in biomass, landings, and body condition of
60 commercially important sardines and anchovies in the Mediterranean Sea, particularly
61 in the Gulf of Lion, while the abundance of non-marketable sprats was increasing
62 (Saraux et al., 2019; Van Beveren et al., 2014). Previous studies have hypothesized
63 that the decline of these small zooplanktivorous pelagic fishes could be due to
64 bottom-up control linked to changes in the availability or quality of their food, which
65 mainly consists of mesozooplankton from 200 μm to 1000 μm in size (Costalago et al.,
66 2012; Le Bourg et al., 2015). This hypothesis thus highlighted the interest of studying
67 the biochemical composition of size-fractionated zooplankton in order to better
68 estimate the amount of energy provided by different size classes to upper-level
69 consumers in the pelagic food webs of the NW Mediterranean Sea.

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

77 aspects of plankton chemistry has developed, and more studies have dealt with the
78 organic and calorific content of marine zooplankton species (Båmstedt, 1986;
79 Barroeta et al., 2017; Omori and Ikeda, 1992). The protein and carbohydrate
80 composition of zooplankton organisms is known to be species- and size-specific
81 (Guisande, 2006; Ventura, 2006). Similarly, the lipid content of marine zooplankton
82 differs among species, and can range from a few percent to more than 60% of
83 individual dry weight (Lee et al., 2006). Works on organic fraction analysis have often
84 been restricted to variations in only certain biochemical compounds (lipids separately
85 from proteins, or nothing about carbohydrates) (Norrbin and Båmstedt, 1984), but few
86 have recorded the three compound types simultaneously (Ventura, 2006). However,
87 quantifying the protein, carbohydrate and lipid content of organisms enables
88 determination of their energy content, which provides information on their nutritional
89 quality as prey for upper-level predators (Harmelin-Vivien et al., 2019; Spitz et al.,
90 2010). Despite the complex structure and functions of zooplankton (Steinberg and
91 Landry, 2017), previous biochemical studies considered zooplankton as a
92 homogenous compartment (Goswami et al., 1981; Kumari et al., 1993), or
93 investigated only a few plankton groups, especially large organisms (Choe et al., 2003;
94 Harmelin-Vivien et al., 2012). However, body size determines potential predator-prey
95 interactions (Emmerson and Raffaelli, 2004), rates of production (Banse and Mosher,
96 1980), energy requirements and metabolism (Brown et al., 2004), and mortality rates
97 (Hirst and Kiorboe, 2002). A size-based approach has thus been proposed as a useful
98 means to obtain a better insight into the structure and functioning of marine food webs,
99 and used in plankton studies (Carlotti et al., 2008; Rolff, 2000; Saiz et al., 2007).

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

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

118 The main aims of this study were thus (1) to determine the biochemical
119 composition of different zooplankton size-fractions (80-200 μm , 200-300 μm ,
120 300-500 μm , 500-1000 μm , 1000-2000 μm and >2000 μm size classes) and of some
121 important zooplankton taxonomic groups (copepods, decapod larvae, chaetognaths,
122 salps, siphonophores, teleost eggs) in the NW Mediterranean Sea, by measuring their
123 concentrations in organic compounds (proteins, carbohydrates and lipids) and their
124 energy content in Joules; (2) to explore the seasonal variations of biomass and
125 biochemical composition of the four dominant zooplankton size classes (from 80 μm
126 to 1000 μm); and (3) to analyze the influence of environmental factors on their
127 biochemical composition. The ultimate objective was to provide more comprehensive

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

130 **2. Material and methods**

131 *2.1. Study site*

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

150

151 *2.2. Sampling and sample treatment*

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

153 March 2018 during the SOMLIT programme in order to benefit from the environmental
154 data corresponding to our samples. Zooplankton was sampled vertically from 50 m
155 depth (close to bottom) to the surface with a 80 μm mesh net (50 cm mouth diameter)
156 towed at 1 m s^{-1} . Five vertical tows were performed to collect enough material for the
157 analyses. Four samples were dedicated to biochemical analyses and one to
158 quantification of plankton biomass. Samples were filtered through six different sieves
159 ranged in a column (2000, 1000, 500, 300, 200 and 80 μm mesh size), allowing the
160 separation of six size classes of zooplankton (80-200 μm , 200-300 μm , 300-500 μm ,
161 500-1000 μm , 1000-2000 μm and >2000 μm).

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

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

183

184 *2.3. Biochemical analyses*

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

204 *Energy content estimation*

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

$$207 E_i = [(C_{Pro} \times 21.4) + (C_{Carb} \times 17.2) + (C_{Lip} \times 35.6)] J \text{ mg}^{-1} \text{ DW},$$

208 where C_{Pro} = concentration of proteins in the size class or group i in $\mu\text{g mg}^{-1} \text{ DW}$,

209 C_{Carb} = concentration of carbohydrates in the size class or group i in $\mu\text{g mg}^{-1} \text{ DW}$,

210 C_{Lip} = concentration of lipids in the size class or group i in $\mu\text{g mg}^{-1} \text{ DW}$. The

211 coefficients 21.4 for proteins, 17.2 for carbohydrates and 35.6 for lipids are the

212 conversion factors in Joules recommended for zooplankton by (Postel et al., 2000).

213 The total amount of energy provided by zooplankton per cubic meter per season

214 ($E_T J \text{ m}^{-3}$) in the 0-50 m water column was calculated as the sum, for the four size

215 classes considered, of the biomass (B_i in mg DW m^{-3}) of each size class at a given

216 season multiplied by its energy value E_i ($J \text{ mg}^{-1} \text{ DW}$): $E_T = \sum(B_i \times E_i)$.

217 Biomasses (mg DW m^{-3}) of the four zooplankton size classes (80-1000 μm)

218 analyzed per season were used to calculate the total amount of energy provided by

219 zooplankton at the Solemio site, as the two largest size classes were not collected in

220 sufficient amount and represented each only a low percentage of the biomass sampled

221 with this kind of plankton net.

222

223 *2.4. Statistical analyzes*

224 The effect of size and group on the biochemical composition of zooplankton was

225 tested by means of one-way ANOVA or non-parametric Kruskal-Wallis tests after

226 testing for normality and homogeneity of variances, followed by appropriate paired

227 comparison tests, using the software R (R Core Team, 2017). A redundancy analysis

228 (RDA) was used to relate the biochemical characteristics of zooplankton (biochemical

229 concentrations and energy content) to environmental and zooplankton factors (size,

230 season, T, S, O_2 , NO_3 , PO_4 , Chl a , POC, PON, and SPM). RDA is a constrained

231 ordination analysis that enables the representation of multivariate data in a reduced
232 number of axes of the greatest variability (Legendre and Legendre, 2012). As
233 variables were expressed in different units ($\mu\text{g mg}^{-1}$ DW, J mg^{-1} DW, $\mu\text{mol L}^{-1}$, $\mu\text{g L}^{-1}$,
234 etc.), the analysis was done on transformed reduced centered data. *F* tests based on
235 999 permutations were used to test the significance of RDA axes, the overall effect of
236 the environment (all variables taken into account) and the effect of each
237 environmental variable. A forward selection of RDA was then performed for
238 determining the minimum number of explanatory factors that could explain a
239 significant proportion of variation in the biochemical data. The RDA analysis was
240 done using the software R (package 'vegan').

241 **3. Results**

242 *3.1. Biochemical composition and energy value of size classes and groups*

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

256 in the 500-1000 μm size class, but did not significantly differ from that of the smaller
257 size classes (below 500 μm), while the lowest lipid values were recorded in two larger
258 size classes (1000-2000 μm and >2000 μm). The highest energy content was obtained
259 in the 500-1000 μm , 300-500 μm and 200-300 μm size classes in decreasing order of
260 importance, while the lowest energy content was observed in the largest size class
261 (>2000 μm). Energy was mainly linked to the proportion of proteins, which provided
262 around 53.3% (38.1%-60.5% depending on the size class) of the total energy content,
263 while lower energy content was provided by lipids (33.3%, from 24.3% to 42.3%) and
264 carbohydrates (13.4%, from 8.3% to 20.6%).

265 Size classes differed in their taxonomic composition (Table II), which in turn may
266 influence their biochemical composition. The smallest size class (80-200 μm) was
267 generally composed of a mixture of phytoplankton (diatoms such as *Chaetoceros* spp.,
268 dinobionts such as *Gymnodinium* spp., *Heterocapsa* spp.), detritus and
269 microzooplankton (mostly bivalves and gastropod larvae, and young copepod stages
270 such as eggs, nauplii and copepodites), in decreasing order of importance. The 200-300
271 μm size class was dominated by small copepods (copepodites of Paracalanidae,
272 Oithonidae, Oncaeidae, *Microsetella* spp. and Corycaeidae), the 300-500 and
273 500-1000 μm size classes were dominated by larger copepods (Paracalanidae, *Acartia*
274 spp., Centropagidae, *Candacia* spp., *Calanus* spp. and *Paraeuchaeta* spp.), which
275 differed in size and developmental stages in the different size classes. These size
276 classes were also composed of different crustaceans, such as decapod larvae
277 (including zoea, and megalops), cladocerans (*Evadne* spp., *Pleopis* spp., *Penilia* spp.)
278 and euphausiids. The 500-1000 and 1000-2000 μm size classes also contained eggs of
279 teleosts and crustaceans, with a larger proportion of teleost eggs. The two largest size
280 classes (> 1000 μm) were dominated by gelatinous zooplankton (salps such as
281 blastozoid or oozoid generations of Thaliacea, calyphoran siphonophores such as

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

285 The different groups analyzed (copepods, decapod larvae, chaetognaths, salps,
286 siphonophores, and teleost eggs) differed in their biochemical composition (Table III).
287 All groups contained a high percentage of ash (>47%), the highest proportions being
288 found in siphonophores and salps (89% and 83% respectively). Decapod larvae and
289 copepods presented the highest protein (>410 $\mu\text{g mg}^{-1}$ DW) and energy content (>11 J
290 mg^{-1} DW), but did not differ significantly from those of chaetognaths and eggs.
291 Conversely, siphonophores and salps presented significantly lower protein (<220 μg
292 mg^{-1} DW) and energy content (<7 J mg^{-1} DW). The highest carbohydrate content (>42
293 $\mu\text{g mg}^{-1}$ DW) was observed in salps, and the lowest (<13 $\mu\text{g mg}^{-1}$ DW) in chaetognaths.
294 The highest lipid content (>61 $\mu\text{g mg}^{-1}$ DW) was observed in decapod larvae, and the
295 lowest (<24 $\mu\text{g mg}^{-1}$ DW) in salps. Thus, the energy-rich copepods, decapod larvae,
296 chaetognaths and eggs contrasted with the energy-poor salps and siphonophores. The
297 energy content of these organisms was mainly issued from proteins (73.4%), then lipids
298 (21.5%) and carbohydrates (5.1%). The relative importance of proteins in energy
299 peaked in chaetognaths, copepods, and decapod larvae (77-79%), in eggs for lipids
300 (30.8%) and in salps for carbohydrates (18.5%).

301

302 3.2. Seasonal variation of zooplankton biomass and community composition

303 The total biomass represented by the 80-1000 μm zooplankton varied with season,
304 with higher values (>6.0 mg DW m^{-3}) in spring and summer 2017, and lower values
305 (<4.4 mg DW m^{-3}) in autumn 2016 and 2017, and winter 2017 and 2018 (Table IV).
306 The mean biomass of the two smaller size classes (80-200 μm and 200-300 μm)
307 peaked in summer 2017, while those of the two largest ones (300-500 μm and

308 500-1000 μm) peaked in spring 2017, although these differences were not significant.
309 Whatever the size class, the biomass of zooplankton decreased from autumn 2016 to
310 winter 2017, increased abruptly in spring and summer 2017, and finally decreased
311 again in autumn 2017 and winter 2018. However, the biomass of the three largest size
312 classes was higher in autumn 2017 and winter 2018 than in autumn 2016 and winter
313 2017, resulting in higher total biomass of zooplankton in those seasons the second year
314 of study, even if the difference was not statistically significant.

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

333

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

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

362

363 *3.4. Influence of environmental factors*

364 The mean values of environmental parameters by season used to run the RDA
365 were presented in Table VI. The RDA axes overall explained 57.6% of the inertia of
366 the biochemical composition of zooplankton at Solemio (permutations F test, $p <$
367 0.001). The forward selection procedure of the RDA retained eight variables (three
368 size classes: 80-200 μm , 200-300 μm and 300-500 μm , salinity, temperature, oxygen,
369 nitrate and phosphate concentrations) that explained significantly the variations in the
370 biochemical composition of zooplankton (Fig. 3). The RDA indicated that salinity
371 was the most important factor, followed by size (200-300 μm) and nitrate
372 concentration, in explaining the biochemical composition of zooplankton on axis
373 RDA 1 (28.1% of the total variance), while oxygen, temperature, phosphate
374 concentration and size (80-200 μm and 300-500 μm) were associated with axis RDA
375 2 (21.6% of the total variance). PON, POC, chlorophyll a , and SPM concentrations
376 were not significant to explain the variation in biochemical composition of
377 zooplankton. Proteins, lipids, and energy were all positively correlated with the
378 medium size classes (200-300 μm and 300-500 μm), and negatively with the smallest
379 size class (80-200 μm). Higher lipid, energy and protein content was positively
380 associated with higher salinity, oxygen, nitrate and phosphate concentrations, and
381 lower sea temperature. Two groups of seasons can be distinguished, since most points
382 of autumn 2016 and 2017, summer 2017 and winter 2017 were positioned on the
383 positive part of axis RDA 2, while those of spring 2017 and winter 2018 were
384 positioned on the negative part. Zooplankton of the first group, characterized by low
385 protein, lipid and energy content, corresponded to an environment characterized by

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

389

390 **4. Discussion**

391 The biochemical composition of marine organisms is an important parameter to
392 apprehend their nutritional quality as prey for upper-level consumers (Spitz et al.,
393 2010). The present study is the first to explore the quality, quantified by the
394 biochemical composition and energy content, of different zooplankton size classes
395 and taxonomic groups in the NW Mediterranean Sea. Our results indicated that
396 zooplankton quality was strongly influenced by size class, taxonomic composition
397 and season. The comparison of our data with those of the literature is often difficult
398 because of different plankton sampling methods (net mesh size), measuring methods,
399 units in which results are expressed (wet weigh, dry weight, ash-free dry weight, and
400 carbon weight), and the conversion factors used to calculate the energy content
401 (Postel et al., 2000; Yebra et al., 2017), as discussed by Harmelin-Vivien et al. (2019).
402 To facilitate comparisons, concentration values of biochemical components in the
403 literature were transformed and expressed as percentages of total organic components
404 when values of proteins, carbohydrates and lipids were available.

405

406 *4.1. Biochemical composition of zooplankton*

407 In this study, proteins were always identified as the dominant organic compounds
408 in zooplankton communities, representing a mean percentage of 58.3% of organic
409 components in the Mediterranean Sea, which reflected their prominent role as
410 structural components for growth and metabolic reserve (Carlotti et al., 1993). Lipids
411 (21.4% in the present study) formed the second most important component, while

412 carbohydrates represented generally lower concentrations (but 20.3% here). These
413 results were consistent with those recorded for zooplankton in tropical (Goswami et
414 al., 1981; Kumar et al., 2013; Kumari et al., 1993), temperate (Chen and Chen, 1992;
415 Jo et al., 2017; Kuroshima et al., 1987; Mayzaud and Martin, 1975; Raymont et al.,
416 1971) and polar zones (Percy and Fife, 1981; Torres et al., 1994; Yun et al., 2015),
417 although there are regional differences. Harmelin-Vivien et al. (2019) found 63.1% of
418 proteins and 27.8% of lipids in zooplankton from subantarctic Kerguelen waters in
419 springtime, while Goswami et al. (1981) recorded lower proportions of proteins
420 (49.7%) and lipids (11.4%) in the tropical Andaman Sea. The high lipid content of
421 zooplankton organisms in polar regions, particularly before entering in cold seasons,
422 is a well-documented pattern, since lipids form the most energy-rich reserves under
423 extreme cold conditions (Donnelly et al., 1994; Lee et al., 2006). In contrast, the low
424 protein and lipid content of zooplankton in tropical regions is related to low food
425 concentrations available year round to zooplankton in warm waters (Lee et al., 2006).
426 The generally low carbohydrate content of zooplankton suggests that they might not
427 contribute substantially towards body reserves (Kumar et al., 2013).

428

429 *4.2. Effect of size and group composition*

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

438 protein and lipid concentrations of organisms increased with increasing size of
439 individuals (Båmstedt, 1988; Guisande, 2006), particularly in the size range 200-1000
440 μm for many mesozooplanktonic organisms, such as copepods, in temperate regions.
441 The low concentrations of biochemical components in the largest size classes (>1000
442 μm) observed in the Bay of Marseille can be attributed to the dominance of protein-
443 and energy-poor gelatinous organisms, such as salps and siphonophores. These results
444 contrast with those observed in cold high-latitude oceans, where an increase in all
445 component concentrations with zooplankton size classes is generally recorded
446 (Guisande, 2006; Hagen and Auel, 2001; Harmelin-Vivien et al., 2019), and is related
447 to the high abundance of euphausiids and large-sized copepods and amphipods in the
448 $>1000 \mu\text{m}$ size classes, many species having their adult stage far above this size.

449 Concentrations in biochemical composition have been observed to differ widely
450 between taxonomic groups and species of zooplankton (Donnelly et al., 1994;
451 Harmelin-Vivien et al., 2019; Kumar et al., 2013; Lee et al., 2006; Mayzaud and
452 Martin, 1975; Nakai, 1942; Percy and Fife, 1981). In our study, zooplankton species
453 composition in the Bay of Marseille differed between size classes, but the dominant
454 groups were similar to those previously recorded in the Gulf of Lion (Bănaru et al.,
455 2014; Champalbert, 1996; Espinasse et al., 2014). Detritus, micro-phytoplankton and
456 small copepod stages dominated the smallest size class (80-200 μm). Detritus could
457 be related to the proximity of the large city of Marseille, the constant inputs of
458 Cortiou sewage wastewaters and sporadic river water runoff (Bănaru et al., 2014;
459 Millet et al., 2018). Copepods dominated the 200-300 μm , 300-500 μm , and 500-1000
460 μm size classes, while the 1000-2000 μm and $>2000 \mu\text{m}$ size classes were highly
461 diversified and dominated by gelatinous organisms, as previously observed in the
462 Mediterranean Sea (Bănaru et al., 2014; Champalbert, 1996; Razouls and
463 Kouwenberg, 1993; Saiz et al., 2007). Copepods were rich in proteins and lipids

464 (82.7% and 11.4%), as observed in many previous studies (Harmelin-Vivien et al.,
465 2019; Mauchline, 1998; Ventura, 2006). Most copepods convert part of their food to
466 lipids, stored as reserves (Lee et al., 2006), depending on their metabolism and body
467 size (Vogedes et al., 2010; Zarubin et al., 2014). Calanoid copepod species may have
468 higher lipid and thus energy content than cyclopoid copepods (Ventura, 2006). The
469 high contribution of energy from lipid in eggs (30.8%) compared to other groups
470 reflects the importance of reserve investment for offspring development (Guisande
471 and Harris, 1995). In contrast, gelatinous zooplankton, such as salps and
472 siphonophores, contain a high percentage of water, less protein but higher proportions
473 of carbohydrates, as previously observed in other regions (Harmelin-Vivien et al.,
474 2019; Lucas, 2009). However, among gelatinous organisms, chaetognaths are
475 characterized by a high protein content, which may be linked to their predatory
476 behavior, particularly upon copepods (Terazaki, 1995). The higher percentage of
477 chaetognaths in the 1000-2000 μm size class in Marseille may explain the higher
478 protein and lipid content of this size class, compared to the >2000 μm size class
479 dominated by salps and siphonophores.

480

481 *4.3. Seasonal variability of zooplankton biochemical composition*

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

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

497 García-Comas et al. (2011) demonstrated that low temperature and high salinity
498 correlate with high zooplankton populations in the Mediterranean Sea, due to a higher
499 phytoplankton production favored by an increase in nutrients induced by winter water
500 mixing and convection. Similarly, Donoso et al. (2017) observed an increase in
501 zooplankton biomass in spring after a winter convection episode. High nutrient inputs
502 in the Bay of Marseille favor particularly the development of picophytoplankton and
503 nanophytoplankton (Grégori et al., 2001), which have recently been recognized as the
504 main zooplankton food resources in the Mediterranean Sea (Hunt et al., 2017). The
505 amount of food available would in turn increase the organic matter and energy content
506 of zooplankton organisms, as observed for *Calanus finmarchicus* by Comita et al.
507 (1966). These authors indicated that if a spring increase in *Calanus* calorific value is
508 observed in all years, an additional peak in winter may occur when environmental and
509 feeding conditions are favorable, as observed here during the second winter studied.

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

515 However the two peaks of zooplankton energy differed in their biochemical

516 composition and the underlying mechanisms. In spring 2017, the peak of energy was
517 due to a conspicuous increase of lipid concentrations, while the high energy content in
518 winter 2018 was related to very high protein concentrations, along with rather high
519 lipid content. While in both seasons, the main part of the mesozooplankton biomass
520 (72%) was provided by the 300-500 and 500-1000 μm size classes, with a high
521 abundance of energy-rich copepods, their size and composition differed. In spring
522 2017, there was a huge abundance of small-sized species (Oncaeidae, Oithonidae) and
523 nauplii stages, linked to rapid reproduction processes of copepods in that season
524 (Gaudy, 1985), along with numerous lipid-rich crustacean and teleost eggs. Eggs
525 provided a large part of the lipids quantified, but there was also probably an increase
526 in structural polar lipids, due to the high individual abundance. This hypothesis was
527 supported by the fact that lipids increased in all size classes, and not only in larger
528 ones, in which eggs were more abundant. Conversely, in winter 2018, zooplankton
529 abundance was twice lower than in spring 2017, with much larger individuals (data
530 not shown), particularly of large-sized protein-rich Calanidae and bivalve larvae.
531 Båmstedt (1988) demonstrated that protein content in copepods is better correlated
532 with individual length than lipids, which display a higher individual variability. The
533 protein/lipid ratio increases then with the size of copepods (Båmstedt, 1988). Thus, the
534 high protein concentrations recorded in winter 2018 could be linked to the larger size
535 of copepod individuals and the presence of protein-rich groups such as mollusk
536 larvae.

537

538 *4.4. Influence of zooplankton energy content on food web functioning*

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

542 DW to 12.5 J mg^{-1} DW in the Andaman Sea (Kumar et al., 2013), 9.4 J mg^{-1} DW in
543 Kerguelen waters (Harmelin-Vivien et al., 2019), 9.6 J mg^{-1} DW in the Chukchi Sea
544 (Yun et al., 2015), and 13.4 J mg^{-1} DW in the Japan Sea (Jo et al., 2017). Our results
545 indicated that the energy density of zooplankton peaked in the medium size classes
546 (200-1000 μm) and varied widely with seasons and years depending on environmental
547 factor variations, among which temperature, salinity and nutrient inputs seemed to
548 prevail. Many studies highlight the feeding selectivity of predators, which generally
549 target specific prey sizes and species to fulfill their metabolic requirements, as
550 observed in the Gulf of Lion (Mellon-Duval et al., 2017), but prey quality is also of
551 crucial importance for consumers (Albo-Puigserver et al., 2017; Spitz et al., 2010).
552 Stomach content analyses have revealed the importance of mesozooplankton (mostly
553 200-1000 μm) as the main prey of small planktivorous fish species, such as sardine
554 and anchovy (Le Bourg et al., 2015), and the quality of their food has been questioned to
555 be a possible cause of their decline in body condition (Brosset et al., 2016; Saraux et
556 al., 2019). We demonstrated in the present paper that these fishes indeed select the
557 most energy-rich zooplankton size classes (200-1000 μm) and taxonomic groups
558 (copepods), but that the quality of these prey may vary widely over time (and
559 probably also in space, as demonstrated in the Bay of Biscay by Dessier et al. (2018)).
560 Thus, the metabolism of sardine and anchovy in the NW Mediterranean Sea could
561 have been constrained by the low quality of their prey in previous years, resulting in
562 the observed poor body condition. A better understanding, on the basis of further
563 studies combining stable isotope analyses with a modelling approach, of how
564 environmental factors influence the blooms of the different phytoplankton size classes
565 and microheterotrophs, and their trophic pathways up to the different zooplankton size
566 classes (Hunt et al., 2017), and of the resulting effects on their biochemical and
567 energy content, would be necessary to fully understand the small pelagic fishery crisis

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

572

573 **5. Conclusion**

574 The biochemical composition of size-fractionated zooplankton was analyzed
575 seasonally at the Solemio site in the Bay of Marseille, NW Mediterranean Sea. The
576 biochemical composition of zooplankton differed with size classes and groups, and
577 varied between seasons and years according to environmental factor variations. We
578 observed that (1) the highest energy content was recorded in the 300-500 μm and
579 500-1000 μm mesozooplankton size classes, which were dominated by copepods, the
580 most energy-rich group of organisms analyzed; (2) the largest zooplankton size class
581 ($>2000 \mu\text{m}$) presented the lowest energy content, due to the high importance of
582 energy-poor salps and siphonophores; and (3) high seasonal variations of the
583 biochemical composition occurred, with higher energy content in all zooplankton size
584 classes in low temperature - high oxygen and nutrient-rich waters (generally spring
585 and winter). Our results, obtained over 18 months, clearly suggested that community
586 composition strongly determined the biochemical content of each zooplankton size
587 class. The inter-annual variability obtained for two successive autumn-winter periods
588 appeals for repeating this study over multiyear period. In the Bay of Marseille, the
589 high energy level of mesozooplankton may result for certain years from nutrient
590 enrichment by winter convection events and/or from terrestrial inputs linked to
591 precipitations during the cold seasons. Therefore, decadal observations of the
592 atmospheric and anthropic forcing conditions on the planktonic food web functioning
593 and in fine on the quantity and quality of the zooplankton are required. Such further

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

597

598

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614

615

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