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Núria Viladrich, Lorenzo Bramanti, Georgios Tsounis, Martina Coppari, Carlos Dominguez-Carrió, Audrey Pruski, Sergio Rossi

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1 **Estimations of FFA as a reliable proxy for larval performance**  
2 **in Mediterranean octocoral species**

3 Viladrich N<sup>1,2\*</sup>, Bramanti L<sup>2</sup>, Tsounis G<sup>3</sup>, Coppari M<sup>1,4</sup>, Dominguez-Carrió C<sup>5,6</sup>, Pruski  
4 AM<sup>2</sup>, Rossi S<sup>1,7,8</sup>

5  
6 <sup>1</sup> Institut de Ciència i Tecnologia Ambientals (Universitat Autònoma de Barcelona),  
7 Edifici Z Campus UAB, Cerdanyola del Vallès, 08193, Spain

8 <sup>2</sup> Sorbonne Université, CNRS, Laboratoire d'Ecogéochimie des Environnements  
9 Benthiques, LECOB, 66500 Banyuls-sur-Mer, France

10 <sup>3</sup> California State University, Northridge, USA

11 <sup>4</sup> Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica  
12 delle Marche, 60131, Ancona, Italy

13 <sup>5</sup> Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain

14 <sup>6</sup> Instituto de Investigação em Ciências do Mar - Okeanos, Universidade dos Açores,  
15 Horta, Portugal

16 <sup>7</sup> DiSTeBA, Università del Salento, Lecce 73100, Italy

17 <sup>8</sup> Instituto de Ciências do Mar (Labomar), Universidade Federal do Ceará, Fortaleza,  
18 Ceará, Brazil

19 \* Corresponding author: viladrich.nuria@gmail.com

20  
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22  
23 **Abstract**

24 The survival, behaviour and competence period of lecithotrophic larvae depends  
25 not only on the energy allocation transferred by maternal colonies, but also on the amount

26 of energy consumed to sustain embryonic, larval and post-larval development. The  
27 objective of the present work is to understand the effect of energy consumption on the  
28 performance of lecithotrophic larvae. To this aim, we analysed free fatty acid (FFA)  
29 content and composition of the larvae of three Mediterranean octocorals (*Corallium*  
30 *rubrum*, *Eunicella singularis* and *Paramuricea clavata*) as a proxy for energy  
31 consumption. Results showed that *C. rubrum* larvae consume more FFA than *P. clavata*,  
32 whereas the energy consumed by *E. singularis* larvae is high but highly variable. These  
33 results are in accordance with the larval behaviour of these three species, since *C. rubrum*  
34 larvae are characterized by their high swimming activity frequency, *P. clavata* larvae are  
35 almost inactive, and the swimming activity frequency of *E. singularis* larvae is high,  
36 although variable. The differences in FFA composition of the larvae suggest contrasting  
37 energetic strategies that could explain the differences in survival and recruitment rates.  
38 In fact, a high dispersal and recruitment capacities for *E. singularis* larvae can be inferred  
39 from the FFA composition, whereas the high spatial and temporal variability of  
40 recruitment observed in *C. rubrum* may be related to the non-selective transfer of FA  
41 from maternal colonies. Finally, the high recovery rates after mass mortality events  
42 observed in *P. clavata* could be favoured by the presence of a specific FA (22:6(n-3))  
43 related to adaptation mechanisms under environmental stresses during the first  
44 developmental stages.

45

## 46 **Introduction**

47 Maternal energy investment in lecithotrophic larvae of marine invertebrates  
48 mainly consists in the allocation of lipids (Richmond, 1987; Arai *et al.*, 1993), being wax  
49 esters, triacylglycerols, sterols and polar lipids the most abundant (Figueiredo *et al.*,  
50 2012). Some symbiotic species also present photosynthetic dinoflagellates of the family

51 Symbiodiniaceae (*sensu* LaJeunesse *et al.*, 2018) in their larvae, which may provide  
52 additional energy to the offspring during development (Kopp *et al.*, 2016; Mies *et al.*,  
53 2017). Differences in the energetic allocation from maternal colonies to larvae, or in the  
54 capacity to obtain energy during the planktonic phase, may influence larvae survival rates.  
55 For example, it is commonly assumed that symbiotic larvae have higher survival rates  
56 than non-symbiotic ones (Yakovleva *et al.*, 2009; Harii *et al.*, 2010). However, survival,  
57 behaviour and the competence period of larvae also depends on the amount of energy  
58 consumed to sustain embryonic, larval and post-larval development (Holland & Spencer,  
59 1973; Gallager & Mann, 1986; Pechenik, 1990; Qian *et al.*, 1990). For instance, since the  
60 metabolic demands in lecithotrophic larvae are related with swimming behaviour, the  
61 actively swimming larvae are expected to have a high energy consumption (Okubo *et al.*,  
62 2008). Moreover, after the swimming phase, the recruitment success of sessile organisms  
63 may also involve a high energy consumption for substrate recognition, competition for  
64 space and early growth (Adjeroud *et al.*, 2017).

65         The study of free fatty acid (FFA) content in the larval stage may be used as a  
66 proxy for the amount of lipid used at specific times, since FFAs are obtained from the  
67 oxidation of lipid reserves (Gurr *et al.*, 2002), which in turn are beta-oxidized to provide  
68 a source of highly efficient energy (high ATP/Fatty Acid (FA) molecule; Sargent *et al.*,  
69 1988). Therefore, the FFA content is directly related to the energy consumed at a specific  
70 time. Moreover, studying the FFA composition may help to understand the nature of  
71 energetic requirements, such as the attainment and maintenance of optimal health and  
72 physiological functions. This approach has been thoroughly used in the fish culture  
73 industry (Bell & Sargent, 1996; Izquierdo, 1996; Copeman *et al.*, 2002; Bransden *et al.*,  
74 2005) and has recently been applied to understand some important ecological process on  
75 coral species (Viladrich *et al.*, 2016; Conlan *et al.*, 2017; Grinyó *et al.*, 2018). Studies

76 that looked at total FA showed that a dietary deficiency in some FAs can reduce the  
77 nutritional condition and growth in adult colonies (Latyshev *et al.*, 1991; Imbs, 2013;  
78 Radice *et al.*, 2019), as well as lower swimming activity and the survival of the larvae  
79 (Figueiredo *et al.*, 2012), which can eventually lead to lower recruitment rates (Conlan *et al.*,  
80 *et al.*, 2017). Hence, the different types of FAs (Saturated Fatty Acids, SFA;  
81 MonoUnsaturated Fatty Acids, MUFA and PolyUnsaturated Fatty Acids, PUFA) could  
82 be a good proxy for larval condition. In general, when FAs are catabolized, SFA and  
83 MUFA are preferentially consumed and PUFA are selectively retained (Rainuzzo *et al.*,  
84 1994; Tocher, 2003). Therefore, the availability of large amounts of certain PUFA is  
85 considered essential for larval development and health status (DeMott & Muller-Navarra,  
86 1997; Wen *et al.*, 2002; Figueiredo *et al.*, 2012).

87         Among benthic sessile invertebrates, gorgonians play a paramount role as  
88 ecosystem engineers in many benthic communities around the world (Gili & Coma, 1998;  
89 Wild *et al.*, 2011; Velásquez & Sánchez, 2015), and are considered one of the main three-  
90 dimensional constituents of the “marine animal forests” (*sensu* Rossi, 2013). Gorgonians  
91 exhibit three different strategies for sexual reproduction: (1) broadcast spawning: sperm  
92 and oocytes are released in the water column and fertilization is external, (2) surface  
93 brooding: oocytes/zygotes are retained by mucous material on the surface of the female  
94 colonies during larval development, although it is unclear if fertilization is internal or  
95 external and (3) internal brooding: the oocytes are internally fertilized and the female  
96 colonies retain the zygotes and embryos within their body during their development  
97 (Kahng *et al.*, 2011).

98         In the Mediterranean Sea, the non-symbiotic gorgonians *Corallium rubrum* and  
99 *Paramuricea clavata*, together with the symbiotic *Eunicella singularis*, are characteristic  
100 species of shallow benthic communities in coastal areas (Weinberg, 1979a). They are

101 gonochoric, releasing lecithotrophic larvae once a year during summer months (Coma *et*  
102 *al.*, 1995; Santangelo *et al.*, 2003; Ribes *et al.*, 2007). The gorgonians *C. rubrum* and *E.*  
103 *singularis* are internal brooders, whereas *P. clavata* is a surface brooder (Coma *et al.*,  
104 1995; Santangelo *et al.*, 2003; Ribes *et al.*, 2007). While both *C. rubrum* and *P. clavata*  
105 release non-symbiotic ciliated larvae (planulae), *E. singularis* larvae, such as the adult  
106 colonies, contain *Symbiodinium* of the clade A' (Forcioli *et al.*, 2011; Weinberg, 1979b).  
107 The larval competence (i.e., the period during which pelagic larvae are able to settle) is  
108 approximately 8 days for *E. singularis*, 11 days for *P. clavata* and 27 days for *C. rubrum*  
109 (Zelli *et al.*, 2020).

110         The aim of the present work is to explore the link between energy consumption,  
111 and larval performance in the three most characteristic and widely distributed shallow-  
112 water Mediterranean octocoral species. To achieve this objective, FFA were considered  
113 as a proxy for energy consumption, and their content and composition were analysed in  
114 the larvae of the three species just after release. The FFA content was used to quantify  
115 the energy consumed in the pelagic phase, whereas the FFA composition was compared  
116 between larval species to explore different energy requirements, in the light of data on  
117 larval performances (i.e., swimming activity rates and settlement rates) of the three  
118 species. These results can provide clues to understand how the energy used by larvae  
119 relates to their performance.

120

## 121 **Materials and methods**

### 122 ***Sampling procedure***

123         Larvae of *P. clavata* (surface brooder) were collected at 25-30 m depth by scuba  
124 diving at Punta s'Oliguera in Cap de Creus on June 22<sup>nd</sup> 2012 (NW Mediterranean, 42°  
125 17'03'' N; 003° 17'95'' E; Fig. 1). Larvae of the two internal brooder species (*C. rubrum*

126 and *E. singularis*) are difficult to obtain *in situ*. For this reason, 5 female colonies of *C.*  
127 *rubrum* collected at 25-30 m depth and 5 female colonies of *E. singularis* collected at 15-  
128 20 m depth at Punta s'Oliguera were maintained separately in 8L tank at  $20 \pm 1.0$  °C for  
129 1-2 days until larval release. A chiller (Tank chiller line TK 2000) was used to keep a  
130 constant seawater temperature, and the water was filtered using a biological filter (SERA  
131 fil bioactive 250+UV). Larvae of *E. singularis* were collected on July 19<sup>th</sup> 2012 and larvae  
132 of *C. rubrum* on July 27<sup>th</sup> 2012.

133 For each species, three replicates of 30 different larvae were fixed on pre-  
134 combusted GF/F filters, cold shocked with liquid nitrogen and stored at -80°C. Filters  
135 were then freeze-dried for 24h at -110°C and a pressure of 100 mbar. The freezer-dried  
136 material was stored at -20°C for further analyses.

137

#### 138 ***Free fatty acid (FFA) content and composition in larvae***

139 FFA content and composition were assessed for the three replicates of each  
140 species according to the method described by Viladrich *et al.*, (2016). Each filter with 30  
141 larvae was dissolved in dichloromethane:methanol (3:1) spiked with an internal standard  
142 (2-octyldodecanoic acid and 5 $\beta$ -cholanic acid) in order to estimate recuperation. The  
143 extraction solvent was eluted through an aminopropyl glass column resulting in 3  
144 fractions (neutral lipids, FFAs and polar lipids). In this study, the FFA fraction was  
145 methylated using a solution of 20% boron trifluoride-methanol reagent heated at 90°C for  
146 1 h.

147 The methyl esters of FA (FAMES) were separated and analysed by gas  
148 chromatography with mass spectrometry detection (GC/MS, 7820A GC from Agilent  
149 Technologies) equipped with a DB-5ms Agilent column (60 m length, 0.25 mm internal  
150 diameter and 0.25  $\mu$ m phase thickness). Hydrogen was used as a carrier gas at 30

151 mL·min<sup>-1</sup>. The high compound numbers in the samples and the similarity of retention  
152 required a complex method of temperature ramps, with the oven temperature  
153 programmed to increase from 50°C to 160°C at 20°C min<sup>-1</sup>, from 160°C to 188°C at 0.5°C  
154 min<sup>-1</sup>, from 188°C to 229°C at 20°C min<sup>-1</sup>, from 229°C to 235°C at 2°C min<sup>-1</sup> and, finally,  
155 from 235°C to 300°C at 5°C min<sup>-1</sup>. The injector and detector temperatures were 300°C  
156 and 320°C, respectively. FAMES were identified by comparing their retention times with  
157 those of commercial standards of FA (Supelco 37 Component FAME Mix and Supelco®  
158 Mix C<sup>4</sup>-C<sup>24</sup>) and were quantified by integrating areas under peaks in the chromatograms  
159 (Chromquest 4.1 software) using calibration curves derived from the Supelco 37  
160 Component FAME Mix. The results are presented in µg FFA larvae<sup>-1</sup> and in percentage  
161 of Saturated Free Fatty Acids (free SFA), Mono Unsaturated Free Fatty Acids (free  
162 MUFA) and Poly Unsaturated Free Fatty Acids (free PUFA), besides each FFA  
163 component percentage.

164

### 165 *Statistical analyses*

166 Differences in FFA content and in percentage of free SFA, free MUFA and free  
167 PUFA between larvae of the different species were tested using a one-way ANOVA.  
168 Before performing the ANOVAs, normality of data residuals and variance homogeneity  
169 were tested using the Shapiro-Wilk and Bartlett tests (functions “shapiro.test” and  
170 “bartlett.test” of the package *stats* of the R environment, respectively). One-way ANOVA  
171 tests were performed with the function “aov” of the package *stats* (Chambers and Hastie  
172 1992).

173 A correspondence analysis (CA) was used to check for associations among FFA  
174 composition of larvae using the function “ca” of the *ca* package in the R environment  
175 (Nenadic & Greenacre, 2007). Before performing the CA, FFA compounds that  
176 represented less than 2% of the total concentration were eliminated and percentages



177 recalculated so that the sum was equal to 100%. After this transformation, the CA was  
178 performed on a cross table containing 13 FFA compounds and 9 samples of larvae (3 per  
179 species).

180

## 181 **Results**

### 182 ***Free fatty acid (FFA) content in larvae***

183 FFA content was  $0.098 \pm 0.015$   $\mu\text{g}$  per larvae (mean  $\pm$  SD) for *C. rubrum*,  $0.045$   
184  $\pm 0.005$  for *P. clavata* and  $0.125 \pm 0.07$  for *E. singularis* (Fig. 2a). ANOVA results  
185 showed significant differences between *C. rubrum* and *P. clavata* (one-way ANOVA,  
186  $p < 0.005$ ), whereas no differences were observed between *E. singularis* and *C. rubrum*  
187 or *P. clavata* larvae, possibly due to the high variability of the *E. singularis* values (one-  
188 way ANOVA,  $p > 0.1$ ; Fig. 2a). ANOVA on free SFA, expressed as a percentage of total  
189 fatty acids, did not show significant differences between species (one-way ANOVA,  
190  $p > 0.5$ ; Fig. 2b), being  $18.39 \pm 4.37$  % for *C. rubrum*,  $14.45 \pm 1.48$  % for *P. clavata* and  
191  $26.98 \pm 11.60$  % for *E. singularis* (mean  $\pm$  SD). The percentage of free MUFA was  
192 significantly higher in *C. rubrum* ( $65.61 \pm 6.32$  %) than in *P. clavata* and *E. singularis*  
193 larvae ( $31.21 \pm 15.26$  % and  $26.77 \pm 17.40$ , respectively) (one-way ANOVA,  $p < 0.05$ ;  
194 Fig. 2b), while free PUFA percentage was significantly higher in *P. clavata* and *E.*  
195 *singularis* ( $54.33 \pm 13.87$  % and  $46.26 \pm 6.17$ , respectively) than in *C. rubrum* larvae  
196 ( $15.98 \pm 2.08$  %) (one-way ANOVA,  $p < 0.01$ ; Fig. 2b).

197

### 198 ***Free fatty acid (FFA) composition in larvae***

199 The first two dimensions explained 86% of the total variance of the CA. As it can  
200 be observed in the biplot, Dimension 1 (44% of total inertia) separates *C. rubrum* larvae  
201 from those of *P. clavata* and *E. singularis* based on the FFA descriptors (Fig. 3).  
202 Dimension 2 (42% of total inertia) shows that there are also differences between *P.*

203 *clavata* and *E. singularis* larvae, indicating that larvae from the three species displayed  
204 distinct FFA compositions (Fig. 3). Samples of *C. rubrum* larvae, which all lay very close  
205 to one another, are characterized by the presence of 18:1(n-9). *P. clavata* samples form a  
206 second and less compact group, with 20:4(n-6), 20:5(n-3) and 22:6(n-3) as dominant FFA.  
207 Finally, the samples of *E. singularis* larvae are widely spread on the biplot and associated  
208 to different FFA, with 18:3(n-3), 18:4(n-3), 20:2(n-6), 16:1 and 16:0 being the principal  
209 markers.

210

## 211 **Discussion**

212 This study shows, for the first time, that FFA content and composition could be  
213 used to understand lecithotrophic larvae performance. Until now, FFA portion in coral  
214 larvae had been largely neglected, probably due to their low concentration (Figueiredo *et*  
215 *al.*, 2012; Conlan *et al.*, 2017). However, our results underscore the importance of FFAs  
216 during the larval period of *C. rubrum*, *P. clavata* and *E. singularis*, despite their low  
217 concentrations ( $< 0.12 \mu\text{g}$  per larva; Fig. 2a).

218 According to our results, *C. rubrum* larvae had a 2-fold higher FFA content than  
219 *P. clavata*, whereas the FFA content in *E. singularis* was highly variable ( $\pm 57 \%$ ). Since  
220 FFA are the main sources of energy for ATP production, these values represent a measure  
221 of larvae energetic consumption (Gurr *et al.*, 2002). Moreover, as seawater temperature  
222 at the time of release was the same for the three species (Viladrich *et al.*, 2016), the  
223 influence of temperature on the observed variability can be excluded, and larval energetic  
224 consumption can be related to the active movement of the larvae (Okubo *et al.*, 2008).  
225 Therefore, on the basis of FFA content, we would expect *C. rubrum* larvae to be highly  
226 mobile, whereas *P. clavata* quite passive, and *E. singularis* larval motility high and also  
227 highly variable. Measurements of larval swimming activity frequency (i.e. percentage of

228 time during which active swimming or crawling behaviour is displayed by larvae) for the  
229 three species are in accordance with the values of FFA content measured, with 77%  
230 activity for *C. rubrum* (Martinez-Quintana *et al.*, 2015), 5% for *P. clavata* and 20-90%  
231 for *E. singularis* (Guizien *et al.*, 2020). Therefore, energy consumption may be a good  
232 proxy to estimate coral larvae swimming activity frequency.

233 As previously highlighted, the energy used depends on the specific metabolic  
234 requirements (Viladrich *et al.*, 2016, 2017; Grinyó *et al.*, 2018), and therefore, a detailed  
235 study of the FFA composition during the larval stage in different species may also help  
236 understanding the mechanisms underlying larval performance. In *E. singularis*, the high  
237 quantity of FFA 18:4(n-3) (Fig. 3) support the hypothesis that the Symbiodiniaceae are  
238 transferred from the mother colonies to the larvae, as previously proposed by Weinberg  
239 and Weinberg (1979). This fatty acid, in fact, is a robust tracer of the photosynthetic  
240 activity of symbiotic dinoflagellates (Papina *et al.*, 2003; Treignier *et al.*, 2008; Pupier *et*  
241 *al.*, 2021), since it cannot be synthesized *de novo* by heterotrophs (Volkman *et al.*, 1989;  
242 Dalsgaard *et al.*, 2003). Therefore, the presence of 18:4(n-3) indicates a surplus of energy  
243 which can increase survival rates and the competency of larvae (Ben-David-Zaslow &  
244 Benayahu, 1998; Harii *et al.*, 2010; Figueredo *et al.*, 2012). Interestingly, this is in line  
245 with the lack of genetic structuring at large spatial scales observed for this species  
246 (Costantini *et al.*, 2016). The high variability of this FFA (18:4(n-3)) observed in *E.*  
247 *singularis* larvae could be caused by the number of symbionts per larva, which varies  
248 according to the supply from mother colonies (Gaither & Rowan, 2010; Roth *et al.*, 2013).  
249 Nevertheless, not all the symbiotic larvae obtain an energy surplus from symbiosis, as  
250 showed by Kopp *et al.*, (2016) and Mies *et al.*, (2017). Future studies should focus on the  
251 role of Symbiodiniaceae in *E. singularis* larvae energetic budget. Finally, large amounts  
252 of 18:3(n-3) in *E. singularis* larvae could explain the higher recruitment rates of this

253 species with respect to *C. rubrum* and *P. clavata* (Bramanti *et al.*, 2005; Linares *et al.*,  
254 2008; Linares *et al.*, 2012) since this marker may have beneficial effects on larval  
255 settlement, following a trend already observed in bivalve and polychaete larvae (Pawlik  
256 & Faulkner, 1986; Jonsson *et al.*, 1999; da Costa *et al.*, 2011).

257         In the case of *C. rubrum*, our results suggest that the nutritional status of the adult  
258 colonies may directly affect the viability of the larvae due to a non-selective transfer of  
259 the most conspicuous FA, as previously suggested for different marine organisms (Qian  
260 & Chia, 1991; Harland *et al.*, 1993; Dalsgaard *et al.*, 2003; Figueredo *et al.*, 2012). In  
261 fact, 18:1(n-9) is the main component for both *C. rubrum* larvae (62%) and maternal  
262 colonies (30%) (Fig. 3) (Viladrich *et al.*, 2016), and it has been associated to detritus  
263 (Schultz & Quinn, 1973; Fahl & Kettner, 1993), which is the main food source for this  
264 species (Tsounis *et al.*, 2006). This non-selective transfer of lipids from maternal colonies  
265 to larvae may result in a dependence of recruitment on the nutritional condition of the  
266 mother colonies (Lasker, 1990; Yoshioka, 1996; Dunstan & Johnson, 1998), which in  
267 turn is affected by environmental conditions (Rossi & Tsounis, 2007). Indeed, the high  
268 spatial and temporal variability of recruitment rates observed in *C. rubrum* (Garrabou &  
269 Harmelin, 2002; Santangelo *et al.*, 2012) may be partially explained by the spatial and  
270 temporal variability of environmental conditions. Moreover, the high variability of  
271 recruitment rates could also be related to the low free PUFA content in *C. rubrum* larvae  
272 (Fig. 2b), since PUFA are mainly devoted to growth and resistance to stress conditions,  
273 thus enhancing larval metamorphosis and survival (Bell & Sargent, 1996; Pond *et al.*,  
274 1996; Sargent *et al.*, 1997; Albessard *et al.*, 2001; Rossi *et al.*, 2006; Figueredo *et al.*,  
275 2012; Conlan *et al.*, 2017). Therefore, small changes in the energy storage reserves of  
276 mother colonies may have serious consequences on the following generations.

277            Interestingly, some FFAs may be key to understand the resilience of some species  
278 after thermal stress episodes. Previous studies showed higher recruitment rates of *P.*  
279 *clavata* after a mass mortality events caused by increased temperature, if compared to *C.*  
280 *rubrum* and *E. singularis* (Coma *et al.*, 2006; Cupido *et al.*, 2009; Santangelo *et al.*, 2015).  
281 High recruitment rates after disturbances are associated to high resilience, attributed  
282 either to the high reproductive output of local *P. clavata* survivors (Cupido *et al.*, 2009),  
283 or to migration from distant populations (Padron *et al.*, 2018). However, it is possible that  
284 such high recruitment rates in *P. clavata* could also be concurrently favoured by the  
285 presence of the FFAs 22:6(n-3), 20:4(n-6) and 20:5 (n-3) (Fig. 3), which are key  
286 components of lipids for larval performance. Indeed, 22:6(n-3) is known to influence  
287 membrane fluidity and permeability, which can have a positive impact on enzyme  
288 activity, immune functions and adaptation to heat stress, among many other cellular  
289 processes (Dratz & Holte, 1992; Hall *et al.*, 2000; Bergé & Barnathan 2005; Kneeland *et*  
290 *al.*, 2013). Furthermore, the FFA 20:4(n-6) and 20:5(n-3) are essential fatty acids for  
291 larval development, which have been related to the production of biologically active  
292 eicosanoids under stress or unfavourable conditions (Sargent *et al.*, 1999), supporting the  
293 immune system functioning and osmoregulation (Chapelle, 1986; Mazonra *et al.*, 2003).

294            The present results are thus in accordance with the literature on larval performance  
295 of the studied species. The FFA content in larvae might then be used to predict the  
296 swimming activity frequency of different sessile invertebrate species, and their  
297 composition might also give a more detailed understanding of the mechanisms underlying  
298 larval performances. This new approach paves the path for a new approach to the study  
299 of early life-history stages of lecithotrophic larvae.

300

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308

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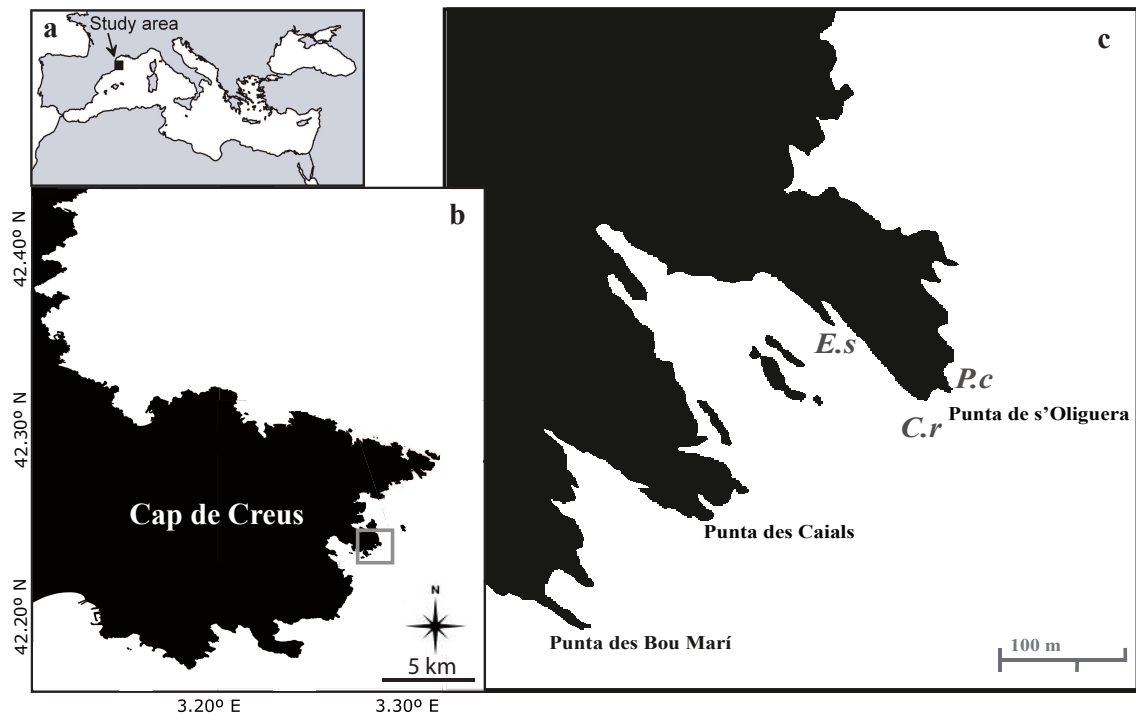
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691 **Figures**

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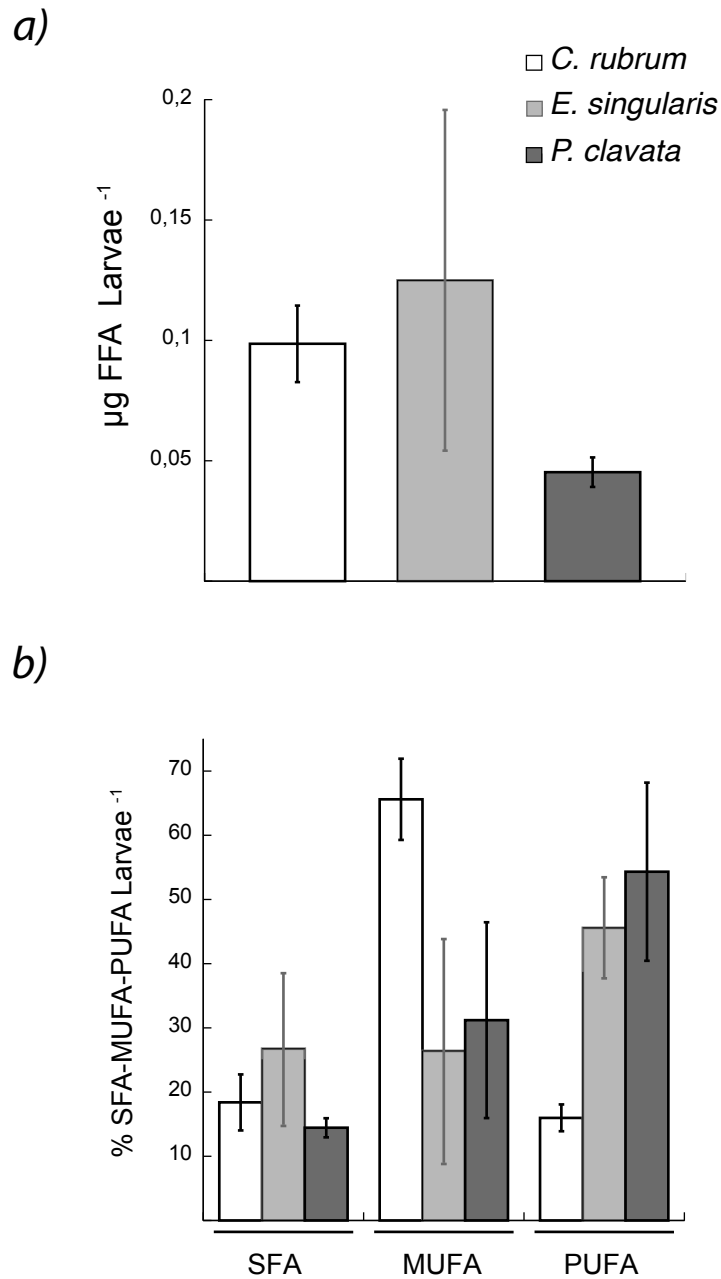
693 **Fig. 1** Location of the study area in NW Mediterranean (a, b) and sampling sites on the  
694 eastern coast of Cap de Creus (c). *C.r.*, *E.s* and *P.c* indicate the position of the *Corallium*  
695 *rubrum* (25-30 m depth), *Eunicella singularis* (15-20 m depth) and *Paramuricea clavata*  
696 (25-30 m depth) populations, respectively.

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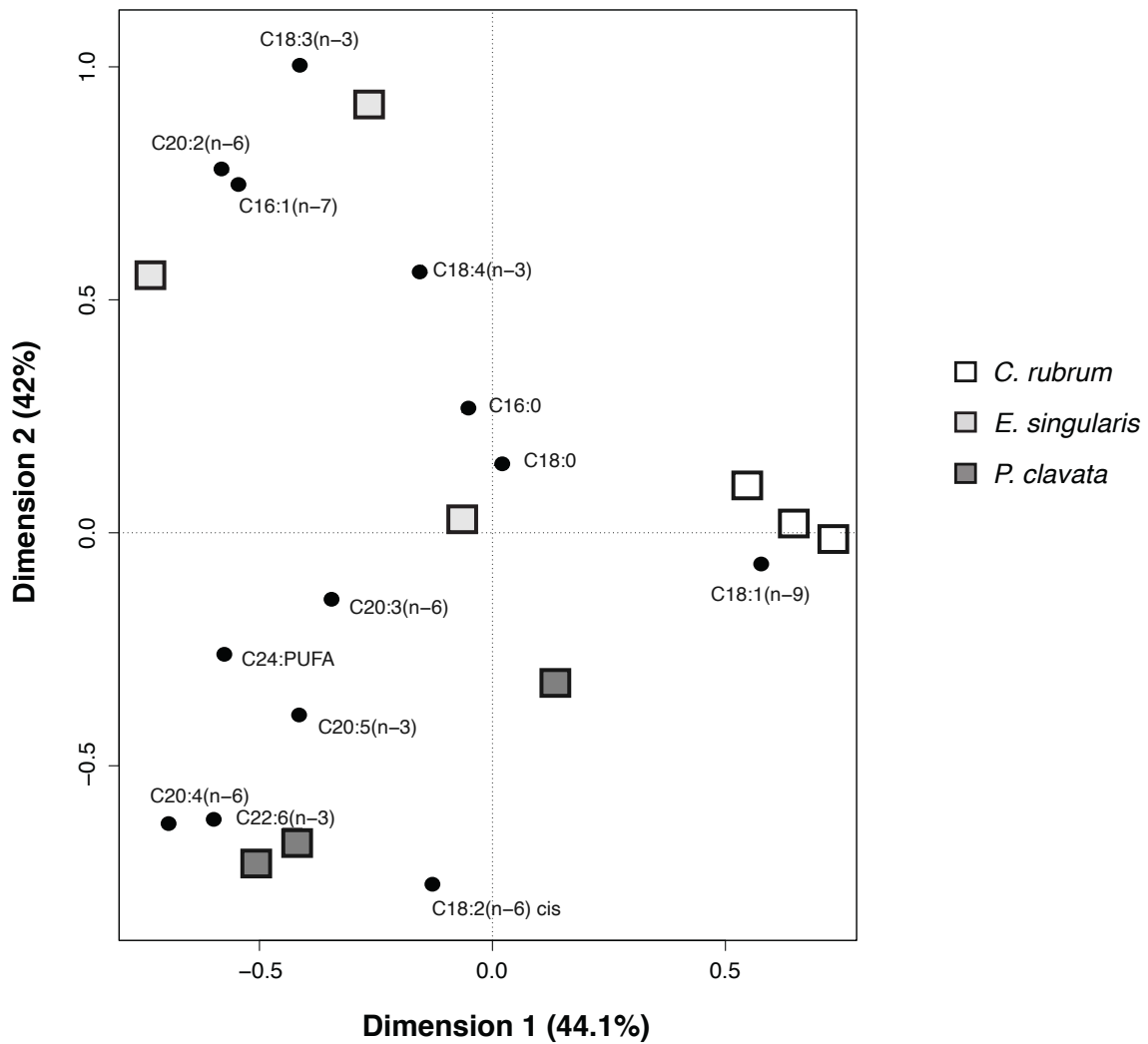
699 **Fig. 2** Free fatty acid content (mean  $\pm$  SD) in *Corallium rubrum* larvae (white), *Eunicella*  
 700 *singularis* larvae (grey), and *Paramuricea clavata* larvae (dark grey) expressed as (a)  $\mu\text{g}$   
 701 larvae<sup>-1</sup> and (b) percentage of Saturated Fatty Acids (SFA), MonoUnsaturated Fatty  
 702 Acids, (MUFA) and PolyUnsaturated Fatty Acids (PUFA). N = 3.



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704 **Fig. 3** Correspondence analysis (CA) biplot illustrating the ordering of the studied larvae  
 705 in the first two dimensions with regard to their free fatty acid composition (black points).  
 706 *Corallium rubrum* in white, *Eunicella singularis* in grey, and *Paramuricea clavata* in  
 707 dark grey.

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718 **Electronic Supplementary Material**

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721 **Table. 1.** Mean values and standard deviation (SD) of Free Fatty Acid (FFA) markers

722 and Saturated (SFA), MonoUnsaturated (MUFA) and PolyUnsaturated Fatty Acids

723 (PUFA) for *Corallium rubrum*, *Eunicella singularis* and *Paramuricea clavata* larvae.

	<i>Corallium rubrum</i>		<i>Eunicella singularis</i>		<i>Paramuricea clavata</i>	
	Mean	SD	Mean	SD	Mean	SD
C12:0	0.04	0.07	0.12	0.01	0.07	0.06
C13:0	0.00	0.00	0.02	0.03	0.00	0.00
C14:1(n-5)	0.00	0.00	0.04	0.06	0.00	0.00
C14:0	0.50	0.13	0.57	0.17	0.49	0.06
C15:1(n-5)	0.00	0.00	0.05	0.08	0.00	0.00
C15:0	0.23	0.07	0.27	0.07	0.10	0.09
C16:1(n-7)	0.66	0.22	5.73	1.56	1.01	0.05
C16:0	10.12	2.82	12.12	2.33	6.57	1.05
C17:1(n-7)	0.00	0.00	0.00	0.00	0.00	0.00
C17:0	0.51	0.10	0.37	0.13	0.18	0.16
C18:3(n-6)	0.00	0.00	1.08	0.70	0.00	0.00
C18:4(n-3)	2.37	0.80	1.75	2.71	0.14	0.12
C18:2(n-6)	4.30	0.94	0.73	0.82	9.44	3.88
C18:3(n-3)	5.39	1.22	17.29	14.82	2.40	1.11
C18:1(n-9)	62.44	6.70	19.09	16.57	27.86	14.71
C18:1(n-7)	0.00	0.00	0.00	0.00	0.00	0.00
C18:0	3.92	0.84	3.49	0.74	2.95	0.45
C20:4(n-6)	1.69	0.09	8.42	6.14	25.89	9.93
C20:5(n-3)	0.49	0.43	1.54	0.80	3.32	0.58
C20:3(n-6)	0.59	0.52	2.16	0.39	3.23	1.42
C20:4(n-3)	0.00	0.00	0.24	0.14	0.57	0.14
C20:2(n-6)	0.49	0.09	5.51	1.43	0.27	0.23
C20:1(n-9)	0.75	0.04	1.06	0.31	0.50	0.44
C20:0	0.17	0.02	0.12	0.03	0.08	0.07
C21:0	0.35	0.05	0.41	0.26	0.62	0.58
C22:5	0.00	0.00	0.08	0.08	0.14	0.12
C22:6(n-3)	0.00	0.00	2.15	1.93	4.59	1.36
C22:4	0.30	0.09	0.22	0.28	0.29	0.25
C22:5(n-x)	0.00	0.00	0.05	0.09	0.00	0.00
C22:2(n-6)	1.08	0.10	0.35	0.20	0.96	0.69
C22:1(n-9)	0.00	0.00	0.60	0.07	0.63	0.55
C22:0	0.67	0.05	8.59	14.16	1.46	1.32
C23:0	0.94	0.33	0.45	0.27	0.97	0.39
C24:PUFA	0.36	0.09	2.58	1.29	3.43	0.62
C24:1(n-9)	0.69	0.08	0.45	0.15	0.88	0.32
C24:0	0.94	0.12	0.45	0.11	0.96	0.47
SFA	18.39	4.37	26.98	11.60	14.45	1.48
MUFA	65.61	6.32	26.77	17.40	31.21	15.26
PUFA	15.98	2.08	46.26	6.17	54.33	13.87