



UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

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

This is the peer reviewed version of the following article:

Original

Survey of the allelopathic potential of Mediterranean macroalgae: production of long-chain polyunsaturated aldehydes (PUAs) / Pezzolesi, L.; Accoroni, S.; Rindi, F.; Samorì, C.; Totti, C; Pistocchi, R.. - In: PHYTOCHEMISTRY. - ISSN 0031-9422. - ELETTRONICO. - 189:(2021).
[10.1016/j.phytochem.2021.112826]

Availability:

This version is available at: 11566/290659 since: 2024-03-26T16:55:29Z

Publisher:

Published

DOI:10.1016/j.phytochem.2021.112826

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

1 1 **Survey of the allelopathic potential of Mediterranean macroalgae: production of long-**
2
3 2 **chain polyunsaturated aldehydes (PUAs)**
4

5 3
6
7 4 Laura Pezzolesi^{1*}, Stefano Accoroni², Fabio Rindi², Chiara Samorì³, Cecilia Totti², Rossella
8
9 5 Pistocchi¹
10

11 6
12
13 7 ¹ Dipartimento di Scienze Biologiche, Geologiche e Ambientali - Università di Bologna, via
14
15 8 Sant'Alberto 163, 48123 Ravenna (Italy)

16 9 ² Dipartimento di Scienze della Vita e dell'Ambiente - Università Politecnica delle Marche, via
17
18 10 Breccie Bianche, 60131 Ancona (Italy)

19
20 11 ³ Dipartimento di Chimica "Giacomo Ciamician" - Università di Bologna, via Selmi 2, 40126
21
22 12 Bologna (Italy)

23
24 13
25 14
26 15
27 16
28
29 17
30
31 18 *Corresponding Author

32 19 Dr. Laura Pezzolesi

33
34 20 Tel. +39 (0)544 937373; fax: +39 (0)544 937411

35
36 21 laura.pezzolesi@unibo.it
37
38 22
39
40 23
41 24
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

24 Abstract

1
25 Chemical interactions between macroalgae and other organisms play an important role in determining
3
4 species compositions and dominance patterns, and can explain the widespread success of some
5
6 species in establishing their predominant populations in a specific coastal area. Allelopathy could act
7
8 as a self-regulatory strategy of the algal community, being not only a succession regulator but also an
9
10 active mechanism maintaining the species diversity especially in a delimited environment, such as
11
12 the benthic ecosystem. Polyunsaturated aldehydes (PUAs) are among the most studied allelopathic
13
14 compounds and are commonly released into the aquatic environment by different phytoplankton
15
16 species in response to environmental stressors (e.g. wounding, grazing, or competition for nutrients).
17
18 Diatom- released PUAs were observed to affect phytoplankton community dynamics and structure,
19
20 and showed inhibitory effects on reproduction and development of marine invertebrates. As for
21
22 macroalgae, there are only a few reports that attest the production of PUAs, and mostly refer to *Ulva*
23
24 spp. In this study, the production of PUAs by a number of Mediterranean macroalgae was investigated
25
26 at different sampling times, aiming at providing the first evidence of potential allelochemical activity.
27
28 Results highlighted the potential production by macroalgae of a variety of aldehydes, among which
29
30 some never reported so far. Some species (i.e. *D. polypodioides* and *U. cf. rigida*) were found to
31
32 produce higher PUAs amounts than others, and even a wider variety of structures (e.g. length of the
33
34 carbon chain); these species might exert strong effects on epiphytic species or other organisms of the
35
36 benthic community, especially considering the differential sensitivities of the various taxa. A high
37
38 dPUA concentrations (order of μM) potentially due to the release of PUAs by algal species was found,
39
40 and might affect the population dynamics of the epiphytic organisms (e.g. microalgae, meiofauna),
41
42 of grazers, as well as of the microbial community.

43

44 Keywords

45
46 *Ulva*; *Dictyopteris polypodioides*; Ulvaceae; Dictyotaceae; macroalgae; GC-MS; polyunsaturated
47
48 aldehydes; benthic ecosystem.

49

50

51

52 1. Introduction

53
54 Marine algae belong to several phylogenetic lineages, and often cohesist in similar niches from an
55
56 ecological standpoint, being subjected to herbivory and competition. Herbivory is considered a high
57
58 stress for these organisms and can account for a 60-100% loss of total algal production (Slattery and
59
60 Gochfeld, 2009); as result, some algal species respond to herbivory by enhancing the synthesis of a
61
62 pool of defensive metabolites that can vary among individuals within a population, also temporally
63
64 and/or spatially.

65

66

67

68

58 Most investigations ascribe the success of bloom-forming green macroalgae over other coastal
1
259 species mostly to their physiological characteristics (e.g. rapid nutrient uptake, fast growth, and
3
460 environmental tolerance) (Lotze and Schramm, 2000); however, chemical interactions between
5
661 macroalgae and other organisms play an important role in determining species compositions and
7
862 dominance patterns, and can explain the widespread success of some species in establishing their
9
1063 predominant populations in a specific coastal area. Fouling by microalgae can also be directly
11
1264 inhibited by macroalgal metabolites; several studies reported how seagrasses and macroalgae
13
1465 inhibited biofilm growth (e.g. Gette-Bouvarot et al., 2015; Ben Gharbia et al., 2017), suggesting that
15
1666 they might produce and release allelochemicals acting as biological mitigation agents, although
17
1867 benthic species seem to be more resistant than planktonic ones to the biological effects of
19
2068 allelochemicals. Allelopathic compounds isolated from marine organisms include low-molecular-
21
2269 weight peptides, phenols, alkaloids, fatty acids and their derivatives, including oxylipins such as
23
2470 aldehydes. Several ecological functions have been ascribed to allelopathy, such as intrapopulation
25
2671 signals, feeding deterrence or antagonism, and grazers repellence. Organisms able to produce and
27
2872 secrete allelopathic compounds may have an advantage over competitors under the same
29
3073 environment; thus, allelopathy could act as a self-regulatory strategy of the algal community (Hay,
31
3274 2009), being not only a succession regulator but also an active mechanism maintaining the species
33
3475 diversity especially in a delimited environment, such as the benthic ecosystem. Reports on
35
3676 interactions between macroalgae and phytoplankton highlighted as living tissues or extracts from
37
3877 *Ulva*, *Corallina*, and *Sargassum* spp. inhibited the growth of various microalgae, such as the
39
4078 dinoflagellate *Prorocentrum donghaiense* (R. Wang et al., 2007) or other planktonic species i.e.
41
4279 *Heterosigma akashiwo*, *Alexandrium tamarense*, *Skeletonema costatum*, *Amphidinium carterae*,
43
4480 *Scrippsiella trochoidea* (Y. Wang et al., 2007; Nan et al., 2008). Additionally, fresh and/or dried thalli
45
4681 of *Ulva lactuca* inhibited the growth of several common microalgal blooming species (e.g.
47
4882 *Margalefidinium polykrikoides*, *Karlodinium veneficum*, *Chattonella marina*, *Prorocentrum*
49
5083 *cordatum*, *Karenia brevis*, *Pseudo-nitzschia multiseriis*, and *Aureococcus anophagefferens*) in a
51
5284 dose-dependent manner, demonstrating that this species produces heat-stable allelochemicals (Tang
53
5485 and Gobler, 2011).

55
5686 Aldehydes are among the most studied allelopathic compounds identified in the aquatic environment
57
5887 (Dabrowska and Nawrocki, 2013). They can originate from a variety of processes like oxidation,
59
6088 photochemical transformations, and decomposition of organic matter. In particular, polyunsaturated
61
6289 aldehydes (PUAs) are volatile compounds commonly released into the aquatic environment (at
63
6490 nanomolar concentrations) by different phytoplankton species (Wichard et al., 2005a) in response to
65
6691 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
1
93 Ortega, 2013). Experimental works demonstrated that PUAs can induce changes in microzooplankton
2
3
94 growth dynamic and community structure (Franzè et al., 2018), have a teratogenic effect on copepods
4
5
95 (Romano et al., 2010) and inhibitory effects on reproduction and development of marine
6
7
96 invertebrates, thus they are considered an anti-grazer defense (Miralto et al., 1999; Poulet et al.,
8
9
97 2007; Lauritano et al., 2012; Brugnano et al., 2016). In field studies, differential effects of PUAs on
10
11
98 the bacterial community in terms of growth and metabolic activity (Balestra et al., 2011; Paul et al.,
12
13
99 2012) and on ciliates (Lavrentyev et al., 2015) were reported. In addition, diatom-released PUAs
14
100 were observed to affect phytoplankton community dynamics and structure by suppressing the growth
15
16
101 of other species (Casotti et al., 2005; Ribalet et al., 2007). Despite the attention on these compounds,
17
18
102 their role and ecological significance remain poorly understood.

19
20
103 More recently, attention has been posed also to the benthic environment as benthic primary producers
21
22
104 represent an important source of organic carbon in some littoral areas. Some studies evidenced PUAs
23
24
105 production by different benthic diatoms (Jüttner et al., 2010; Scholz and Liebezeit, 2012; Pezzolesi
25
26
106 et al., 2017), demonstrating as allelopathic benthic species could affect community composition. As
27
28
107 for macroalgae, there are only a few reports that attest the production of PUAs, and mostly refer to
29
30
108 *Ulva* spp.: Kajiwara et al. (1996) described long-chain aldehydes (e.g. pentadecanal, (8Z)-
31
32
109 heptadecenal, (8Z, 11Z)-heptadecadienal and (8Z, 11Z, 14Z)-heptadecatrienal) as odor components
33
34
110 of the essential oil prepared from the green alga *Ulva pertusa*; Akakabe et al. (2003) highlighted the
35
36
111 presence of 2,4-decadienal in *Ulva conglobata*; Alsufyani et al. (2014) documented the presence of
37
38
112 C₁₀-PUAs (e.g. 2,4,7-decatrienal and 2,4-decadienal, deriving from omega-3 and omega-6
39
40
113 polyunsaturated fatty acids (PUFAs) with 20 or 18 carbon atoms) in damaged tissue of *Ulva* spp. All
41
42
114 these studies demonstrated that the amount and structural diversity of released PUAs, as well as of
43
44
115 other oxylipins (Barbosa et al., 2016), can vary depending on the species and also on the
45
46
116 environmental conditions.

47
48
117 In this study, the production of PUAs by a number of Mediterranean macroalgae was investigated at
49
50
118 different sampling times, aiming at providing the first evidence of this allelochemical activity in other
51
52
119 species besides *Ulva* spp. The Mediterranean Sea is a hotspot of marine biodiversity, counting a
53
54
120 variety of macroalgal species that show widely different distribution patterns. Seaweed communities
55
56
121 are important coastal ecosystems that modify their structure and composition in space and time,
57
58
122 responding to environmental stressors or changes. In particular, the Conero Riviera located in the
59
60
123 Adriatic Sea is a rocky area in proximity of the city of Ancona, on the central Adriatic shore of Italy,
61
62
124 and its long-term changes in the macroalgal flora have been well documented (Rindi et al., 2020). As
63
64
125 a result, this area was selected for its interesting macroalgal community.

126
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2. Results and discussion

2.1 PUAs dissolved in seawater

Analyses of seawater collected in August and September 2015 in the Piscinetta del Passetto (Conero Riviera, Adriatic Sea, Italy) revealed the presence of high concentrations of total dissolved polyunsaturated aldehydes (dPUAs), ranging from 0.16 to 0.25 μM (corresponding to 162 and 248 nM) (Fig. 1). This amount is much higher than previously observed in the planktonic environment both in the Adriatic Sea (order of nM) by Ribalet et al. (2014) and in Atlantic seawater (Spain) by Bartual and Ortega (2013), but comparable to values found in a work performed in freshwater (Dabrowska et al., 2017). The high dPUA concentrations found in the present study could be explained considering the low hydrodynamism typical of confined benthic environments, and the presence of a well-developed phytobenthic community, characterized by several PUA producers (i.e. both micro- and macroalgae). In addition, Bartual et al. (2020) attested that μM ranges of dPUAs are expected to occur in the immediate vicinity of the producer cell, as occurred in this benthic environment, but disperse rapidly, consistently with pM to nM concentrations of dPUAs quantified in marine environments. A variety of aldehydes was putatively identified based on the mass spectra obtained at the three sampling times (Fig. 1), as short, medium, or long-chain aldehydes. In particular, C15:3 resulted among the most abundant PUAs (about 57.2 nM, corresponding to the 11-26% of the total aldehydes), together with C9:4 (about 44.6 nM, corresponding to 18-31% in August), followed by C6:2 (24.9 nM) and C8:1 (33.0 nM), whose relative abundances were up to 11 and 13%, respectively (Table 1). Contrarily to the other times, in the sampling performed at the end of August a fraction of these compounds remained unidentified (34%). dPUAs could be derived either by diatoms and macroalgae, as suggested by the high concentrations and the qualitative profile reported, as well as by a previous study performed in the same area that attested the potential production of a variety of low and medium-chain aldehydes, including some detected in the present work, by three benthic diatoms (Pezzolesi et al., 2017), and also by other studies performed on several phytoplankton species (i.e. Wichard et al., 2005a). It is worth considering that most of the previous studies focused only on the detection of three PUAs, i.e. heptadienal (C7:2), octadienal (C8:2), and decadienal (C10:2) (Vidoudez et al., 2011; Morillo-García et al., 2014; Ribalet et al., 2014; Wu and Li, 2016; Bartual et al., 2018; Bartual et al., 2020). Together with octatrienal (C8:3), C7:2 and C8:2 resulted the most abundant dPUA in coastal areas of the Adriatic Sea (Vidoudez et al., 2011; Ribalet et al., 2014); C7:2, C8:2, and C10:2 were also the most abundant in the northern South China Sea (Wu and Li, 2016), as similarly reported by Bartual et al. (2018) in southwestern Spain. These authors attested 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 μM 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 μM 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 nmol g^{-1} for *D. polypodioides* and between 477 and 623
190 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 nmol g^{-1} for all macroalgae, except for
193 *Hypnea musciformis* (Wulfen) J.V.Lamouroux 1813 in July, where about 582 nmol g^{-1} of aldehydes

194 were detected, and for *Dictyota dichotoma* (Hudson) J.V.Lamouroux 1809 in April (about 330 nmol
195 g⁻¹). 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⁻¹ for C14:5 and from 332 to 553
241 nmol g⁻¹ 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⁻¹
252 compared to 2.3 nmol g⁻¹ 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 (μM). 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 μM 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 μM) 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
297
298
299
300
301
302
303
304
305

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.

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 μM , based on a
344 benzaldehyde curve in the range from 5 up to 1250 μM ($R^2 = 0.9979$).

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 μL 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^{-1} 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_2SO_4 , as described in (Wichard et al., 2005b).
356 Samples were then treated and analyzed as previously described.

358 Acknowledgements

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

362 Note

363 The authors declare no competing financial interest.

1
2
364
3
4
365
6
7
366
8
367
10
368
12
369
14
15
370
16
17
371
18
19
372
20
21
373
22
23
374
24
25
375
26
27
376
28
29
377
30
31
378
32
33
379
35
380
37
381
38
39
382
40
41
383
43
384
45
385
46
47
386
48
387
49
50
51
388
52
53
54
389
55
56
57
58
59
60
392
61
62
63
64
65

References

- Accoroni, S., Glibert, P.M., Pichierri, S., Romagnoli, T., Marini, M., Totti, C., 2015a. A conceptual model of annual *Ostreopsis* cf. *ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients. *Harmful Algae* 45, 14–25. <https://doi.org/10.1016/j.hal.2015.04.002>
- Accoroni, S., Percopo, I., Cerino, F., Romagnoli, T., Pichierri, S., Perrone, C., Totti, C., 2015b. Allelopathic interactions between the HAB dinoflagellate *Ostreopsis* cf. *ovata* and macroalgae. *Harmful Algae* 49, 147–155. <https://doi.org/10.1016/j.hal.2015.08.007>
- Adolph, S., Poulet, S.A., Pohnert, G., 2003. Synthesis and biological activity of $\alpha,\beta,\gamma,\delta$ -unsaturated aldehydes from diatoms. *Tetrahedron* 59, 3003–3008. [https://doi.org/10.1016/S0040-4020\(03\)00382-X](https://doi.org/10.1016/S0040-4020(03)00382-X)
- Akakabe, Y., Matsui, K., Kajiwara, T., 2003. 2,4-Decadienals are produced via (R)-11-HPITE from arachidonic acid in marine green alga *Ulva conglobata*. *Bioorganic Med. Chem.* 11, 3607–3609. [https://doi.org/10.1016/S0968-0896\(03\)00364-X](https://doi.org/10.1016/S0968-0896(03)00364-X)
- Alsufyani, T., Engelen, A.H., Diekmann, O.E., Kuegler, S., Wichard, T., 2014. Prevalence and mechanism of polyunsaturated aldehydes production in the green tide forming macroalgal genus *Ulva* (Ulvales, Chlorophyta). *Chem. Phys. Lipids* 183, 100–109. <https://doi.org/10.1016/j.chemphyslip.2014.05.008>
- Arunkumar K., Sivakumar S.R., R.R., 2010. Review on bioactive potential in seaweeds (marine macroalgae): a special emphasis on bioactivity of seaweeds against plant pathogens. *Asian J. Plant Sci.* 9, 227–240.
- Balestra, C., Alonso-Sáez, L., Gasol, J.M., Casotti, R., 2011. Group-specific effects on coastal bacterioplankton of polyunsaturated aldehydes produced by diatoms. *Aquat. Microb. Ecol.* 63, 123–131. <https://doi.org/10.3354/ame01486>
- Barbosa, M., Valentão, P., Andrade, P.B., 2016. Biologically active oxylipins from enzymatic and nonenzymatic routes in macroalgae. *Mar. Drugs* 14, 23. <https://doi.org/10.3390/md14010023>
- Bartual, A., Hernanz-Torrijos, M., Sala, I., Ortega, M.J., González-García, C., Bolado-Penagos, M., 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
- 397 Bartual, A., Morillo-García, S., Ortega, M.J., Cózar, A., 2018. First report on vertical distribution of
398 dissolved polyunsaturated aldehydes in marine coastal waters. *Mar. Chem.* 204, 1–10.
399 <https://doi.org/10.1016/j.marchem.2018.05.004>
400
- 401 Bartual, A., Ortega, M.J., 2013. Temperature differentially affects the persistence of
402 polyunsaturated aldehydes in seawater. *Environ. Chem.* 10, 403–408.
403 <https://doi.org/10.1071/EN13055>
404
- 405 Ben Gharbia, H., Yahia, O.K.D., Cecchi, P., Masseret, E., Amzil, Z., Herve, F., Rovillon, G., Nouri,
406 H., M'Rabet, C., Couet, D., Triki, H.Z., Laabir, M., 2017. New insights on the species-specific
407 allelopathic interactions between macrophytes and marine HAB dinoflagellates. *PLoS One* 12.
408 <https://doi.org/10.1371/journal.pone.0187963>
409
- 410 Brugnano, C., Granata, A., Guglielmo, L., Minutoli, R., Zagami, G., Ianora, A., 2016. The
411 deleterious effect of diatoms on the biomass and growth of early stages of their copepod
412 grazers. *J. Exp. Mar. Bio. Ecol.* 476, 41–49. <https://doi.org/10.1016/j.jembe.2015.11.015>
413
- 414 Casotti, R., Mazza, S., Brunet, C., Vantrepotte, V., Ianora, A., Miralto, A., 2005. Growth inhibition
415 and toxicity of the diatom aldehyde 2-trans, 4-trans-decadial on *Thalassiosira weissflogii*
416 (*Bacillariophyceae*). *J. Phycol.* 41, 7–20. <https://doi.org/10.1111/j.1529-8817.2005.04052.x>
417
- 418 Dabrowska, A., Nawrocki, J., 2013. Aldehyde concentrations in wet deposition and river waters.
419 *Sci. Total Environ.* 452–453, 1–9. <https://doi.org/10.1016/j.scitotenv.2013.02.037>
420
- 421 Dabrowska, A., Sztuka, N., Dabrowski, A., 2017. Polyunsaturated aldehydes as bioactive secondary
422 metabolites in the aquatic environment. 15th International Conference on Environmental
423 Science and Technology Rhodes, Greece, 31 August - 2 September 2017.
424
- 425 Dittami, S.M., Wichard, T., Malzahn, A.M., Pohnert, G., Boersma, M., Wiltshire, K.H., 2010.
426 Culture conditions affect fatty acid content along with wound-activated production of
427 polyunsaturated aldehydes in *Thalassiosira rotula* (*Coscinodiscophyceae*). *Nov. Hedwigia*,
428 *Beihefte* 136, 231–248. <https://doi.org/10.1127/1438-9134/2010/0136-0231>
429
- 430 Edwards, B.R., Bidle, K.D., Van Mooy, B.A.S., 2015. Dose-dependent regulation of microbial
431 activity on sinking particles by polyunsaturated aldehydes: Implications for the carbon cycle.
432 *Proc. Natl. Acad. Sci. U. S. A.* 112, 5909–5914. <https://doi.org/10.1073/pnas.1422664112>
433
434
435

- 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
- 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
- 430 Hansen, E., Ernstsens, 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
- 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
- 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
- 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
- 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>
444
- 444 Kumari, P., Kumar, M., Reddy, C.R.K., Jha, B., 2014. Nitrate and phosphate regimes induced
445 lipidomic and biochemical changes in the intertidal macroalga *Ulva lactuca* (Ulvophyceae,
446 Chlorophyta). *Plant Cell Physiol.* 55, 52–63. <https://doi.org/10.1093/pcp/pct156>
447
- 447 Lauritano, C., Carotenuto, Y., Miralto, A., Procaccini, G., Ianora, A., 2012. Copepod population-
448 specific response to a toxic diatom diet. *PLoS One* 7, e47262.
449 <https://doi.org/10.1371/journal.pone.0047262>
450
- 450 Lavrentyev, P.J., Franzè, G., Pierson, J.J., Stoecker, D.K., 2015. The effect of dissolved
451 polyunsaturated aldehydes on microzooplankton growth rates in the Chesapeake Bay and
452 Atlantic coastal waters. *Mar. Drugs* 13, 2834–2856. <https://doi.org/10.3390/md13052834>
453

- 454 Lotze, H.K., Schramm, W., 2000. Ecophysiological traits explain species dominance patterns in
1
455 macroalgal blooms. *J. Phycol.* 36, 287–295. <https://doi.org/10.1046/j.1529-8817.2000.99109.x>
3
- 456 Miralto, A., Barone, G., Romano, G., Poulet, S.A., Ianora, A., Russo, G.L., Buttino, I., Mazzarella,
5
457 G., Laabl, M., Cabrini, M., Glacobbe, M.G., 1999. The insidious effect of diatoms on copepod
7
458 reproduction. *Nature* 402, 173–176. <https://doi.org/10.1038/46023>
9
- 459 Morillo-García, S., Valcañel-Pérez, N., Cózar, A., Ortega, M.J., Maciás, D., Ramírez-Romero, E.,
11
460 García, C.M., Echevarría, F., Bartual, A., 2014. Potential polyunsaturated aldehydes in the
13
461 Strait of Gibraltar under two tidal regimes. *Mar. Drugs* 12, 1438–1459.
15
462 <https://doi.org/10.3390/md12031438>
17
- 463 Nan, C., Zhang, H., Lin, S., Zhao, G., Liu, X., 2008. Allelopathic effects of *Ulva lactuca* on
19
464 selected species of harmful bloom-forming microalgae in laboratory cultures. *Aquat. Bot.* 89,
21
465 9–15. <https://doi.org/10.1016/j.aquabot.2008.01.005>
23
- 466 Paul, C., Reunamo, A., Lindehoff, E., Bergkvist, J., Mausz, M.A., Larsson, H., Richter, H.,
25
467 Wängberg, S.Å., Leskinen, P., Bañstedt, U., Pohnert, G., 2012. Diatom derived
27
468 polyunsaturated aldehydes do not structure the planktonic microbial community in a
29
469 mesocosm study. *Mar. Drugs* 10, 775–792. <https://doi.org/10.3390/md10040775>
31
- 470 Pezsolesi, L., Pichierri, S., Samorì, C., Totti, C., Pistocchi, R., 2017. PUFAs and PUAs production
33
471 in three benthic diatoms from the northern Adriatic Sea. *Phytochemistry* 142, 85–91.
35
472 <https://doi.org/10.1016/j.phytochem.2017.06.018>
37
- 473 Pichierri, S., Pezsolesi, L., Vanucci, S., Totti, C., Pistocchi, R., 2016. Inhibitory effect of
39
474 polyunsaturated aldehydes (PUAs) on the growth of the toxic benthic dinoflagellate *Ostreopsis*
41
475 cf. *ovata*. *Aquat. Toxicol.* 179, 125–133. <https://doi.org/10.1016/j.aquatox.2016.08.018>
43
- 476 Pierson, J.J., Frost, B.W., Leising, A.W., 2007. The lost generation of *Calanus pacificus*: Is the
46
477 diatom effect responsible? *Limnol. Oceanogr.* 52, 2089–2098.
48
478 <https://doi.org/10.4319/lo.2007.52.5.2089>
50
- 479 Pohnert, G., 2000. Wound-activated chemical defense in unicellular planktonic algae. *Angew.*
52
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)
54
481
56
- 482 Poulet, S.A., Cueff, A., Wichard, T., Marchetti, J., Dancie, C., Pohnert, G., 2007. Influence of
58
483 diatoms on copepod reproduction. III. Consequences of abnormal oocyte maturation on
60
484 reproductive factors in *Calanus helgolandicus*. *Mar. Biol.* 152, 415–428.
62
63
64
65

- 485 <https://doi.org/10.1007/s00227-007-0701-5>
1
- 486 Ribalet, F., Bastianini, M., Vidoudez, C., Acri, F., Berges, J., Ianora, A., Miralto, A., Pohnert, G.,
3
487 Romano, G., Wichard, T., Casotti, R., 2014. Phytoplankton cell lysis associated with
5
488 polyunsaturated aldehyde release in the northern Adriatic Sea. PLoS One 9, 9(1): e85947.
7
489 <https://doi.org/10.1371/journal.pone.0085947>
9
- 490 Ribalet, F., Berges, J.A., Ianora, A., Casotti, R., 2007. Growth inhibition of cultured marine
11
491 phytoplankton by toxic algal-derived polyunsaturated aldehydes. Aquat. Toxicol. 85, 219–227.
13
492 <https://doi.org/10.1016/j.aquatox.2007.09.006>
15
- 493 Ribalet, F., Intertaglia, L., Lebaron, P., Casotti, R., 2008. Differential effect of three
17
494 polyunsaturated aldehydes on marine bacterial isolates. Aquat. Toxicol. 86, 249–255.
19
495 <https://doi.org/10.1016/j.aquatox.2007.11.005>
21
- 496 Rindi, F., Gavio, B., Díaz-Tapia, P., Di Camillo, C.G., Romagnoli, T., 2020. Long-term changes in
23
497 the benthic macroalgal flora of a coastal area affected by urban impacts (Conero Riviera,
25
498 Mediterranean Sea). Biodivers. Conserv. 29, 2275–2295. <https://doi.org/10.1007/s10531-020-01973-z>
27
- 499
29
500 Romano, G., Miralto, A., Ianora, A., 2010. Teratogenic effects of diatom metabolites on sea urchin
31
501 *Paracentrotus lividus* embryos. Mar. Drugs 8, 950–967. <https://doi.org/10.3390/md8040950>
33
34
- 502 Scholz, B., Liebezeit, G., 2012. Screening for competition effects and allelochemicals in benthic
36
503 marine diatoms and cyanobacteria isolated from an intertidal flat (southern North Sea).
38
504 Phycologia 51, 432–450. <https://doi.org/10.2216/11-80.1>
40
- 505 Slattery M., G.D.J., 2009. Chemical Ecology of Marine Bacteria and Algae, in: Jorg D. Hardege
42
506 (Ed.), Chemical Ecology. UNESCO-EOLSS, p. 572.
44
- 507 Sommer, U., 2009. Copepod growth and diatoms: Insensitivity of *Acartia tonsa* to the composition
46
508 of semi-natural plankton mixtures manipulated by silicon:nitrogen ratios in mesocosms.
48
509 Oecologia 159, 207–215. <https://doi.org/10.1007/s00442-008-1193-9>
50
- 51
510 Tang, Y.Z., Gobler, C.J., 2011. The green macroalga, *Ulva lactuca*, inhibits the growth of seven
53
511 common harmful algal bloom species via allelopathy. Harmful Algae 10, 480–488.
54
512 <https://doi.org/10.1016/j.hal.2011.03.003>
56
- 513 Taylor, R.L., Caldwell, G.S., Olive, P.J.W., Bentley, M.G., 2012. The harpacticoid copepod *Tisbe*
59
514 *holothuriae* is resistant to the insidious effects of polyunsaturated aldehyde-producing diatoms.
61
62
63
64
65

- 515 J. Exp. Mar. Bio. Ecol. 413, 30–37. <https://doi.org/10.1016/j.jembe.2011.11.024>
1
- 516 Vidoudez, C., Casotti, R., Bastianini, M., Pohnert, G., 2011. Quantification of dissolved and
3
517 particulate polyunsaturated aldehydes in the Adriatic Sea. Mar. Drugs 9, 500–503.
5
518 <https://doi.org/10.3390/md9040500>
7
- 519 Wang, R., Xiao, H., Wang, Y., Zhou, W., Tang, X., 2007. Effects of three macroalgae, *Ulva linza*
8
520 (Chlorophyta), *Corallina pilulifera* (Rhodophyta) and *Sargassum thunbergii* (Phaeophyta) on
10
521 the growth of the red tide microalga *Prorocentrum donghaiense* under laboratory conditions. J.
11
522 Sea Res. 58, 189–197. <https://doi.org/10.1016/j.seares.2007.03.002>
13
15
- 523 Wang, Y., Yu, Z., Song, X., Tang, X., Zhang, S., 2007. Effects of macroalgae *Ulva pertusa*
16
524 (Chlorophyta) and *Gracilaria lemaneiformis* (Rhodophyta) on growth of four species of
17
525 bloom-forming dinoflagellates. Aquat. Bot. 86, 139–147.
18
526 <https://doi.org/10.1016/j.aquabot.2006.09.013>
19
20
21
22
23
- 527 Wichard, T., Gerecht, A., Boersma, M., Poulet, S.A., Wiltshire, K., Pohnert, G., 2007. Lipid and
24
528 fatty acid composition of diatoms revisited: Rapid wound-activated change of food quality
25
529 parameters influences herbivorous copepod reproductive success. ChemBioChem 8, 1146–
26
530 1153. <https://doi.org/10.1002/cbic.200700053>
27
28
29
30
31
- 531 Wichard, T., Poulet, S.A., Boulesteix, A.L., Ledoux, J.B., Lebreton, B., Marchetti, J., Pohnert, G.,
32
532 2008. Influence of diatoms on copepod reproduction. II. Uncorrelated effects of diatom-
33
533 derived $\alpha,\beta,\gamma,\delta$ -unsaturated aldehydes and polyunsaturated fatty acids on *Calanus*
34
534 *helgolandicus* in the field. Prog. Oceanogr. 77, 30–44.
35
535 <https://doi.org/10.1016/j.pocean.2008.03.002>
36
37
38
39
40
41
- 536 Wichard, T., Poulet, S.A., Halsband-Lenk, C., Albaina, A., Harris, R., Liu, D., Pohnert, G., 2005a.
42
537 Survey of the chemical defence potential of diatoms: Screening of fifty species for $\alpha,\beta,\gamma,\delta$ -
43
538 unsaturated aldehydes. J. Chem. Ecol. 31, 949–958. [https://doi.org/10.1007/s10886-005-3615-
45
46
47
48
49](https://doi.org/10.1007/s10886-005-3615-
44
539 z)
- 540 Wichard, T., Poulet, S.A., Pohnert, G., 2005b. Determination and quantification of $\alpha,\beta,\gamma,\delta$ -
50
541 unsaturated aldehydes as pentafluorobenzyl-oxime derivatives in diatom cultures and natural
51
542 phytoplankton populations: Application in marine field studies. J. Chromatogr. B Anal.
52
543 Technol. Biomed. Life Sci. 814, 155–161. <https://doi.org/10.1016/j.jchromb.2004.10.021>
53
54
55
56
57
- 544 Wu, Z., Li, Q.P., 2016. Spatial distributions of polyunsaturated aldehydes and their biogeochemical
58
545 implications in the Pearl River Estuary and the adjacent northern South China Sea. Prog.
59
60
61
62
63
64
65

546
1
~~2~~
547
3
4
~~5~~
548
6
7
549
8
9
~~10~~
11
12
551
13
14
~~15~~
16
17
553
18
19
~~20~~
21
22
555
23
24
~~25~~
26
27
557
28
29
~~30~~
31
32
~~33~~
34
~~35~~
36
37
~~38~~
39
40
562
41
42
~~43~~
44
45
564
46
47
~~48~~
49
50
566
51
52
~~53~~
54
55
568
56
57
~~58~~
59
60
61
62
63
64
65

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

576

577

578

579

580

581

582

583

584

585

586

587

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

604

605

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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				x
	<i>Chondria capillaris</i>	(Hudson) M.J.Wynne 1991	Florideophyceae				x
	<i>Chondria dasyphylla</i>	(Woodward) C.Agardh 1817	Florideophyceae				x
	<i>Gracilaria bursa-pastoris</i>	(S.G.Gmelin) P.C.Silva 1952	Florideophyceae				x
	<i>Gracilaria</i> sp.		Florideophyceae	x	x		
	<i>Hypnea musciformis</i>	(Wulfen) J.V.Lamouroux 1813	Florideophyceae	x	x	x	
	<i>Palisada perforata</i>	(Bory) K.W.Nam 2007	Florideophyceae	x	x	x	
	<i>Cystoseira compressa</i>	(Esper) Gerloff & Nizamuddin 1975	Phaeophyceae	x	x	x	
	<i>Dictyopteris polypodioides</i>	(A.P.De Candolle) J.V.Lamouroux 1809	Phaeophyceae	x	x	x	
	<i>Dictyota dichotoma</i>	(Hudson) J.V.Lamouroux 1809	Phaeophyceae				x
	<i>Padina pavonica</i>	(Linnaeus) Thivy 1960	Phaeophyceae	x	x	x	
	<i>Ulva</i> cf. <i>rigida</i>	C.Agardh 1823	Ulvophyceae	x	x	x	x

580 **Figure captions**

1

2
581

3

4

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

6

8

584 Figure 2 – Total aldehydes production (nmol g⁻¹) in Mediterranean macroalgae (data are mean ± st.
10 dev. of 3 replicates).

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131

132

133

134

135

136

137

138

139

140

141

142

143

144

145

146

147

148

149

150

151

152

153

154

155

156

157

158

159

160

161

162

163

164

165

166

167

168

169

170

171

172

173

174

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

226

227

228

229

230

231

232

233

234

235

236

237

238

239

240

241

242

243

244

245

246

247

248

249

250

251

252

253

254

255

256

257

258

259

260

261

262

263

264

265

266

267

268

269

270

271

272

273

274

275

276

277

278

279

280

281

282

283

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

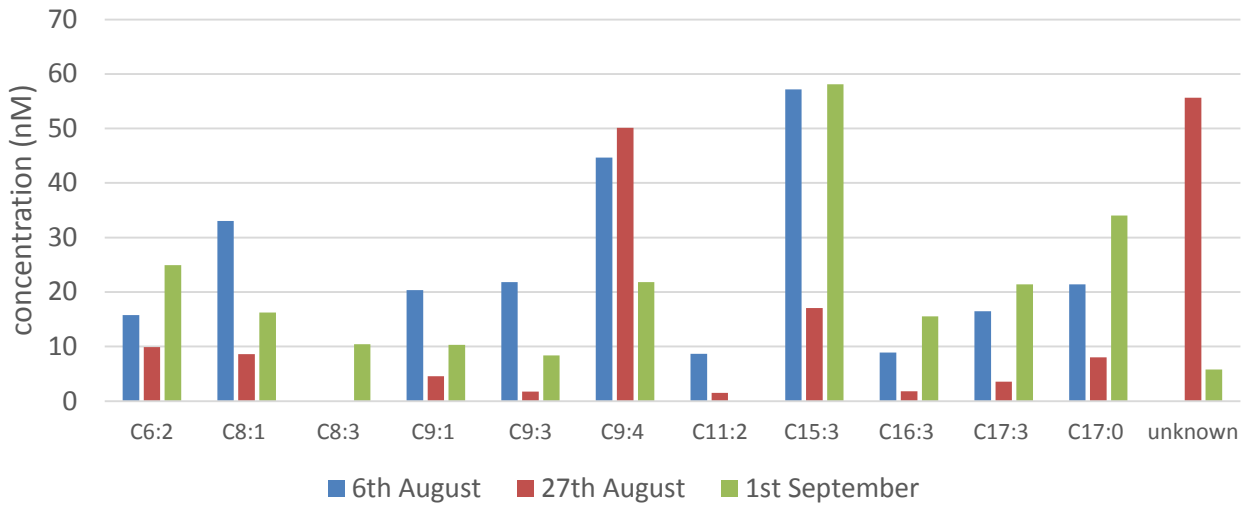
347

348

349

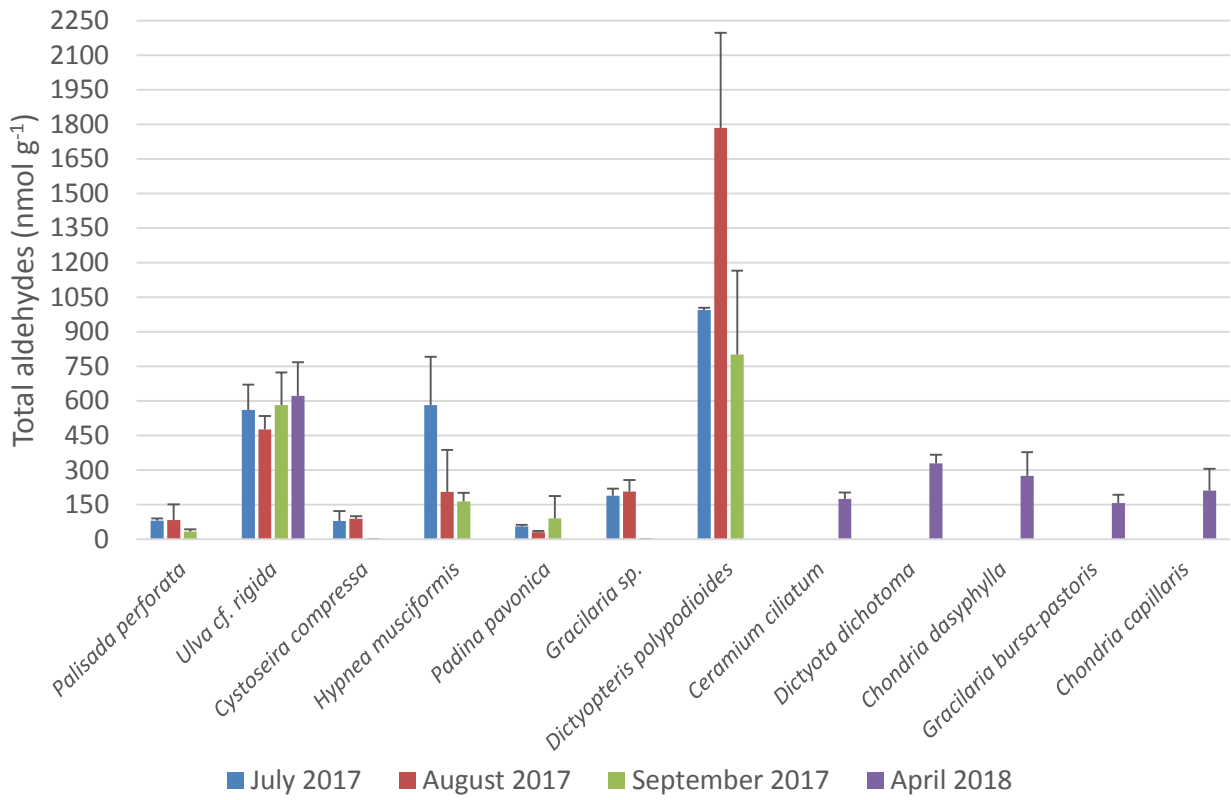
<

609 Figure 1



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

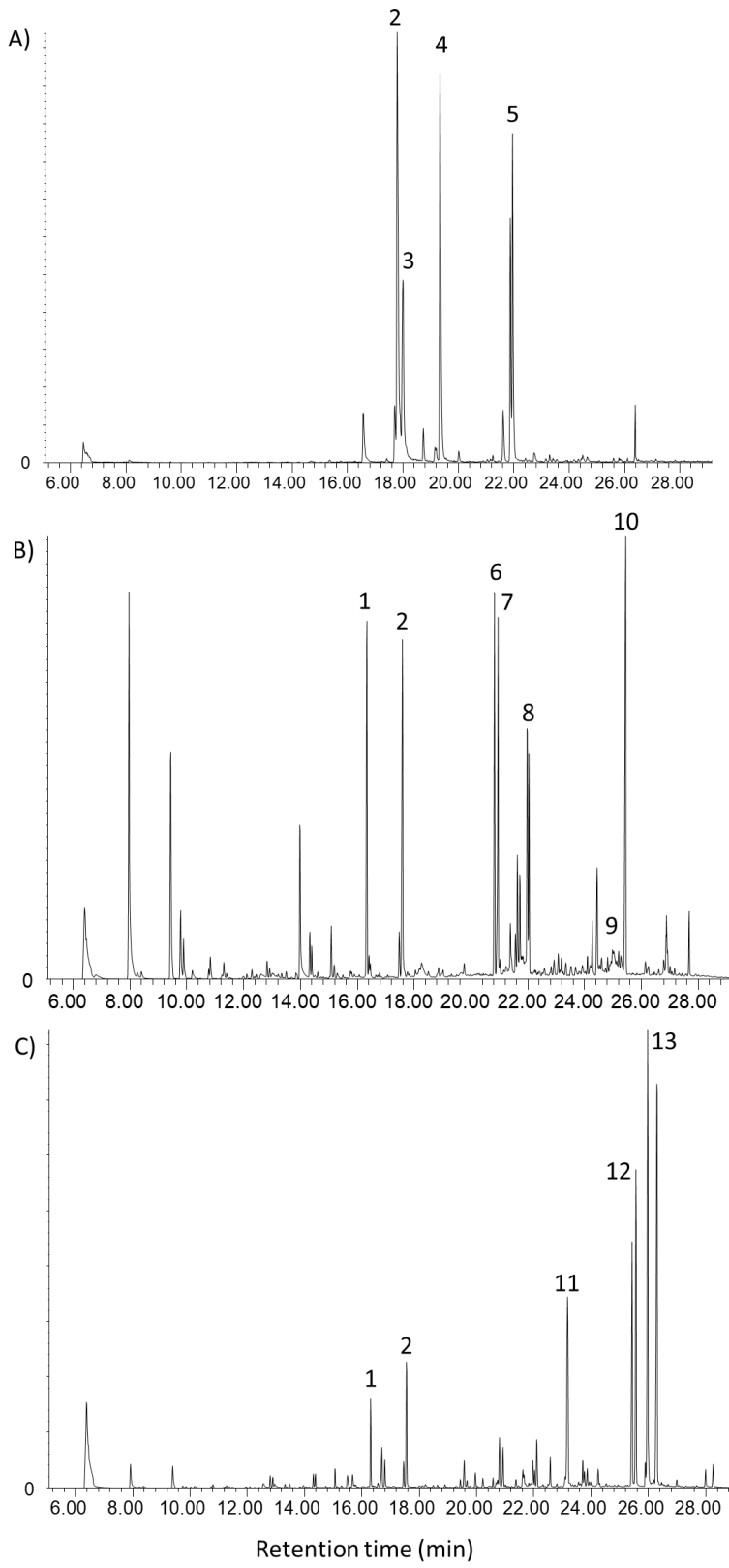
628 Figure 2



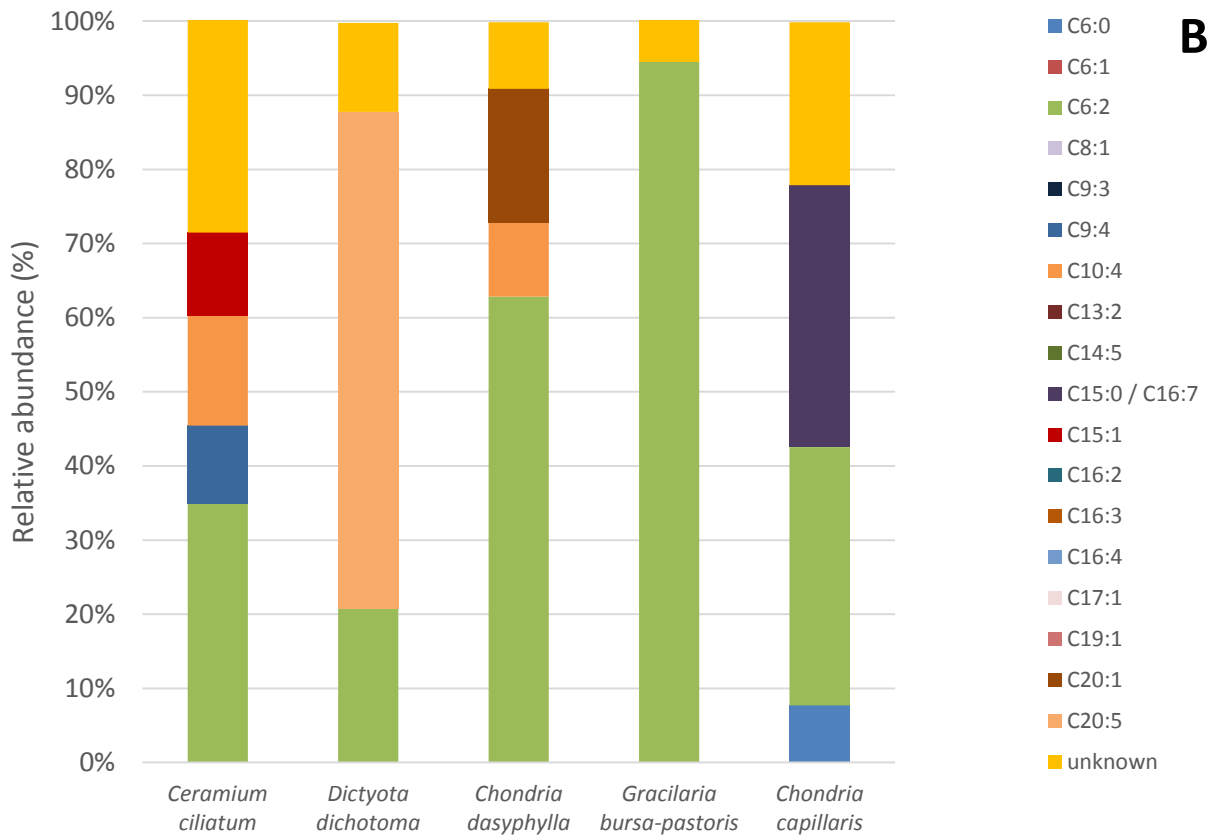
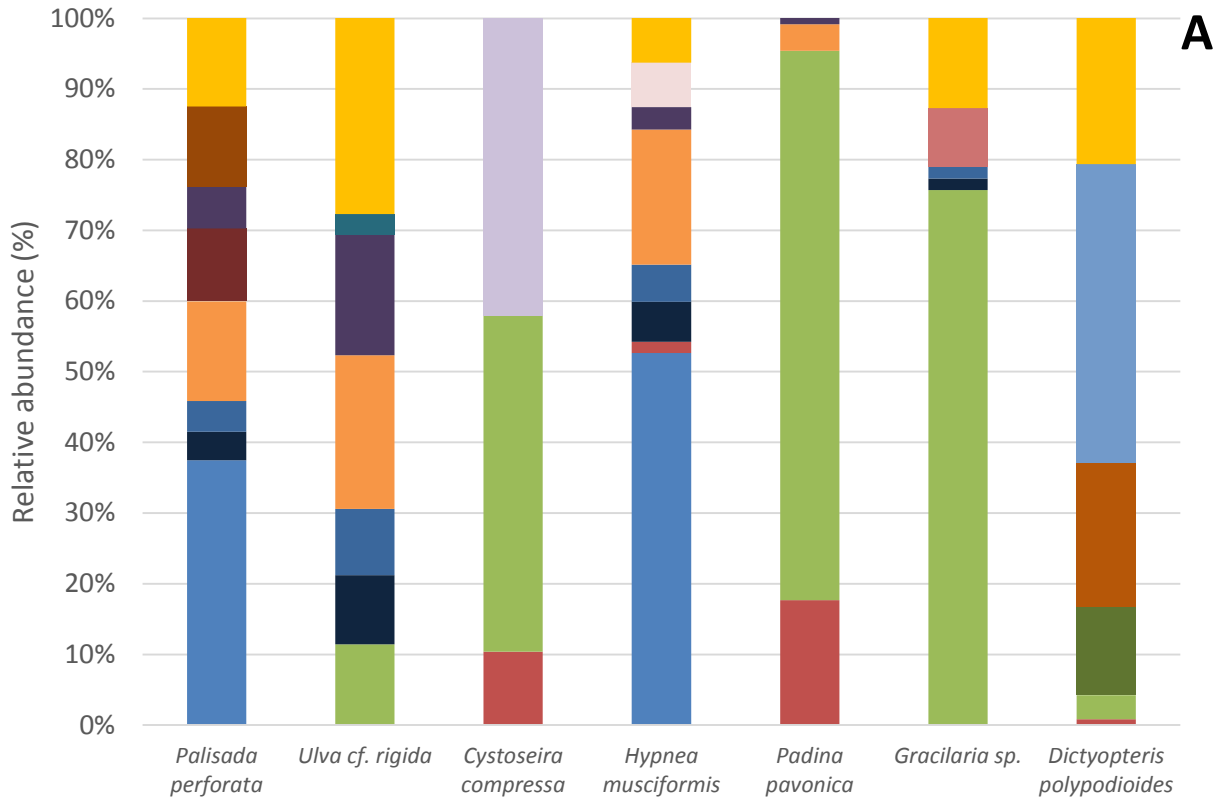
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

639 Figure 3

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65



643 Figure 4

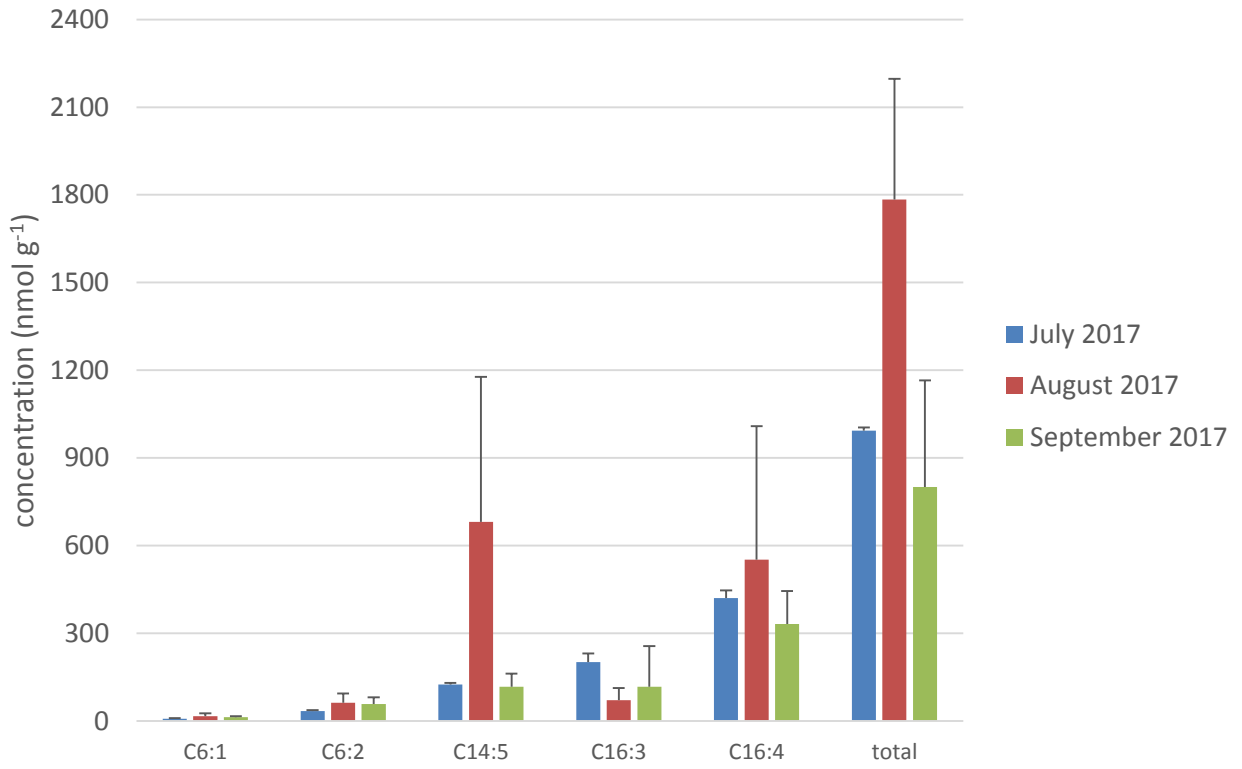


644

645

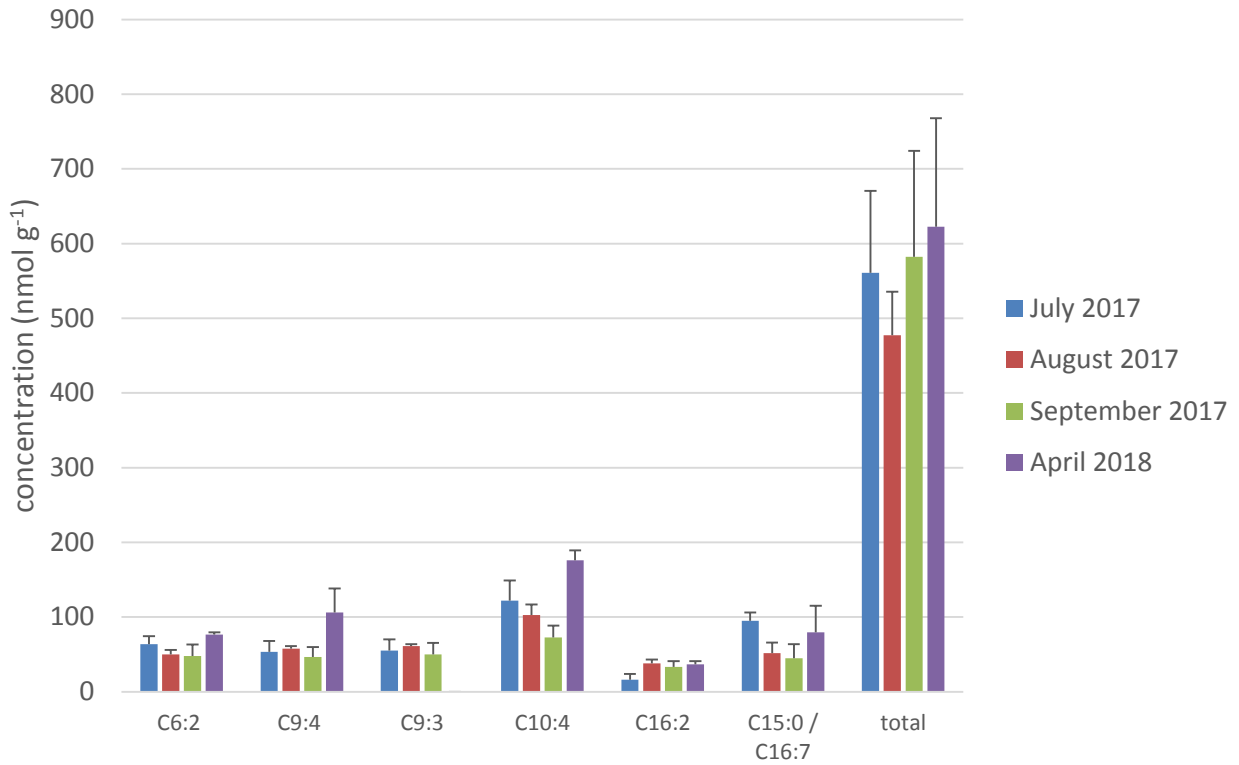
61
62
63
64
65

646 Figure 5

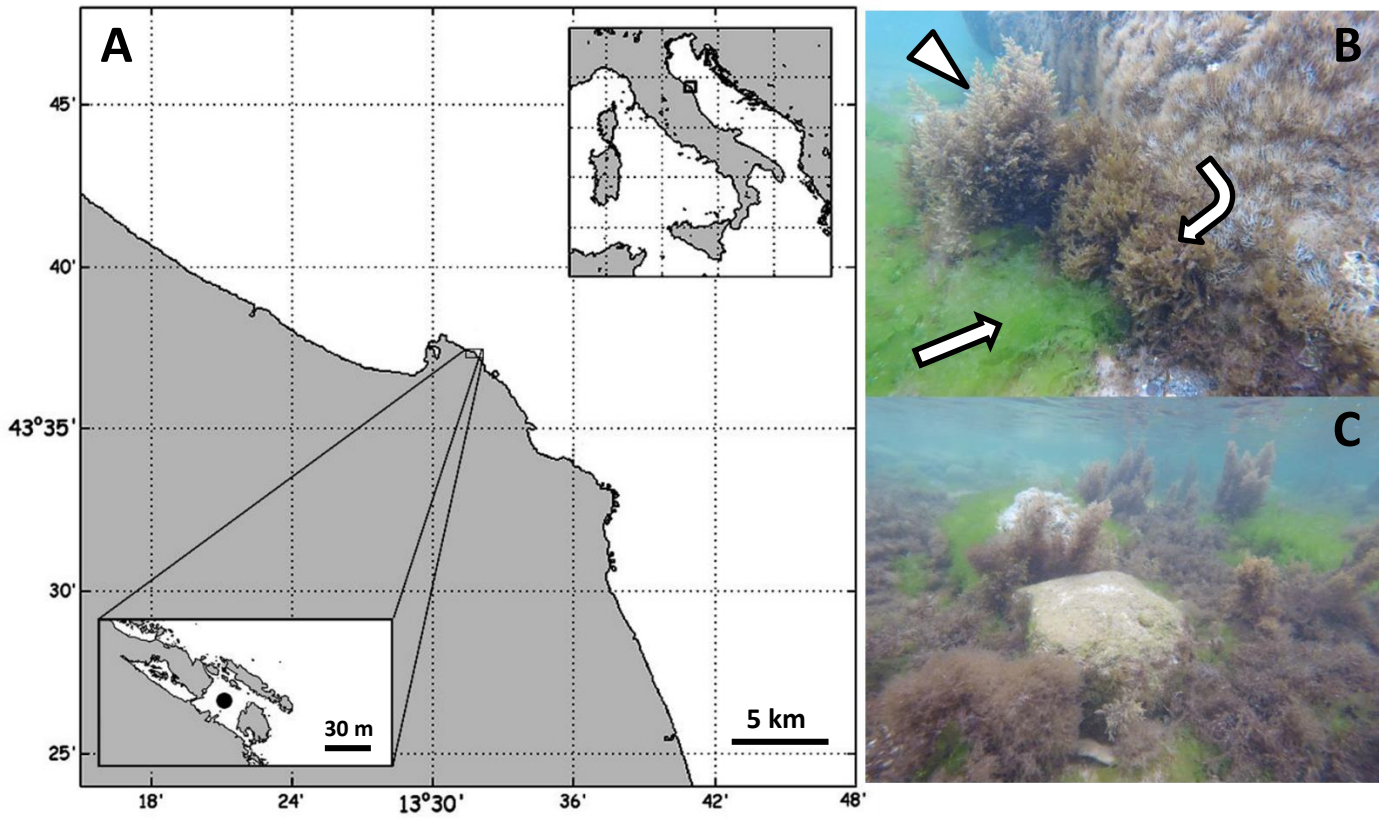


649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665

661 Figure 6



676 Figure 7



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65



Click here to access/download
Supplementary Material
supplementary.pdf