



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE  
DELLA RICERCA

## Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Comparative life cycle assessment of microalgae cultivation for non-energy purposes using different carbon dioxide sources

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Porcelli, R., Dotto, F., Pezzolesi, L., Marazza, D., Greggio, N., Righi, S. (2020). Comparative life cycle assessment of microalgae cultivation for non-energy purposes using different carbon dioxide sources. *SCIENCE OF THE TOTAL ENVIRONMENT*, 721, 1-12 [10.1016/j.scitotenv.2020.137714].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/768462> since: 2020-08-17

*Published:*

DOI: <http://doi.org/10.1016/j.scitotenv.2020.137714>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

1 **Survey of the allelopathic potential of Mediterranean macroalgae: production of long-**  
2 **chain polyunsaturated aldehydes (PUAs)**

3  
4 Laura Pezzolesi<sup>1\*</sup>, Stefano Accoroni<sup>2</sup>, Fabio Rindi<sup>2</sup>, Chiara Samorì<sup>3</sup>, Cecilia Totti<sup>2</sup>, Rossella  
5 Pistocchi<sup>1</sup>

6  
7 <sup>1</sup> Dipartimento di Scienze Biologiche, Geologiche e Ambientali - Università di Bologna, via  
8 Sant'Alberto 163, 48123 Ravenna (Italy)

9 <sup>2</sup> Dipartimento di Scienze della Vita e dell'Ambiente - Università Politecnica delle Marche, via  
10 Breccie Bianche, 60131 Ancona (Italy)

11 <sup>3</sup> Dipartimento di Chimica "Giacomo Ciamician" - Università di Bologna, via Selmi 2, 40126  
12 Bologna (Italy)

13  
14  
15  
16  
17  
18 \*Corresponding Author

19 Dr. Laura Pezzolesi

20 Tel. +39 (0)544 937373; fax: +39 (0)544 937411

21 laura.pezzolesi@unibo.it

22  
23

24 **Abstract**

25 Chemical interactions between macroalgae and other organisms play an important role in determining  
26 species compositions and dominance patterns, and can explain the widespread success of some  
27 species in establishing their predominant populations in a specific coastal area. Allelopathy could act  
28 as a self-regulatory strategy of the algal community, being not only a succession regulator but also an  
29 active mechanism maintaining the species diversity especially in a delimited environment, such as  
30 the benthic ecosystem. Polyunsaturated aldehydes (PUAs) are among the most studied allelopathic  
31 compounds and are commonly released into the aquatic environment by different phytoplankton  
32 species in response to environmental stressors (e.g. wounding, grazing, or competition for nutrients).  
33 Diatom-released PUAs were observed to affect phytoplankton community dynamics and structure,  
34 and showed inhibitory effects on reproduction and development of marine invertebrates. As for  
35 macroalgae, there are only a few reports that attest the production of PUAs, and mostly refer to *Ulva*  
36 spp. In this study, the production of PUAs by a number of Mediterranean macroalgae was investigated  
37 at different sampling times, aiming at providing the first evidence of potential allelochemical activity.  
38 Results highlighted the potential production by macroalgae of a variety of aldehydes, among which  
39 some never reported so far. Some species (i.e. *D. polypodioides* and *U. cf. rigida*) were found to  
40 produce higher PUAs amounts than others, and even a wider variety of structures (e.g. length of the  
41 carbon chain); these species might exert strong effects on epiphytic species or other organisms of the  
42 benthic community, especially considering the differential sensitivities of the various taxa. A high  
43 dPUA concentrations (order of  $\mu\text{M}$ ) potentially due to the release of PUAs by algal species was found,  
44 and might affect the population dynamics of the epiphytic organisms (e.g. microalgae, meiofauna),  
45 of grazers, as well as of the microbial community.

46

47 **Keywords**

48 *Ulva*; *Dictyopterus polypodioides*; Ulvaceae; Dictyotaceae; macroalgae; GC-MS; polyunsaturated  
49 aldehydes; benthic ecosystem.

50

51 **1. Introduction**

52 Marine algae belong to several phylogenetic lineages, and often cohesist in similar niches from an  
53 ecological standpoint, being subjected to herbivory and competition. Herbivory is considered a high  
54 stress for these organisms and can account for a 60-100% loss of total algal production (Slattery and  
55 Gochfeld, 2009); as result, some algal species respond to herbivory by enhancing the synthesis of a  
56 pool of defensive metabolites that can vary among individuals within a population, also temporally  
57 and/or spatially.

58 Most investigations ascribe the success of bloom-forming green macroalgae over other coastal  
59 species mostly to their physiological characteristics (e.g. rapid nutrient uptake, fast growth, and  
60 environmental tolerance) (Lotze and Schramm, 2000); however, chemical interactions between  
61 macroalgae and other organisms play an important role in determining species compositions and  
62 dominance patterns, and can explain the widespread success of some species in establishing their  
63 predominant populations in a specific coastal area. Fouling by microalgae can also be directly  
64 inhibited by macroalgal metabolites; several studies reported how seagrasses and macroalgae  
65 inhibited biofilm growth (e.g. Gette-Bouvarot et al., 2015; Ben Gharbia et al., 2017), suggesting that  
66 they might produce and release allelochemicals acting as biological mitigation agents, although  
67 benthic species seem to be more resistant than planktonic ones to the biological effects of  
68 allelochemicals. Allelopathic compounds isolated from marine organisms include low-molecular-  
69 weight peptides, phenols, alkaloids, fatty acids and their derivatives, including oxylipins such as  
70 aldehydes. Several ecological functions have been ascribed to allelopathy, such as intrapopulation  
71 signals, feeding deterrence or antagonism, and grazers repellence. Organisms able to produce and  
72 secrete allelopathic compounds may have an advantage over competitors under the same  
73 environment; thus, allelopathy could act as a self-regulatory strategy of the algal community (Hay,  
74 2009), being not only a succession regulator but also an active mechanism maintaining the species  
75 diversity especially in a delimited environment, such as the benthic ecosystem. Reports on  
76 interactions between macroalgae and phytoplankton highlighted as living tissues or extracts from  
77 *Ulva*, *Corallina*, and *Sargassum* spp. inhibited the growth of various microalgae, such as the  
78 dinoflagellate *Prorocentrum donghaiense* (R. Wang et al., 2007) or other planktonic species i.e.  
79 *Heterosigma akashiwo*, *Alexandrium tamarense*, *Skeletonema costatum*, *Amphidinium carterae*,  
80 *Scrippsiella trochoidea* (Y. Wang et al., 2007; Nan et al., 2008). Additionally, fresh and/or dried thalli  
81 of *Ulva lactuca* inhibited the growth of several common microalgal blooming species (e.g.  
82 *Margalefidinium polykrikoides*, *Karlodinium veneficum*, *Chattonella marina*, *Prorocentrum*  
83 *cordatum*, *Karenia brevis*, *Pseudo-nitzschia multiseriis*, and *Aureococcus anophagefferens*) in a  
84 dose-dependent manner, demonstrating that this species produces heat-stable allelochemicals (Tang  
85 and Gobler, 2011).

86 Aldehydes are among the most studied allelopathic compounds identified in the aquatic environment  
87 (Dabrowska and Nawrocki, 2013). They can originate from a variety of processes like oxidation,  
88 photochemical transformations, and decomposition of organic matter. In particular, polyunsaturated  
89 aldehydes (PUAs) are volatile compounds commonly released into the aquatic environment (at  
90 nanomolar concentrations) by different phytoplankton species (Wichard et al., 2005a) in response to  
91 environmental stressors (e.g. wounding, grazing, or competition for nutrients) (Dittami et al., 2010;

92 Ribalet et al., 2014), and can persist in the water even after phytoplankton bloom decline (Bartual and  
93 Ortega, 2013). Experimental works demonstrated that PUAs can induce changes in microzooplankton  
94 growth dynamic and community structure (Franzè et al., 2018), have a teratogenic effect on copepods  
95 (Romano et al., 2010) and inhibitory effects on reproduction and development of marine  
96 invertebrates, thus they are considered an anti-grazer defense (Miralto et al., 1999; Poulet et al., 2007;  
97 Lauritano et al., 2012; Brugnano et al., 2016). In field studies, differential effects of PUAs on the  
98 bacterial community in terms of growth and metabolic activity (Balestra et al., 2011; Paul et al.,  
99 2012) and on ciliates (Lavrentyev et al., 2015) were reported. In addition, diatom-released PUAs were  
100 observed to affect phytoplankton community dynamics and structure by suppressing the growth of  
101 other species (Casotti et al., 2005; Ribalet et al., 2007). Despite the attention on these compounds,  
102 their role and ecological significance remain poorly understood.

103 More recently, attention has been posed also to the benthic environment as benthic primary producers  
104 represent an important source of organic carbon in some littoral areas. Some studies evidenced PUAs  
105 production by different benthic diatoms (Jüttner et al., 2010; Scholz and Liebezeit, 2012; Pezzolesi  
106 et al., 2017), demonstrating as allelopathic benthic species could affect community composition. As  
107 for macroalgae, there are only a few reports that attest the production of PUAs, and mostly refer to  
108 *Ulva* spp.: Kajiwara et al. (1996) described long-chain aldehydes (e.g. pentadecanal, (8Z)-  
109 heptadecenal, (8Z, 11Z)-heptadecadienal and (8Z, 11Z, 14Z)-heptadecatrienal) as odor components  
110 of the essential oil prepared from the green alga *Ulva pertusa*; Akakabe et al. (2003) highlighted the  
111 presence of 2,4-decadienal in *Ulva conglobata*; Alsufyani et al. (2014) documented the presence of  
112 C<sub>10</sub>-PUAs (e.g. 2,4,7-decatrienal and 2,4-decadienal, deriving from omega-3 and omega-6  
113 polyunsaturated fatty acids (PUFAs) with 20 or 18 carbon atoms) in damaged tissue of *Ulva* spp. All  
114 these studies demonstrated that the amount and structural diversity of released PUAs, as well as of  
115 other oxylipins (Barbosa et al., 2016), can vary depending on the species and also on the  
116 environmental conditions.

117 In this study, the production of PUAs by a number of Mediterranean macroalgae was investigated at  
118 different sampling times, aiming at providing the first evidence of this allelochemical activity in other  
119 species besides *Ulva* spp. The Mediterranean Sea is a hotspot of marine biodiversity, counting a  
120 variety of macroalgal species that show widely different distribution patterns. Seaweed communities  
121 are important coastal ecosystems that modify their structure and composition in space and time,  
122 responding to environmental stressors or changes. In particular, the Conero Riviera located in the  
123 Adriatic Sea is a rocky area in proximity of the city of Ancona, on the central Adriatic shore of Italy,  
124 and its long-term changes in the macroalgal flora have been well documented (Rindi et al., 2020). As  
125 a result, this area was selected for its interesting macroalgal community.

126

## 127 **2. Results and discussion**

### 128 *2.1 PUAs dissolved in seawater*

129 Analyses of seawater collected in August and September 2015 in the Piscinetta del Passetto (Conero  
130 Riviera, Adriatic Sea, Italy) revealed the presence of high concentrations of total dissolved  
131 polyunsaturated aldehydes (dPUAs), ranging from 0.16 to 0.25  $\mu\text{M}$  (corresponding to 162 and 248  
132 nM) (Fig. 1). This amount is much higher than previously observed in the planktonic environment  
133 both in the Adriatic Sea (order of nM) by Ribalet et al. (2014) and in Atlantic seawater (Spain) by  
134 Bartual and Ortega (2013), but comparable to values found in a work performed in freshwater  
135 (Dabrowska et al., 2017). The high dPUA concentrations found in the present study could be  
136 explained considering the low hydrodynamism typical of confined benthic environments, and the  
137 presence of a well-developed phytobenthic community, characterized by several PUA producers (i.e.  
138 both micro- and macroalgae). In addition, Bartual et al. (2020) attested that  $\mu\text{M}$  ranges of dPUAs are  
139 expected to occur in the immediate vicinity of the producer cell, as occurred in this benthic  
140 environment, but disperse rapidly, consistently with pM to nM concentrations of dPUAs quantified  
141 in marine environments. A variety of aldehydes was putatively identified based on the mass spectra  
142 obtained at the three sampling times (Fig. 1), as short, medium, or long-chain aldehydes. In particular,  
143 C15:3 resulted among the most abundant PUAs (about 57.2 nM, corresponding to the 11-26% of the  
144 total aldehydes), together with C9:4 (about 44.6 nM, corresponding to 18-31% in August), followed  
145 by C6:2 (24.9 nM) and C8:1 (33.0 nM), whose relative abundances were up to 11 and 13%,  
146 respectively (Table 1). Contrarily to the other times, in the sampling performed at the end of August  
147 a fraction of these compounds remained unidentified (34%). dPUAs could be derived either by  
148 diatoms and macroalgae, as suggested by the high concentrations and the qualitative profile reported,  
149 as well as by a previous study performed in the same area that attested the potential production of a  
150 variety of low and medium-chain aldehydes, including some detected in the present work, by three  
151 benthic diatoms (Pezzolesi et al., 2017), and also by other studies performed on several phytoplankton  
152 species (i.e. Wichard et al., 2005a). It is worth considering that most of the previous studies focused  
153 only on the detection of three PUAs, i.e. heptadienal (C7:2), octadienal (C8:2), and decadienal  
154 (C10:2) (Vidoudez et al., 2011; Morillo-García et al., 2014; Ribalet et al., 2014; Wu and Li, 2016;  
155 Bartual et al., 2018; Bartual et al., 2020). Together with octatrienal (C8:3), C7:2 and C8:2 resulted  
156 the most abundant dPUA in coastal areas of the Adriatic Sea (Vidoudez et al., 2011; Ribalet et al.,  
157 2014); C7:2, C8:2, and C10:2 were also the most abundant in the northern South China Sea (Wu and  
158 Li, 2016), as similarly reported by Bartual et al. (2018) in southwestern Spain. These authors attested  
159 that averaged concentration values of total dPUA in the water column were significantly higher under

160 stratification compared with mixing conditions (0.370 vs. 0.189 nM) and that concentrations of total  
161 dPUAs reached values up to 1.87 nM in the water column. These ranges of PUAs agreed with those  
162 reported for coastal waters in the Adriatic Sea (up to 2.53 nM) (Vidoudez et al., 2011; Ribalet et al.,  
163 2014) and in the northern China Sea (from 0.10 to 0.37 nM) (Wu and Li, 2016). Interestingly,  
164 Edwards et al. (2015) showed that dPUA doses of 1-10  $\mu\text{M}$  may stimulate respiration, organic matter  
165 hydrolysis, and bacterial growth on the sinking particles; these dPUAs amounts, which are  
166 comparable to those found in the present study in the Adriatic benthic environment, could be  
167 reasonably present in the micro-environment surrounding the phytoplankton-derived sinking  
168 particles, which are hot spots for PUA production and contain considerably higher concentrations  
169 than in the bulk seawater. Thus, the  $\mu\text{M}$  levels of dPUAs are consistent with nM levels of dPUAs in  
170 the surrounding water on the basis of a simple molecular diffusion model as explained in previous  
171 studies (Ribalet et al., 2008; Bartual et al., 2018). A study by Lavrentyev et al. (2015) showed that  
172 levels of dPUAs (i.e. octadienal and heptadienal) between 0.5 and 2 nM may impair the growth of  
173 common microzooplankters. It is known that harmful effects of dPUAs on phytoplanktonic species  
174 could be directly dependent on the average concentrations of dPUAs and their persistence, and that  
175 native phytoplankton strains from areas with PUA concentrations at nM levels would be less sensitive  
176 to PUA effects than other areas where PUA producers are not common. This could explain also the  
177 higher resistance of benthic species than planktonic ones to potential allelochemicals (Ben Gharbia  
178 et al., 2017).

### 179 2.3 PUAs production by macroalgae

180 During the sampling period, the sampling site was characterized by generally calm and warm waters  
181 (Table S1) ranging in temperature from 16.0 to 27.8 °C and salinity levels from 33 to 38, in agreement  
182 with those observed at the same site in previous studies (e.g. Accoroni et al., 2015a). The macroalgal  
183 species collected in the Piscinetta del Passetto are among the most common present in the area (Rindi  
184 et al., 2020). Quantitative analysis of PUAs in the macroalgal thalli demonstrated different  
185 productions of these compounds by the algal tissue in the macroalgal species (Fig. 2). PUAs in fact  
186 are produced from an oxidative degradation of free polyunsaturated fatty acids (PUFAs) that occurs  
187 after cell disruption (Pohnert, 2000). The highest aldehyde production was detected in *Dictyopteris*  
188 *polypodioides* (A.P.De Candolle) J.V.Lamouroux 1809 and *Ulva* cf. *rigida* C.Agardh 1823:  
189 concentrations varied between 801 and 1784  $\text{nmol g}^{-1}$  for *D. polypodioides* and between 477 and 623  
190  $\text{nmol g}^{-1}$  for *U. cf. rigida*. Production of aldehydes resulted constant in *U. cf. rigida* during the whole  
191 sampling period, while in *D. polypodioides* a higher amount was reported in August compared to the  
192 other times. For the other species, values were below 300  $\text{nmol g}^{-1}$  for all macroalgae, except for  
193 *Hypnea musciformis* (Wulfen) J.V.Lamouroux 1813 in July, where about 582  $\text{nmol g}^{-1}$  of aldehydes

194 were detected, and for *Dictyota dichotoma* (Hudson) J.V.Lamouroux 1809 in April (about 330 nmol  
195 g<sup>-1</sup>). Results highlighted a high allelopathic potential by various macroalgal species, capable to  
196 produce a variety of bioactive compounds apart PUAs. Phaeophyceae are known to produce  
197 polyphenols and terpenes. Terpenoids are almost ubiquitous in macroalgae, including *Dictyopteris*,  
198 often showing antibacterial or antiviral activity (Arunkumar K. and Sivakumar S.R., 2010). The genus  
199 *Ulva* is capable to alter the metabolic pathways involved in lipid biosynthesis, including fatty acids  
200 and oxylipins, especially as adaptation strategies to nutrient imbalance (Kumari et al., 2014).  
201 Although algal oxylipins are considered to play a role in controlling interactions with other organisms  
202 and with the environment, promoting algal survival (Barbosa et al., 2016), the occurrence and  
203 distribution of these compounds are unpredictable and differ among species and in relation to growth  
204 conditions, as documented also in the present study.

205

#### 206 2.4 Qualitative profile of PUAs produced by macroalgae

207 Qualitative analysis of the aldehyde profile of the different macroalgal species was carried out to  
208 understand the different allelopathic potential that the various macroalgae could have towards the  
209 other organisms of the benthic ecosystem. For example, a positive relationship between the length  
210 and the biological activity of the molecule has been described (i.e. long-chain aldehydes are more  
211 toxic than the short ones) (Adolph et al., 2003) and confirmed by toxicological studies performed on  
212 phytoplankton and invertebrate species (Hansen et al., 2004; Ribalet et al., 2007; Pichierri et al.,  
213 2016). A variety of aldehydes, including short-, middle-, but also long-chain compounds was  
214 observed here, including aldehydes whose presence in algal species was never reported so far (Table  
215 S2). Most of the derivatives caused two or more peaks reported to be stereoisomers of the PUAs (Fig.  
216 3) by Wichard et al. (2005b); derivatized aldehydes were identified by their molecular ions and  
217 fragmentation patterns (Fig. S1). Specifically, the relative amount (%) of the different compounds  
218 produced by species sampled in July and April was reported (Fig. 4A and 4B). The short-chain PUA  
219 C6:2 resulted the main compound in different species (i.e. *Ceramium ciliatum* (J.Ellis) Ducluzeau  
220 1806, *Chondria dasyphylla* (Woodward) C.Agardh 1817, *Chondria capillaris* (Hudson) M.J.Wynne  
221 1991, *Padina pavonica* (Linnaeus) Thivy 1960, *Cystoseira compressa* (Esper) Gerloff & Nizamuddin  
222 1975, *Gracilaria* sp. and *Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva 1952), accounting up to  
223 78% and 94% in *P. pavonica* and *G. bursa-pastoris*, respectively. Among the middle-chain PUAs,  
224 C9:4 and C10:4 had a relative abundance of 11% and 15% in *C. ciliatum*, and 9% and 22% in *Ulva*  
225 cf. *rigida*, respectively. For the long-chain aldehydes, their production was detected in most algal  
226 species, such as *Ceramium ciliatum*, *Palisada perforata* (Bory) K.W.Nam 2007, *Hypnea*  
227 *musciformis*, *Dictyota dichotoma*, *U. cf. rigida*, *Gracilaria* sp., *Chondria* spp. and *Dictyopteris*



228 *polypodioides*; however, their relative abundances were high only in *D. dichotoma* and *D.*  
229 *polypodioides*, corresponding to the 67% for C20:5 in *D. dichotoma* in April and to 13%, 20% and  
230 42% for C14:5, C16:3 and C16:4 in *D. polypodioides* in July, respectively. The ability of macroalgal  
231 species to produce long-chain PUAs was already identified by Kajiwara et al. (1996), which reported  
232 C15-, C16- and C17-unsaturated aldehydes in essential oils of edible seaweeds (i.e. green algae *Ulva*  
233 *pertusa* and *Enteromorpha clathrata*), attested as responsible for the “characteristic seaweeds or algae  
234 odor”. Moreover, these authors investigated the presence of specific enzymes involved in the long-  
235 chain aldehydes biosynthesis, discovering that production of long-chain aldehydes was widespread  
236 in a wide range of green, red and brown seaweeds, including also some species belonging to genera  
237 analyzed in the present study (i.e. *Ulva pertusa*, *Dictyota dichotoma*, *Dictyopteris divaricata*,  
238 *Cystoseira hakodatensis*, *Gracilaria asiatica* and *Chondria crassicaulis*). It has to be considered that  
239 *D. polypodioides* resulted the greatest PUA-producer among the species analyzed, thus its long-chain  
240 PUAs production resulted high, ranging from 117 to 682 nmol g<sup>-1</sup> for C14:5 and from 332 to 553  
241 nmol g<sup>-1</sup> for C16:4 (Fig. 5). Contrarily to *U. cf. rigida*, the qualitative profile differed among sampling  
242 times (Fig. 6), attesting that the algal growth cycle or the environmental conditions may affect the  
243 allelopathic potential. As for *Ulva* spp., middle and long-chain PUAs had already been described,  
244 specifically C7:2, C10:2, C10:3 (Alsufyani et al., 2014), as well as C16:3, C17:2, C17:3 in *U. pertusa*  
245 (Kajiwara et al., 1996; Akakabe et al., 2003). *U. cf. rigida* resulted the best PUA-producer (C7- and  
246 C10-aldehydes) in a set of *Ulva* species (including *U. rotundata*, *U. ohnoi*, *U. compressa*, *U.*  
247 *mutabilis*) collected in Portugal (Alsufyani et al., 2014); however, the qualitative profile obtained  
248 here for the Mediterranean *Ulva cf. rigida* resulted quite different (Fig. 6). Results of the present  
249 study highlighted that this algal species could produce a great range of aldehydes, some of them never  
250 reported so far (i.e. C6 and C9-aldehydes), and their quantitative amount was higher than the data  
251 previously reported; for instance, decatetraenal concentration ranged between 73 and 176 nmol g<sup>-1</sup>  
252 compared to 2.3 nmol g<sup>-1</sup> reported for C10-PUAs (i.e. decatrienal plus decadienal) by Alsufyani et al.  
253 (2014). This discrepancy could depend on the high plasticity in PUA production observed in *Ulva*  
254 spp., as well as by the different algal conditions and morphotypes that are known to affect the  
255 biosynthesis of these compounds, and oxylipins in general (Barbosa et al., 2016; Alsufyani et al.,  
256 2014). PUAs are biosynthetically derived from free polyunsaturated fatty acids (PUFAs) that are  
257 released from phospho- and galactolipids after cell disruption, and it was assessed as fatty acid  
258 depletion is directly linked to the production of PUAs (Wichard et al., 2007), thus stress responses  
259 and changes in PUFAs content could address to variation in the PUAs amount and profile as well  
260 (Alsufyani et al., 2014). In addition, previous studies performed with standard PUAs ranging from 7  
261 to 10 carbon atoms attested a positive correlation between the length of PUAs carbon chain and their

262 bioactivity (Adolph et al., 2003; Ribalet et al., 2007; Pichierri et al., 2016), thus the long-chain  
263 compounds found in the present work could have considerable effects on the benthic community, in  
264 particular at the concentrations observed in seawater ( $\mu\text{M}$ ). PUAs, in fact, showed effects on bacterial  
265 growth and metabolic activity (Balestra et al., 2011), as well as on zooplanktonic organisms (Franzè  
266 et al., 2018; Ianora et al., 2003; Wichard et al., 2008), in  $\mu\text{M}$  concentrations. However, it is import to  
267 consider that the extent of deleterious effects varies widely among copepod species and experiments  
268 (e.g. Pierson et al., 2007; Sommer, 2009; Taylor et al., 2012).

269

### 270 **3. Conclusions**

271 To the best of our knowledge, this is one of the few studies that reports a qualitative and quantitative  
272 PUAs profile in several macroalgae, and highlights the potential production of a variety of aldehydes,  
273 among which some never reported so far, that could be involved in allelopathic interactions.

274 Some macroalgal species (i.e. *D. polypodioides* and *U. cf. rigida*) were found to produce higher PUAs  
275 amounts than others, and even a wider variety of structures (e.g. length of the carbon chain); these  
276 species might exert strong effects on epiphytic species or other organisms of the benthic community,  
277 especially considering the differential sensitivities of the various taxa. The high dPUA concentrations  
278 (order of  $\mu\text{M}$ ) found in the present study, potentially due to the release of PUAs by algal species,  
279 might affect the population dynamics of the epiphytic organisms (e.g. microalgae, meiofauna), of  
280 grazers, as well as of the microbial community.

281 More exploratory studies are required to understand the complex relationships among organisms in  
282 the benthic marine environment, to increase the knowledge about allelopathy and species succession  
283 dynamics, and to understand how to preserve biodiversity.

284

### 285 **4. Experimental**

#### 286 *4.1 Reagents*

287 All reagents were purchased from Sigma-Aldrich (Milan, Italy) and used without further purification.

288

#### 289 *4.2 Sampling area and procedure*

290 The study site was the station locally called Piscinetta del Passetto (Conero Riviera, Italy, northern  
291 Adriatic Sea: 43°37'09" N, 13°31'54" E, Fig. 7), a semi-enclosed and shallow (mean depth 1.5 m)  
292 inlet in the urban area of Ancona, sheltered by a natural reef and with a rocky bottom partially covered  
293 by cobbles.

294 Water samples for the analysis of dissolved aldehydes (dPUAs) were collected by snorkeling in the  
295 proximity of the phytobenthic community in August and September 2015 using polyethylene bottles

296 (2 L). Bottles were placed closed to the macroalgal species and water was collected, avoiding  
297 resuspension phenomena. Water samples were kept refrigerated during transportation, than in  
298 laboratory water was filtered using GF/F Whatman filters (0.7 µm porosity, 47 mm) and stored at -  
299 22 °C until the analyses. Subsequently, several macroalgal samplings were carried out at four  
300 different times (July, August, September 2017, and April 2018). In total, twelve different species of  
301 macroalgae (Table 2) were collected: *Ceramium ciliatum* (J.Ellis) Ducluzeau 1806 (Ceramiaceae),  
302 *Chondria capillaris* (Hudson) M.J.Wynne 1991 Rhodomelaceae (Florideophyceae), *Chondria*  
303 *dasyphylla* (Woodward) C.Agardh 1817 (Rhodomelaceae), *Gracilaria bursa-pastoris* (S.G.Gmelin)  
304 P.C.Silva 1952 (Gracilariaceae), *Gracilaria* sp. (Gracilariaceae), *Hypnea musciformis* (Wulfen)  
305 J.V.Lamouroux 1813 (Cystocloniaceae), *Palisada perforata* (Bory) K.W.Nam 2007  
306 (Rhodomelaceae), *Cystoseira compressa* (Esper) Gerloff & Nizamuddin 1975 (Sargassaceae),  
307 *Dictyopteris polypodioides* (A.P.De Candolle) J.V.Lamouroux 1809 (Dictyotaceae), *Dictyota*  
308 *dichotoma* (Hudson) J.V.Lamouroux 1809 (Dictyotaceae), *Padina pavonica* (Linnaeus) Thivy 1960  
309 (Dictyotaceae), and *Ulva* cf. *rigida* (C.Agardh 1823 (Ulvaceae). Each species was collected in three  
310 replicates each time at approximately 0.5 m depth in 50 mL polyethylene tubes, depending on the  
311 presence at the sampling time. Surface temperature and salinity were measured with a CTD, Model  
312 30 Handheld Salinity, Conductivity and Temperature System, YSI (Yellow Spring, OH USA).  
313 Macroalgae were identified based on their main morphological characters, then small portions of  
314 thalli were treated in order to remove all benthic organisms, including those strongly attached,  
315 following the method described in Accoroni et al. (2015b), with some modifications. Briefly, thalli  
316 were carefully washed in filtered sea water (FSW) containing 1% of surfactant to remove epiphytic  
317 cells and bacteria, and then observed under a stereo-microscope in order to mechanically remove the  
318 epiphytes with scalpels and tweezers, then the thalli were rinsed with FSW and vigorously washed  
319 several times to ensure their total cleanliness. The seaweed thalli were checked for the succesful removal  
320 of all epiphytes, then dried with absorbent paper, weighed to determine the fresh weight (g fr.wt.),  
321 and finally stored at -80 °C in new tubes.

322

### 323 4.3 PUAs analysis

#### 324 4.3.1 Aldehydes in the macroalgae

325 The quantitative analysis of PUAs in seaweeds was carried out following the method of Wichard et  
326 al. (2005b). A portion of thallus (about 0.2-0.8 g fr.wt.) of each seaweed was shredded with mortar  
327 and pestle, in liquid nitrogen. The powder thus obtained was transferred into 10 mL tubes. Then the  
328 samples were prepared and analyzed by gas chromatography-mass spectrometry (GC-MS), using

329 benzaldehyde as internal standard (stock solution 1 mM) and a 6850 Agilent HP gas chromatograph  
330 connected to a 5975 Agilent HP quadrupole mass spectrometer, as described in Pezzolesi et al. (2017).  
331 After derivatization of the polyunsaturated aldehydes with O-(2,3,4,5,6-  
332 pentafluorobenzyl)hydroxylamine hydrochloride solution (PFBHA HCl), aldehyde quantification  
333 was performed based on the internal standard (i.e. benzaldehyde) and assuming a response factor of  
334 1 for each compound, while identification was done by comparison of retention times and mass  
335 spectra with those of commercial standards, when possible: propionaldehyde, 2,4-heptadienal,  
336 octanal, 2-octenal, 2,4-octadienal, 6-nonenal, 2,6- nonadienal, 4-decenal, 2,4-decadienal, undecanal,  
337 8-undecenal and 2,4-undecadienal. If PUAs standards were not available, the identification was  
338 performed by comparison with NIST libraries, or putatively identified based on their mass spectra.  
339 Data were expressed as total aldehydes production considering all the putatively identified  
340 compounds in the selected ion monitoring (SIM) scanning mode (m/z 181) derived from the analysis  
341 of algal tissues. Mass spectra reported a typical aldehydes fragmentation pattern (analytical fragments  
342 for the PFBO-derivatives were 181 and the molecular ion  $M^{+}$  or M-1), including either saturated,  
343 monounsaturated and polyunsaturated aldehydes. LOD was calculated as 8  $\mu$ M, based on a  
344 benzaldehyde curve in the range from 5 up to 1250  $\mu$ M ( $R^2 = 0.9979$ ).

345

#### 346 4.3.2 Dissolved aldehydes in seawater

347 Concentration of dissolved aldehydes was determined following the protocol described by Vidoudez  
348 and Pohnert (2008). After determination of the exact volume of the filtered seawater (about 2 L), 1  
349 mL of a 25mM O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride solution (PFBHA HCl)  
350 in Tris-HCl 100 mM pH 7.2, and 50  $\mu$ L of internal standard (benzaldehyde, 1 mM in methanol) were  
351 added. The solution was then immediately charged on a C18 cartridge (Waters sep-pak plus) at 250  
352 mL h<sup>-1</sup> for derivatizing the dissolved aldehydes. The derivatized aldehydes were eluted from the  
353 cartridge using 5 mL of 25 mM PFBHA in methanol. The eluates were incubated for 1 h at room  
354 temperature to ensure complete derivatization. PUA derivatives were then extracted with a mixture  
355 of 2:1:2 water:methanol:hexane and acidified with H<sub>2</sub>SO<sub>4</sub>, as described in (Wichard et al., 2005b).  
356 Samples were then treated and analyzed as previously described.

357

#### 358 Acknowledgements

359 This research did not receive any specific grant from funding agencies in the public, commercial, or  
360 not-for-profit sectors.

361

#### 362 Note

363 The authors declare no competing financial interest.

364

## 365 **References**

- 366 Accoroni, S., Glibert, P.M., Pichierri, S., Romagnoli, T., Marini, M., Totti, C., 2015a. A conceptual  
367 model of annual *Ostreopsis* cf. *ovata* blooms in the northern Adriatic Sea based on the  
368 synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients.  
369 *Harmful Algae* 45, 14–25. <https://doi.org/10.1016/j.hal.2015.04.002>
- 370 Accoroni, S., Percopo, I., Cerino, F., Romagnoli, T., Pichierri, S., Perrone, C., Totti, C., 2015b.  
371 Allelopathic interactions between the HAB dinoflagellate *Ostreopsis* cf. *ovata* and macroalgae.  
372 *Harmful Algae* 49, 147–155. <https://doi.org/10.1016/j.hal.2015.08.007>
- 373 Adolph, S., Poulet, S.A., Pohnert, G., 2003. Synthesis and biological activity of  $\alpha,\beta,\gamma,\delta$ -unsaturated  
374 aldehydes from diatoms. *Tetrahedron* 59, 3003–3008. <https://doi.org/10.1016/S0040->  
375 [4020\(03\)00382-X](https://doi.org/10.1016/S0040-4020(03)00382-X)
- 376 Akakabe, Y., Matsui, K., Kajiwara, T., 2003. 2,4-Decadienals are produced via (R)-11-HPITE from  
377 arachidonic acid in marine green alga *Ulva conglobata*. *Bioorganic Med. Chem.* 11, 3607–  
378 3609. [https://doi.org/10.1016/S0968-0896\(03\)00364-X](https://doi.org/10.1016/S0968-0896(03)00364-X)
- 379 Alsufyani, T., Engelen, A.H., Diekmann, O.E., Kuegler, S., Wichard, T., 2014. Prevalence and  
380 mechanism of polyunsaturated aldehydes production in the green tide forming macroalgal  
381 genus *Ulva* (Ulvales, Chlorophyta). *Chem. Phys. Lipids* 183, 100–109.  
382 <https://doi.org/10.1016/j.chemphyslip.2014.05.008>
- 383 Arunkumar K., Sivakumar S.R., R.R., 2010. Review on bioactive potential in seaweeds (marine  
384 macroalgae): a special emphasis on bioactivity of seaweeds against plant pathogens. *Asian J.*  
385 *Plant Sci.* 9, 227–240.
- 386 Balestra, C., Alonso-Sáez, L., Gasol, J.M., Casotti, R., 2011. Group-specific effects on coastal  
387 bacterioplankton of polyunsaturated aldehydes produced by diatoms. *Aquat. Microb. Ecol.* 63,  
388 123–131. <https://doi.org/10.3354/ame01486>
- 389 Barbosa, M., Valentão, P., Andrade, P.B., 2016. Biologically active oxylipins from enzymatic and  
390 nonenzymatic routes in macroalgae. *Mar. Drugs* 14, 23. <https://doi.org/10.3390/md14010023>
- 391 Bartual, A., Hernanz-Torrijos, M., Sala, I., Ortega, M.J., González-García, C., Bolado-Penagos, M.,  
392 López-Urrutia, A., Romero-Martínez, L., Lubián, L.M., Bruno, M., Echevarría, F., García,

- 393 C.M., 2020. Types and distribution of bioactive polyunsaturated aldehydes in a gradient from  
394 mesotrophic to oligotrophic waters in the Alborán Sea (Western Mediterranean). *Mar. Drugs*  
395 18, 159. <https://doi.org/10.3390/md18030159>
- 396 Bartual, A., Morillo-García, S., Ortega, M.J., Cózar, A., 2018. First report on vertical distribution of  
397 dissolved polyunsaturated aldehydes in marine coastal waters. *Mar. Chem.* 204, 1–10.  
398 <https://doi.org/10.1016/j.marchem.2018.05.004>
- 399 Bartual, A., Ortega, M.J., 2013. Temperature differentially affects the persistence of  
400 polyunsaturated aldehydes in seawater. *Environ. Chem.* 10, 403–408.  
401 <https://doi.org/10.1071/EN13055>
- 402 Ben Gharbia, H., Yahia, O.K.D., Cecchi, P., Masseret, E., Amzil, Z., Herve, F., Rovillon, G., Nouri,  
403 H., M'Rabet, C., Couet, D., Triki, H.Z., Laabir, M., 2017. New insights on the species-specific  
404 allelopathic interactions between macrophytes and marine HAB dinoflagellates. *PLoS One* 12.  
405 <https://doi.org/10.1371/journal.pone.0187963>
- 406 Brugnano, C., Granata, A., Guglielmo, L., Minutoli, R., Zagami, G., Ianora, A., 2016. The  
407 deleterious effect of diatoms on the biomass and growth of early stages of their copepod  
408 grazers. *J. Exp. Mar. Bio. Ecol.* 476, 41–49. <https://doi.org/10.1016/j.jembe.2015.11.015>
- 409 Casotti, R., Mazza, S., Brunet, C., Vantrepotte, V., Ianora, A., Miralto, A., 2005. Growth inhibition  
410 and toxicity of the diatom aldehyde 2-trans, 4-trans-decadienal on *Thalassiosira weissflogii*  
411 (Bacillariophyceae). *J. Phycol.* 41, 7–20. <https://doi.org/10.1111/j.1529-8817.2005.04052.x>
- 412 Dabrowska, A., Nawrocki, J., 2013. Aldehyde concentrations in wet deposition and river waters.  
413 *Sci. Total Environ.* 452–453, 1–9. <https://doi.org/10.1016/j.scitotenv.2013.02.037>
- 414 Dabrowska, A., Sztuka, N., Dabrowski, A., 2017. Polyunsaturated aldehydes as bioactive secondary  
415 metabolites in the aquatic environment. 15th International Conference on Environmental  
416 Science and Technology Rhodes, Greece, 31 August - 2 September 2017.
- 417 Dittami, S.M., Wichard, T., Malzahn, A.M., Pohnert, G., Boersma, M., Wiltshire, K.H., 2010.  
418 Culture conditions affect fatty acid content along with wound-activated production of  
419 polyunsaturated aldehydes in *Thalassiosira rotula* (Coscinodiscophyceae). *Nov. Hedwigia*,  
420 Beihefte 136, 231–248. <https://doi.org/10.1127/1438-9134/2010/0136-0231>
- 421 Edwards, B.R., Bidle, K.D., Van Mooy, B.A.S., 2015. Dose-dependent regulation of microbial  
422 activity on sinking particles by polyunsaturated aldehydes: Implications for the carbon cycle.  
423 *Proc. Natl. Acad. Sci. U. S. A.* 112, 5909–5914. <https://doi.org/10.1073/pnas.1422664112>

- 424 Franzè, G., Pierson, J.J., Stoecker, D.K., Lavrentyev, P.J., 2018. Diatom-produced allelochemicals  
425 trigger trophic cascades in the planktonic food web. *Limnol. Oceanogr.* 63, 1093–1108.  
426 <https://doi.org/10.1002/lno.10756>
- 427 Gette-Bouvarot, M., Mermillod-Blondin, F., Lemoine, D., Delolme, C., Danjean, M., Etienne, L.,  
428 Volatier, L., 2015. The potential control of benthic biofilm growth by macrophytes-A  
429 mesocosm approach. *Ecol. Eng.* 75, 178–186. <https://doi.org/10.1016/j.ecoleng.2014.12.001>
- 430 Hansen, E., Ernstsén, A., Eilertsen, H.C., 2004. Isolation and characterisation of a cytotoxic  
431 polyunsaturated aldehyde from the marine phytoplankter *Phaeocystis pouchetii* (Hariot)  
432 Lagerheim. *Toxicology* 199, 207–217. <https://doi.org/10.1016/j.tox.2004.02.026>
- 433 Hay, M.E., 2009. Marine chemical ecology: Chemical signals and cues structure marine  
434 populations, communities, and ecosystems. *Ann. Rev. Mar. Sci.* 1, 193–212.  
435 <https://doi.org/10.1146/annurev.marine.010908.163708>
- 436 Ianora, A., Poulet, S.A., Miralto, A., 2003. The effects of diatoms on copepod reproduction: A  
437 review. *Phycologia* 42, 351–363. <https://doi.org/10.2216/i0031-8884-42-4-351.1>
- 438 Jüttner, F., Messina, P., Patalano, C., Zupo, V., 2010. Odour compounds of the diatom *Cocconeis*  
439 *scutellum*: Effects on benthic herbivores living on *Posidonia oceanica*. *Mar. Ecol. Prog. Ser.*  
440 400, 63–73. <https://doi.org/10.3354/meps08381>
- 441 Kajiwara, T., Matsui, K., Akakabe, Y., 1996. Biogenesis of volatile compounds via oxylipins in  
442 edible seaweeds, in: Takeoka, G.R., Teranishi, R., Williams, P.J., Kobayashi, A. (Ed.), ACS  
443 Symposium Series. Washington D. C., pp. 146–166. <https://doi.org/10.1021/bk-1996-0637.ch014>
- 445 Kumari, P., Kumar, M., Reddy, C.R.K., Jha, B., 2014. Nitrate and phosphate regimes induced  
446 lipidomic and biochemical changes in the intertidal macroalga *Ulva lactuca* (Ulvophyceae,  
447 Chlorophyta). *Plant Cell Physiol.* 55, 52–63. <https://doi.org/10.1093/pcp/pct156>
- 448 Lauritano, C., Carotenuto, Y., Miralto, A., Procaccini, G., Ianora, A., 2012. Copepod population-  
449 specific response to a toxic diatom diet. *PLoS One* 7, e47262.  
450 <https://doi.org/10.1371/journal.pone.0047262>
- 451 Lavrentyev, P.J., Franzè, G., Pierson, J.J., Stoecker, D.K., 2015. The effect of dissolved  
452 polyunsaturated aldehydes on microzooplankton growth rates in the Chesapeake Bay and  
453 Atlantic coastal waters. *Mar. Drugs* 13, 2834–2856. <https://doi.org/10.3390/md13052834>

- 454 Lotze, H.K., Schramm, W., 2000. Ecophysiological traits explain species dominance patterns in  
455 macroalgal blooms. *J. Phycol.* 36, 287–295. <https://doi.org/10.1046/j.1529-8817.2000.99109.x>
- 456 Miralto, A., Barone, G., Romano, G., Poulet, S.A., Ianora, A., Russo, G.L., Buttino, I., Mazzarella,  
457 G., Laabl, M., Cabrini, M., Glacobbe, M.G., 1999. The insidious effect of diatoms on copepod  
458 reproduction. *Nature* 402, 173–176. <https://doi.org/10.1038/46023>
- 459 Morillo-García, S., Valcaárcel-Pérez, N., Cózar, A., Ortega, M.J., Maciás, D., Ramírez-Romero, E.,  
460 García, C.M., Echevarría, F., Bartual, A., 2014. Potential polyunsaturated aldehydes in the  
461 Strait of Gibraltar under two tidal regimes. *Mar. Drugs* 12, 1438–1459.  
462 <https://doi.org/10.3390/md12031438>
- 463 Nan, C., Zhang, H., Lin, S., Zhao, G., Liu, X., 2008. Allelopathic effects of *Ulva lactuca* on  
464 selected species of harmful bloom-forming microalgae in laboratory cultures. *Aquat. Bot.* 89,  
465 9–15. <https://doi.org/10.1016/j.aquabot.2008.01.005>
- 466 Paul, C., Reunamo, A., Lindehoff, E., Bergkvist, J., Mausz, M.A., Larsson, H., Richter, H.,  
467 Wängberg, S.Å., Leskinen, P., Bañstedt, U., Pohnert, G., 2012. Diatom derived  
468 polyunsaturated aldehydes do not structure the planktonic microbial community in a  
469 mesocosm study. *Mar. Drugs* 10, 775–792. <https://doi.org/10.3390/md10040775>
- 470 Pezolesi, L., Pichierri, S., Samorì, C., Totti, C., Pistocchi, R., 2017. PUFAs and PUAs production  
471 in three benthic diatoms from the northern Adriatic Sea. *Phytochemistry* 142, 85–91.  
472 <https://doi.org/10.1016/j.phytochem.2017.06.018>
- 473 Pichierri, S., Pezolesi, L., Vanucci, S., Totti, C., Pistocchi, R., 2016. Inhibitory effect of  
474 polyunsaturated aldehydes (PUAs) on the growth of the toxic benthic dinoflagellate *Ostreopsis*  
475 cf. *ovata*. *Aquat. Toxicol.* 179, 125–133. <https://doi.org/10.1016/j.aquatox.2016.08.018>
- 476 Pierson, J.J., Frost, B.W., Leising, A.W., 2007. The lost generation of *Calanus pacificus*: Is the  
477 diatom effect responsible? *Limnol. Oceanogr.* 52, 2089–2098.  
478 <https://doi.org/10.4319/lo.2007.52.5.2089>
- 479 Pohnert, G., 2000. Wound-activated chemical defense in unicellular planktonic algae. *Angew.*  
480 *Chemie - Int. Ed.* 39, 4352–4354. [https://doi.org/10.1002/1521-3773\(20001201\)39:23<4352::AID-ANIE4352>3.0.CO;2-U](https://doi.org/10.1002/1521-3773(20001201)39:23<4352::AID-ANIE4352>3.0.CO;2-U)
- 482 Poulet, S.A., Cueff, A., Wichard, T., Marchetti, J., Dancie, C., Pohnert, G., 2007. Influence of  
483 diatoms on copepod reproduction. III. Consequences of abnormal oocyte maturation on  
484 reproductive factors in *Calanus helgolandicus*. *Mar. Biol.* 152, 415–428.



485 <https://doi.org/10.1007/s00227-007-0701-5>

486 Ribalet, F., Bastianini, M., Vidoudez, C., Acri, F., Berges, J., Ianora, A., Miralto, A., Pohnert, G.,  
487 Romano, G., Wichard, T., Casotti, R., 2014. Phytoplankton cell lysis associated with  
488 polyunsaturated aldehyde release in the northern Adriatic Sea. PLoS One 9, 9(1): e85947.  
489 <https://doi.org/10.1371/journal.pone.0085947>

490 Ribalet, F., Berges, J.A., Ianora, A., Casotti, R., 2007. Growth inhibition of cultured marine  
491 phytoplankton by toxic algal-derived polyunsaturated aldehydes. Aquat. Toxicol. 85, 219–227.  
492 <https://doi.org/10.1016/j.aquatox.2007.09.006>

493 Ribalet, F., Intertaglia, L., Lebaron, P., Casotti, R., 2008. Differential effect of three  
494 polyunsaturated aldehydes on marine bacterial isolates. Aquat. Toxicol. 86, 249–255.  
495 <https://doi.org/10.1016/j.aquatox.2007.11.005>

496 Rindi, F., Gavio, B., Díaz-Tapia, P., Di Camillo, C.G., Romagnoli, T., 2020. Long-term changes in  
497 the benthic macroalgal flora of a coastal area affected by urban impacts (Conero Riviera,  
498 Mediterranean Sea). Biodivers. Conserv. 29, 2275–2295. [https://doi.org/10.1007/s10531-020-](https://doi.org/10.1007/s10531-020-01973-z)  
499 [01973-z](https://doi.org/10.1007/s10531-020-01973-z)

500 Romano, G., Miralto, A., Ianora, A., 2010. Teratogenic effects of diatom metabolites on sea urchin  
501 *Paracentrotus lividus* embryos. Mar. Drugs 8, 950–967. <https://doi.org/10.3390/md8040950>

502 Scholz, B., Liebezeit, G., 2012. Screening for competition effects and allelochemicals in benthic  
503 marine diatoms and cyanobacteria isolated from an intertidal flat (southern North Sea).  
504 Phycologia 51, 432–450. <https://doi.org/10.2216/11-80.1>

505 Slattery M., G.D.J., 2009. Chemical Ecology of Marine Bacteria and Algae, in: Jorg D. Hardege  
506 (Ed.), Chemical Ecology. UNESCO-EOLSS, p. 572.

507 Sommer, U., 2009. Copepod growth and diatoms: Insensitivity of *Acartia tonsa* to the composition  
508 of semi-natural plankton mixtures manipulated by silicon:nitrogen ratios in mesocosms.  
509 Oecologia 159, 207–215. <https://doi.org/10.1007/s00442-008-1193-9>

510 Tang, Y.Z., Gobler, C.J., 2011. The green macroalga, *Ulva lactuca*, inhibits the growth of seven  
511 common harmful algal bloom species via allelopathy. Harmful Algae 10, 480–488.  
512 <https://doi.org/10.1016/j.hal.2011.03.003>

513 Taylor, R.L., Caldwell, G.S., Olive, P.J.W., Bentley, M.G., 2012. The harpacticoid copepod *Tisbe*  
514 *holothuriae* is resistant to the insidious effects of polyunsaturated aldehyde-producing diatoms.

- 515 J. Exp. Mar. Bio. Ecol. 413, 30–37. <https://doi.org/10.1016/j.jembe.2011.11.024>
- 516 Vidoudez, C., Casotti, R., Bastianini, M., Pohnert, G., 2011. Quantification of dissolved and  
517 particulate polyunsaturated aldehydes in the Adriatic Sea. *Mar. Drugs* 9, 500–503.  
518 <https://doi.org/10.3390/md9040500>
- 519 Wang, R., Xiao, H., Wang, Y., Zhou, W., Tang, X., 2007. Effects of three macroalgae, *Ulva linza*  
520 (Chlorophyta), *Corallina pilulifera* (Rhodophyta) and *Sargassum thunbergii* (Phaeophyta) on  
521 the growth of the red tide microalga *Prorocentrum donghaiense* under laboratory conditions. *J.*  
522 *Sea Res.* 58, 189–197. <https://doi.org/10.1016/j.seares.2007.03.002>
- 523 Wang, Y., Yu, Z., Song, X., Tang, X., Zhang, S., 2007. Effects of macroalgae *Ulva pertusa*  
524 (Chlorophyta) and *Gracilaria lemaneiformis* (Rhodophyta) on growth of four species of  
525 bloom-forming dinoflagellates. *Aquat. Bot.* 86, 139–147.  
526 <https://doi.org/10.1016/j.aquabot.2006.09.013>
- 527 Wichard, T., Gerecht, A., Boersma, M., Poulet, S.A., Wiltshire, K., Pohnert, G., 2007. Lipid and  
528 fatty acid composition of diatoms revisited: Rapid wound-activated change of food quality  
529 parameters influences herbivorous copepod reproductive success. *ChemBioChem* 8, 1146–  
530 1153. <https://doi.org/10.1002/cbic.200700053>
- 531 Wichard, T., Poulet, S.A., Boulesteix, A.L., Ledoux, J.B., Lebreton, B., Marchetti, J., Pohnert, G.,  
532 2008. Influence of diatoms on copepod reproduction. II. Uncorrelated effects of diatom-  
533 derived  $\alpha,\beta,\gamma,\delta$ -unsaturated aldehydes and polyunsaturated fatty acids on *Calanus*  
534 *helgolandicus* in the field. *Prog. Oceanogr.* 77, 30–44.  
535 <https://doi.org/10.1016/j.pocean.2008.03.002>
- 536 Wichard, T., Poulet, S.A., Halsband-Lenk, C., Albaina, A., Harris, R., Liu, D., Pohnert, G., 2005a.  
537 Survey of the chemical defence potential of diatoms: Screening of fifty species for  $\alpha,\beta,\gamma,\delta$ -  
538 unsaturated aldehydes. *J. Chem. Ecol.* 31, 949–958. [https://doi.org/10.1007/s10886-005-3615-](https://doi.org/10.1007/s10886-005-3615-z)  
539 *z*
- 540 Wichard, T., Poulet, S.A., Pohnert, G., 2005b. Determination and quantification of  $\alpha,\beta,\gamma,\delta$ -  
541 unsaturated aldehydes as pentafluorobenzyl-oxime derivatives in diatom cultures and natural  
542 phytoplankton populations: Application in marine field studies. *J. Chromatogr. B Anal.*  
543 *Technol. Biomed. Life Sci.* 814, 155–161. <https://doi.org/10.1016/j.jchromb.2004.10.021>
- 544 Wu, Z., Li, Q.P., 2016. Spatial distributions of polyunsaturated aldehydes and their biogeochemical  
545 implications in the Pearl River Estuary and the adjacent northern South China Sea. *Prog.*

546 Oceanogr. 147, 1–9. <https://doi.org/10.1016/j.pocean.2016.07.010>

547

548

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566

567

568

569

570 Table 1 – Relative abundance (%) and total concentration ( $\mu\text{g L}^{-1}$ ) of dissolved PUA (dPUAs) in  
 571 seawater in August and September 2015.

Relative abundance (%)	6th August 2015	27th August 2015	1st September 2015
C6:2	6%	6%	11%
C8:1	13%	5%	7%
C8:3	-	-	5%
C9:1	8%	3%	5%
C9:3	9%	1%	4%
C9:4	18%	31%	7%
C11:2	3%	1%	-
C15:3	23%	11%	26%
C16:3	4%	1%	7%
C17:3	7%	2%	9%
C17:0	9%	5%	15%
unknown aldehydes	0%	34%	5%
total concentration ( $\mu\text{g L}^{-1}$ )	26.3	17.2	24.1

572

573

574

575 Table 2 – Macroalgal species sampled in the present study in the Piscinetta del Passetto site (Adriatic Sea, Italy).

	Authority	Class	Family	July 2017	August 2017	September 2017	April 2018
<i>Ceramium ciliatum</i>	(J.Ellis) Ducluzeau 1806	Florideophyceae	Ceramiaceae				x
<i>Chondria capillaris</i>	(Hudson) M.J.Wynne 1991	Florideophyceae	Rhodomelaceae				x
<i>Chondria dasyphylla</i>	(Woodward) C.Agardh 1817	Florideophyceae	Rhodomelaceae				x
<i>Gracilaria bursa-pastoris</i>	(S.G.Gmelin) P.C.Silva 1952	Florideophyceae	Gracilariaceae				x
<i>Gracilaria</i> sp.		Florideophyceae	Gracilariaceae	x	x		
<i>Hypnea musciformis</i>	(Wulfen) J.V.Lamouroux 1813	Florideophyceae	Cystocloniaceae	x	x	x	
<i>Palisada perforata</i>	(Bory) K.W.Nam 2007	Florideophyceae	Rhodomelaceae	x	x	x	
<i>Cystoseira compressa</i>	(Esper) Gerloff & Nizamuddin 1975	Phaeophyceae	Sargassaceae	x	x	x	
<i>Dictyopteris polypodioides</i>	(A.P.De Candolle) J.V.Lamouroux 1809	Phaeophyceae	Dictyotaceae	x	x	x	
<i>Dictyota dichotoma</i>	(Hudson) J.V.Lamouroux 1809	Phaeophyceae	Dictyotaceae				x
<i>Padina pavonica</i>	(Linnaeus) Thivy 1960	Phaeophyceae	Dictyotaceae	x	x	x	
<i>Ulva</i> cf. <i>rigida</i>	C.Agardh 1823	Ulvophyceae	Ulvaceae	x	x	x	x

576

577

578

579

580 **Figure captions**

581

582 Figure 1 – Concentration (nM) of dissolved PUA (dPUAs) in seawater in August and September  
583 2015.

584 Figure 2 – Total aldehydes production (nmol g<sup>-1</sup>) in Mediterranean macroalgae (data are mean ± st.  
585 dev. of 3 replicates).

586 Figure 3 – Representative chromatograms (m/z 181) of standard compounds and algal extracts: (A)  
587 standards; (B) *Ulva cf. rigida*; (C) *Dictyopteris polypodioides*. The numbers 1–13 correspond to the  
588 oxime derivates of the putatively identified substances: (1) hexadienal; (2) benzaldehyde; (3) 2,4-  
589 heptadienal; (4) 2,4-octadienal; (5) 2,4-decadienal; (6) nonatetraenal; (7) nonatrienal; (8)  
590 decatetraenal; (9) hexadecadienal; (10) pentadecanal/ hexadecaheptaenal; (11) tetradecapentaenal;  
591 (12) hexadecatrienal; (13) hexadecatetraenal.

592 Figure 4 – Relative abundance (%) of aldehydes in macroalgae sampled in July 2017 (A) and April  
593 2018 (B).

594 Figure 5 - Aldehydes production (nmol g<sup>-1</sup>) by *Dictyopteris polypodioides* at the different sampling  
595 times (data are mean ± st. dev. of 3 replicates).

596 Figure 6 - Aldehydes production (nmol g<sup>-1</sup>) by *Ulva cf. rigida* at the different sampling times (data  
597 are mean ± st. dev. of 3 replicates).

598 Figure 7 – A: Map indicating the position of the sampling site (Piscinetta del Passetto). B, C: details  
599 of the phytobenthic community in July 2017. In Figure 6B arrowhead indicates *Cystoseira*  
600 *compressa*, curved arrow *Dictyopteris polypodioides*, and straight arrow *Ulva cf. rigida*, three of the  
601 species used for extraction of PUAs.

602

603

604

605

606

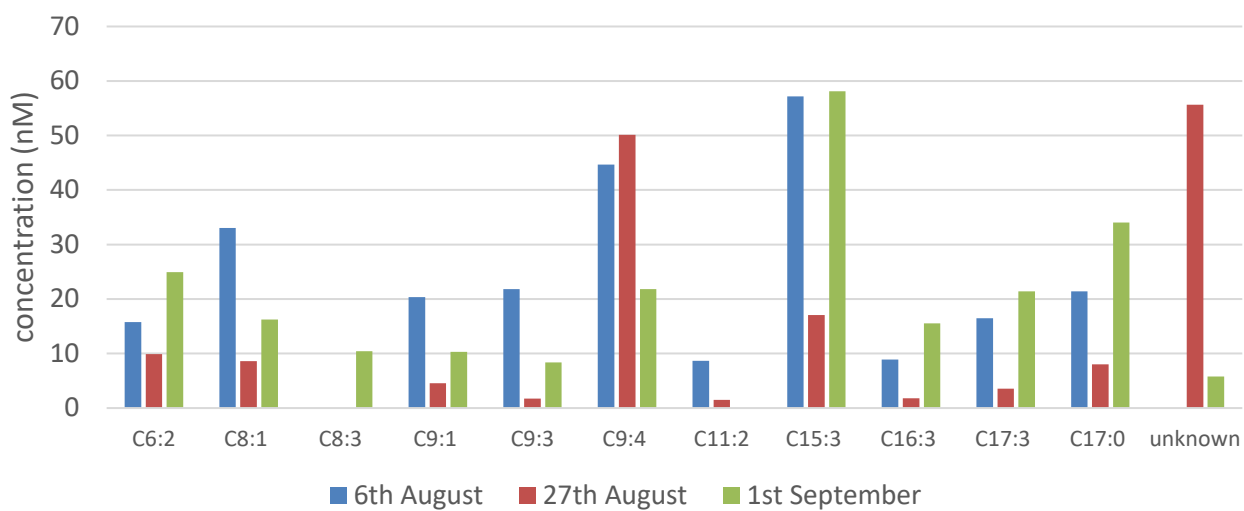
607

608

609 Figure 1

610

611



612

613

614

615

616

617

618

619

620

621

622

623

624

625

626

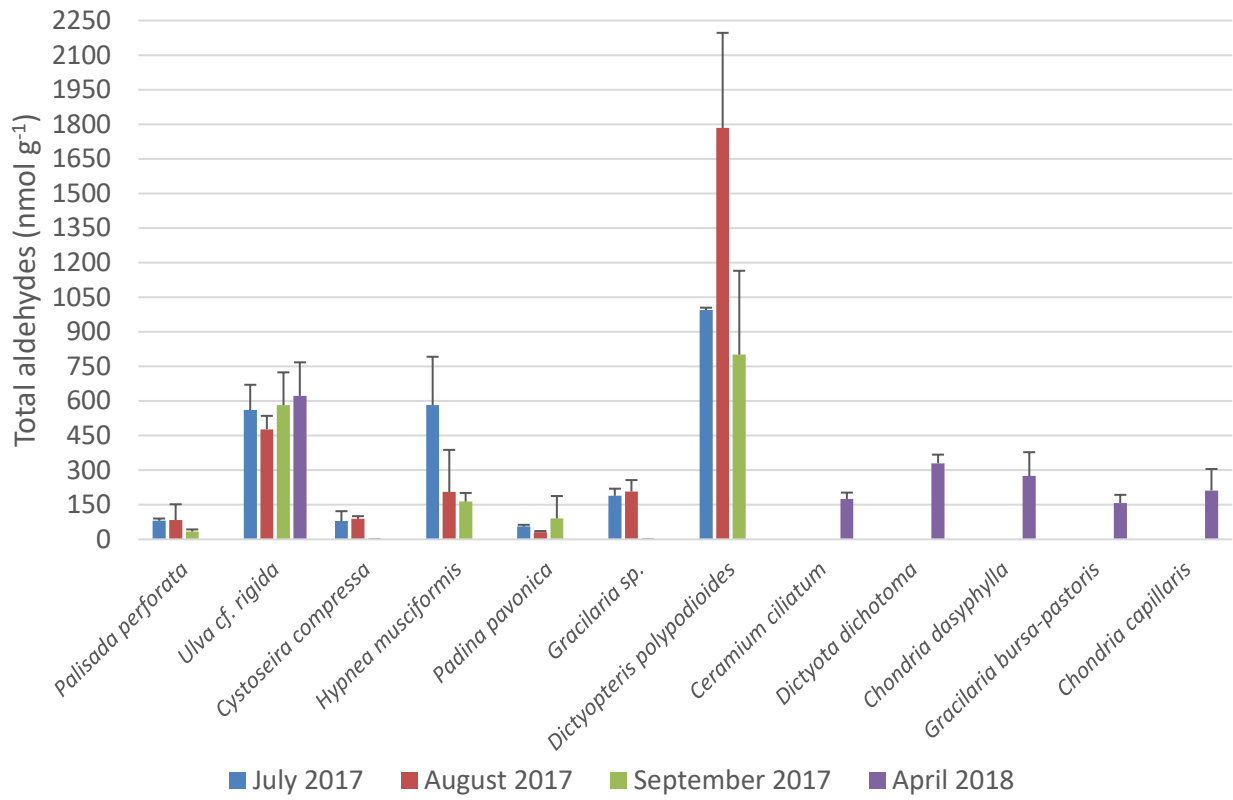
627

628 Figure 2

629

630

631



632

633

634

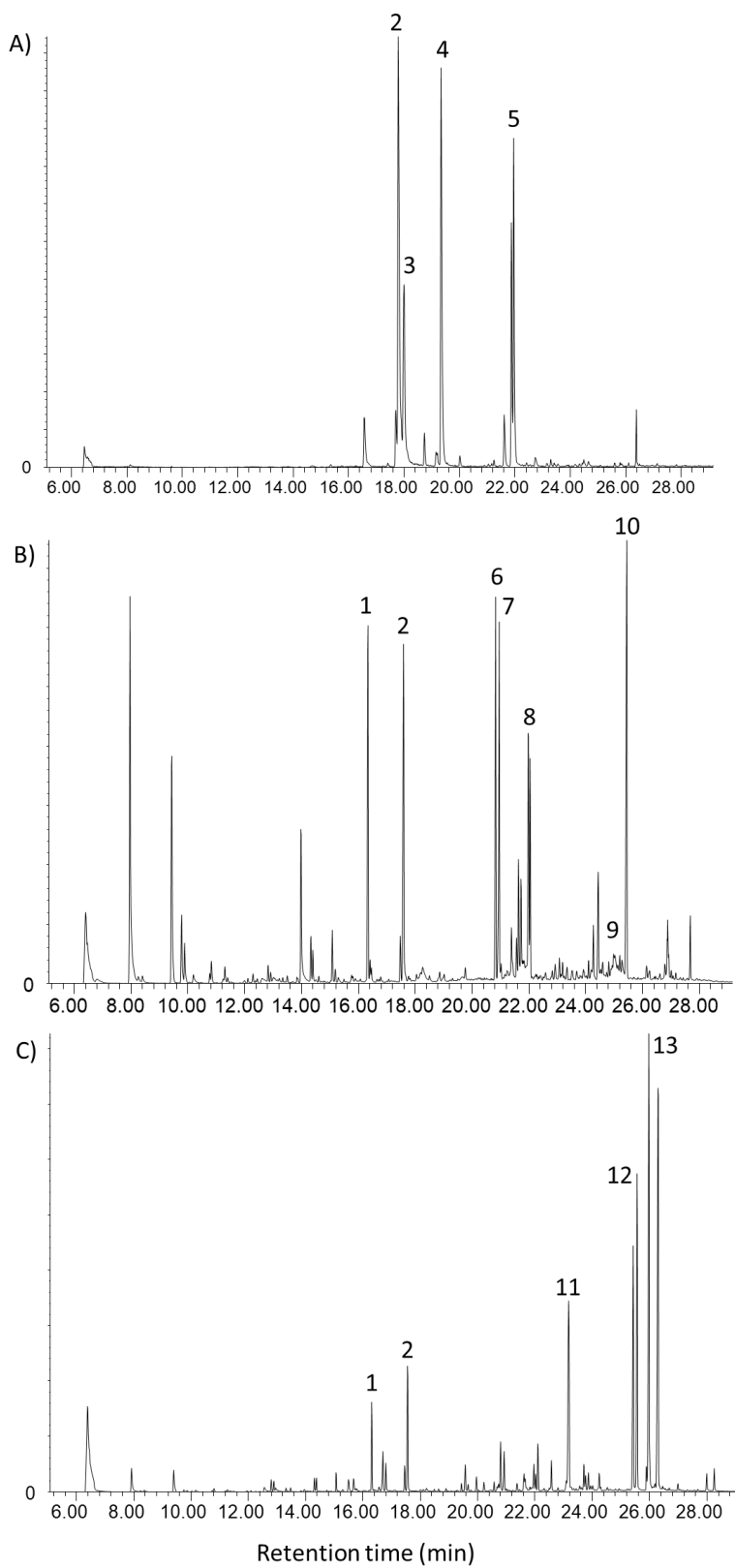
635

636

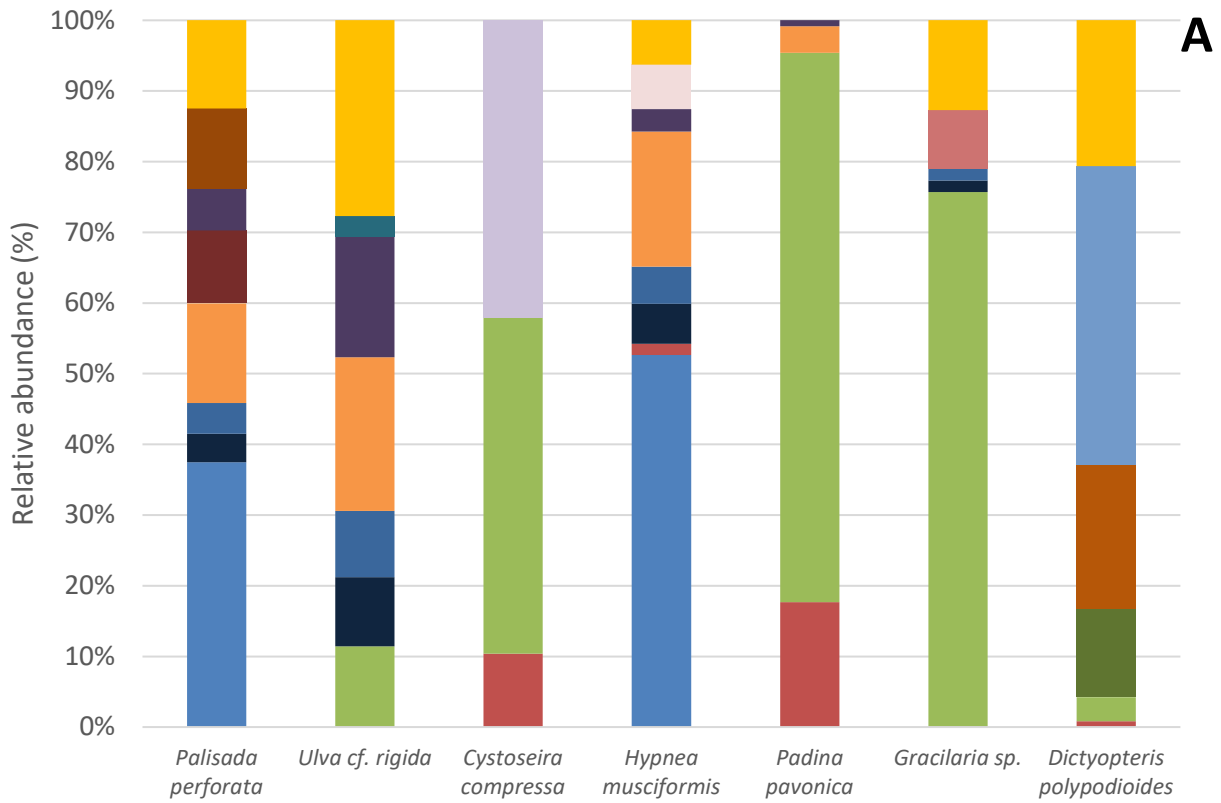
637

638

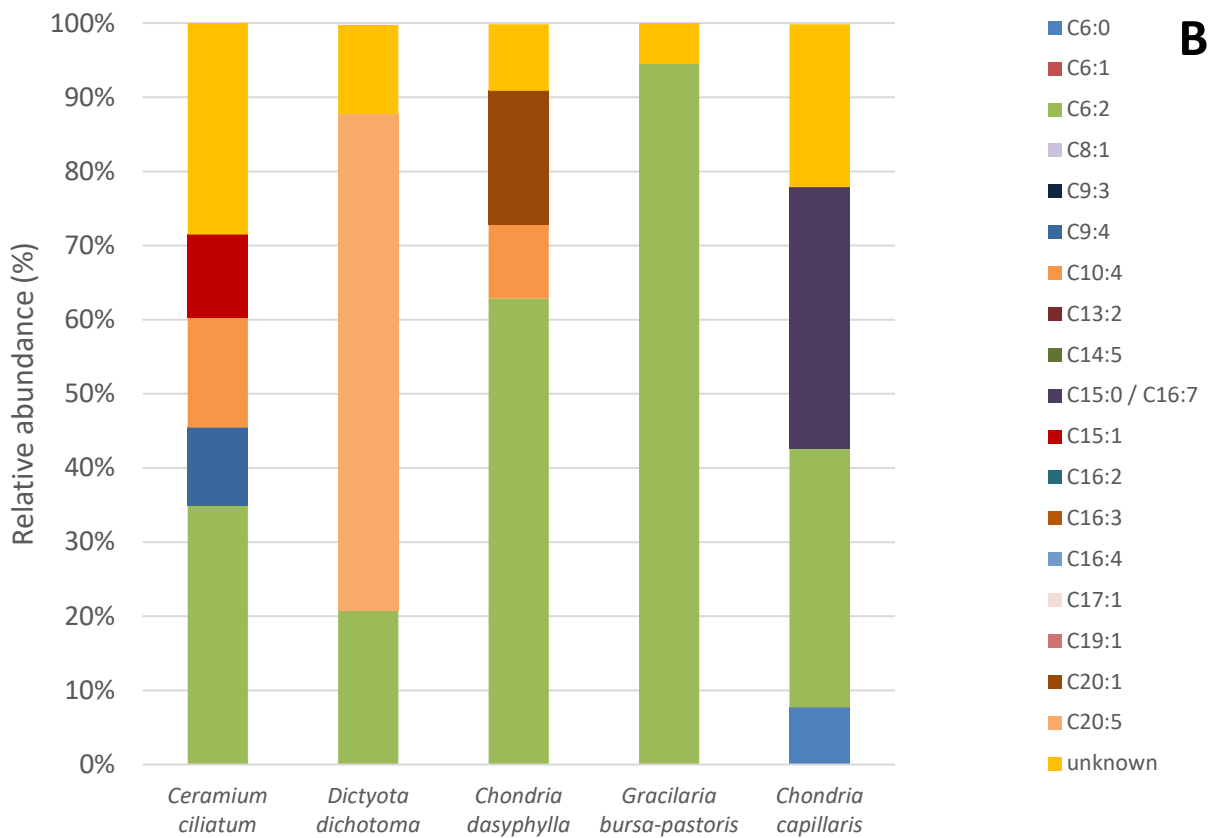




643 Figure 4



644

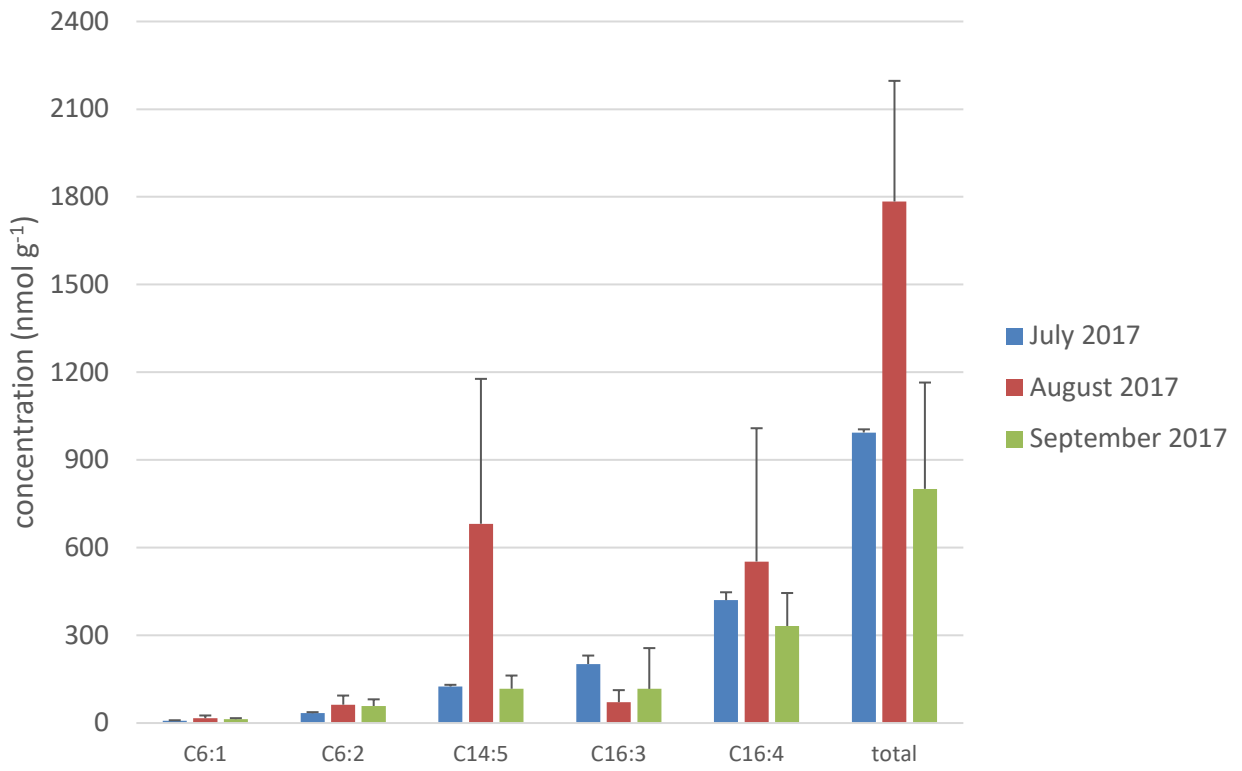


645

646 Figure 5

647

648



649

650

651

652

653

654

655

656

657

658

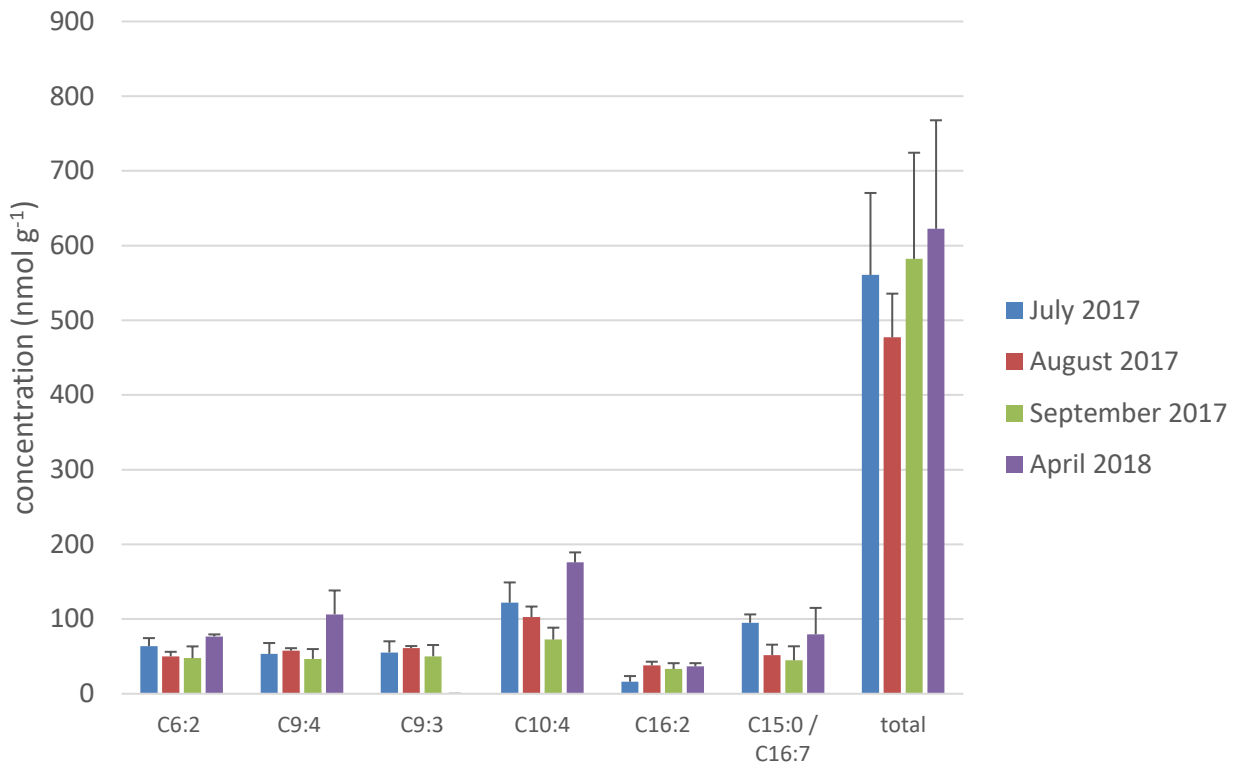
659

660

661 Figure 6

662

663



664

665

666

667

668

669

670

671

672

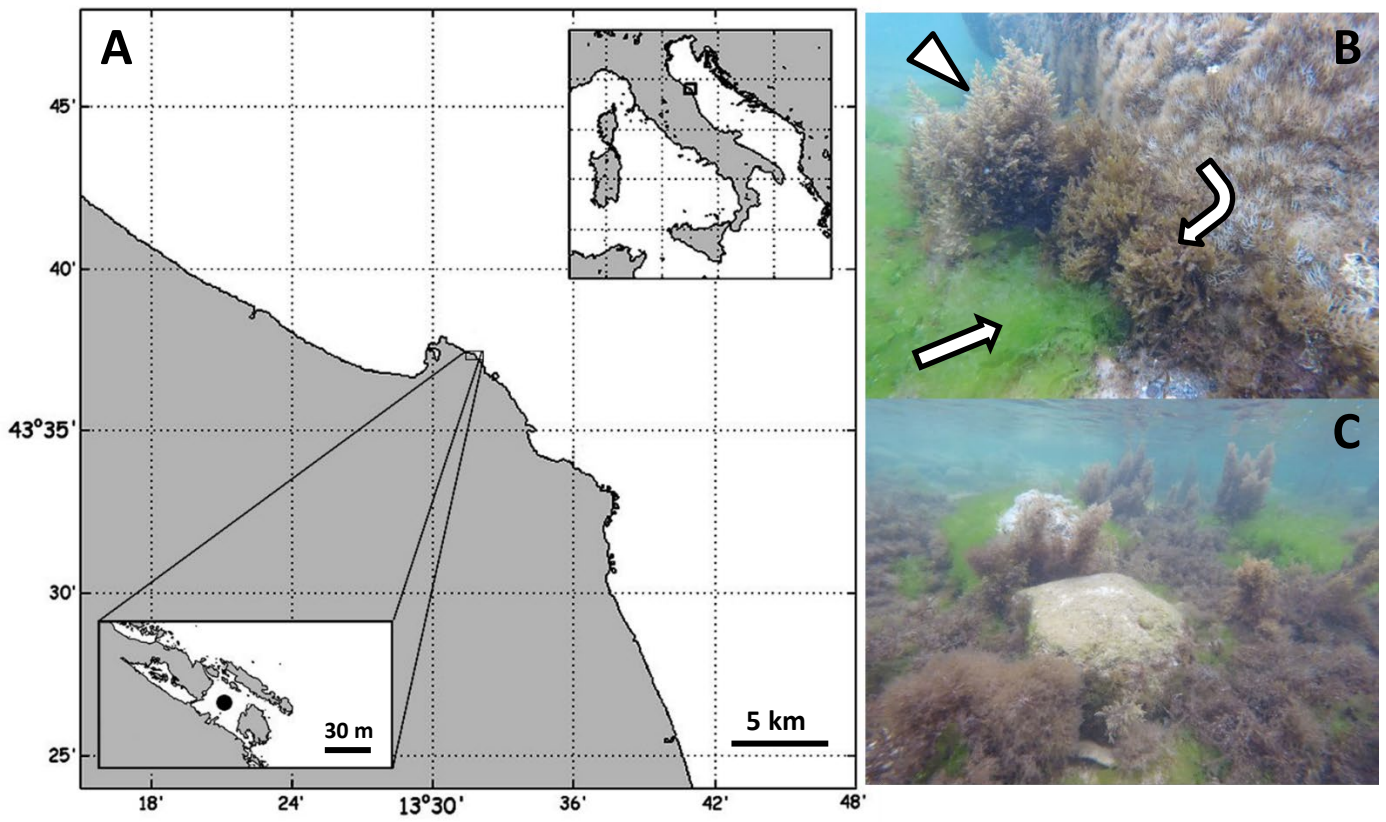
673

674

675

676 Figure 7

677



678

679