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Photo-protective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs)

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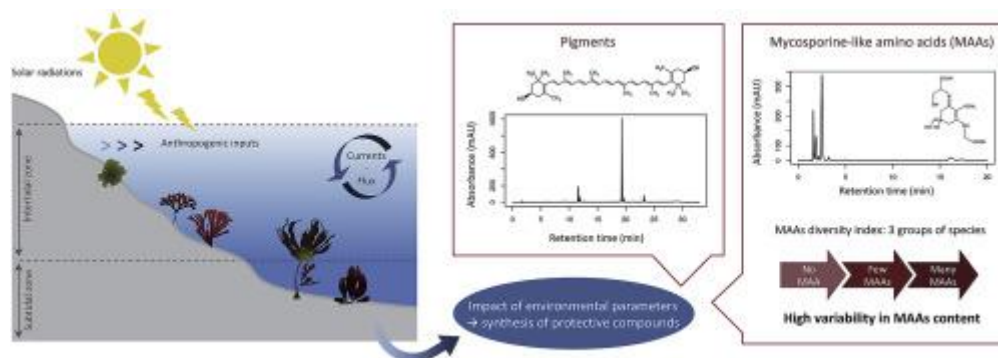
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Abstract :

To cope with the biotic and abiotic stresses experienced within their environment, marine macroalgae have developed certain defence mechanisms including the synthesis of photo-protective molecules against light and particularly harmful UV radiation. The aim of this study was to screen selected red algae, a highly diverse phylogenetic group, for the production of photo-protective molecules. The pigment content and composition (i.e. chlorophyll-a, phycobiliproteins and carotenoids) and the composition of mycosporine-like amino acids (MAAs) were studied in 40 species of red macroalgae collected in Brittany (France), at two distinct periods (i.e. February and July 2017). A high inter-specific variability was demonstrated in terms of pigment content and MAA composition. Twenty-three potential MAAs were detected by HPLC, and six were identified by LC-MS (i.e. shinorine, palythine, asterina-330, porphyra-334, usurijene and palythene). This is the first study to report on the composition of pigments and MAAs in a diverse group of red seaweeds from Brittany, including some species for which the MAA composition has never been studied before. Nevertheless, the results suggested that some species of red algae are more likely to cope with high levels of light radiation since those species such as *Bostrychia scorpioides*, *Porphyra dioica*, *Gracilaria vermiculophylla* and *Vertebrata lanosa* are living in environments exposed to higher levels of irradiation, and had various MAAs in addition to their photo-protective pigments.

Graphical abstract



Highlights

► Detection of 23 potential MAAs in 40 Rhodophyta species, including 6 already identified (shinorine, palythine, asterina-330, porphyra-334, usurijene, palythene). ► First report on the MAAs composition for some red seaweeds. ► High variability in MAAs content and composition between the different species, with no link with phylogeny, morphology, position on the shore or sampling site. ► A MAAs extraction method using 70% ethanol being less toxic than conventional methanol, and giving potential valorisable extracts.

Keywords : Algae, Diversity, HPLC, MAAs, Metabolites, Photo-protection, Pigments, Rhodophyta, Screening

25 1. Introduction

26 Macroalgae are known to play a key role in coastal ecosystems as being at the
27 basis of the food web and providing refugium for many animal species (Lüning, 1990).
28 In particular, seaweeds produce diverse compounds under natural conditions, which
29 could potentially be used in health, cosmetic, or food sectors (Holdt and Kraan, 2011;
30 Stengel et al., 2011; Stiger-Pouvreau and Guérard, 2018; Surget et al., 2017). However,
31 both the concentration and composition of these bioactive compounds can fluctuate
32 within individuals, with their habitat and with season. In the context of climate change,
33 the environmental parameters impacting particularly intertidal seaweeds are in a state of
34 flux. To cope with changing environmental conditions, seaweeds have developed
35 particular adaptive, metabolic responses and are thus able to resist and adapt to different
36 abiotic stresses (Davison and Pearson, 1996; Gantt, 1990).

37
38 Irradiance is one of the factors which can change over the intertidal range in
39 coastal areas, in term of quantity and quality because it varies during the day and with
40 the tide, the turbidity of the water, the climate, the depth or seasons (Sagert et al., 1997).
41 Light is also very important for seaweeds as algae are photosynthetic organisms (Gantt,
42 1990; Lobban and Harrison, 1994) with some living in the intertidal zone and thus
43 exposed directly to the sunlight during emersion phases. One of the consequences of
44 climate change is the amount of ultraviolet (UV) radiation reaching the Earth's surface
45 (Bischof et al., 2006; Thomas et al., 2012). UV radiations are notably at the origin of
46 DNA alterations, the synthesis of reactive oxygen species (ROS) and, in seaweeds, the
47 photo-inhibition mechanism or the degradation of photosynthetic pigments (Karentz,
48 2001; Karsten, 2008; Van de Poll et al., 2001). However, due to human activities and

49 ozone depletion, the protective filter that constitutes the atmosphere gradually loses its
50 effectiveness, which means that the amount of UV to which the organisms are exposed
51 tends to increase (Carreto et al., 2018; McKenzie et al., 2007). Although some authors
52 agree that the ozone layer will probably not fully recover before several decades, the
53 hole in this layer would be resorbing at mid-latitudes due to the implementation of some
54 policies tending to reduce greenhouse gas emissions, but this would not be the case at
55 high latitudes and the evolution of the UV level remains complex (McKenzie et al.,
56 2011; Wilmouth et al., 2018).

57
58 Different types of macroalgae have developed various strategies to protect
59 themselves from harmful UV radiation. These include the synthesis of photo-protective
60 molecules (Bhatia et al., 2011; Le Lann et al. 2016; Rastogi et al., 2010; Sinha et al.,
61 2007). Among the algal photo-protective compounds, this study focuses on pigments
62 and mycosporine-like amino acids (MAAs), synthesized by red algae (Rhodophyta).
63 MAAs were discovered in 1965 in the terrestrial fungus *Ascochyta pisi* (Leach, 1965).
64 Since then, the presence of MAAs has been demonstrated in a wide range of marine
65 organisms from bacteria to fish (Bandaranayake, 1998; La Barre et al., 2014; Rosic et
66 al., 2015), including different species of algae (Bedoux et al., 2014; Sinha et al., 2007).
67 MAAs are low molecular weight molecules (< 400 Da), soluble in water, with a high
68 molar extinction coefficient (between 28,000 - 50,000 M⁻¹.cm⁻¹) and a maximum of
69 absorption (λ_{\max}) between 310 - 362 nm (Rastogi et al., 2010). In addition, it has been
70 demonstrated that red algae, which constitute a well-marked phylogenetic group, have
71 the greatest diversity and the highest proportion of MAAs (Carreto and Carignan, 2011;
72 Karentz, 2001; Sinha et al., 2007). Until now, about 20 MAAs have been identified in

73 various red macroalgae (Sinha et al., 2007). However, as some recent studies have
74 shown (Briani et al., 2018; Hartmann et al., 2016), many MAAs have yet to be
75 identified. The major role of MAAs in photo-protection against UV radiation has
76 already been widely demonstrated (Bedoux et al., 2014; Huovinen et al., 2004; Singh et
77 al., 2008), and functionality could be linked to their individual structures (Wolley et al.,
78 2018). It appears that some MAAs may also have various functions such as anti-
79 oxidants (De la Coba et al., 2009; Torres et al., 2018; Wada et al., 2015), osmolytes,
80 nitrogen storage, or protective agents against desiccation or temperature variations
81 (Bhatia et al., 2011; Oren and Gunde-Cimerman, 2007).

82

83 Pigments are part of a second significant group of photo-protective compounds
84 in macroalgae. There are three main families of pigments: chlorophylls, carotenoids and
85 phycobiliproteins (reviewed in Stengel et al., 2011). They do not all have a role in
86 photo-protection, such as chlorophylls and phycobiliproteins which harvest light for
87 photosynthesis. Among the chlorophylls, there is only chlorophyll-*a* in red algae
88 (Stengel et al., 2011). One characteristic of red algae is the presence of
89 phycobiliproteins (reviewed by Dumay et al., 2014). These are “secondary pigments”
90 which also capture solar energy and transfer it to chlorophyll *a* for the photosynthesis;
91 they are particularly effective under low light conditions (Gantt, 1990; Lobban and
92 Harrison, 1994). There are 3 main groups of phycobiliproteins in the Rhodophyta:
93 phycoerythrin (PE, $\lambda_{\max} = 540\text{-}570$ nm) which provides their red colour, phycocyanin
94 (PC, $\lambda_{\max} = 610\text{-}620$ nm) and allophycocyanin (APC, $\lambda_{\max} = 650\text{-}655$ nm) which both
95 have a blueish hue (Dumay et al., 2014). Finally, there are two groups of carotenoids:
96 carotenes (α -carotene, β -carotene in red algae) and xanthophylls (i.e. zeaxanthin,

97 antheraxanthin, violaxanthin and lutein in red algae) which convey a yellowish
98 colouration. These last pigments have several physiological roles (Karsten, 2008;
99 Young and Frank, 1996): they take part in photosynthesis through light harvesting in
100 thylakoids (Hashimoto et al., 2016) but they also have an important role in photo-
101 protection (Mimuro and Akimoto, 2003). Indeed, they also may participate in the
102 dissipation of solar energy (Young et al., 1997) and to the de-activation of reactive
103 oxygen species (ROS) (Rastogi et al., 2010).

104 According to the nature, quality and quantity of photo-protective compounds,
105 and also their morphology (blade, filamentous, crustose) and life cycle, different species
106 of macroalgae are then more or less sensitive to UV radiation (Davison and Pearson,
107 1996; Roleda et al., 2004). In this context, the aim of the present study was to
108 investigate the inter-specific variations in red algal photo-protective compounds within
109 a diverse, representative group of temperate and local red seaweeds. Thus, a screening
110 was carried out on a large number of red seaweeds sampled from the coast of Brittany
111 (France). In order to study the maximum number of species, seaweeds were sampled
112 during two distinctly different seasons, i.e. winter and summer, as some species occur
113 only during a part of the year. Candidate species for analysis were also collected from
114 the upper subtidal zone (i.e. emersed only at spring tides for maximum 1 h; Ar Gall and
115 Le Duff, 2014) and along the intertidal zone (i.e. with different maximum emersion
116 times from 1 h in the lower intertidal zone to 12 h in the upper intertidal zone during
117 spring tides; Ar Gall and Le Duff, 2014). A MAA diversity index was determined for
118 each species, in order to compare and provide hierarchical comparisons within the red
119 macroalgae collected in this study. Moreover, in order to test the hypotheses of a
120 relationship between the diversity of MAAs and individual red algal taxonomy, various

121 orders/families of red macroalgae were specifically targeted for collection.

122

123 **2. Materials and methods**

124 *2.1 Sampling sites*

125 Macroalgae were collected at low tides on four different sites in Brittany (France):

126 Porspoder (48°28'58''N – 4°46'5''W), Portsall (48°33'53''N – 4°42'5''W), Saint-Pabu

127 (48°34'34''N – 4°38'45''W) and Le Faou (48°17'44''N – 4°10'56''W). Portsall,

128 exposed North-West, is a sheltered and mainly rocky site, surrounded by sand and

129 intertidal pools. Despite its western exposure, Porspoder remains sheltered due to the

130 presence of two large rocky over-hangs. The site chosen at Saint-Pabu, was exposed to

131 the North; it is characterized by sandy dunes. Finally, Le Faou differs from the three

132 previous sites because a river flows there into the Bay of Brest, forming a sheltered but

133 muddy estuary at low tide. Two samplings periods were carried out: in February

134 (winter) and July (summer) 2017.

135

136 *2.2 Macroalgal samples*

137 Forty species of red macroalgae were collected across the four different sites: 21 species

138 were found both in winter and summer and 19 species were found on the shore only for

139 one season. All species belong to the class of Florideophyceae, except *Porphyra dioica*

140 (Bangiophyceae). These included species from different morpho-anatomical groups (i.e.

141 MAG, according to Steneck and Dethier, 1994): filamentous algae (MAG 2); corticated

142 or polysiphonous filaments algae (MAG 2.5); foliose algae (MAG 3); corticated foliose

143 algae (MAG 3.5); corticated algae (MAG 4); and articulated calcareous algae (MAG 6)

144 (Appendix 1).

145 After collection, algal samples were brought back to the laboratory into sampling bags.
146 Samples were stored in a fridge and washed within 4 hours of sampling with filtered
147 seawater to remove residual sediments and salt; visible epiphytes were removed by
148 hand. Seaweeds were then stored in the freezer before being freeze-dried (β 1-8 LD plus
149 Christ) and the entire thallus was milled into a fine powder (MM400 Retsch). The dried
150 powder was kept in darkness at room temperature until analyses.

151

152 *2.3 Extraction and assay of chlorophyll-a and carotenoids*

153 Pigments were extracted from 75 mg dry weight (DW) of algal powder in
154 750 μ L of 90% acetone, according to Schmid and Stengel (2015). Two successive
155 extractions of 30 min and 4 h were carried out at 4 °C, under magnetic agitation.
156 Samples were then centrifuged at 10,000 rpm for 5 min (miniSpin plus, Eppendorf), and
157 the supernatants combined and filtered for HPLC (High Pressure Liquid
158 Chromatography) analysis (Dionex Ultimate 3000, ThermoScientific). Pigments were
159 separated using an ACE C₁₈ column (150 x 4.6 mm, 3 μ m) with a guard-column, an
160 injection volume of 6 μ L and a run-time of 33 min per sample. Before injection, each
161 sample was automatically diluted $\frac{3}{4}$ with ammonium acetate buffer (0.5 M, pH 7.2).
162 Separation was achieved using a solvent gradient described in Table 1, delivered at a
163 flow rate of 1.0 mL.min⁻¹. A photo-diode array detector (DAD3000, ThermoScientific)
164 was used for the detection of pigments at 435, 470 and 650 nm. The identification and
165 quantification of each pigment was based on spectral comparisons and calibration using
166 commercial standards: chlorophyll *a* (Sigma, USA), and α -carotene, β -carotene, lutein,
167 zeaxanthin, antheraxanthin, violaxanthin (DHI, Denmark). Only peaks with an area
168 larger than 0.4 mAU.min for α - and β -carotenes and 1 mAU.min for the other pigments

169 were considered in the analysis. HPLC data were collected using Chromeleon 0.7
170 software (Thermo Scientific Dionex, France).

171 *2.4 Extraction and assay of phycobiliproteins*

172 Tissue concentrations of phycoerythrin (PE) and phycocyanin (PC) were
173 determined using a method adapted from Sun et al. (2009). Two successive extractions
174 of 15 min at 4 °C were performed from 75 mg DW of algal powder, with 1.5 mL of
175 phosphate buffer (0.1 M, pH 6.8). Subsequently, samples were centrifuged for 20 min at
176 10,000 rpm (miniSpin plus, Eppendorf) and supernatants were used to measure
177 absorbances at 455, 565, 592, 618 and 645 nm using a spectrophotometer (POLARStar
178 Omega, BMG LABTECH). The concentrations of PE and PC ($\text{mg}\cdot\text{g}^{-1}$ DW) were
179 estimated using the following equations from Beer and Eshel (1985):

$$180 \text{ [PE]} = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$181 \text{ [PC]} = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] \times 0.15$$

182

183 *2.5 Mycosporine-like Amino Acids (MAAs) extraction and assay*

184 20 mg DW of finely ground algae were extracted for 2 h with 2 mL of 70%
185 aqueous ethanol (v/v) at 45 °C with magnetic stirring. After centrifugation (centrifuge
186 5810, Eppendorf) at 1,500 rpm for 10 min, the supernatant was recovered. The pellet
187 was re-extracted twice following the same procedure and the combined supernatants
188 were evaporated to dryness under vacuum (miVac, Genevac, France) at 45 °C. The
189 evaporated extracts were then stored at -20 °C before analysis.

190 Prior to HPLC analysis, extracts were dissolved in 500 μL of 2.5% aqueous
191 methanol with 0.1% acetic acid, centrifuged for 5 min at 10,000 rpm (miniSpin plus,

192 Eppendorf), and filtered through 0.2 μm -pore syringe filters. MAAs analyses were
193 performed using a Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany)
194 equipped with a diode-array detector (DAD). MAA separation was performed using a
195 Zorbax Eclipse XDB C₁₈ column, 5 μm , 4.6 \times 250 mm (Agilent, USA) equipped with a
196 guard-column. For one analysis, 20 μL of extract were injected and the operating
197 parameters were as follow: 0.1% acetic acid in Milli-Q water as the mobile phase; a
198 flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$; a column temperature of 25 ± 1 $^{\circ}\text{C}$; an injector temperature of
199 5 ± 1 $^{\circ}\text{C}$; a run time of 20 min. MAAs were detected at 320, 330, 334 and 360 nm.
200 HPLC data were collected using Chromeleon 0.7 software (Thermo Scientific Dionex,
201 France). Individual peaks were identified by online absorption spectra and retention
202 time (Rt).

203

204 For identification, MAAs extracts were also analyzed by LC-MS using a LC-
205 ESI-Q-TOF-MS (Dionex, Ultimate 3000, Bruker, micrOTOF-QII) system (Bruker
206 Daltonik GmbH, Bremen, Germany). The same LC method was used, only the flow was
207 reduced to 0.5 $\text{mL}\cdot\text{min}^{-1}$. Source parameters were: positive mode; source temperature,
208 200 $^{\circ}\text{C}$; capillary voltage, 4.5 kV; nebulizer gas (N_2) at 2.8 bars and dry gas (N_2) at 12
209 $\text{L}\cdot\text{min}^{-1}$. Mass spectra acquisition was set at 0.5 Hz from m/Z 50 to 1000. MS/MS
210 analyses were performed with a collision energy of 30 eV and an isolation width of 2
211 m/Z . All raw data were collected with Compass dataAnalysis Version 4.1. The
212 quantification of the identified MAAs was accomplished using their molar extinction
213 coefficients ϵ at the wavelengths of maximum absorption, according to Pelillo et al.
214 (2004), and the molar extinction coefficient of Karsten et al. (1998b). For MAAs whose

215 molecular structure has not yet been elucidated, their quantification was not established.

216 Each MAA was then numbered in the order in which it appeared during the run.

217

218 Finally, the diversity of MAAs within each seaweed species tested was
219 estimated by the development of a new index. The presence and absence of each MAA
220 for each species was coded in a table, as 0 or 1 respectively. A distance matrix was then
221 calculated by comparing species two by two using the following index:

$$Index = \frac{M}{M + N}$$

222 where 'M' is the number of matches between the two species (presence and absence) and
223 'N' the total number of MAAs which were present in only one of the two species. The
224 closer the index was to 1, the more similar the two species were in terms of the
225 composition of their MAAs. A species with many weak indices thus represented a
226 specific composition. A dendrogram was made from this matrix with the R program (R
227 development core team, 2008) to represent species that had similar MAA compositions
228 (hclust with single method, hclust package). In order to highlight any links between the
229 composition of MAAs by algal species with their phylogeny, a phylogenetic analysis
230 was performed using *rbcL* and *cox1* genes sequences, as retrieved from GenBank
231 (Accession numbers for GenBank sequences are listed in Appendix 1 and are available
232 on <https://www.ncbi.nlm.nih.gov>). Sequences were aligned using the ClustalW
233 programme in the BioEdit alignment editor (Hall, 1999). A phylogenetic tree was built
234 using MEGA version 7 (Kumar et al., 2016), based on the Neighbor-Joining clustering
235 method. Evolutionary distances were computed using the Maximum Composite
236 Likelihood. The tree was subjected to a bootstrap test (5000 replicates) to estimate
237 robustness at each branch (pvclust, pvclust package).

238

239 *2.6 Statistical analyses*

240 All data were statistically analyzed with the R program (R development core team,
241 2008). All extractions were performed in triplicate, and results expressed as average \pm
242 standard deviation (SD). Comparisons were carried out using a Student's t-test (2
243 samples) or an ANOVA (more than 2 samples). Beforehand, the conditions of
244 application were controlled using the Shapiro test for compliance with a normal
245 distribution, and the F-test or Bartlett's test for the homogeneity of variance (Dytham,
246 2011). The non-parametric Wilcoxon test (2 samples) or Scheirer-Ray-Hare test (SHR,
247 more than 2 samples) were used when at least one of the conditions of application was
248 not met (to replace the t-test or the ANOVA, respectively). If possible, when significant
249 results were highlighted, *post-hoc* tests (i.e. multiple comparisons) were performed: a
250 Tukey *post-hoc* test after an ANOVA (TukeyHSD), or a non-parametric *post-hoc* test
251 after a Scheirer-Ray test (kruskalmc, pirms package) (Dytham, 2011). Principal
252 Component Analysis (PCA) was carried out (package FactoMineR) to highlight species
253 which stood out.

254

255 **3. Results**256 *3.1 Pigments: chlorophyll a, carotenoids and phycobiliproteins*

257 The chlorophyll *a* content (Fig. 1) varied significantly among the analyzed red algal
258 species (SHR test, p -value < 0.0001). In some species, such as *Mastocarpus stellatus* or
259 *Furcellaria lumbricalis*, chlorophyll *a* concentrations did not exceed $1 \text{ mg.g}^{-1} \text{ DW}$ -
260 whatever the season. However, *Callithamnion tetragonum* and *Plumaria plumosa* for
261 example contained in winter 8.60 ± 0.26 and $8.37 \pm 0.21 \text{ mg.g}^{-1} \text{ DW}$ of chlorophyll *a*,

262 respectively. Moreover, a seasonal variability was demonstrated with chlorophyll *a*
263 concentrations decreasing in summer (SHR test, p-value = 0.0040). For example,
264 *Palmaria palmata* contained 7.02 ± 0.31 mg.g⁻¹ DW of chlorophyll *a* in February, and
265 1.50 ± 0.27 mg.g⁻¹ DW in July. Conversely, *Bostrychia scorpioides* was the only
266 species with a significantly increased concentration in summer (i.e. 5.44 ± 0.43 , as
267 compared to 4.32 ± 0.09 mg.g⁻¹ DW in winter).

268 Similarly, total concentration of carotenoids changed with species (SHR test, p-value <
269 0.0001) (Fig. 2). For example, *Chondrus crispus* and *F. lumbricalis* contained less than
270 0.2 mg.g⁻¹ DW of carotenoids, whereas *Vertebrata lanosa*, *Porphyra dioica* or *B.*
271 *scorpioides* contained up to 0.78, 0.84 and 0.65 mg.g⁻¹ DW of total carotenoids,
272 respectively. A difference in term of composition was also observed (Fig. 2): overall,
273 lutein was the most common carotenoid in 31 species out of 40. However, the amount
274 of lutein remained low as compared to chlorophyll *a*, which constituted 90.1% on
275 average of the total liposoluble pigment content. Moreover, there was no seasonal
276 variation in the carotenoid composition as a whole, but the concentration was seen to
277 decrease in summer (SHR test, p-value = 0.0012) (Fig. 2), except for *V. lanosa* in which
278 the concentration increased (e.g. 0.78 ± 0.01 and 0.66 ± 0.04 mg.g⁻¹ DW in summer and
279 winter, respectively). *Gigartina pistillata*, *Metacallophyllis laciniata* and
280 *Membranoptera alata* did not contain any detectable levels of carotenoids in summer.
281 However, although there were seasonal variations in chlorophyll *a* and overall
282 carotenoid amounts, the ratio of carotenoids / chlorophyll *a* appeared to be constant
283 between both seasons (SHR test, p-value = 0.4919) (data not shown).

284 The different studied red algae contained variable levels of phycobiliproteins
285 similarly to their chlorophyll *a* or carotenoid amounts (SHR test, p-value < 0.0001)

286 (Fig. 3). For example, *Bornetia secundiflora* and *Membranoptera alata* contained 27.39
287 ± 3.13 and 19.67 ± 0.15 mg.g⁻¹ DW of PE in winter, respectively, whereas *C. crispus*,
288 *Mastocarpus stellatus* or *V. lanosa* contained less than 5.0 mg.g⁻¹ DW (Fig. 3).
289 *P. plumosa* was more differentiated from the other species with high levels of PE in
290 both seasons (23.18 ± 0.49 mg.g⁻¹ DW in winter and 13.58 ± 1.36 mg.g⁻¹ DW in
291 summer). Moreover, it would appear that, on the whole, the PE content decreased in
292 summer (SHR test, p-value < 0.0001). *Bostrychia scorpioides* was the only species for
293 which the PE concentration really increased in summer (6.62 ± 0.22 mg.g⁻¹ DW in
294 summer, compared to 5.32 ± 0.50 mg.g⁻¹ DW in winter). The results were similar for
295 phycocyanin (data not shown).

296

297 A Principal Component Analysis (PCA) of all pigments (Fig. 4a, 4b) pointed out
298 six species: *B. scorpioides*, *Porphyra dioica*, *V. lanosa*, *Gracilaria vermiculophylla*,
299 *Gracilaria gracilis* and *Plumaria plumosa*. This last species contained the highest
300 concentration of chlorophyll *a* and phycoerythrin. The five other species were the only
301 ones containing zeaxanthin with a concentration of 0.5 $\mu\text{g.g}^{-1}$ DW or greater. *G. gracilis*
302 also contained antheraxanthin. Finally, the species belonging to similar MAG showed
303 different pigment content (chlorophyll *a*, carotenoids and phycoerythrin) and
304 composition. Moreover, no link between pigment composition of the red seaweeds
305 studied here and their phylogeny, morphology, position on the shore or sampling site
306 could be demonstrated.

307

308 3.2 *Mycosporine-like Amino Acids*

309 HPLC analyses showed 23 different peaks that may correspond to MAAs (Fig. 5
310 and Appendix 2), and six (i.e. shinorine, palythine, asterina-330, porphyra-334,
311 usujirene and palythene) were elucidated by LC-MS. The composition and
312 concentration of various MAAs showed a difference between the various species of red
313 macroalgae sampled in this study. These observations were independent of the sampling
314 season. Amongst the 21 species of algae commonly occurring in both seasons
315 considered, six did not contain any MAA (these included: *F. lumbricalis*,
316 *Metacallophyllis laciniata*, *Polyides rotunda*, *Osmundea pinnatifida*, *Membranoptera*
317 *alata* and *Plumaria plumosa*). For the eight species which could be collected only in
318 summer, no MAAs were detected in four and for those harvested only in winter, five did
319 not contain any detectable MAAs. At the same time, *Mastocarpus stellatus* contained a
320 high concentration of only shinorine (i.e. 3.12 ± 0.26 and 1.62 ± 0.07 mg.g⁻¹ DW,
321 respectively in winter and in summer). One should note that three species, i.e. *Palmaria*
322 *palmata*, *B. scorpioides* and *G. vermiculophylla*, contained eight different MAAs,
323 visible as separate peaks on the chromatograms (data not shown). A chromatogram of
324 the MAAs analysed from *P. palmata* (Fig. 6) showed the presence of six identified
325 MAAs (i.e. shinorine, palythine, asterina-330, porphyra-334, usujirene and palythene)
326 and two other molecular structures that may correspond to MAAs. Moreover, some
327 unidentified MAAs were present in only one algal species, such as *B. scorpioides*,
328 which contained four unidentified (new) MAAs (i.e. MAA_4, MAA_13, MAA_18 and
329 MAA_23), *G. pistillata* which had MAA_14 and MAA_22, and *V. lanosa* MAA_1
330 (Fig. 5).

331

332 Based on HPLC chromatograms, shinorine was the predominant MAA identified
333 among all sampled red macroalgae in this study. Indeed, with the exception of
334 *V. lanosa*, shinorine was the only MAA common to all of those species which appeared
335 able to produce MAAs. Shinorine was found to be in sizeable quantities in *M. stellatus*,
336 *G. gracilis*, and *Ceramium nodulosum* with 3.12 ± 0.26 , 4.00 ± 0.06 and 1.92 ± 0.11
337 mg.g^{-1} DW, respectively for species harvested in winter (February). These amounts
338 were approximately halved in July with 1.62 ± 0.07 , 1.47 ± 0.10 and 0.86 ± 0.06 mg.g^{-1}
339 DW, respectively for the same algae (t test, p-value = 0.0004). The next most common
340 MAA was palythine which was found in 19 different species.

341 Moreover, no large modifications in the level of MAAs (Wilcoxon test,
342 p-value = 0.7804) and their composition was observed between both sampling periods
343 in the different species (Fig. 5). However, some changes were noticed such as in *O.*
344 *pinnatifida* with the presence of asterina-330, usujirene and palythene in those thalli
345 collected in winter (February), but not in those collected in summer (July). The same
346 was observed for *G. pistillata* with the presence of two new MAAs (i.e. MAA_14 and
347 MAA_22) in the winter samples.

348

349 In winter and summer, the analysis of the diversity indices for the MAAs
350 highlighted three groups of species (Fig. 7A and Appendix 3A): those with no MAA
351 (Group 1), those with few MAAs (between 1 and 5; Group 2) and those with many
352 MAAs (6 or more; Group 3). Only *B. scorpioides* seemed to stand out and did not
353 correspond to any group, even though it contained eight different MAAs. Shinorine was
354 present in all species of Groups 2 and 3, except in *V. lanosa* which was also the only
355 species having MAA_1. Moreover, asterina-330 and palythine were also two MAAs

356 found in some species from Group 2, and in all species from Group 3 (with the
357 exception of *V. lanosa* and *B. scorpioides*). Finally, there were some MAAs which were
358 only found in Group 3, notably usujirene and palythene which were present in six of the
359 nine species of the Group 3 in winter. The high diversity in MAAs did not seem to be
360 related to phylogeny (Fig. 7B and Appendix 3B), anatomy and morphology, the height
361 on the shore nor the sampling site (Appendix 1). Hence, species which are close
362 phylogenetically (i.e. *G. vermiculophylla* and *G. gracilis*, or *Callithamnion tetricum* and
363 *C. tetragonum* in our study), morphologically (*M. stellatus* and *C. crispus*), or the only
364 two species harvested from a muddy habitat (i.e. *B. scorpioides* and *G. vermiculophylla*)
365 did not have the same MAAs profile (see Appendix 1 and Fig. 5). No link was also
366 found between the MAAs and the fact that a species was introduced or indigenous to
367 Brittany (data not shown).

368

369 4. Discussion

370 As intertidal macroalgae are organisms directly exposed to solar radiation, they
371 have developed some protective mechanisms in order to survive, such as the synthesis
372 of photo-protective compounds. In this sense, the objective of this study was to collect
373 many different red algal species from a temperate shore in order to have a first overview
374 of the specific distribution of these compounds, and more particularly of mycosporine-
375 like amino acids for which data are not actually available for some species.

376

377 Firstly, a high inter-specific variability has been demonstrated in terms of
378 pigment composition and concentration (Figs 1-3). Some species had a high content of
379 chlorophyll-*a* (*P. plumosa* or *Callithamnion* sp.) while others had more carotenoids

380 (*P. dioica*, *B. scorpioides*, *V. lanosa*), or others had low concentrations of both pigments
381 (*C. crispus* or *G. pistillata*). Three groups were observed from the carotenoids
382 composition, as suggested by Schubert et al. (2006): species rich in lutein, species rich
383 in zeaxanthin, and those with violaxanthin or antheraxanthin (Fig. 2). Lutein was the
384 major carotenoid, as already illustrated (Esteban et al., 2009; Marquardt and Hanelt,
385 2004).

386

387 Furthermore, the quantity of photosynthetic pigment (i.e. chlorophyll-*a* and
388 phycobiliproteins) decreased in the summer samples (Figs. 1, 3) due to an increase in
389 irradiance. Indeed, the increase in light energy availability leads species to reduce their
390 pigment complement to harvest the same quantity of light (Ak and Yücesan, 2012;
391 Ramus et al., 1976). Another explanation would be linked to the nitrogenous nature of
392 chlorophyll-*a* and phycobiliproteins (Huovinen et al., 2006; Lüning, 1990; Parjikolaie et
393 al., 2013). They are used for nitrogen storage in winter and as a nitrogen source when
394 limitations occur in summer (Barufi et al., 2011; Lapointe and Duke, 1984; Surget et al.,
395 2017). Stack et al. (2017) demonstrated that the protein content of *Porphyra dioica*
396 doubled in winter months, perhaps because of phycobiliproteins acting as a nitrogen
397 storage. In general, the impact of N concentration on pigments has already been widely
398 demonstrated in many species such as in *Palmaria palmata* (Corey et al., 2013;
399 Parjikolaei et al., 2013).

400

401 The aim of the second part of this study was to elucidate the MAAs composition
402 of 40 red macroalgae. The extraction method in 70% ethanol, coupled with the extract
403 analysis by HPLC, provided reliable data, and had the advantage of being non-toxic,

404 unlike using methanol which is commonly used in the literature (e.g. Briani et al., 2018;
405 Gröniger et al., 2000; Guihéneuf et al., 2018; Pandey et al., 2017; Yuan et al., 2009).
406 The use of a less-toxic solvent such as ethanol allowed for the valorization of extracted
407 MAAs as they have a significant potential for biotechnology applications in for example
408 human health and cosmetics (Chrapusta et al., 2017; Lawrence et al., 2018; Pangestuti
409 et al., 2018). For example, Helioguard 365 (Mibelle group, Switzerland) is a
410 commercial formulation used to protect the skin or hair from UV radiation and based on
411 the MAAs shinorine and porphyra-334 extracted from the red macroalga *Porphyra*
412 *umbilicalis* (La Barre et al., 2014; Schmid et al., 2006).

413 The results of the screening revealed a high diversity in MAA composition in
414 the 40 studied species, including some species for which the MAA composition had not
415 been reported before, e.g. *Plumaria plumosa*, *Bornetia secundiflora*, *Dilsea carnosa*,
416 *Gastroclonium ovatum*, *Hypoglossum hypoglossoides*, *Champia parvula* and
417 *Chylocladia verticillata*. It was already demonstrated that some species of the
418 Rhodophyta have the capacity to accumulate a high degree of diversity and
419 concentration of MAAs (e.g. Rastogi et al., 2010; Sinha et al., 2007), but this present
420 study is the first to report on MAA profiles from as many species of red alga from a
421 temperate region, here from Brittany. Based on their absorption maxima and retention
422 time, 23 potential MAAs were identified by HPLC analyses in the 40 red seaweeds
423 harvested along the Brittany coasts. Shinorine and palythine were found to be the most
424 common MAAs in this study (Fig. 5), which is in agreement with Karentz (2001). The
425 composition in MAAs of each species coincided with the literature, even for poorly
426 studied species such as *Asparagopsis armata* (Figueroa et al., 2008). For example, in
427 accordance with the bibliography, shinorine and porphyra-334 are found in *P. dioica*

428 (Guihéneuf et al., 2018); shinorine, palythine and porphyra-334 in *C. nodulosum*; or
429 species contained no MAA such as *Phycodrys rubens* and *F. lumbricalis* (Karsten et al.,
430 1998b).

431 The development of a diversity index for MAAs allowed for the identification of
432 three groups of species (Fig. 7A): i.e. species without MAA (e.g. *Plumaria plumosa*,
433 *H. plumosa*, *M. alata*, *Polyides rotunda* or *F. lumbricalis*), species with a few MAAs
434 (e.g. *Porphyra dioica*, *Gracilaria gracilis*, *C. tetricum*), and some with more than six
435 MAAs (e.g. *Palmaria palmata*, *B. scorpioides*, *Gracilaria vermiculophylla*, *V. lanosa*).
436 These observations suggested a huge diversity of mycosporine-like amino acids among
437 different members of Rhodophyta, with no apparent link with phylogeny, or
438 morphology, position on the shore or sampling site. Similarly to our study, the
439 distribution of various MAAs in other publications is not related to a phylogenetic
440 pattern (Karentz et al., 1991; McClintock and Karentz, 1997), but more to an
441 acclimatization to environmental variations (Briani et al., 2018).

442 Furthermore, 23 MAAs of which some potentially new and as yet unidentified
443 were observed in the 40 species knowing that approximately 20 MAAs have been
444 reported so far in red algae, suggesting that there may be in this study some MAAs that
445 have never been yet identified. *B. scorpioides* was the species that stood out the most
446 because it had four, unique, as yet un-identified MAAs (i.e. MAA_4, MAA_13,
447 MAA_18 and MAA_23). This species also did not fit into any of the three groups based
448 on the diversity index (Fig. 7A). Only six MAAs were identified by LC-MS in the
449 studied species (i.e. shinorine, palythine, porphyra-334, asterina-330, usurijene,
450 palythene). In addition, we propose that MAA_11 corresponded to palythinol by
451 comparing the results with the literature: indeed, palythinol is a MAA often observed in

452 many red macroalgae in the literature (Sinha et al., 2007) and MAAs_11 is the fifth
453 most common MAA in the red algae studied here. MAA_11 is present for example in
454 *P. palmata*, which should contain palythanol according to the literature (Gröniger et al.,
455 2000; Karsten and Wiencke, 1999; Yuan et al., 2009). It is difficult to identify MAAs
456 based only on the absorbance spectra and retention times of known MAAs in the
457 bibliography, especially since some MAAs are present in very small quantities which
458 makes it impossible to detect them in LC-MS. The lack of commercial standards makes
459 this study particularly complex, in addition to making impossible the quantification of
460 the different MAAs. In addition, some peaks have similar absorption maxima and
461 retention times. For example, usujirene and palythene only differ by their cis- or trans-
462 conformation and less than one minute separated them (Carreto and Carignan, 2011). In
463 order to identify other MAAs, more biomass for the extractions (if the available
464 biomass allowed it), or the development of a purification protocol to concentrate each
465 MAA would therefore be necessary.

466 Moreover, there was no large seasonal difference in terms of the composition
467 and concentration of MAAs (Fig. 5). This is an unexpected result as the role of photo-
468 protection by MAAs has been widely demonstrated (e.g. Conde et al., 2000; Singh et
469 al., 2008). We expected that the concentration of total MAAs would increase in relation
470 to the intensity of radiation in the marine ecosystem (Karsten et al., 1998a; Torres et al.,
471 2016). However, it is possible that a possible increase in MAAs content occurred
472 between the two sampling periods, February and July, indicating that two sampling
473 periods are not enough to study seasonal variation in MAAs content. This is suggested
474 by Guihéneuf et al. (2018), who found that the concentration of MAAs increased
475 between February and May in *P. palmata* and *C. crispus* collected on Irish shores and

476 not during summer as expected. Another explanation is that, as MAAs are nitrogenous
477 compounds, they are used in summer as a source of nitrogen (Karentz, 2001). In this
478 sense, a number of studies have already demonstrated the positive effect of nitrogen on
479 concentrations of MAAs (Figuerola et al., 2014; Korbee et al., 2005; Peinado et al.,
480 2004).

481 Thus, the variability observed in pigments and MAAs composition suggests that
482 all species do not seem to have the same protective capabilities against solar radiation.
483 In an attempt to obtain a first explanation of this diversity, we tried to relate this
484 composition to phylogeny, or morphology, position on the shore or sampling site, which
485 was not successful. To date, few studies have examined the link between pigment
486 composition and phylogeny, and no clear relationship has been found (Vandervelde,
487 1973; Wang et al., 2018). The MAAs composition would also not be related to a
488 phylogenetic pattern (Karentz et al., 1991; McClintock and Karentz, 1997). Thus there
489 is currently no explanation for the composition (presence/absence) in MAAs between
490 the different species that seems to be random. However, it has been experimentally
491 demonstrated that an increase in UV radiation could have an impact on the levels of
492 MAAs, for example in *Palmaria palmata* or *Chondrus crispus* (Hoyer et al., 2002;
493 Kräbs et al., 2004); or that the MAAs concentration depended on the season (Guihéneuf
494 et al., 2018), nutrient concentrations and pH (Briani et al., 2018), latitude (Karsten et al.
495 1998b) or depth (Franklin et al., 1999; Karsten and Wiencke, 1999). For example, the
496 total MAA concentration in *P. palmata* and *Devalerea ramentaceae* collected at
497 different depths decrease with sampling depth, although the composition remains the
498 same (Hoyer et al., 2001; Karsten et al., 1999).

499

500 In our study, four species stand out due to their diversified compositions of
501 MAAs: *B. scorpioides*, *G. vermiculophylla*, *V. lanosa* and *P. dioica*, which are the
502 sampled species the most exposed to solar radiation due to their relatively high position
503 on the shore. Indeed, *B. scorpioides* lives at the top of muddy estuaries (Sanchez de
504 Pedro et al., 2014); *G. vermiculophylla* lives at the surface of the intertidal muddy
505 estuaries (Roleda et al., 2012; Surget et al., 2017); *P. dioica* is located on the upper
506 intertidal, and *V. lanosa* is an obligate epiphyte on the brown macroalga *Ascophyllum*
507 *nodosum* (Garbary et al., 2014) which has a wide range of coverage on intertidal
508 rockyshores at mid-tide level. These four red algal species were characterized by the
509 presence of several MAAs and a high concentration of carotenoids, in particular
510 zeaxanthin, a pigment synthesized under higher light conditions (Rmiki et al., 1996;
511 Schubert and Garcia-Mendoza, 2008). Thus, they stood out from the others on the
512 Principal Component Analysis of pigments (Fig. 4). Another species, *G. gracilis*, also
513 stood out the PCA since it contained antheraxanthin, in accordance with Rmiki et al.
514 (1996) and Schubert et al. (2006). Conversely, *P. plumosa*, with no observed MAA, was
515 the single species with the highest concentrations of chlorophyll *a* and phycoerythrin.
516 This alga lives in shaded areas (Yakovleva et al., 1998), which may explain its necessity
517 to have high concentrations of pigments which absorb light energy, rather than act as
518 photoprotectants.

519

520 5. Conclusions

521 This study highlights the occurrence of numerous MAAs in red macroalgae from
522 Brittany, with variations in the number and level of MAAs per species. Thus, this
523 research contributes to reinforce the few existing databases on the MAAs composition

524 of red algae as described by Gröniger et al. (2000) and Sinha et al. (2007), knowing that
525 the identification of these molecules is difficult due to the lack of commercial standards.
526 Here we attempted to identify the factors controlling this MAAs diversity: MAAs
527 composition was no related to a phylogenetic pattern; however, the algal species most
528 exposed to light radiation were those with a high level and diversity of photoprotective
529 compounds which could protect their photosynthetic mechanisms against UV-radiation.
530 Nevertheless, it remains complex to provide conclusions about the actual impact of the
531 different biotic and abiotic factors on the synthesis of each MAA. Although MAAs
532 seem to be photo-protective, they may play other and as yet unknown roles in
533 macroalgal metabolism. Subsequently, it is necessary to select few species and carry out
534 macroalgal cultures under controlled conditions in order to understand factors
535 responsible for the production of each MAAs. Thus, the impact of temperature, UV
536 radiation, nutrients or pH could be tested one by one or in combination, allowing to
537 highlight a change in the ratio of MAAs for example. This would result in a better
538 understanding of the synthesis and function of these compounds.

539

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546

547 **Authors' contributions**

548 F.L. performed the analysis of the samples collected in winter, drafted the manuscript
549 and conducted the statistical analyses. S.L. performed the analysis of the samples
550 collected in summer and contributed to the writing of the manuscript. G.B. and L.T.
551 performed the LC-MS analyses. V.S-P designed the diversity index, helped in carrying
552 out the phylogenetic analyses and contributed to the interpretation of the results. S.C.
553 designed and supervised the project. All authors reviewed and approved the final
554 manuscript.

555

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Table 1. HPLC solvent gradient. (A) methanol:ammonium acetate buffer 0.5 M (80:20), (B) acetonitrile:milliQ water (87.5:13.5), (C) ethylacetate 100%.

Time (min)	% A	% B	% C
0	90	10	0
1	0	100	0
11	0	78	22
24	0	25	75
26	0	25	75
27	0	100	0
28	90	10	0
33	90	10	0

Appendix 1. List of the 40 red macroalgal species collected in February and/or July 2017, with their associated GenBank accession numbers (for the genes *rbcL* and *cox1*), morpho-anatomical group (i.e. MAG; according to Steneck and Dethier, 1994), localisation on the shore, substrate and sampling site. The different MAGs are: (2) filamentous algae; (2.5) corticated or polysiphonous filaments algae; (3) foliose algae; (3.5) corticated foliose algae; (4) corticated algae and (6) articulated calcareous algae.

Species	Accession numbers		MAG	Localisation	Substrate	Sampling sites
	<i>rbcL</i>	<i>cox1</i>				
<i>Ahnfeltiopsis devoniensis</i>	KU640212.1	KF641876.1	4	Lower intertidal	Rocky	Portsall
<i>Asparagopsis armata</i>	GQ337068.1	KJ960343.1	2.5	Lower intertidal	Rocky	Portsall
<i>Bonnemaisonia hamifera</i>	FJ195604.1	KJ960353.1	2.5	Lower intertidal	Rocky	Portsall
<i>Bornetia secundiflora</i>	No data		2.5	Upper subtidal	Rocky	Portsall
<i>Bostrychia scorpioides</i>	AY920825.1	MF094019.1	2.5	Upper intertidal	Muddy	Le Faou
<i>Calliblepharis jubata</i>	KC121138.1	KJ960410.1	4	Lower intertidal	Rocky	Portsall
<i>Callithamnion tetragonum</i>	AF439301.1	MF447481.1	2	Lower intertidal	Rocky	Portsall
<i>Callithamnion tetricum</i>	AF439300.1	KJ960434.1	2	Lower intertidal	Rocky	Portsall
<i>Ceramium echionotum</i>	AF439313.1	KJ960509.1	2.5	Lower intertidal	Rocky	Portsall
<i>Ceramium nodulosum</i>	No data		2.5	Lower intertidal	Rocky	Portsall

<i>Ceramium secundatum</i>	KT250273.1	KT250269.1	2.5	Lower intertidal	Rocky	Portsall
<i>Champia parvula</i>	EF613312.1	HQ422864.1	2.5	Lower intertidal	Rocky	Portsall
<i>Chondracanthus acicularis</i>	KJ202090.1	KJ202081.1	4	Lower intertidal	Rocky	Portsall
<i>Chondrus crispus</i>	KF026483.1	GU645233.1	4	Lower intertidal	Rocky	Portsall
<i>Chylocladia verticillata</i>	No data		2.5	Intertidal	Rocky	Portsall
<i>Corallina</i> sp.	No data		6	Lower intertidal	Rocky	Porspoder ^a ; St-Pabu ^b
<i>Dilsea carnosa</i>	KT310705.1	KY572820.1	3.5	Upper subtidal	Rocky	Portsall
<i>Furcellaria lumbricalis</i>	No data		4	Intertidal	Rocky	Portsall
<i>Gastroclonium ovatum</i>	KU726714.1	KJ960700.1	2.5	Lower intertidal	Rocky	Portsall
<i>Gelidium corneum</i>	HM629821.1	KJ960706.1	4	Lower intertidal	Rocky	Porspoder
<i>Gigartina pistillata</i>	AY294375.1	KJ960717.1	4	Lower intertidal	Rocky	Porspoder ^a ; Portsall ^b
<i>Gracilaria gracilis</i>	AY049400.1	KF714853.1	4	Lower intertidal	Rocky	Portsall
<i>Gracilaria vermiculophylla</i>	JQ768774.1	JQ794759.1	4	Upper intertidal	Muddy	Le Faou
<i>Griffithsia corallinoides</i>	No data		2.5	Lower intertidal	Rocky	Portsall
<i>Heterosiphonia plumosa</i>	AF083379.1	KJ960780.1	2.5	Upper subtidal	Rocky	Portsall ^a ; St-Pabu ^b
<i>Hypoglossum hypoglossoides</i>	AF257368.1	KJ179930.1	3.5	Lower intertidal	Rocky	Portsall

<i>Lomentaria articulata</i>	KU726701.1	KU707860.1	2.5	Lower intertidal	Rocky	Portsall
<i>Mastocarpus stellatus</i>	U02992.1	KY572683.1	4	Lower intertidal	Rocky	Portsall
<i>Membranoptera alata</i>	JQ864359.1	KJ960846.1	3.5	Lower intertidal	Rocky	Portsall
<i>Metacallophyllis laciniata</i>	No data		3.5	Upper subtidal	Rocky	Portsall
<i>Osmundea hybrida</i>	AF281878.1	KX258831.1	4	Lower intertidal	Rocky	Portsall
<i>Osmundea pinnatifida</i>	JX828140.1	KU566536.1	4	Lower intertidal	Rocky	Porspoder ^a ; Portsall ^b
<i>Palmaria palmata</i>	LN999410.1	KY572816.1	3.5	Lower intertidal	Rocky	Porspoder ^a ; Portsall ^b
<i>Phycodrys rubens</i>	JX110932.1	KY572841.1	3.5	Upper subtidal	Rocky	Porspoder
<i>Plocamium cartilagineum</i>	HQ224543.1	JF271583.1	4	Lower intertidal	Rocky	Portsall
<i>Plumaria plumosa</i>	KU381993.1	HQ412551.1	2.5	Lower intertidal	Rocky	Portsall
<i>Polyides rotunda</i>	No data		4	Lower intertidal	Rocky	Portsall
<i>Porphyra dioica</i>	JN703282.1	JN847313.1	3	Upper intertidal	Rocky	Portsall ^a ; St-Pabu ^b
<i>Sphaerococcus coronopifolius</i>	AY294376.1	KJ961109.1	4	Upper subtidal	Rocky	St-Pabu
<i>Vertebrata lanosa</i>	MF120849.1	KX344122.1	2.5	Intertidal	Rocky	Portsall

^aSamples collected in winter; ^bSamples collected in summer

Figure legends

Figure 1. Chlorophyll *a* concentrations ($\text{mg}\cdot\text{g}^{-1}$ DW) (mean \pm standard deviation, $n=3$) of 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that chlorophyll-*a* level was below the limit of detection or quantification.

Figure 2. Carotenoid concentrations ($\text{mg}\cdot\text{g}^{-1}$ DW) (mean, $n=3$) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that carotenoids level was lower than the limit of detection or quantification.

Figure 3. Phycoerythrin concentrations ($\text{mg}\cdot\text{g}^{-1}$ DW) (mean \pm standard deviation, $n=3$) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that phycoerythrin level was lower than the limit of quantification.

Figure 4. Principal Component Analysis (PCA) of pigment contents, for 21 species of red macroalgae collected in winter and summer (A: variables; B: samples). Ellipses were drawn around species that had a confidence level of 95% or more. Dimension 1 was principally characterised by chlorophyll *a* level and dimension 2 by zeaxanthin presence and level (correlation coefficient = 0.93 and 0.88, respectively).

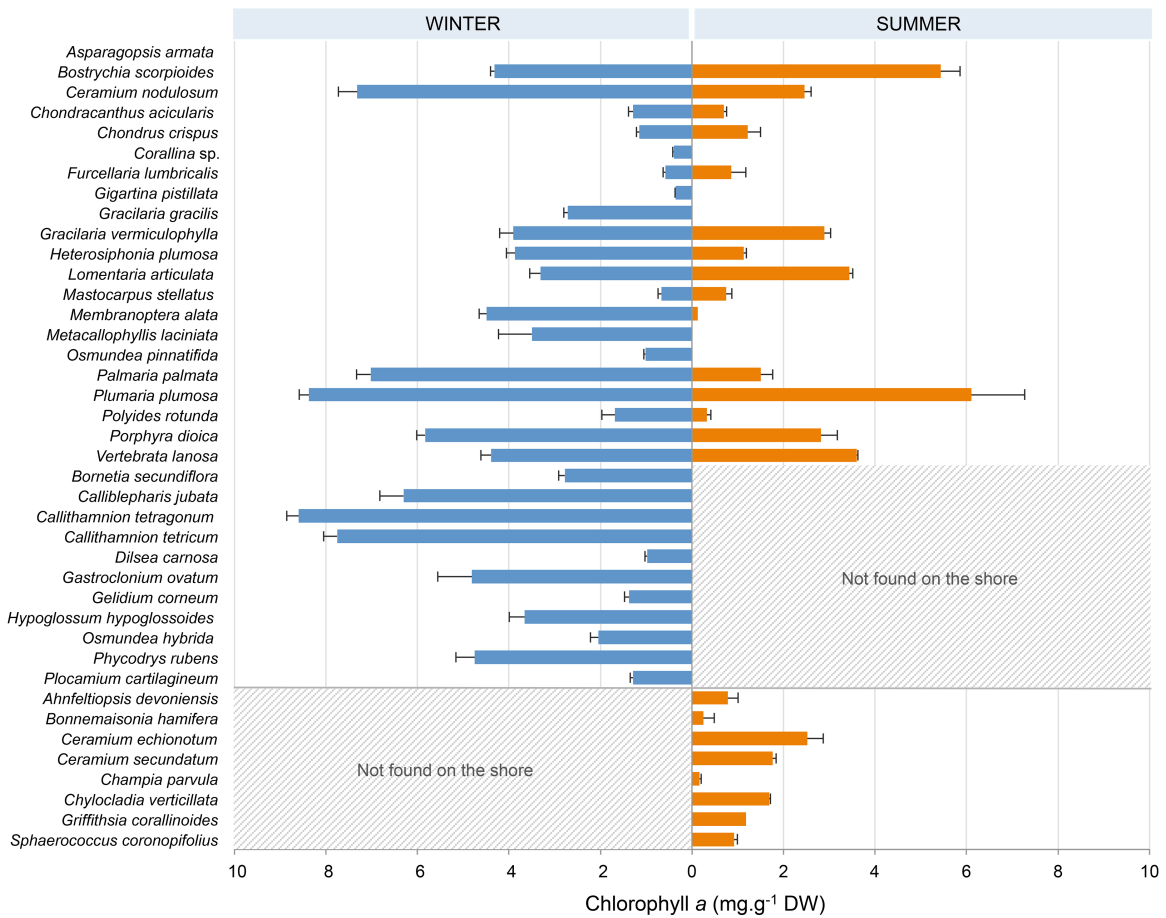
Figure 5. Mycosporine-like amino acids composition (mAU.min) (mean, $n=3$) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that MAAs level was lower than the limit of detection or quantification.

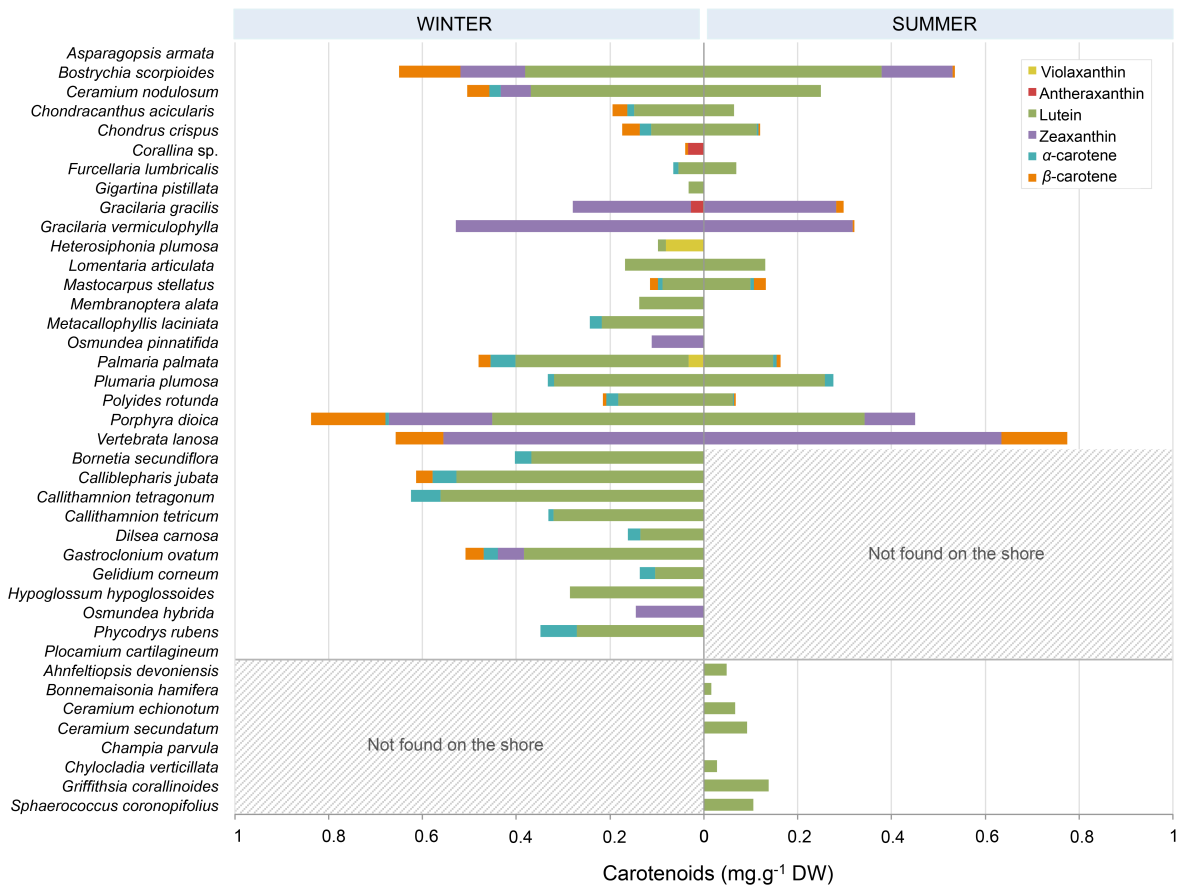
Figure 6. Chromatogram at 330 nm of MAAs extracted from the red macroalga *Palmaria palmata* collected in February 2017 (winter) from Porspoder (Brittany, France).

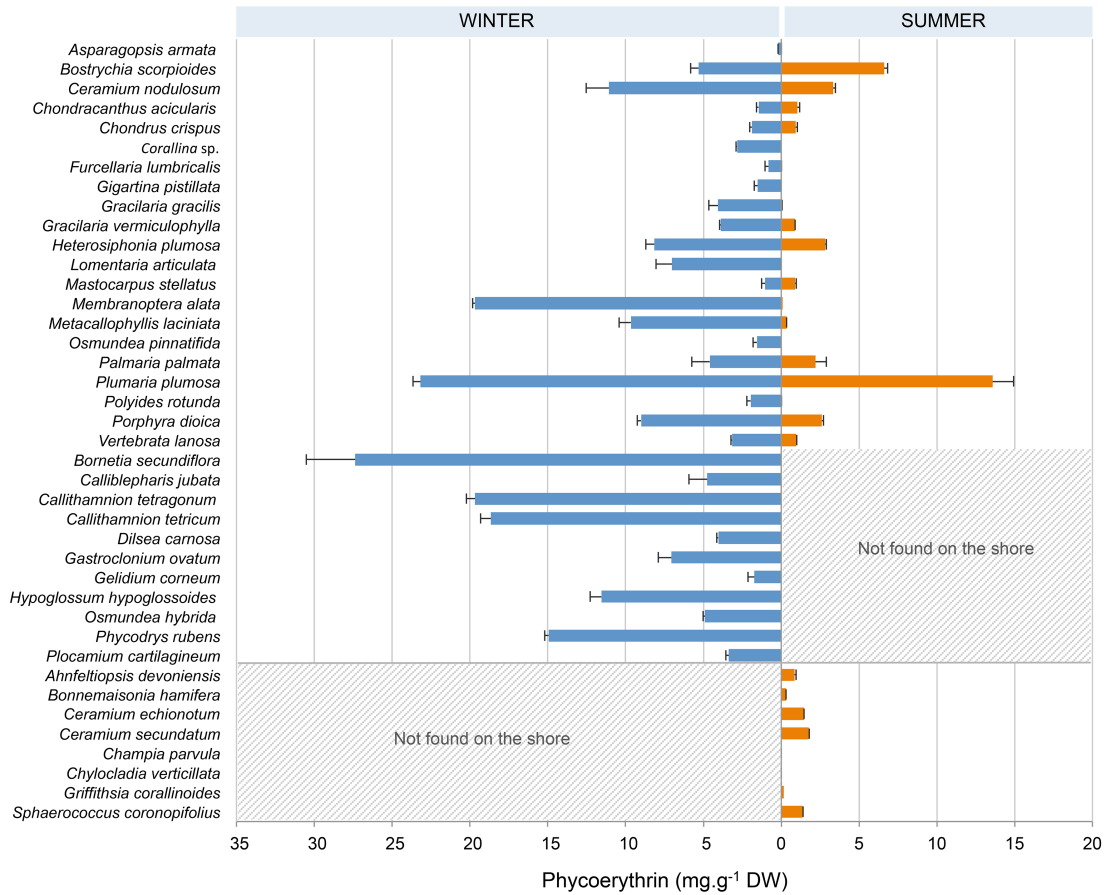
Figure 7. (A) Dendrogram based on the diversity indices of MAAs, obtained from hierarchical cluster analysis (single linkage method); (B) Neighbor-joining phylogenetic tree based on *rbcL* and *cox1* genes sequences, for the 32 macroalgal species collected from Brittany during winter. The most relevant boot-strap values are shown next to the branches. Some species are not represented on the phylogenetic tree due to missing sequences on GenBank (see Appendix 1).

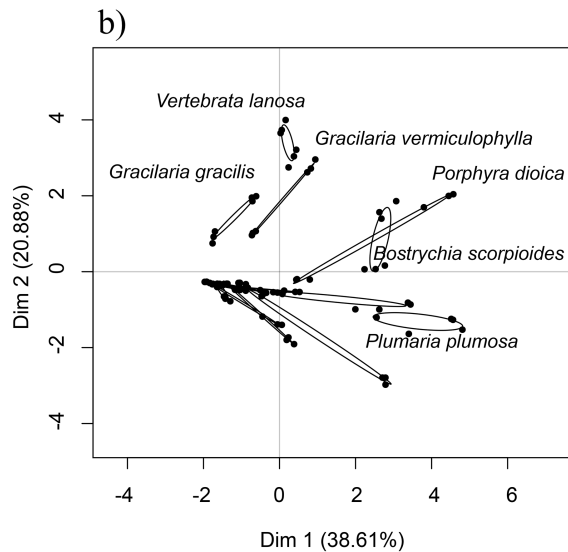
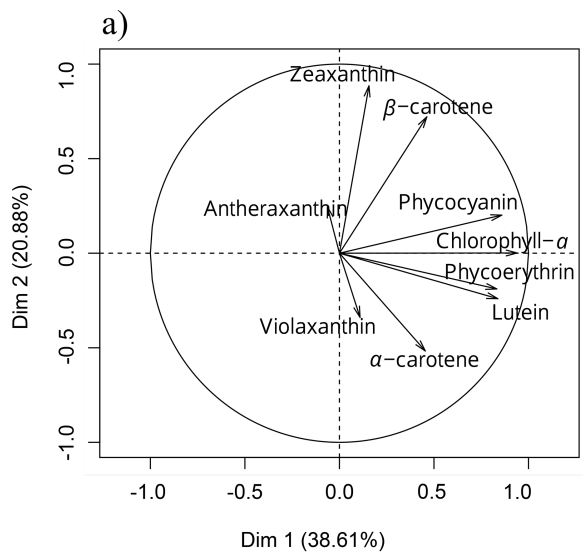
Appendix 2. List of the 23 different peaks analysed in 40 red macroalgae species, that may correspond to MAAs, with their absorption spectra, λ_{\max} and their retention time (Rt).

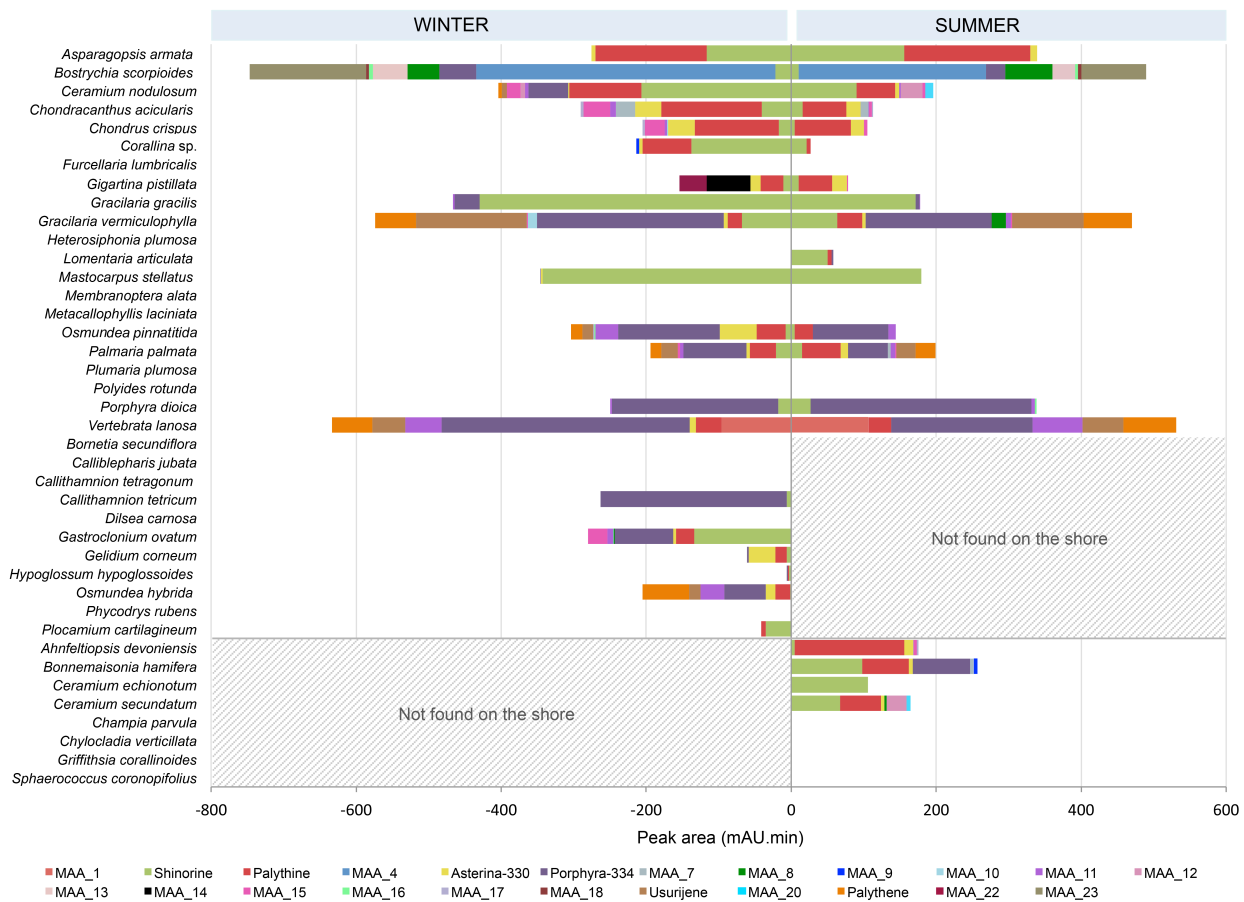
Appendix 3. (A) Dendrogram based on the diversity indices of MAAs, obtained from hierarchical cluster analysis (single linkage method); (B) Neighbor-joining phylogenetic tree based on *rbcL* and *cox1* genes sequences, for the 29 macroalgal species collected in summer. The most relevant boot-strap values are shown next to the branches. Some species are not represented on the phylogenetic tree due to missing sequences on GenBank (see Appendix 1).

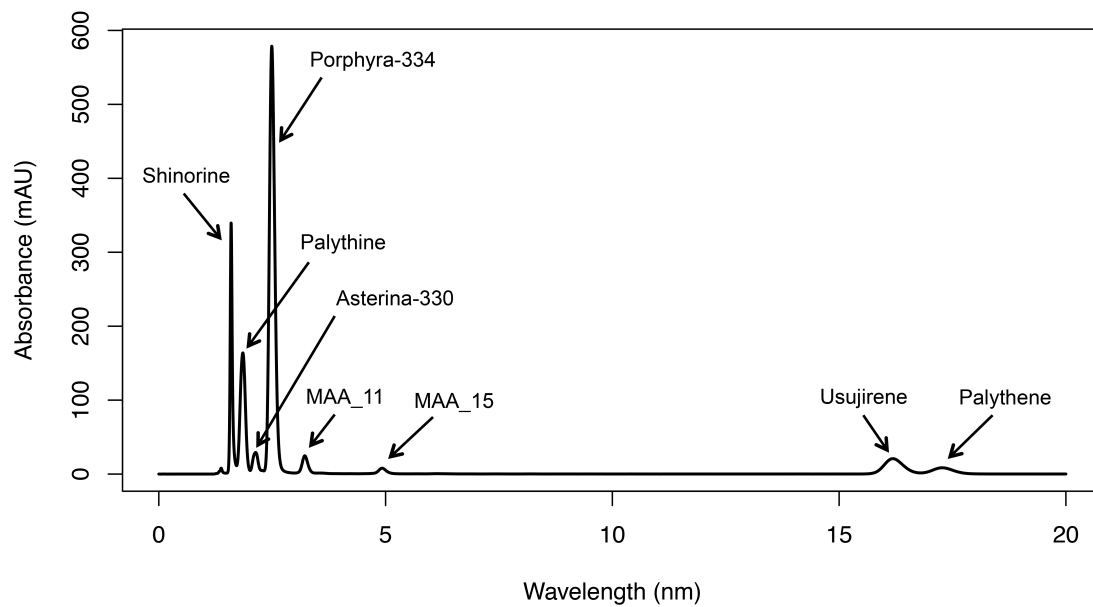






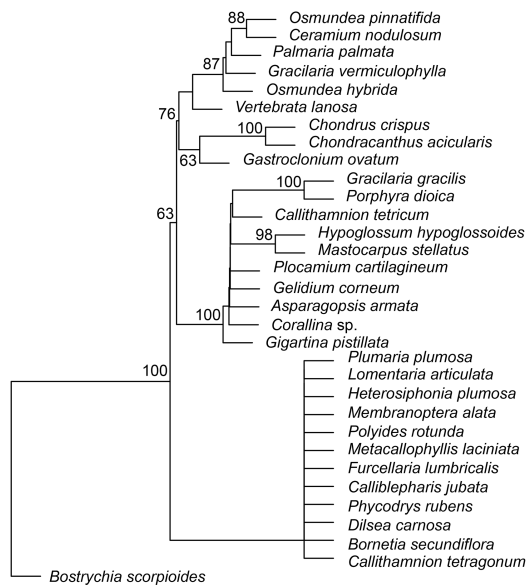




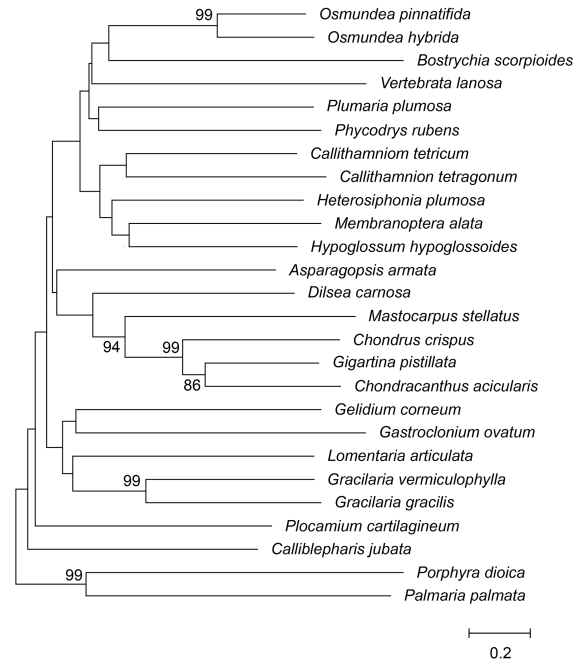


ACCEPTED

a)



b)



Highlights

- Detection of 23 potential MAAs in 40 Rhodophyta species, including 6 already identified (shinorine, palythine, asterina-330, porphyra-334, usurijene, palythene)
- First report on the MAAs composition for some red seaweeds
- High variability in MAAs content and composition between the different species, with no link with phylogeny, morphology, position on the shore or sampling site
- A MAAs extraction method using 70% ethanol being less toxic than conventional methanol, and giving potential valorisable extracts