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

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

Laura Pezzolesi[1*], Stefano Accoroni[2], Fabio Rindi[2], Chiara Samorì[3], Cecilia Totti[2], Rossella Pistocchi[1]

[1] Dipartimento di Scienze Biologiche, Geologiche e Ambientali - Università di Bologna, via Sant'Alberto 163, 48123 Ravenna (Italy)

[2] Dipartimento di Scienze della Vita e dell'Ambiente - Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona (Italy)

[3] Dipartimento di Chimica "Giacomo Ciamician" - Università di Bologna, via Selmi 2, 40126 Bologna (Italy)

*Corresponding Author

Dr. Laura Pezzolesi

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

laura.pezzolesi@unibo.it

Abstract

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

Keywords

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

1. Introduction

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

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

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

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

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

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

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

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

2.4 Qualitative profile of PUAs produced by macroalgae

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

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

4.3.2 Dissolved aldehydes in seawater

Concentration of dissolved aldehydes was determined following the protocol described by Vidoudez and Pohnert (2008). After determination of the exact volume of the filtered seawater (about 2 L), 1 mL of a 25mM O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride solution (PFBHA HCl) in Tris–HCl 100 mM pH 7.2, and 50 µL of internal standard (benzaldehyde, 1 mM in methanol) were added. The solution was then immediately charged on a C18 cartridge (Waters sep-pak plus) at 250 mL h^{-1} for derivatizing the dissolved aldehydes. The derivatized aldehydes were eluted from the cartridge using 5 mL of 25 mM PFBHA in methanol. The eluates were incubated for 1 h at room temperature to ensure complete derivatization. PUA derivatives were then extracted with a mixture of 2:1:2 water:methanol:hexane and acidified with H_2SO_4, as described in (Wichard et al., 2005b). Samples were then treated and analyzed as previously described.

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Note

The authors declare no competing financial interest.

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570 Table 1 – Relative abundance (%) and total concentration (µg 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 (µg L^{-1})	26.3	17.2	24.1

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573

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

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^{-1}) 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^{-1}) by *Dictyopteris polypodioides* at the different sampling
595 times (data are mean ± st. dev. of 3 replicates).

596 Figure 6 - Aldehydes production (nmol g^{-1}) 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.

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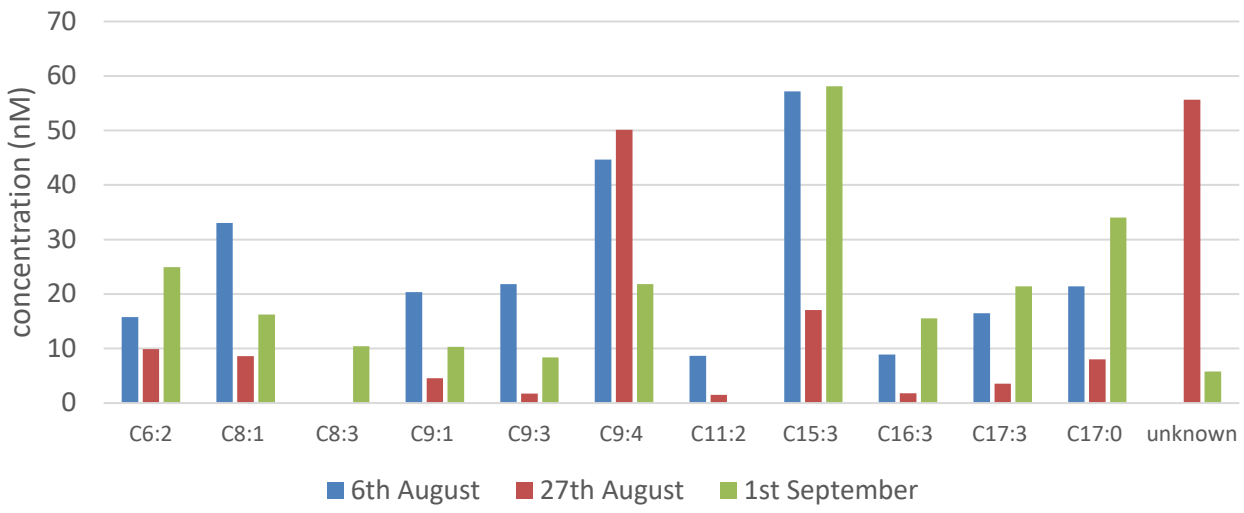
Figure 1


70
60
50
40
30
20
10
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concentration (nM)
C6:2
C8:1
C8:3
C9:1
C9:3
C9:4
C11:2
C15:3
C16:3
C17:3
C17:0
unknown
6th August
27th August
1st September

628 Figure 2

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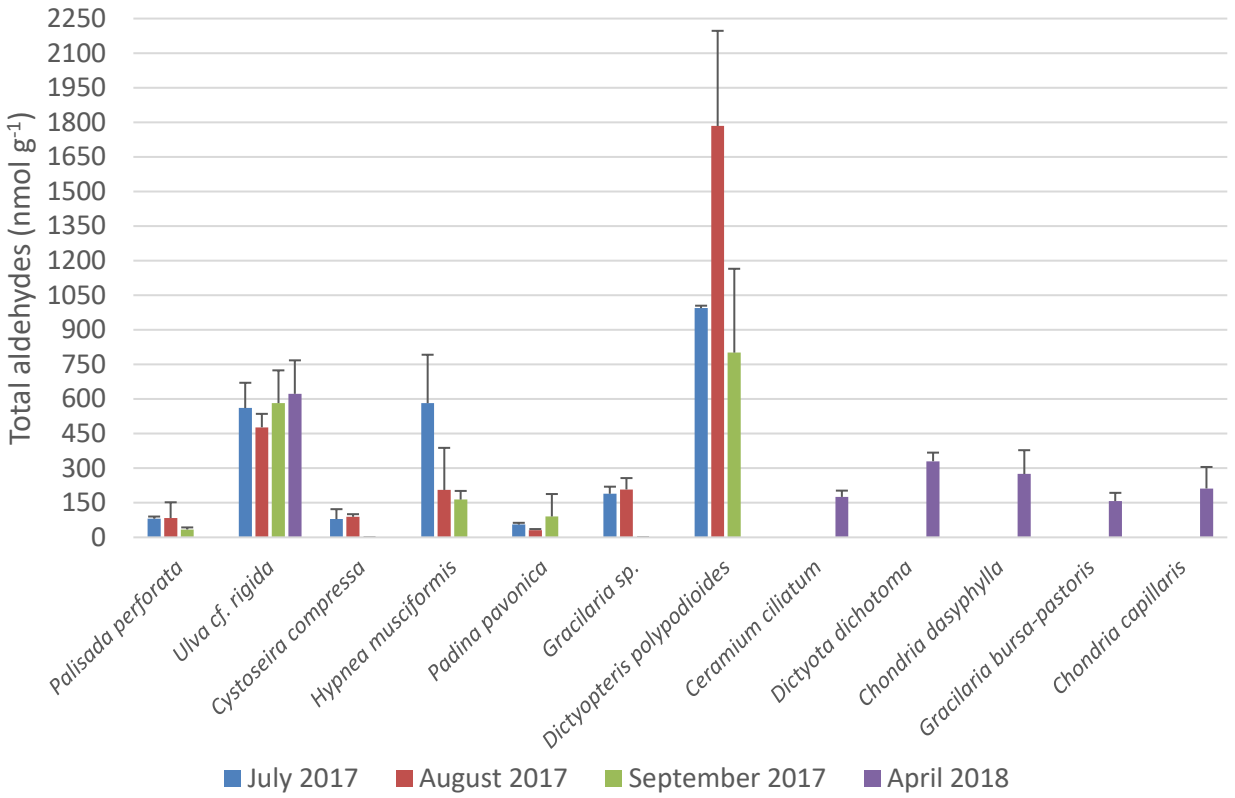

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639 Figure 3

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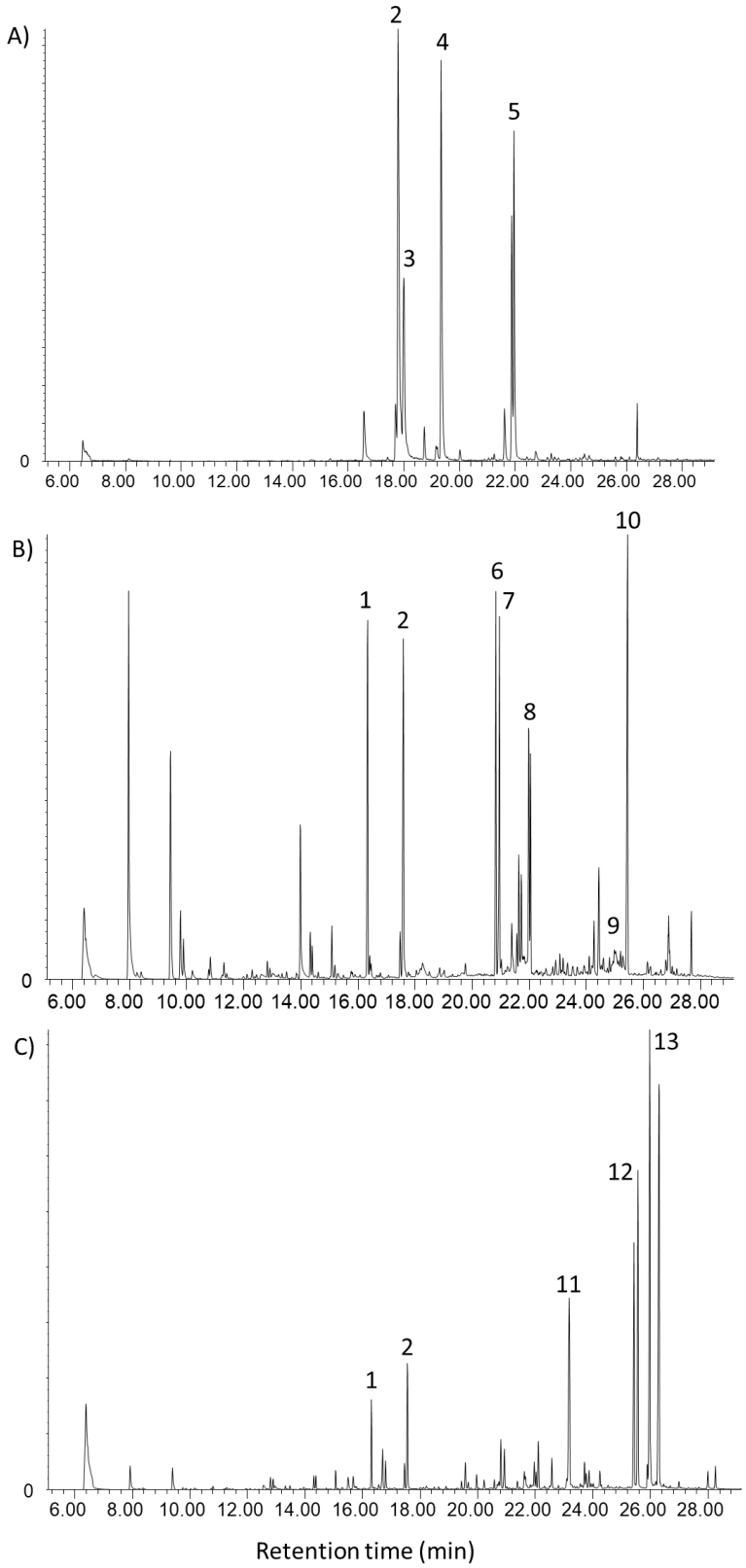

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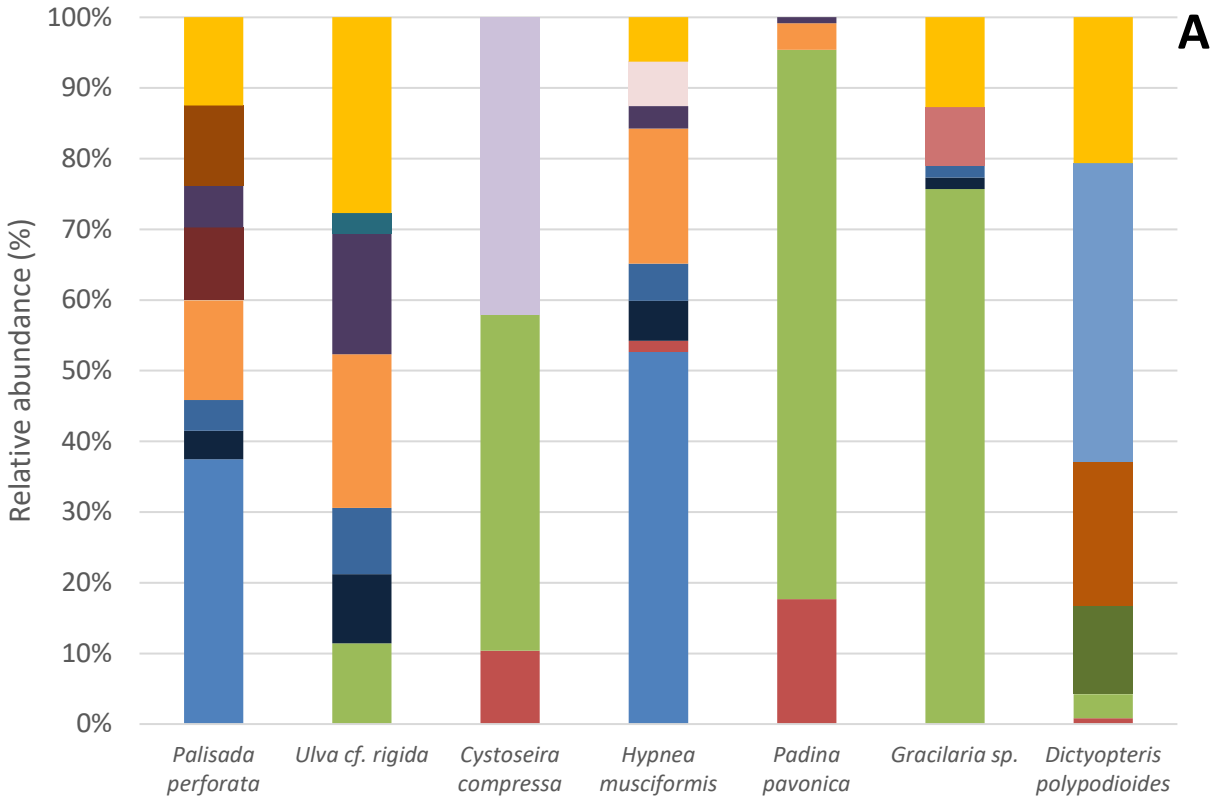
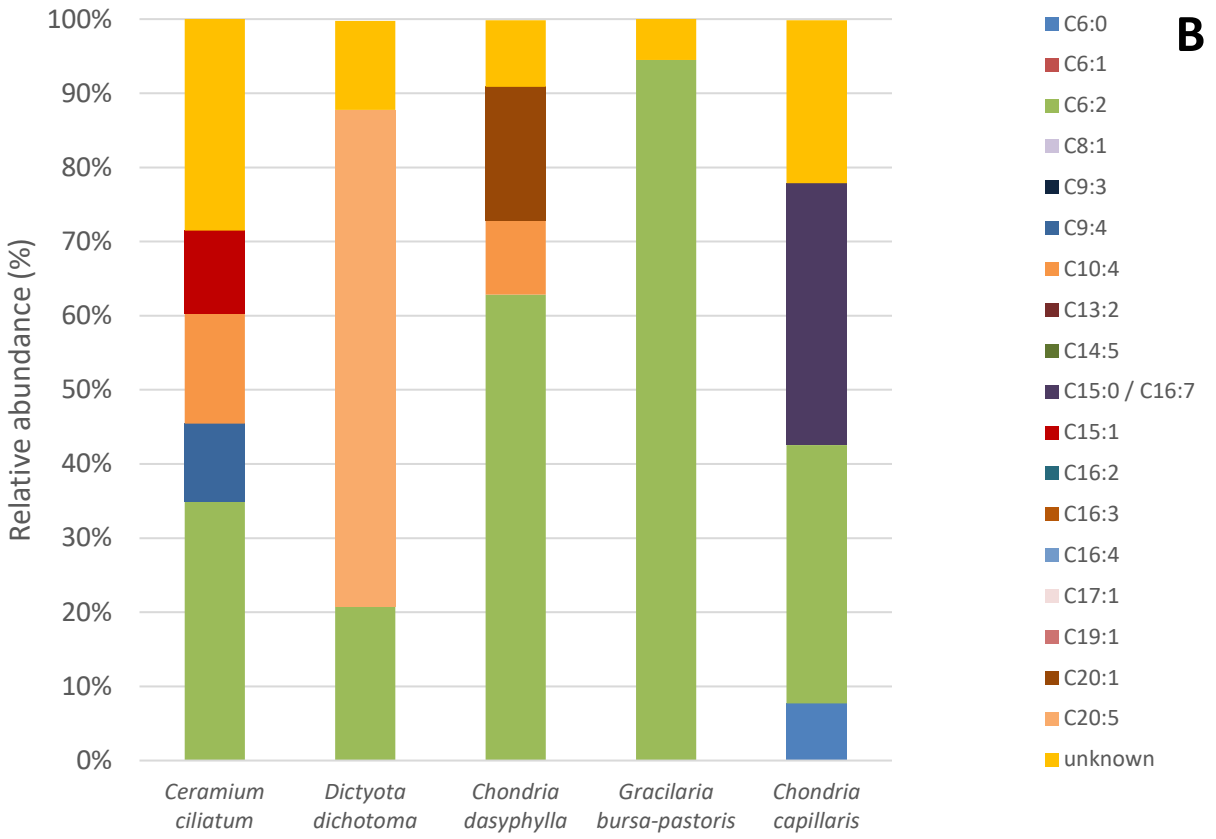
Figure 4


A
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance (%)
Palisada perforata
Ulva cf. rigida
Cystoseira compressa
Hypnea musciformis
Padina pavonica
Gracilaria sp.
Dictyopteris polypodioides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100%
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50%
40%
30%
20%
10%
0%
Relative abundance (%)
Ceramium ciliatum
Dictyota dichotoma
Chondria dasyphylla
Gracilaria bursa-pastoris
Chondria capillaris
C6:0
C6:1
C6:2
C8:1
C9:3
C9:4
C10:4
C13:2
C14:5
C15:0 / C16:7
C15:1
C16:2
C16:3
C16:4
C17:1
C19:1
C20:1
C20:5
unknown

Figure 5

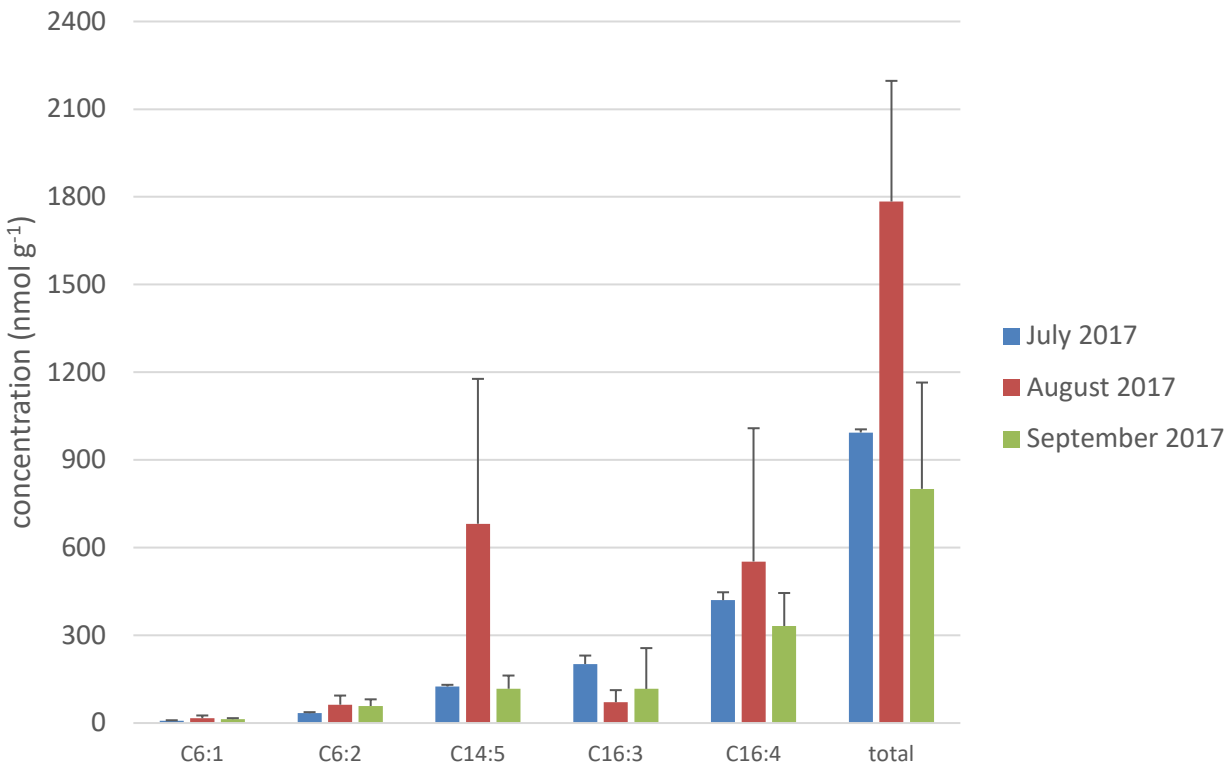
2400
2100
1800
1500
1200
900
600
300
0
concentration (nmol g-1)
C6:1
C6:2
C14:5
C16:3
C16:4
total
July 2017
August 2017
September 2017

Figure 6

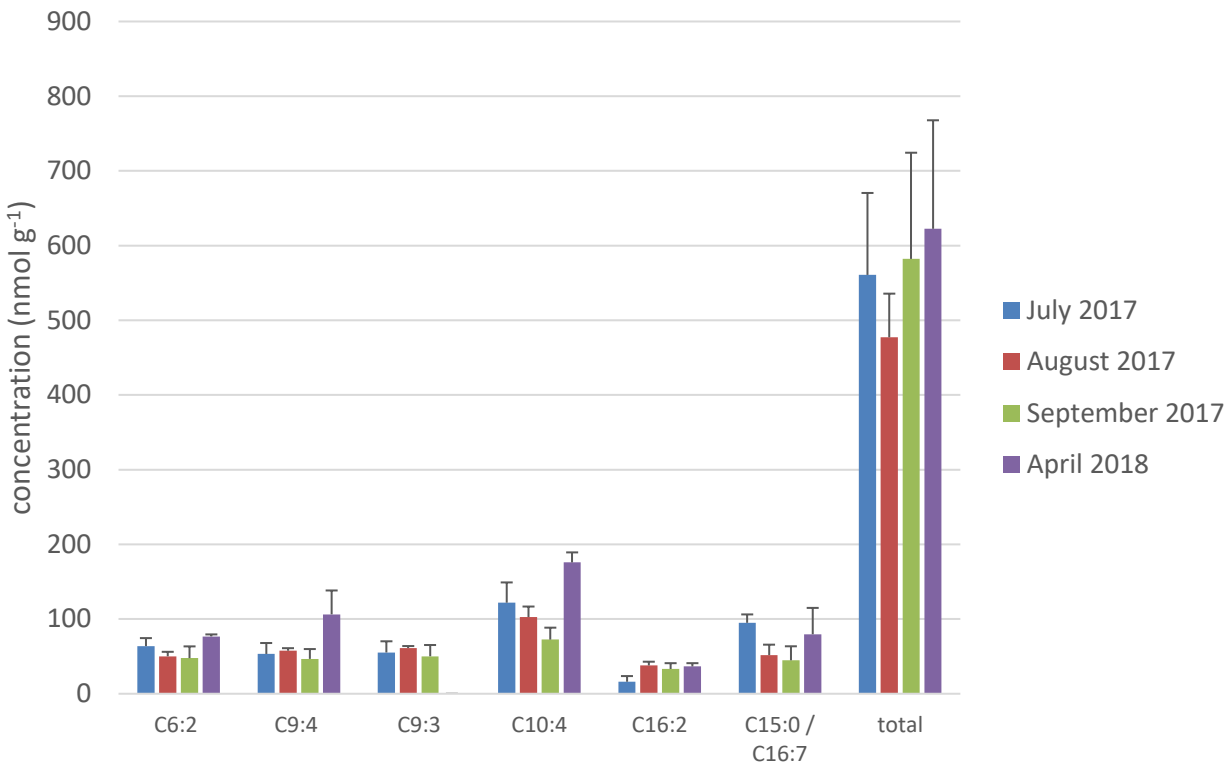
900
800
700
600
500
400
300
200
100
0
concentration (nmol g-1)
C6:2
C9:4
C9:3
C10:4
C16:2
C15:0 / C16:7
total
July 2017
August 2017
September 2017
April 2018

Figure 7