Polyp expansion of passive suspension feeders: a red coral case study

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Polyp activity in passive suspension feeders has been considered to be affected by several environmental factors such as hydrodynamics, water temperature and food concentration. To better elucidate the driving forces controlling polyp expansion in these organisms and the potential role of particle concentration, the octocoral *Corallium rubrum* was investigated in accordance with two approaches: 1) High-frequency *in-situ* observations examining various environmental and biological variables affecting the water column, and 2) Video-recorded flume-controlled laboratory experiments performed under a range of environmental and biological conditions in terms of water temperature, flow speed, chemical signals and zooplankton. In the field, *C. rubrum* polyp expansion correlated positively with particle (seston and zooplankton) concentration and current speed. This observation was confirmed by the flume video records of the laboratory experiments, which showed differences in polyp activity due to changes in temperature and current speed, but especially in response to increasing nutritional stimuli. The maximum activity was observed at the highest level of nutritional stimulus consisting of zooplankton. Zooplankton and water movement appeared to be the main factors controlling polyp expansion. These results suggest that the energy budget of passive suspension feeders (and probably the benthic community as a whole) may rely on their ability to maximise prey capture during food pulses. The latter, which may be described as discontinuous organic matter (dead or alive) input, may be the key to a better understanding of benthic-pelagic coupling processes and trophic impacts on animal forests composed of sessile suspension feeders.

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15 ABSTRACT

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17 several environmental factors such as hydrodynamics, water temperature and food

18 concentration. To better elucidate the driving forces controlling polyp expansion in these

19 organisms and the potential role of particle concentration, the octocoral *Corallium*

20 *rubrum* was investigated in accordance with two approaches: 1) High-frequency *in-situ*

21 observations examining various environmental and biological variables affecting the

22 water column, and 2) Video-recorded flume-controlled laboratory experiments

23 performed under a range of environmental and biological conditions in terms of water

24 temperature, flow speed, chemical signals and zooplankton. In the field, *C. rubrum*

25 polyp expansion correlated positively with particle (seston and zooplankton)

26 concentration and current speed. This observation was confirmed by the flume video

27 records of the laboratory experiments, which showed differences in polyp activity due to

28 changes in temperature and current speed, but especially in response to increasing

29 nutritional stimuli. The maximum activity was observed at the highest level of nutritional

30 stimulus consisting of zooplankton. Zooplankton and water movement appeared to be

31 the main factors controlling polyp expansion.

32 These results suggest that the energy budget of passive suspension feeders (and

33 probably the benthic community as a whole) may rely on their ability to maximise prey

34 capture during food pulses. The latter, which may be described as discontinuous

35 organic matter (dead or live) input, may be the key to a better understanding of benthic-

36 pelagic coupling processes and trophic impacts on animal forests composed of sessile

37 suspension feeders.

38

39

INTRODUCTION

Passive suspension feeders play an important role in energy transfer from the water

column to the benthos (Gili & Coma, 1998). These organisms are important biomass

contributors in benthic communities, being an essential part of the 'animal forest', in

which the main three-dimensional builders are clonal or individual organisms of animal

origin (Rossi et al., 2017a). Seston (live and dead particles present in the water column,

Rossi & Gili 2007) availability, which depends on its abundance, composition and

renewal rate, is one of the most important parameters affecting the distribution, energy

fluxes and biological constraints of suspension feeders (Grémare et al., 1997; Coma et

al., 2001). As such, it is key to expanding our knowledge of community dynamics and

the potential regression, substitution and/or mortality of suspension feeder populations,

processes that have led to profound transformations over the last few decades (Rossi,

2013).

An immediate response of passive suspension feeders to changes in seston availability

and hydrodynamism is to tune their feeding activity, which can also be affected by other

short-term and seasonal environmental and biological changes (Coma et al., 1994;

Rossi & Gili, 2007; Previati et al., 2010). In gorgonians, alcyonarians and zoanthids for

example, feeding activity is reflected in polyp expansion (Dai & Lin, 1993). It has been

demonstrated to vary seasonally (Coma et al., 1994; Garrabou, 1999; Rossi, 2002), with

the frequency of food inputs a potential factor driving polyp expansion (Tsounis et al.,

2006; Rossi & Gili, 2007, Rossi et al., 2017b).

The more variable the water column environmental factors, the more diversified the

mechanisms to enhance prey capture and feeding optimisation, as organisms adopt a

63 range of strategies to take advantage of every potential source of food (Coma et al.,

64 2001). In laboratory experiments, capture rates and polyp expansion among passive

65 suspension feeders have been shown to be related to nutritional stimuli, particle

66 concentration and flow speed (Leversee, 1976; Dai & Lin, 1993; Anthony, 1999), but

67 there is almost no information on how environmental factors affect *in situ* polyp activity

68 during short-term cycles (Rossi & Gili, 2007). Epibenthic water masses and associated

69 plankton and seston concentrations can change rapidly, with particulate organic matter

70 concentration tripling or quadrupling in less than a day (Grémare et al., 2003; Rossi &

71 Gili, 2007; Rossi et al., 2013). These non-continuous food pulses have never been fully

72 studied in relation to the activities of passive suspension feeders and may be a key

73 factor for understanding the overall energy budget of sessile organisms in animal

74 forests.

75 The aim of this study is to achieve a better understanding of which factors drive polyp

76 activity in passive suspension feeders, seeking to determine whether environmental and

77 biological factors act synergistically in polyp expansion, using the red coral (*Corallium*

78 *rubrum*) as a model organism. To achieve this objective, two methodological

79 approaches were used: 1) High-resolution polyp observations in the field (Rossi & Gili,

80 2007), under recorded environmental conditions (i.e. current speed, zooplankton

81 concentration, chlorophyll *a* and protein content of epibenthic seston). This will help to

82 better understand whether there is any pulse-like energy input (Palardy et al., 2006;

83 Tsounis et al., 2006). 2) *Ex-situ* high-resolution flume (closed channel under controlled

84 conditions) experiments to test *C. rubrum* polyp activity in relation to a range of

85 environmental factors (i.e. temperature, current speed, nutritional stimuli and presence

86 of zooplankton). The main aim is to understand how intermittent food supply may

87 influence the energy budgets of passive suspension feeders.

88

89 MATERIALS AND METHODS

90 *Field survey*

91 The field survey was conducted in the Medes Islands, NW Mediterranean (40º 02' 55''N,

92 3º 13' 30''E). Sampling and observations were carried out at 18–20 m depth within a

93 coralligenous community located in a channel. The channel was alternately influenced

94 by northerly and southerly currents, which may reach high speeds (from 2 up to 30 cm

95 s^{-1}, Rossi & Gili, 2007).

96 *C. rubrum* polyp expansion was monitored at a high frequency (i.e. once every 6 hours)

97 by a single SCUBA diver from June 24 to 29, 1997. This period was chosen because

98 pelagic primary production and the frequency of seston pulses is high (Rossi & Gili,

99 2007). Expansion is defined as the maximum aperture of polyps (Sebens, 1987). Polyp

100 activity was observed in ten groups of ten colonies each time by scuba divers.

101 The following parameters were concomitantly monitored: (1) hydrodynamics (using an

102 Aandera SDP® Doppler current meter, moored in the same place as the observed

103 passive suspension feeders, recording currents 0.5 m above the benthic surface), (2)

104 seston concentration and quality determined by assessment of chlorophyll *a* and protein

105 concentrations (see Rossi & Gili, 2007), and (3) zooplankton concentration determined

106 by analysing two samples collected by a scuba diver towing two small plankton nets (22

107 cm in diameter with a mesh size of 100 μm) a distance of 40 m (Coma et al., 1994;

108 Rossi et al., 2004). Wind, wave height and tidal oscillation were recorded every day by

109   the Estartit Meteorological Station in accordance with the protocols of Cebrián et al.

110   (1996).

111

112   *Experimental observations on polyp activity*

113   On March 15, 2007, several small colonies of *C. rubrum* were collected at depths of 28-

114   30 m (water temperature 14ºC) using a crowbar, and were immediately transferred to

115   the Observatoire Océanologique de Banyuls (France) (see a description of the area in

116   Rossi et al. 2003; this area is 40 km from the Medes Islands, where we made the field

117   study). Colonies were placed in small cylinders, 4 cm in diameter (two colonies per

118   cylinder), attaching them with non-toxic rubber, and kept at 15°C in running seawater for

119   two weeks prior to the start of the experiments. Corals were fed three times a week with

120   copepods, ground mussels and *Artemia* nauplii. Running seawater supplied to the tanks

121   was filtered through a 4 μm filter, allowing only pico- and nano-plankton to be present in

122   the aquaria, which is a negligible part of the diet of these species (Tsounis et al., 2006;

123   Picciano & Ferrier-Pagés, 2007).

124   The flume used (Fig. 1) was 10 cm wide X 20 cm high with a total length of 450 cm,

125   resulting in an overall maximum water capacity of 85 litres. For each experiment, filtered

126   (2 μm) seawater was used. Four colonies (two cylinders) were tested in each

127   experiment. The pump used could generate speeds ranging from 1 to 6 cm s^{-1}. A

128   Minilab SD12 ultrasonic current meter (Sensordata, Bergen, Norway) (resolution 1 mm

129   s^{-1}, bandwidth 35 Hz and effective acoustic path length 29 mm) was used to measure

130   water flow during each experiment. Water temperature was recorded to the nearest

131   0.5ºC.

132   The polyps of all colonies were closed at the beginning of each experiment. Colonies

133   were placed in the flume and acclimated for 30 min, with experiments conducted from

134   24 to 72 hours.

135   For assessment of the effects of zooplankton and nutritional stimuli, natural

136   Mediterranean zooplankton was used.

137   Zooplankton was collected from epibenthic waters using 200 μm mesh nets near the

138   coast on the 6th and 14th April, 2007. The samples were transported in a cooler to the

139   main lab. The zooplankton was centrifuged (3000 rpm) and stored at -20ºC until its use

140   in the experimental set-up. An aliquot of 1 ml in 5 replicates from each sampling was

141   fixed with 6% formalin in order to count the number of items added to the flume water in

142   each experiment (Coma et al., 1994).

143   For the chemical signal experiments, a selected volume of zooplankton (40 and 120 ml

144   in 65 litres, corresponding to nutritional stimuli N1 and N2) was gently ground with a

145   glass homogeniser, in order to make a uniform mass of the zooplankton. The

146   homogenised zooplankton was filtered through a 10 μm mesh to remove the solid part

147   and the liquid part was stored 60-90 minutes before the experiment in a cooler. The

148   liquid part was the nutritional stimulus added to the flume. A selected volume (120 ml in

149   65 litres, final concentration 1500 ± 252 ind. m^{-3}) was used directly in the experiment to

150   understand the influence of zooplankton (not ground, the dead particles directly added

151   to the flume) on polyp expansion (N3). In both cases, the zooplankton (filtrate or

152   particles) was placed directly in the flume once the water was running, not before. All

153   the nutritional stimuli experiments were conducted at a temperature of 18ºC with a

154   constant flow speed of 3 cm s^{-1}.

155   The flume was illuminated using LEDs (Lunartec 48 LED white 40W bulbs). A mirror

156   oriented 45° with respect to the flume's main axis was placed downstream from the

157   monitored colonies so that they could be observed through cameras without major

158   disturbance to the water current (Fig.1). A video system with a colour camera (JAI

159   S3200® fitted with a 50 mm objective) was placed next to the flume and used to monitor

160   the colonies. The signal was received using a Falcon Plus® video grabber and

161   transferred to a PC, where the images were recorded in JPEG format (80%

162   compression). Real image size was 104 x 78 mm (Duchêne et al., 2000; Maire et al.,

163   2007) corresponding to 736 X 568 pixels and thus to a resolution of 140 µm pixel^{-1}. The

164   frame capture rate was set at 3 images/min.

165   Maximum polyp expansion was recorded and calculated (taking into account the

166   contracted and fully expanded polyp). The coral's white polyps and tentacle crowns

167   contrast with the black background and red coenenchima, allowing for good image

168   analysis. The JPEG images were assembled into AVI films (SEM VIDEO 1). Image

169   processing was then performed on the films using CVAB software (© J.C. Duchêne).

170   Image analysis allowed calculation of the surface area of open polyps on the coral

171   branches. The surface area of open polyps on each branch was separated and

172   accounted for in every image of each film. Segmentation of the images allowed the

173   open polyps to be separated from the coenenchyme in distinct pixel patches

174   corresponding to existing regions of the coral. The labelled regions were tracked across

175   images in the films, providing information on the activity of the polyps forming the

176   colonies. Two types of information were derived from the observations: (1) the total

177   surface area of the open polyps, and (2) the polyp activity index (taking into account the

178   minimum and maximum expansion of the polyp, as total pixel counting), obtained from

179   subsequent image differentiation. The polyp expansion showed the changes occurring

180   in each region at any given time, including opening and closing polyps and moving

181   tentacles. If a polyp moved its tentacles, the recorded surface area is expected to

182   remain the same, with a null difference between the two successive apparent surface

183   areas. While measuring colony size, the software also allowed to measure the events

184   characterised by low dynamism, such as slow feeding movements, and examination of

185   mesoglea inflation before polyp opening.

186

187   **Statistical analysis**

188   The variability in i) *C. rubrum* polyp expansion, ii) seston composition and iii) zooplankton

189   composition was assessed at various temporal scales by multivariate analyses and

190   correlated with the environmental variables recorded during the sampling. By means of

191   two laboratory experiments, we assessed the variability in *C. rubrum* polyp expansion in

192   response to a range of temperatures, current speeds and nutritional stimuli.

193   *In situ*, to assess differences in *C. rubrum* polyp expansion, the design incorporated two

194   factors: Cycle (Cy, as a fixed factor with 5 levels, each 24 h) and Time (Ti, as a random

195   factor with 4 levels, nested in Cycle, each 6 h), with n = 3. Multivariate analyses of

196   variance (PERMANOVA, Anderson, 2001) considered Euclidean distances based on

197   untransformed polyp expansion data and previously normalised seston composition,

198   using 9,999 random permutations of the appropriate units (Anderson & Braak, 2003).

199   To assess differences in zooplankton composition we performed permutational analyses

200   of variance (PERMANOVA, Anderson, 2001) considering Bray Curtis dissimilarities

201   based on transformed data (fourth root), using 9,999 random permutations of the

202   appropriate units (Anderson & Braak, 2003), adopting a design with one factor, i.e. Cycle

203   (Cy, as a fixed factor with 5 levels, n = 4). In order to detect which taxa contributed most

204   to dissimilarity among the cycles, a similarity percentage (SIMPER) analysis was

205   performed (Clarke, 1993). To examine the generality of patterns in polyp expansion,

206   seston composition and zooplankton composition, we generated MDS plots.

207   In addition, we performed another two laboratory experiments in order to evaluate *C.*

208   *rubrum* polyp expansion under a range of physical conditions and nutritional stimuli. To

209   assess the effect of temperature and current speed on polyp activity, we performed

210   permutational analyses of variance (PERMANOVA, Anderson, 2001) considering

211   Euclidean distances based on untransformed data, using 9,999 random permutations of

212   the appropriate units (Anderson & Braak, 2003), adopting a design with two factors:

213   Temperature (Te, as a fixed factor with 3 levels) and Current (Cu, as a fixed factor with 3

214   levels) with n = 12.

215   Moreover we performed a further experiment to evaluate the effects of nutrient levels,

216   following a design with one factor, i.e. Nutritional Stimuli (Nu, as a fixed factor with 4

217   levels) with n = 8.

218   When significant differences were encountered (p < 0.05), post-hoc pairwise tests were

219   carried out to ascertain the consistency of the differences across the conditions tested.

220   Because of the restricted number of unique permutations in the pairwise tests, *p* values

221   were obtained from Monte Carlo samplings. The analyses were performed using PRIMER

222   v. 6 (Clarke & Gorley, 2006).

223

224 **Results**

225 *Polyp activity in high time-resolution field monitoring*

226

227 The activity rhythms (polyp expansion) of *C. rubrum* between the 24th of June (15:00)

228 and the 29th of June (9:00) 1997 in relation to the environmental variables tested are

229 shown in Figure 2 (A to D). The activity of the 100 colonies varied between 0% (polyps

230 fully closed) and 100% (polyps fully open), the change occurring in only 6 hours in some

231 cases (see for example the transition between 26 of June at 9:00 and 26 of June at

232 15:00). Current speed ranged from 0.2 cm s^{-1} to 30 cm s^{-1}. The mean current speed

233 during the activity rhythm observations was 9.3±9.4 SD cm s^{-1}, with the highest speeds

234 recorded towards the middle and end of the experimental period (Fig. 2A). Zooplankton

235 concentration (mainly copepods and nauplii) varied from 298 individuals m^{-3} to 8437

236 individuals m^{-3}. The mean concentration was 2122±2412 SD individuals m^{-3}.

237 Zooplankton had higher concentrations in the later cycles (Fig. 2B). Chlorophyll *a*

238 concentration varied from 0.28 µg L^{-1} to 0.70 µg L^{-1}, with a mean of 0.4±0.1 SD µg L^{-1},

239 with the highest values recorded at the beginning of the experimental period (Fig. 2C).

240 Protein concentration followed a different tendency (Fig. 2D), ranging from 135 µg L^{-1} to

241 243 µg L^{-1}. The mean concentration was 176±32 SD µg L^{-1}, with the highest

242 concentrations found towards the middle and end of the experimental period. No

243 significant relationships were directly observed between the tested environmental

244 variables and red coral polyp expansion.

245   The results of the PERMANOVA reveal that the percentage of expanded *C. rubrum*

246   polyps varied significantly among sampling times of cycles (Table S1), while pairwise

247   analyses underline the differences within each cycle (Table S2).

248   Following the same experimental design, PERMANOVA analyses on seston composition

249   exhibited significant differences at the investigated temporal scales (Tables S1 and S2).

250   In particular, pairwise analyses of both polyp expansion and seston composition showed

251   significant differences among all sampling times of the first cycle.

252   MSD plots of seston composition in relation to each sampling time show separation of

253   cycles and sampling times (Fig. 3a), indicating the high number of open polyps during the

254   cycles characterized by higher sea water movement. Significant differences among

255   cycles were confirmed by Permanova for zooplankton composition (Table S3).

256

257   The SIMPER analysis revealed the highest dissimilarity in the zooplankton assemblages,

258   reaching 30.74% for C1 vs. C5, followed by C2 vs. C5 (30.12%), C1 vs. C4 (27.02%), C1

259   vs. C3 (25.09%) and C2 vs. C4 (25.06%) (Table S4), highlighting variation between the

260   first and last cycles. The MSD plots confirm the separation of cycles (Fig. 3b).

261

262   **Laboratory experiments**

263   In the experiments, a clear rhythm appears in the opening events of the coral branches

264   (Fig. S1 SEM). This rhythm was most dominant when the colony's polyps are fully

265   extended as could be observed in the records of individual polyp activity (Fig. S2 SEM).

266   The results of the PERMANOVA showed a significant Te x Cu interaction (Table S5 and

267   S6, Fig. 4), demonstrating that *C. rubrum* polyp expansion varied significantly among the

268 tested temperatures and currents. Increased temperature led to a rapid expansion of *C.*

269 *rubrum* expansion in still-water, however the simultaneous interactions between current

270 and temperature resulted in fast polyp expansion when the current speed was maximum

271 and the temperature was low (Fig. 4).

272

273 **Nutritional stimuli**

274 The results of the PERMANOVA (Table S7) and pairwise analyses (Table S8) reveal that

275 *C. rubrum* polyp expansion varied significantly among the different nutritional stimuli

276 tested, except for N1 vs. N2 (Fig. 5). In particular, the analyses showed a rapid expansion

277 of *C. rubrum* polyps when nutrition stimuli increased, reaching a maximal reaction when

278 nutritional stimulus consisted of zooplankton (Fig. 5).

279

280 DISCUSSION

281 The present study represents a first step to provide new insights into the relationship

282 between environmental-biological conditions and the capacity of passive suspension

283 feeders to intercept pulse-like energy inputs. High-resolution observations of polyp

284 activity in the field highlighted the complex combination of environmental variables

285 linked to seawater movement even on the small scale.

286 Ex-situ high-resolution flume experiments showed that polyp expansion accelerates with

287 current speed. In addition, the presence of nutritional stimuli, especially zooplankton,

288 induces a clear response in *C. rubrum* polyp activity, confirming they are sensitive in

289 detecting food availability.

290 The outcomes also contribute to our understanding of the biology and ecology of red

291 coral. *Corallium rubrum* polyp expansion seems to be most affected by water

292 temperature, as observed by Picciano & Ferrier-Pages (2007), and by sea water

293 movement as revealed here. Passive suspension feeding depends on current flow.

294 Nevertheless, given constant seston concentrations, increasing current speed enhance

295 filtration up to a maximum beyond which filtration no longer increased (Wildish, &

296 Kristmanson, 2005). Our *in situ* results suggest complex hydrodynamic conditions act in

297 a complementary way to shape polyp activity. It is clear that different conditions were

298 present in the first and second slot of the week. During the last days, a combination of

299 water movement and seston concentration could be the key to understanding increased

300 activity of *C. rubrum*. Water movement effects are essential to understanding plankton

301 activity and concentration (Sebens & De Reimer, 1977; Palardy et al., 2006). Increasing

302 water movement will increase this plankton activity and concentrations which, in turn,

303 will increase the number of red coral expanded colonies.

304 While the assessment of the effect of drag forces on polyp retention ability is beyond the

305 scope of the current study, we found a clear relationship between polyp activity and

306 current speed, similar to what has been reported in studies of other gorgonian species

307 (Dai & Lin, 1993). The rigid structure of the $CaCO_3$ skeleton of *C. rubrum* creates a

308 highly inflexible structure that has a limited range of movement, unlike other highly

309 flexible gorgonian passive suspension feeders used in previous studies (Dai & Lin,

310 1993). Highly flexible organisms may be able to minimise drag forces by altering colony

311 shape and reducing projected colony area when exposed to increased flow (Vogel,

312 1996). Previous studies of scleractinian corals suggest that current velocity within

313   colonies has an upper limit, or saturation velocity, which is dependent on colony

314   morphology (Chamberlain & Graus, 1975). The current speeds used in our study have

315   no dramatic effect on polyp shape and are considered optimal for polyp particle capture

316   (Leversee, 1976; Sponaugle, 1991). An increase in current speed (from 0 to 6 cm s^{-1})

317   therefore increases particle delivery to the polyps. Dai and Lin (1993) showed that

318   *Acanthogorgia vega* had a broader spectrum of polyp efficiency (as capture rates) at

319   flow speeds ranging from 0 to 15 cm s^{-1} than the other two species tested, probably due

320   to its bushy shape. In fact, the effect of flow on particle capture by polyps is probably a

321   general phenomenon among octocorals (Robbins & Shick, 1980; Patterson, 1991; Dai &

322   Lin, 1993), with polyp capture efficiency falling as the Reynolds number increases. In

323   the present study, *C. rubrum* polyp activity tends to increase with current speed, even in

324   the absence of increased abundance of food.

325   Similar to previous studies of *C. rubrum* polyp activity, the current study found a

326   significant relationship between temperature and polyp activity, with less polyp activity

327   at high temperatures. Previati et al. (2010) showed a significant relationship between

328   oxygen consumption and activity (open polyps) at 18-20ºC and a current of 1 cm s^{-1}

329   (approx.): oxygen consumption and activity increased, but above this temperature

330   oxygen consumption decreased. Studies of other octocorals follow the same trend of

331   closing their polyps at higher temperatures and decreasing oxygen consumption to

332   maintain a decreased metabolic rate in a quasi-dormant stage (Coma et al., 2002;

333   Previati et al., 2010). Although the relationship between temperature and polyp activity

334   might be the result of endogenous rhythms related to an internal clock (Previati et al.,

335   2010), it seems that lack of water flow (decreased current speed) is a key factor in the

336   spontaneous opening of polyps in the absence of external stimuli. In the present study,

337   colonies also decreased their maximum opening frequency as temperature increased in

338   still-water conditions. One hypothesis is that polyp expansion is needed for gas

339   exchange and excretion, however the majority of colonies tend to remain closed as

340   much as possible as temperature increases. When food availability in the water column

341   is low, the increase in temperature and flow seems to increase the response of *C.*

342   *rubrum* (maximum opening frequency), indicating a balance between the need for

343   opening and the current stimulus. Differences between the still-water and current-speed

344   experiments in terms of maximum opening frequency suggest current stimulus has a

345   greater influence than temperature constraints. Anthozoans need to expand their polyps

346   to favour breathing (Previati et al., 2010). Our results suggests that food acquisition

347   (related with water movement) in these passive suspension feeders appears to have

348   priority over gas exchange.

349   Of all the variables used to test for a response in polyp activity in the present study, the

350   addition of zooplankton elicited the fastest response in *C. rubrum* colonies. *C. rubrum* is

351   considered a passive suspension feeder capturing particulate organic matter (POM)

352   from the surrounding environment (Tsounis et al., 2006). Other Mediterranean and

353   tropical asymbiotic octocorals (gorgonians, soft corals) are able to capture POM (Ribes

354   et al., 2003), small zooplankton (Coma et al., 1994; Rossi et al., 2004) and

355   phytoplankton (Widding and Schlichter 2001). *C. rubrum* is also able to feed on

356   bacterioplankton (pico- and nanoplankton) (Picciano & Ferrier-Pages, 2007). A chemical

357   or chemical/physical (zooplankton) stimulus caused a rapid response in terms of polyp

358   activity, in some cases within a few seconds. The rapid response of polyp activity

359   increases with temperature, but at higher food concentrations the response becomes

360   even more rapid (Grémare et al., 2004). Relying completely on heterotrophic inputs

361   from seston, the detection of chemical signals and/or food particles may be more

362   important than other variables (i.e., temperature or current). Although there may be

363   inter-individual variability in the response (Duchêne et al., 2000; Duchêne, 2017), there

364   is clearly less variability in polyp activity when zooplankton stimuli are combined with

365   flow speed than with flow speed alone. This result is not surprising as it has been

366   demonstrated in previous studies that the addition of food to the water column can elicit

367   a response in other taxa (Duchêne & Rosenberg, 2001; Maire et al., 2007; Duchêne,

368   2017). The response to a chemical signal indicating increased zooplankton

369   concentrations has not previously been experimentally tested in octocorals, but in

370   scleractinian corals, particle concentration also elicited a response (Anthony, 1999). It is

371   clear that the synergistic effects of higher current speed and the presence of

372   zooplankton (or its chemical signal) provide a stimulus for the expansion of the polyps of

373   this gorgonian. Therefore, we hypothesise that if food and current stimulate polyp

374   activity, a recurring hydrodynamic parameter (food pulses due to high seston

375   concentration) may cause current speed and particle concentration (dead or alive) to act

376   synergistically.

377   The high-frequency *in situ* monitoring used in this study is currently the only known

378   method for detecting the response in terms of polyp activity to changes in zooplankton

379   and particulate organic matter availability and current flow speeds. In the space of just a

380   few hours, epibenthic seston concentrations may fluctuate dramatically, with large

381   increases and decreases in the concentrations of available zooplankton or seston

382 (Rossi & Gili, 2007; Rossi et al., 2013). A greater frequency of high-speed current

383 episodes may have a synergistic effect on the entire coralligenous community, by both

384 increasing currents and resuspending particulate organic matter. This environment

385 creates optimal conditions for nutrient cycling and capture of crustacean zooplankton

386 and increasing prey capture rates among benthic suspension feeders. A relationship

387 between food pulses and feeding activity was also found in other tide-dominated

388 environments (Naylor, 1976; Naylor, 2005).

389 We consider that in the Mediterranean Sea (and in other benthic systems), food

390 availability is non-continuous for benthic suspension feeders. Increasing the frequency

391 of high current-speed events and hence the quantity of available epibenthic seston may

392 be a driver of pulse-like temporal changes in the particulate organic matter available in

393 the water column for the energy budgets of coralligenous (and other) benthic

394 communities. Many authors have shown the positive relationship between prey capture

395 rates and concentrations of plankton (Sebens & De Reimer, 1977; Coma et al., 1994;

396 Palardy et al., 2006). In intertidal systems, there is a clear relationship between benthic

397 suspension feeding activity and tidal fluxes (Sebens, 1987). In *C. rubrum,* short periods

398 of high seston and zooplankton abundance could be the key to understanding energy

399 input, high-current episodes creating high prey concentrations, leading to maximum

400 particle capture rates.

401 Optimal foraging theory (Hughes, 1980) posits the need to take advantage of favourable

402 feeding pulses as an individual colony but also as a population within a community.

403 Palardy et al. (2006) suggested that the energy budget of passive suspension feeders

404 may be dependent on non-continuous zooplankton availability, and Robbins & Shick

405 (1980) related the activity of *Metridium senile* to tidal flux. It is clear that even if polyp

406 seston capture is an important source of nutrition (being a more constant food source,

407 Ribes et al., 1999) the detected seasonal concentrations may not be sufficient, given

408 the energy constraints of most passive suspension feeders. In the complex

409 coralligenous community, a broad spectrum of energy constraints is shown by the

410 diverse range of activities and behaviours observed during our study period. Many

411 organisms take advantage of the food pulses related to tidal patterns of water

412 movement, resuspension and nutrient recirculation (Robbins & Shick, 1980; Gibson,

413 2003). We hypothesise that the foraging strategy of *C. rubrum* (but also other benthic

414 organisms and communities) is influenced by the frequency of high current-speed

415 events, which are far from homogeneous in different seasons (Rossi & Gili, 2007).

416

417 CONCLUSIONS

418 In this paper we showed that temperature and current speed are essential cues to

419 understand the polyp expansion of passive suspension feeders, however chemical

420 signals have a prevalence in the activity of these organisms. The synergy between

421 currents and zooplankton is thus the key to understand prey capture of benthic

422 suspension feeders in natural environments. Such a combination is not homogeneous

423 through the time, so when we try to understand and quantify the energy balance of

424 these metazoans, we have to consider the available food pulses that may be in phase

425 with tidal rhythm and also affected by strong winds or temporal upwellings. The

426 presence of food pulses is key to understanding global energy inputs and the energy

427   budgets of these organisms, and how these synergistic effects (current speed with

428   particle concentration) bring energy pulses to the benthic communities.

429

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437

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578

579 FIGURE CAPTIONS

580 **Figure 1 The flume used for laboratory experiments.** It consisted of a closed

581 transparent plastic ellipsoid channel placed in a temperature-controlled chamber.

582 **Figure 2 The activity rhythms (polyp expansion) of C. rubrum in relation to the**

583 **environmental variables. (A) Current speed, (B) Zooplankton concentration, (C)**

584 **Chlorophyll *a* concentration, (D) Protein concentration.**

585 **Figure 3 MDS plots for seston composition with Euclidean distances based on**

586 **normalised data; vectors with Pearson's correlation coefficients show (A)**

587 **environmental variables and (B) polyp activity. MDS plots for the zooplankton**

588 **community, with Euclidean distances based on normalised data; vectors with**

589 **Pearson's correlation coefficients show (C) environmental variables and (D) polyp**

590 **activity.**

591 **Figure 4 The influence of temperature and current on *C. rubrum* polyp expansion**

592 **under a range of experimental conditions.** C0= still water; C1= 3 cm s^{-1}; C2= 6 cm s^{-}

593 ^{1}. T1= 13° C; T2=18° C; T3= 25° C. Data are reported as mean values ± S.E.

594 **Figure 5 The influence of nutritional stimuli on *C. rubrum* polyp expansion under a**

595 **range of experimental conditions.** N0= no stimulus; N1= 40 ml; N2= 120 ml;

596 N3=zooplankton (aprox 1500 ind m^{-3}). Data are reported as mean values ± S.E.

597

598   SUPPLEMENTARY ELECTRONIC MATERIAL FIGURE CAPTIONS

599   **Figure S1 SEM: Periodograms from three different colonies.** Example of three

600   periodograms from three colonies (peaks represent polyp expansion), showing

601   endogenous rhythms at 18°C and still-water conditions. On the left the recorded

602   normalised activities (i.e. the number of pixels divided by the maximum polyp expansion

603   for that experiment); on the right the Lomb periodogram with frequencies on the X axis

604   and number of occurrences on the Y axis. Figures close to the peaks indicate the

605   periods. The 3 dashed lines represent the significance of the peaks, 0.1, 0.01 and

606   0.001, the smallest value corresponding to the highest significance.

607   **Figure 2 SEM: Records of individual polyp activity.** (A) The area below the peaks for

608   a given experiment. (B) The derivative of this curve with absolute values (increased or

609   decreased polyp expansion). These records usually show a steeper descent after

610   opening.

611

612   VIDEO RECORDING: *Corallium rubrum* polyp activity at 18°C and 3 cm s^{-1} current

613   speed.

614

615

Figure 1

Figure 1 The flume used for laboratory experiments.

It was a closed transparent plastic ellipsoid channel placed in a temperature-controlled chamber.
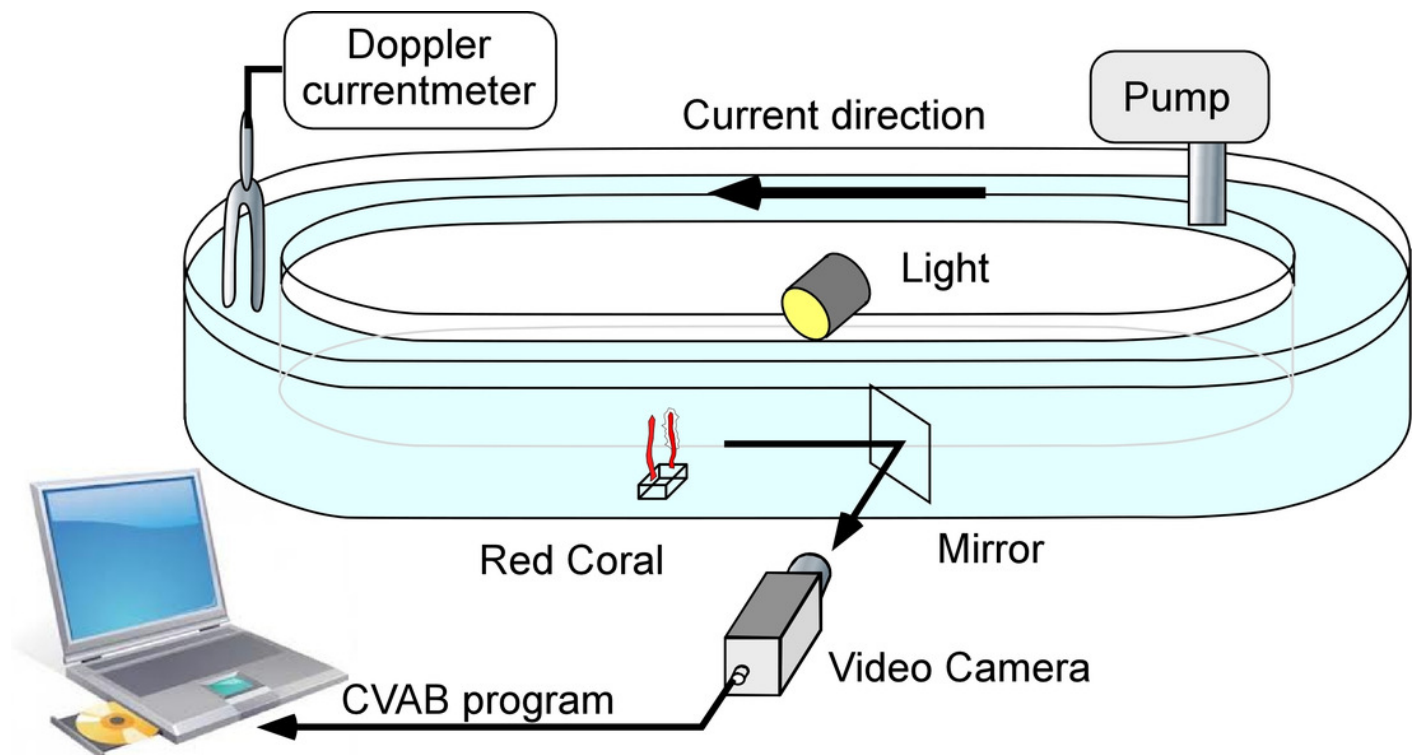
Figure 2

Figure 2 The activity rhythms (polyp expansion) of C. rubrum in relation to the environmental variables.

(A) Current speed (B) Zooplankton concentration(C) Chlorophyll *a* concentration (D) Protein concentration.
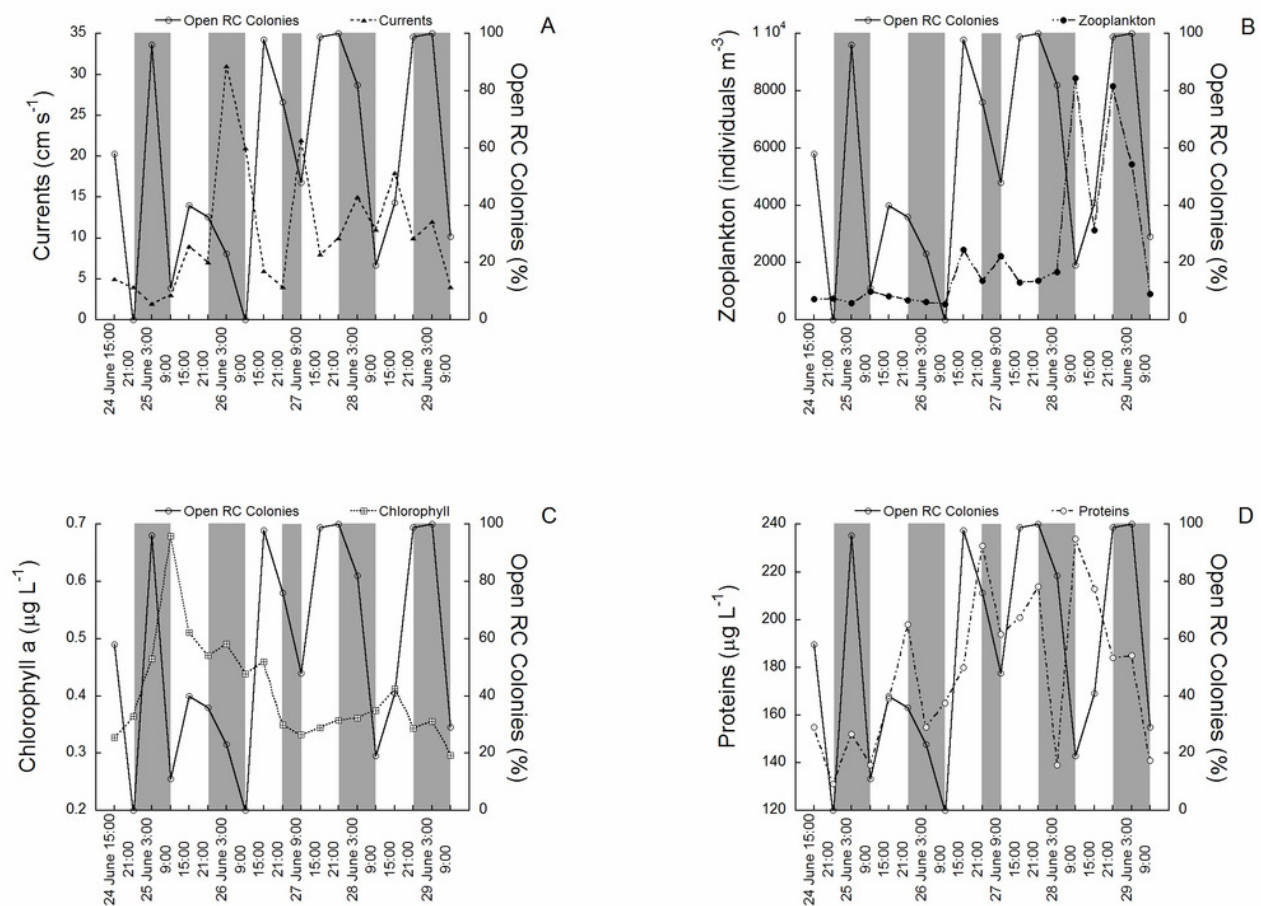
Figure 3

Figure 3 MDS plots for seston composition, with Euclidean distances based on normalised data

vectors with Pearson's correlation coefficients show (A) environmental variables and (B) polyp activity. MDS plots for the zooplankton community, with Euclidean distances based on normalised data; vectors with Pearson's correlation coefficients show (C) environmental variables and (D) polyp activity.
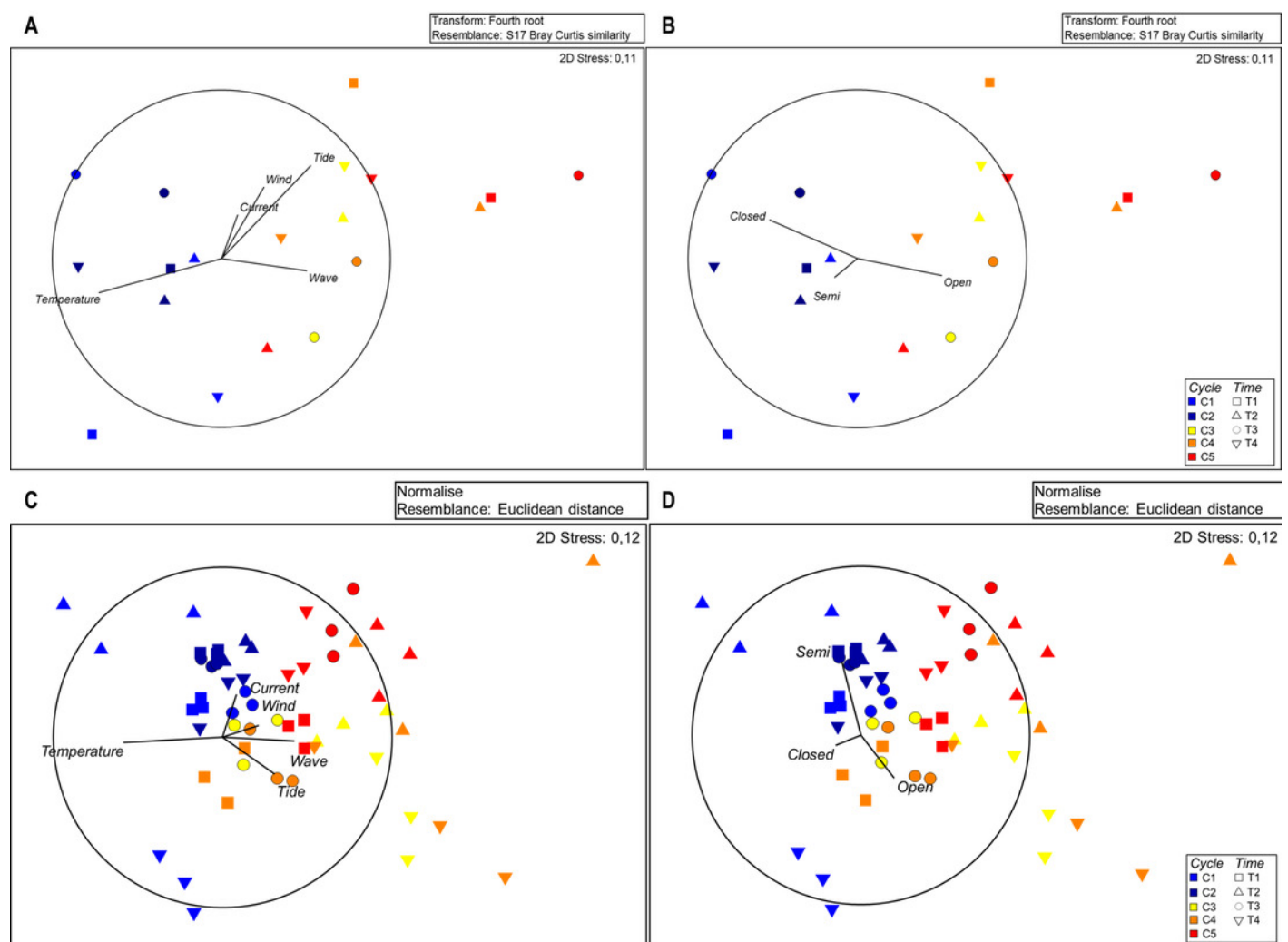
Figure 4

Figure 4 The influence of temperature and current on *C. rubrum* polyp expansion under a range of experimental conditions.

C0= still water; C1= 3 cm s^{-1}; C2= 6 cm s^{-1}. T1= 13° C; T2=18° C; T3= 25° C. Data are reported as mean values ± S.E.
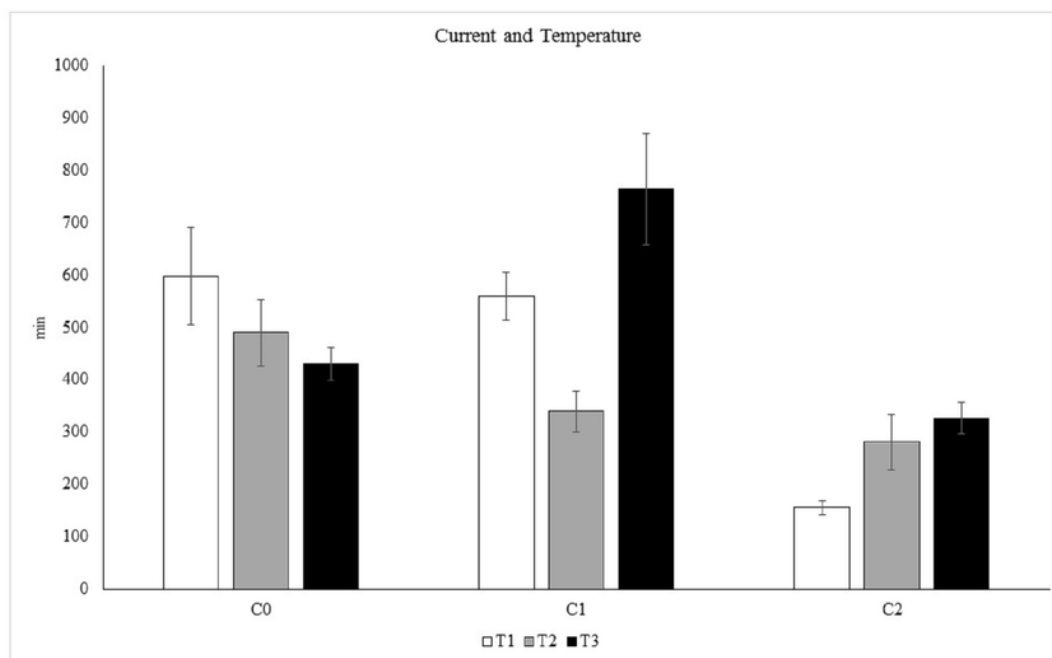
Figure 5

Figure 5 The influence of nutritional stimuli on *C. rubrum* polyp expansion under a range of experimental conditions.

N0= no stimulus; N1= 40 ml; N2= 120 ml; N3=zooplankton (aprox 1500 ind m^{-3}). Data are reported as mean values ± S.E.