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23 **Foraging of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) on invasive**  
24 **allochthonous and autochthonous algae**

25

26 Antonio Pusceddu<sup>✉\*</sup>, Marta Mikhno, Angelica Giglioli, Marco Secci, Viviana Pasquini, Davide  
27 Moccia, Pierantonio Addis

28

29 Department of Life and Environmental Sciences, University of Cagliari, Via T. Fiorelli, 1, 09126  
30 Cagliari, Italy

31

32 <sup>✉</sup> Corresponding Author: [apusceddu@unica.it](mailto:apusceddu@unica.it); Tel. +39 070 6758053

33 **Abstract**

34 Attempts to control marine invasive alien species (IAS) with native predators gained contrasting  
35 results, so far. To explore the feasibility of this approach to control the invasive marine alga *Caulerpa*  
36 *cylindracea*, we investigated the foraging behaviour of the sea urchin *Paracentrotus lividus* on three  
37 native macroalgae (*Ulva* sp., *Penicillus capitatus* and *Cystoseira compressa*) and on *C. cylindracea*.  
38 The consumption rate of *C. cylindracea* fresh biomass resulted larger than that of the other algae,  
39 when offered separately or in combination. *C. cylindracea*, however, was not the most attractive food  
40 item. The larger consumption rates of *C. cylindracea* can be explained by its specific caloric content  
41 (as assessed by its biochemical composition) that is lower than that of the other algae. Our results  
42 confirm that *P. lividus* can feed on *C. cylindracea*, but do not fully support its use to control *C.*  
43 *cylindracea*, unless in conditions where this alga is largely dominant because of other factors.

44

45 **Key words:** Invasive alien species, *Caulerpa cylindracea*, foraging behaviour, *Paracentrotus lividus*

## 46 **1. Introduction**

47 Biological invasions are often promoted by human activities which, accidentally or intentionally,  
48 through multiple vectors, allow the introduction and spreading of allochthonous species in areas that  
49 are well outside their natural distribution range (Mack et al., 2000). Allochthonous species can settle  
50 permanently in the new habitat because of favourable abiotic (e.g., climate) and biotic (e.g., positive  
51 biological interactions or lack of negative ones) conditions. If other factors do not limit their growth  
52 and spreading, they become Invasive Alien Species (IAS). Biological invasions are currently a key  
53 component of global environmental change (Simberloff et al., 2013; Geraldi et al., 2020) and cause  
54 severe damages to the recipient ecosystems and the communities relying on (Butchart et al., 2010).

55 Thanks to their three-dimensional characteristics, the global oceans are increasingly prone to  
56 biological invasions (Williams et al., 2013; Chan and Briski, 2017). Vectors contributing the spread  
57 of marine IAS include maritime transport, ballast waters, aquaculture, illegal trade of ornamental  
58 species, and tourism (Zenetos et al., 2012; Katsanevakis et al., 2013; Tsiamis et al., 2019). IAS can  
59 be vectors of diseases and pests (Crowl et al., 2008), overwhelm native species by competition  
60 (Katsanevakis et al., 2014; Zwerschke et al., 2018), alter food chains (Lavery et al., 2017; Calizza et  
61 al., 2021), decrease biodiversity (Rizzo et al., 2020) and even modify key ecosystem functions (Bax  
62 et al., 2003; Molnar et al., 2008; Rizzo et al., 2017; Palmas et al., 2019).

63 The most recent estimates indicate the presence in the Mediterranean Sea of more than 1000  
64 allochthonous marine species, more than half of which are consolidated and widespread, and almost  
65 120 are invasive (Katsanevakis et al., 2012; Dailianis et al., 2017; Chartosia et al., 2018; Katsanevakis  
66 et al., 2020; Ragkousis et al., 2020; Zenetos and Galanidi, 2020). The large majority of allochthonous  
67 species currently found in the Mediterranean Sea entered the basin through the Suez Canal (namely,  
68 Lessepsian species; Katsanevakis et al., 2014; Galil et al., 2015). Other allochthonous species (e.g.,  
69 the invasive alga *Rugulopteryx okamurae*) entered the Mediterranean through the Strait of Gibraltar  
70 (García Gómez et al., 2020). Secondary vectors of alien species introduction in the Mediterranean  
71 Sea include maritime navigation and transport via ballast waters, which plausibly allowed >300  
72 allochthonous species to enter the basin, and aquaculture, which is estimated to have been the cause  
73 of introduction of about 64 alien species (Katsanevakis et al., 2014). To prevent the spread of  
74 potentially harmful aquatic organisms and pathogens in ships' ballast water, the International  
75 Maritime Organization (IMO) adopted the Ballast Water Management Convention or BWM  
76 Convention which entered into force in 2017.

77 The green alga *Caulerpa cylindracea* (Sonder, 1845), native to south-western Australia, appeared  
78 for the first time in the Mediterranean Sea in 1990. Since then, *C. cylindracea* has rapidly spread into

79 the basin causing significant effects on the recipient ecosystems (Piazzi et al., 2005; Piazzi and Balata,  
80 2008, 2009; Bulleri et al., 2009; Holmer et al., 2009; Hendricks et al., 2010; Žuljević et al. 2011;  
81 Balata et al. 2015; Pusceddu et al., 2016; Rizzo et al., 2017; Gribben et al., 2018), to the point of  
82 being considered one of the most serious biological invasions recorded in the whole basin (Klein and  
83 Verlaque, 2008; Rizzo et al. 2020 and citations therein). Such rapid and widespread expansion can  
84 be ascribed, on the one hand, to the capacity of this alga to thrive in a broad spectrum of environmental  
85 conditions, including either hard or soft substrates, tide pools, dead seagrass beds, detritus sea beds,  
86 coralligenous concretions, at depths ranging from 0 to >70 m (Piazzi et al. 2001; Klein and Verlaque,  
87 2008; Bulleri et al., 2009; Piazzi and Balata, 2009; Pacciardi et al., 2011; Gennaro and Piazzi, 2011;  
88 2014). In addition, *C. cylindracea* is well suited in ecosystems degraded by anthropogenic pressures  
89 (eutrophication, increased sedimentation, habitat destruction and over-exploitation) which help its  
90 successful spread (Bulleri et al., 2011; Ceccherelli et al., 2014; Piazzi et al., 2016).

91 Like other species of the same order (e.g., *Caulerpa taxifolia*) *C. cylindracea* produces secondary  
92 metabolites (caulerpin, caulerpenin, caulerpicin) which are involved in the chemical defence against  
93 herbivores (Sureda et al., 2009; Feline et al., 2012) or competition with other species (Klein and  
94 Verlaque, 2008). Despite this, some herbivore species feed on *C. cylindracea*, including the sparid  
95 fishes *Boops boops* (Ruitton et al. 2006), *Sarpa salpa* (Tomas et al., 2011b), *Diplodus sargus*,  
96 *Diplodus vulgaris* and *Spondylisoma cantharus* (Terlizzi et al., 2011; Feline et al., 2017), and the  
97 sea urchins *Paracentrotus lividus* and *Sphaerechinus granularis* (Ruitton et al., 2006; Tejada et al.,  
98 2013). Sea urchins, being generalist herbivores or omnivores, can have a strong ecological role in  
99 either structuring or altering benthic coastal communities (Lawrence, 1975; Lawrence et al., 2013;  
100 Dayton, 1985; Bonaviri et al. 2011; Agnetta et al., 2015), and are therefore excellent candidates to  
101 investigate their potential role in providing a possible resistance to invasion by allochthonous algae  
102 (Cebrian et al., 2011).

103 Native herbivores can incorporate alien algae in their diets and several experiments have been  
104 carried out to investigate their potential to control invasive algae (Gollan and Wright, 2006). Those  
105 experiments, however, generated contrasting results, particularly for the urchin *P. lividus*. Monteiro  
106 et al. (2009) reported that different herbivore species, including *P. lividus*, preferred native seaweeds  
107 over the invasive brown seaweed *Sargassum muticum*. In contrast, Tomas et al. (2011a) reported that  
108 *P. lividus* exhibited some preference for *Caulerpa racemosa* (now *C. cylindracea*). Most recent  
109 experiments carried out in the field investigated the feeding preferences of *P. lividus* for either  
110 allochthonous (*C. cylindracea* and *C. taxifolia* var. *distichophylla*) or native seaweeds (*Dictyopteris*  
111 *membranacea* and *Cystoseira compressa*) (Noè et al. 2018). Those experiments revealed that *P.*

112 *lividus* could ingest all the combinations of food offered, though it preferentially consumed a mixed  
113 diet including the invasive alien algae.

114 To shed light on the trophic factors possibly modulating the foraging behaviour of a generalist  
115 herbivorous sea urchin towards three native and one invasive algae, we exposed in mesocosms the  
116 sea urchin *P. lividus* to: i) an autochthonous green algae, *Ulva sp.*, used in urchin aquaculture (Prato  
117 et al., 2018); ii) *Penicillus capitatus*, a green alga common in Mediterranean shallow bottoms (Guiry,  
118 2001); iii) the autochthonous brown alga *C. compressa*, common in Mediterranean shallow bottoms  
119 and present in *P. lividus* gut contents (Boudouresque and Verlaque, 2013b; Noè et al., 2018), and iv)  
120 the invasive alien green alga *C. cylindracea*.

121 We tested the following null hypotheses: 1) the sea urchin has no preference for any of the tested  
122 algae; 2) the tested algae have the same biochemical compositions and caloric contents; 3) the feeding  
123 behaviour of *P. lividus* does not change on the tested algae.

124

## 125 **2. Material and methods**

### 126 *2.1 Harvesting and housing of sea urchins and algae*

127 Specimens of *P. lividus* were harvested at a depth of ca. 5 m from the south coast of Sardinia  
128 (39°08'28"N, 9°24'05"E). Within one hour from harvesting, specimens were transported in seawater  
129 at *in situ* temperature (25°C) to the mesocosm facility of the University of Cagliari located at the  
130 Santa Gilla Fish Consortium. Prior to the experiments, the sea urchins were acclimated for two weeks  
131 in circular tanks (150L volume) at  $25 \pm 0.5$  °C and at salinity 35, with forced aeration, a 75-L water  
132 daily exchange rate, and fed with the green alga *Ulva sp.*

133 The algae used as food in the manipulative experiments were harvested in the proximity of the sea  
134 urchins' harvesting sites and included: the green algae *Ulva sp.* and *P. capitatus*, the brown alga *C.*  
135 *compressa*, and the invasive green alga *C. cylindracea*.

136 The algae were maintained, for a maximum of 24 hours before the experiments, in separate tanks  
137 (150 L volume) kept at the same temperature, salinity, aeration, and daily water exchange rates as  
138 those used for the sea urchins, under ambient light conditions.

139

### 140 *2.2 Sea urchin biometrics and pabulum preparation*

141 Before each experiment sea urchins were weighed ( $\pm 0.001$ g) and measured by means of a precision  
142 gauge (height and test diameter in mm). Pabulum consisted of fragments of the algae (see below for

143 details) dried with absorbent paper and weighed ( $\pm 0.001$ g). *P. lividus* specimens used in the different  
144 experiments had a similar size ( $5.0\pm 0.5$  cm in diameter) and weight ( $60\pm 3$  g).

145

### 146 2.3 Experimental set-up and sampling strategy

147 We carried out 4 replicated experiments in which the sea urchins ( $n=3$ ) were exposed, separately, to  
148 each of the algae (Exp1, single item), and 4 replicated experiments in which the sea urchins ( $n=3$ )  
149 were exposed to variable mixtures of the algae (Exp2, multiple items) (**Fig. A.1**).

150 In all experiments, sea urchins were provided with more algae than they could consume. No urchin  
151 consumed  $> 50\%$  of the initial wet algal mass during the 24-h feeding period. All experiments started  
152 at the same time (6 pm) to homogenize for any circadian effect on feeding behaviour.

153 In Exp1, each 50-L tank containing a single sea urchin was added with 8 g (wet weight) of the  
154 algae. In Exp2, a total of 9 g (wet weight) of three equally represented algae (i.e., 3 g per species),  
155 were introduced in each of the three 50-L tanks (containing a single sea urchin specimen); each of  
156 these experiments contained a different combination of algal species (**Fig. A.1**).

157 At the beginning of the experiment, the food items (single or mixture) were divided into three  
158 portions each positioned along the outer edge of the tank. The sea urchin was placed in the centre of  
159 the tank, at the same distance from each of the individual food portions (**Fig. A.2a**). The movements  
160 of the sea urchins during each of the 48 h ongoing experiments were recorded by means of a 4K  
161 digital camera (GoPro Hero3 Black) in time-lapse mode (one frame per minute), positioned on top of  
162 the manipulation tanks (**Fig. A.2b**).

163

### 164 2.4 Biochemical composition and food quality of algae

165 Stems and ramuli of *C. cylindracea*, stems and apices of *P. capitatus* and the whole thalli of *Ulva sp.*  
166 and *C. compressa* were analysed spectrophotometrically in terms of protein, carbohydrate, and lipid  
167 contents, using a UV-Visible VARIAN Cary 50 spectrophotometer. More specifically: proteins were  
168 determined according to Hartree (1972), as modified by Lowry et al. (1951) and Rice (1982). The  
169 procedure proposed by Gerchacov and Hatcher (1972) was used to determine carbohydrates, whereas  
170 lipids were determined by the Bligh and Dyer (1959) and Marsh and Wenstein (1966) methods.

171 For each analysis, approximately 0.15 g (wet weight) of each alga were homogenized by a manual  
172 potter in 2 mL of distilled water. For each analytical replicate, 200  $\mu$ L of the homogenate were used  
173 for each of the biochemical analyses. The protein, carbohydrate and lipid contents were normalized

174 to the fresh weight of the algae, after transformation into equivalents of C, using the conversion  
175 factors 0.49, 0.40, 0.75 mgC mg<sup>-1</sup> and their sum reported as biopolymeric C (BPC; Fabiano et al.,  
176 1995).

177 Since nitrogen is the most limiting factor for nutrition and proteins are rich in N, while  
178 carbohydrates of macroalgae are often structural in nature and therefore pre-eminently refractory for  
179 heterotrophs (Pusceddu et al., 2009), we used the protein to carbohydrate ratio as a synthetic  
180 descriptor of the nutritional quality of the food items along with their caloric content, estimated  
181 through the following equation (Pusceddu and Fabiano, 1996):

$$182 \quad \text{Kcal g}^{-1} = 0.041\% \text{ CHO} + 0.055\% \text{ PRT} + 0.095\% \text{ LIP}$$

183 where CHO, PRT, LIP are the percentage fractions of carbohydrates, proteins, and lipids over their  
184 sum, respectively.

185

## 186 *2.5 Feeding behaviour*

187 Since sea urchins may be attracted to food but not stimulated to eat nor ingest it, the combined  
188 assessment of attraction (by studying movement) and ingestion (by measuring consumption) are the  
189 usual means of evaluating the response of sea urchins to food, ultimately providing insights about  
190 their food preference (Lawrence et al., 2013). Therefore, we investigated the feeding behaviour of the  
191 sea urchins considering new different behavioural proxies: a) the rate and speed of encounter, as  
192 proxies of food attraction (only in experiments with algal mixtures); b) the wet and dry biomass  
193 consumption rates; c) the tortuosity of the route to the food item; d) the time and speed to reach the  
194 food item, as corollary proxies of food preference.

195 The food attraction was estimated considering the order by which the animal chose the food in the  
196 first three movements towards the available items. For each algal species (either in experiments with  
197 separate or mixed items) and for each experiment, the value 0 was assigned if the urchin did not  
198 encounter the item, 1 was assigned to the first species encountered, 2 to the second, and 3 to the third.  
199 The reciprocal (1/x) of the average of the first three movements for each algal species and for each  
200 experiment (ranging from 0, no attraction, to 1, maximum attraction) was considered as an estimate  
201 of the food attraction.

202 At the end of each experiment, uneaten algae were siphoned out and collected with a 120 µm  
203 mesh-net. Fragments were dried with absorbent paper and weighed to obtain a wet weight (± 0.001g).  
204 Subsequently, the dry mass of the fragments was determined gravimetrically after drying in an oven



205 at 60° C for 48 hours. Wet mass of consumed algae was calculated as the difference in algal fragments  
206 wet weight measured before and the end of the experiment.

207 During the experiments we did not include a concomitant control (tanks with algae but without  
208 sea urchins). To avoid any possible bias in consumption rates estimates, we assessed changes in algal  
209 biomass of the four algae during separate experiments carried out under the same conditions as those  
210 used in the foraging experiments. The fresh algal biomass changes after 24 h without grazers did  
211 never exceed 0.5% of the initial biomass for each of the four algae (range 0.08-0.43%) and, thus, the  
212 potential overestimation of daily consumption did not exceed 1.0% (**Fig. A.3**).

213 Dry mass of consumed algae was calculated as the difference in algal fragments dry weight  
214 measured before and at the end of the experiment. The dry weight of the algal fragments was  
215 estimated from a calibration linear curve obtained by plotting the weight of dry vs wet replicate  
216 fragments (n=10 per alga). The wet and dry mass consumption rates for each of the algae were  
217 obtained by normalizing the weight of consumed algae per specimens and day (g of wet or dry  
218 biomass consumed ind<sup>-1</sup> d<sup>-1</sup>).

219 The tortuosity of the sea urchin's route to the food item was determined as the ratio between the  
220 distance (cm) travelled to reach the item and the shorter distance (cm) between the centre of the tank  
221 and the position of the targeted item at the edge of the tank.

222 The time (min) needed to reach the item was estimated as the average of the time spent to reach  
223 the first item and those of all the moves from one item to the following one during the whole  
224 experiment.

225 The speed (cm min<sup>-1</sup>) to the target was determined as the ratio between the mean time to the target  
226 and the actual distance travelled to reach it during the different movements recorded during the  
227 experiment.

228

## 229 *2.6 Data analysis*

230 Differences in food consumption rates, tortuosity of the route to the food item, time and speed to  
231 reach the items of the sea urchins exposed to the different algae and their mixtures were determined  
232 on Euclidean distance-based matrixes of untransformed data in either univariate or multivariate  
233 settings. The tests considered the algae as a fixed factor with 4 levels in experiments with exposure  
234 to single algae or 3 levels in experiments with exposure to the different mixtures of the algae. Post-  
235 hoc tests were conducted for all significant interactions.

236 Differences in protein, carbohydrate and lipid contents, values of the protein to carbohydrate ratio,  
237 caloric content, and gross biochemical composition among the four algae were determined by  
238 permutational analysis of variance (PERMANOVA) on Euclidean distance-based matrices of  
239 untransformed data in univariate and multivariate (biochemical composition) settings. The species or  
240 their parts were used as the unique source of variance (fixed factor with 6 levels): the whole thalli of  
241 *Ulva sp.* and *C. compressa*, stems and ramuli of *C. cylindracea*, stems and apices of *P. capitatus*.  
242 Post-hoc tests were conducted for all significant interactions.

243 All statistical analyses were carried out using the PRIMER 7 software, under the PERMANOVA  
244 routine.

245

### 246 **3. RESULTS**

#### 247 *3.1 Feeding behaviour of P. lividus exposed separately to the four algae*

248 When sea urchins were exposed separately to each of the four food items, daily consumption rates of  
249 the invasive algae *C. cylindracea* fresh biomass were significantly higher (**Table 1**) than those of the  
250 other three autochthonous species (**Fig. 1A**). Daily consumption rates of dry biomass of *Ulva sp.* were  
251 significantly lower (**Table 1**) than those of both *P. capitatus* and *C. cylindracea* (**Fig. 1B**). The  
252 tortuosity of the route and speed to the food target did not vary significantly among the four algae  
253 when exposed separately to the sea urchins (**Table 1**), whereas the time to reach *C. compressa* was  
254 significantly higher (**Table 1**) than that needed to reach *Ulva sp.* and *P. capitatus*, but similar to that  
255 needed to reach *C. cylindracea* (**Fig. 1C**).

256 The results of the multivariate test carried out in the multivariate context, considering altogether  
257 the tortuosity of the route, the time and speed to the food item, indicate that *P. lividus* had a different  
258 feeding behaviour on the four algae (**Table 1**). The results of the post-hoc test (**Table 2**) also revealed  
259 that *P. lividus* exhibited a similar (i.e., not statistically different) feeding behaviour on *Ulva sp.* and  
260 *P. capitatus* (**Fig. 2**).

261

#### 262 *3.2 Feeding behaviour of P. lividus exposed to algal mixtures*

263 Fresh algal biomass consumption rates by sea urchins varied significantly among algae in different  
264 combination trials (**Table 3**). In all trials, consumption rates of fresh biomass of *C. cylindracea*, when  
265 present, were significantly higher than those of the other species (**Table 4**). In all experiments, the  
266 significantly lowest consumption rates of fresh algal biomass were recorded for *Ulva sp.* (**Fig. 3A**).

267 Dry algal biomass consumption rates of *P. capitatus*, when present, were significantly higher (Table  
268 4) than those on *C. cylindracea* and *C. compressa* (Fig. 3B).

269 The *P. lividus* food attraction for the four algae varied in the different trials (Table 3). *C.*  
270 *cylindracea*, when present, was as attractive as or less attractive than the other items, whereas the  
271 significantly most attractive item varied in the different trials, with *C. compressa* showing  
272 cumulatively the significantly highest attraction, followed by *P. capitatus* and *Ulva sp.* (Fig. 4).

273 Sea urchins' route tortuosity, time and speed to the food item did not differ among algal species  
274 in almost all the trials with ternary combinations of algae (Table 3), with the unique exception for  
275 the trial including *P. capitatus*, *Ulva sp.* and *C. cylindracea*, during which the time and the speed to  
276 reach *P. capitatus* were significantly higher and lower, respectively, than those needed to reach the  
277 other two algae (Table 4).

278

### 279 3.3 Biochemical composition and food quality of algae

280 The four algae showed significantly different biochemical compositions (Table 5A). The results of  
281 the post-hoc tests showed that the biochemical composition varied significantly either among the four  
282 species or between different parts of *C. cylindracea* and *P. capitatus* (Table 5B). These differences  
283 are corroborated by the results of the CAP, from which it emerges that *P. capitatus* is characterized  
284 by an intraspecific variability in the biochemical composition that is significantly far larger than that  
285 of the other three species (Fig. 5). More in details, *P. capitatus* and *Ulva sp.* are characterized by a  
286 significantly and generally larger carbohydrate content than the two other algae, *C. compressa* shows  
287 the significantly highest protein content, and *C. cylindracea* is characterized by the significantly  
288 highest lipid content (Fig. 6). *P. capitatus* is characterized by the significantly lowest values of the  
289 protein to carbohydrate ratio (on average 0.16), whereas the stem of *C. cylindracea* (0.52) and the  
290 whole thallus of *C. compressa* (0.48) showed the highest ones (Fig. A.4).

291 The results of the PERMANOVA test reveal that the four species are characterized by  
292 significantly different caloric contents (Pseudo-F = 363.8;  $p < 0.001$ ) with the significantly highest  
293 values measured in the *P. capitatus* stem and the lowest ones in both ramuli and stem of *C.*  
294 *cylindracea* (Fig. A.5).

295

## 296 4. Discussion

297 Biological invasions are widely recognized as a serious threat to the ecological integrity of marine  
298 ecosystems, with potentially enormous economic and social impacts (Mack et al., 2000; Occhipinti

299 Ambrogi, 2011; Katsanevakis et al., 2014). Though unpredictably, some features of invasive alien  
300 species, including life traits, geographical origin, and the absence of natural predators, can increase  
301 the likelihood of a successful invasion (Boudouresque and Verlaque, 2002). In the case of invasive  
302 seaweeds, their proliferation may be due to the limited grazing effects by native herbivores (the so-  
303 called Enemy Release Hypothesis; Elton, 1958; Cacabelos et al., 2010). Early attempts to eradicate  
304 locally invasive alien species at sea have often failed (Ceccherelli and Piazzini 2005; Klein and  
305 Verlaque, 2007), but succeed partially with physical methods based on "hand removal" on very large  
306 areas, which, ultimately are much expensive (Marks et al. 2017). These difficulties appear even more  
307 stringent in the case of the invasive algae *C. cylindracea*, among the most widespread invaders in the  
308 Mediterranean Sea (Piazzini et al., 2005). This species, able to modify benthic communities and reduce  
309 biodiversity over a broad range of spatial scales, can in fact quickly spread through asexual  
310 fragmentation thanks to its ability to regenerate from even small tissue fragments (Smith, 2016;  
311 Bulleri et al., 2018), (Bulleri et al., 2010; Piazzini and Balata, 2009; Pusceddu et al., 2016; Tamburello  
312 et al., 2015; Gribben et al., 2018). In the last two decades, some attempts have been made to test the  
313 reliability of controlling invasive alien species at sea through autochthonous predators, but most of  
314 them gained minor or null success (Secord, 2003). More specifically, the biocontrol of the invasive  
315 green alga *Caulerpa taxifolia* was attempted using herbivorous sea slugs in the Mediterranean Sea,  
316 though with limited success (Thibaut and Meinesz, 2000; Thibaut et al., 2001).

317 *P. lividus*, is a highly efficient generalist herbivorous species (Boudouresque and Verlaque, 2013a;  
318 2013b; Agnetta et al., 2013), whose grazing activity can be as intense as to constitute, in some  
319 conditions, even a threat to benthic macroalgal biodiversity. One of these conditions is typically the  
320 result of the decrease or loss of sea urchin natural predators (e.g., fish, humans), which ultimately  
321 determines a massive proliferation of this highly efficient herbivorous, a dramatic loss of benthic  
322 biodiversity (Bianchelli et al., 2016), and the formation and persistence of sterile "barrens" (Pinnegar  
323 et al., 2000; Guidetti et al. 2003; Bianchelli and Danovaro, 2021). The feeding behaviour of *P. lividus*  
324 is also characterized by a food choice that is dependent upon the relative abundance of available  
325 resources. More specifically, the "apostatic" or "switching" behaviour of *P. lividus* allows this species  
326 to move from a rare but preferred resource to a less preferred but abundant one (Harper, 1969;  
327 Lawrence 2001 and literature therein). Accordingly, recent studies reported that *P. lividus* also  
328 consumes the invasive seaweed *C. cylindracea* (Bulleri et al., 2009; Cebrian et al., 2009; Tomas et  
329 al. 2011a; Noé et al. 2018), that has become a dominant component of benthic macroalgal biodiversity  
330 in several coastal sectors of the Mediterranean Sea.

331 Although we must acknowledge that the assessment of the actual food preference would imply a  
332 deeper analysis of other associated functional traits including, for instance, assimilation and egestion

333 rates, the results of our experiments indicate that the consumption rate of fresh biomass of *C.*  
334 *cylindracea* by *P. lividus* is larger than that of the other four algae, when offered separately. This  
335 result is partially consistent with a previous study (Noè et al., 2017), that showed that *P. lividus* could  
336 ingest several combinations of natural and allochthonous algae, though it preferentially consumed the  
337 mixture composed by the invasive *C. cylindracea* and the native *Dictyopteris membranacea*. Based  
338 on our results, we investigated whether such a preferential consumption (as assessed by concomitant  
339 positive attraction and a discrete consumption) could be explained by the different biochemical  
340 composition and, thus, by the caloric content of the different algae. By comparing the consumption  
341 rates of *P. lividus* of the four algae (when offered separately) and their caloric content, we indeed  
342 found that the highest consumption rates occur for the alga with the lowest caloric content (*C.*  
343 *cylindracea*), whereas the lowest ones are observed for the remaining three algae ,each with a caloric  
344 content exceeding 1.3 Kcal g<sup>-1</sup> (Fig. 7). These results indicate that *P. lividus* exposed to *C. cylindracea*  
345 would be pushed to ingest a greater quantity of this alga to obtain the same amount of energy  
346 otherwise obtained from other algae, to meet its energetic demand. However, we must stress here that  
347 this result is obtained only considering outcomes of the experiments carried out when algae are  
348 provided separately, a condition that is unlikely in nature. When the consumption rates are assessed  
349 in terms of dry biomass, *C. cylindracea* is not the most consumed food item. This apparent  
350 discrepancy can be explained by the much larger water content of *C. cylindracea* thalli when  
351 compared with that of the other three algae, and ultimately explains the much more diluted caloric  
352 content of this alga.

353 The results of the second set of experiments confirmed that the consumption rates of fresh biomass  
354 of *C. cylindracea* by *P. lividus* exceeded that of the other algal species, regardless of the offered  
355 combination of the species (Noé et al., 2017). Nevertheless, the analysis of all of the other descriptors  
356 used to estimate the food preference of *P. lividus* exposed to the different combinations of the four  
357 algae, indicate that *C. cylindracea* is never the first species reached by the sea urchin and that, when  
358 other native algae are present, it does not represent the preferential (or most attractive) food target for  
359 *P. lividus*.

360 Our results, therefore, while confirming that the invasive alga *C. cylindracea* can be part of the *P.*  
361 *lividus* diet, do not fully support the possible use of sea urchins to control the further spreading of this  
362 invasive alga, unless in conditions where the presence of *C. cylindracea* is largely dominant due to  
363 other disturbance factors that keep much low the biomass of other native algae (like in barrens;  
364 Ceccherelli et al., 2014; Bianchelli et al., 2016) or is present in association with other invasive algae  
365 (e.g., *Caulerpa taxifolia* var. *distichophylla*; Noè et al., 2018). Finally, we must anticipate that this  
366 latter eventual option needs further verification, as we cannot in principle exclude that the expansion

367 or uncontrolled introduction of this sea urchin could become a deterrent to native algal forests  
368 recolonization, as previously reported (Caronni et al., 2015).

369

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374

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640 **Table 1.** Results of the PERMANOVA tests conducted to assess differences in consumption rates of fresh and  
 641 dry algal biomass, tortuosity of the route, time and speed to the target and behaviour (multivariate response)  
 642 in the experiments conducted separately with the four algae. df = degrees of freedom; MS= mean squares;  
 643 Pseudo-F = permutational F; P = probability after Monte Carlo simulations. ns = not significant; \*\*\* = P  
 644 <0.001; \*\* = P> 0.01; \* = P <0.05.  
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<b>Response variable</b>	<b>df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>P(MC)</b>
<b>Fresh mass consumption</b>	3	18.466	46.983	***
<b>Dry mass consumption</b>	3	0.039	7.645	***
<b>Tortuosity of the route</b>	3	0.125	3.977	ns
<b>Time to target</b>	3	12.576	4.385	*
<b>Speed to target</b>	3	6.223	3.631	ns
<b>Behaviour (Multivariate response)</b>	3	37.429	7.469	***

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648 **Table 2.** Post-hoc pairwise tests conducted to assess differences in the consumption rates of fresh and dry algal  
649 biomass, time to the food target and behaviour (multivariate response) in the experiments conducted separately  
650 with the four algae.  $t$  =  $t$  value; P(MC) = probability after Monte Carlo simulations. ns = not significant; \*\*\*  
651 =  $P < 0.001$ ; \*\* =  $P > 0.01$ ; \* =  $P < 0.05$ . Reported are only tests for significant effects as from the results  
652 reported in Table 1.  
653

Response variable	Contrast	$t$	P(MC)
Fresh mass consumption	<i>Ulva, Penicillus</i>	0,835	ns
	<i>Ulva, Cystoseira</i>	0,602	ns
	<i>Ulva, Caulerpa</i>	9,604	***
	<i>Penicillus, Cystoseira</i>	0,068	ns
	<i>Penicillus, Caulerpa</i>	8,875	**
	<i>Cystoseira, Caulerpa</i>	7,358	***
Dry mass consumption	<i>Ulva, Penicillus</i>	4,602	*
	<i>Ulva, Cystoseira</i>	1,254	ns
	<i>Ulva, Caulerpa</i>	5,556	***
	<i>Penicillus, Cystoseira</i>	2,426	*
	<i>Penicillus, Caulerpa</i>	1,262	ns
	<i>Cystoseira, Caulerpa</i>	1,811	ns
Time to the target	<i>Ulva, Penicillus</i>	0,862	ns
	<i>Ulva, Cystoseira</i>	3,037	*
	<i>Ulva, Caulerpa</i>	1,277	ns
	<i>Penicillus, Cystoseira</i>	2,861	*
	<i>Penicillus, Caulerpa</i>	1,045	ns
	<i>Cystoseira, Caulerpa</i>	1,478	ns
Behaviour (Multivariate response)	<i>Ulva, Penicillus</i>	0,672	ns
	<i>Ulva, Cystoseira</i>	2,703	*
	<i>Ulva, Caulerpa</i>	2,939	*
	<i>Penicillus, Cystoseira</i>	2,686	*
	<i>Penicillus, Caulerpa</i>	3,136	*
	<i>Cystoseira, Caulerpa</i>	2,663	*

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656 **Table 3.** Results of the PERMANOVA tests carried out to assess differences in the consumption rates of fresh  
657 and dry algal biomass mass, food attraction, tortuosity of the route, time and speed to the food target in  
658 experiments carried out exposing *P. lividus* to ternary combinations of the four algal species. df = degrees of  
659 freedom; MS = sum of squares; Pseudo-F = permutational F; P = probability level after Monte Carlo  
660 simulations. ns = not significant; \*\*\* = P <0.001; \*\* = P > 0.01; \* = P <0.05. Cy = *Cystoseira compressa*, U =  
661 *Ulva rigida*, Ca = *Caulerpa cylindracea*, Pe = *Penicillus capitatus*.  
662

Combination	Variable	df	MS	Pseudo-F	P(MC)
CyUCa	Fresh biomass consumption	2	0,73	28,467	**
	Dry biomass consumption	2	0,02	11,238	*
	Food Attraction	2	0,02	11,238	*
	Tortuosity of the route	2	0,53	2,106	ns
	Time to the target	2	3721,7	1,579	ns
	Speed to the target	2	1,60	1,185	ns
CyPeCa	Fresh biomass consumption	2	0,94	119,830	***
	Dry biomass consumption	2	0,09	9,593	*
	Food Attraction	2	0,46	383,250	***
	Tortuosity of the route	2	0,62	2,315	ns
	Time to the target	2	1647,40	2,389	ns
	Speed to the target	2	21,29	2,801	ns
CyPeU	Fresh biomass consumption	2	0,37	16,585	**
	Dry biomass consumption	2	0,08	18,704	**
	Food Attraction	2	0,14	7,021	*
	Tortuosity of the route	2	0,67	2,085	ns
	Time to the target	2	1273,00	1,978	ns
	Speed to the target	2	7,74	0,940	ns
PeUCa	Fresh biomass consumption	2	0,81	24,201	**
	Dry biomass consumption	2	0,04	31,527	***
	Food Attraction	2	0,04	2,269	ns
	Tortuosity of the route	2	1,47	4,685	ns
	Time to the target	2	1757,20	11,107	*
	Speed to the target	2	61,64	5,467	*

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**Table 4.** Post-hoc tests carried out to assess the significance of the differences in the rates of consumption of fresh and dry mass, tortuosity of the route to, time and speed of achievement of the food target in the experiments conducted while simultaneously offering ternary combinations of algal species. P = probability after Monte Carlo simulations. ns = not significant; \*\*\* = P < 0.001; \*\* = P > 0.01; \* = P < 0.05. Cy = *Cystoseira compressa*, U = *Ulva rigida*, Ca = *Caulerpa cylindracea*, Pe = *Penicillus capitatus*.

Combination	Variable	Contrast	t	P(MC)
CyUCa	Fresh biomass consumption	<i>Cystoseira, Ulva</i>	3,679	*
		<i>Cystoseira, Caulerpa</i>	3,079	*
		<i>Ulva, Caulerpa</i>	11,102	***
	Dry mass consumption	<i>Cystoseira, Ulva</i>	3,823	*
		<i>Cystoseira, Caulerpa</i>	0,933	ns
		<i>Ulva, Caulerpa</i>	8,285	**
	Food Attraction	<i>Cystoseira, Ulva</i>	3,311	*
		<i>Cystoseira, Caulerpa</i>	1,452	ns
		<i>Ulva, Caulerpa</i>	1,835	ns
	Tortuosity of the route	<i>Cystoseira, Ulva</i>	0,269	ns
		<i>Cystoseira, Caulerpa</i>	1,447	ns
		<i>Ulva, Caulerpa</i>	1,541	ns
	Time to the target	<i>Cystoseira, Ulva</i>	1,486	ns
		<i>Cystoseira, Caulerpa</i>	1,084	ns
		<i>Ulva, Caulerpa</i>	1,402	ns
Speed to the target	<i>Cystoseira, Ulva</i>	0,129	ns	
	<i>Cystoseira, Caulerpa</i>	1,117	ns	
	<i>Ulva, Caulerpa</i>	1,291	ns	
CyPeCa	Fresh biomass consumption	<i>Cystoseira, Penicillus</i>	2,948	*
		<i>Cystoseira, Caulerpa</i>	14,456	***
		<i>Penicillus, Caulerpa</i>	13,425	***
	Dry mass consumption	<i>Cystoseira, Penicillus</i>	3,591	*
		<i>Cystoseira, Caulerpa</i>	2,240	ns
		<i>Penicillus, Caulerpa</i>	2,703	ns
	Food Attraction	<i>Cystoseira, Penicillus</i>	16,454	***
		<i>Cystoseira, Caulerpa</i>	16,454	***
		<i>Penicillus, Caulerpa</i>	5,196	**
	Tortuosity of the route	<i>Cystoseira, Penicillus</i>	2,755	*
		<i>Cystoseira, Caulerpa</i>	0,110	ns
		<i>Penicillus, Caulerpa</i>	1,773	ns
	Time to the target	<i>Cystoseira, Penicillus</i>	2,035	ns
		<i>Cystoseira, Caulerpa</i>	1,346	ns
		<i>Penicillus, Caulerpa</i>	1,713	ns
Speed to the target	<i>Cystoseira, Penicillus</i>	3,632	*	
	<i>Cystoseira, Caulerpa</i>	1,331	ns	
	<i>Penicillus, Caulerpa</i>	0,746	ns	
CyPeU	Fresh biomass consumption	<i>Cystoseira, Penicillus</i>	2,019	ns
		<i>Cystoseira, Ulva</i>	5,495	**
		<i>Penicillus, Ulva</i>	5,363	**
	Dry mass consumption	<i>Cystoseira, Penicillus</i>	3,284	**
		<i>Cystoseira, Ulva</i>	5,096	*
		<i>Penicillus, Ulva</i>	5,167	**
	Food Attraction	<i>Cystoseira, Penicillus</i>	3,829	*
		<i>Cystoseira, Ulva</i>	1,083	ns
		<i>Penicillus, Ulva</i>	2,231	ns
	Tortuosity of the route	<i>Cystoseira, Penicillus</i>	2,391	ns
		<i>Cystoseira, Ulva</i>	0,582	ns
		<i>Penicillus, Ulva</i>	1,387	ns
	Time to the target	<i>Cystoseira, Penicillus</i>	1,830	ns
		<i>Cystoseira, Ulva</i>	0,947	ns
		<i>Penicillus, Ulva</i>	1,422	ns
Speed to the target	<i>Cystoseira, Penicillus</i>	1,271	ns	
	<i>Cystoseira, Ulva</i>	0,105	ns	
	<i>Penicillus, Ulva</i>	1,518	ns	

PeUCa	Fresh biomass consumption	<i>Penicillus, Ulva</i>	6,625	**
		<i>Penicillus, Caulerpa</i>	3,318	*
		<i>Ulva, Caulerpa</i>	5,964	**
	Dry mass consumption	<i>Penicillus, Ulva</i>	7,959	**
		<i>Penicillus, Caulerpa</i>	3,435	*
		<i>Ulva, Caulerpa</i>	4,857	**
	Food Attraction	<i>Penicillus, Ulva</i>	2,232	ns
		<i>Penicillus, Caulerpa</i>	0,939	ns
		<i>Ulva, Caulerpa</i>	1,138	ns
	Tortuosity of the route	<i>Penicillus, Ulva</i>	2,402	ns
		<i>Penicillus, Caulerpa</i>	2,132	ns
		<i>Ulva, Caulerpa</i>	0,794	ns
	Time to the target	<i>Penicillus, Ulva</i>	3,787	*
		<i>Penicillus, Caulerpa</i>	3,148	*
		<i>Ulva, Caulerpa</i>	1,047	ns
	Speed to the target	<i>Penicillus, Ulva</i>	4,066	*
		<i>Penicillus, Caulerpa</i>	2,585	ns
		<i>Ulva, Caulerpa</i>	0,416	ns

672 **Table 5.** Results of PERMANOVA (A) and post-hoc pairwise tests (B) carried out to assess differences in the  
 673 biochemical composition of the different structures of the four algal species: thalli for all the four species,  
 674 stems for *P. capitatus* and apex of *C. cylindracea*. df = degrees of freedom; MS = mean squares; Pseudo-F =  
 675 permutational F; P = probability level after Monte Carlo simulations; ns = not significant; \*\*\* = P <0.001; \*\*  
 676 = P > 0.01; \* = P <0.05.

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 678 **A) PERMANOVA test**  
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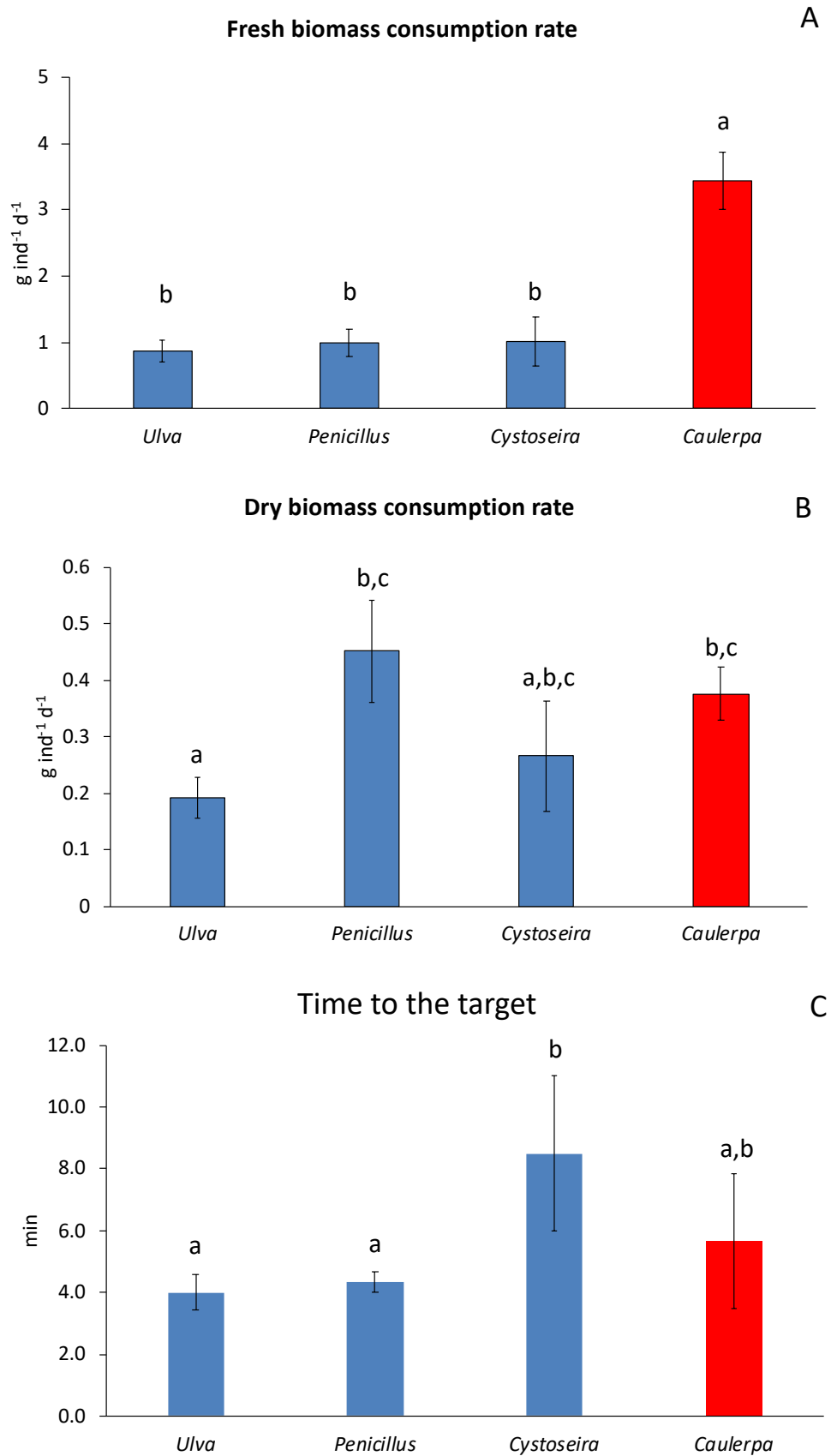
Source	Source	df	MS	Pseudo-F	P
Biochemical composition	Algal parts	5	1270,9	76,77	***
	Residuals	12	16.553		

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 682 **B) Post-hoc pairwise tests**  
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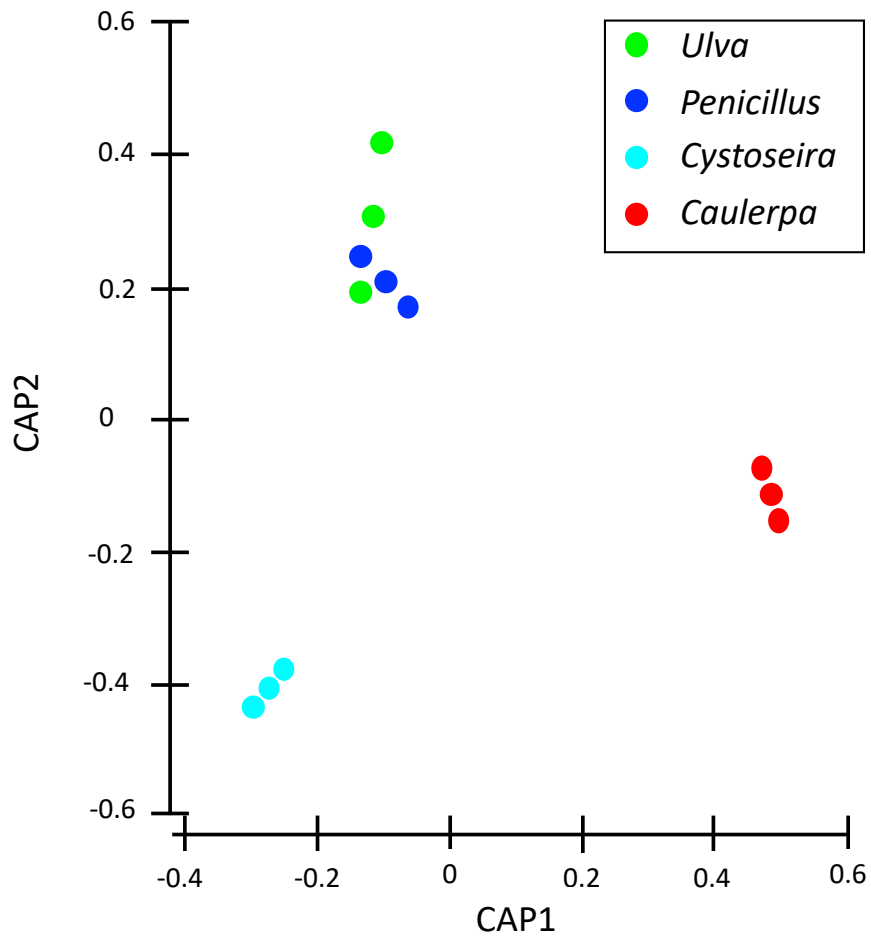
Contrast	t	P(MC)
<i>Penicillus apex, Penicillus stem</i>	6,9423	**
<i>Penicillus apex, Caulerpa stem</i>	9,9645	***
<i>Penicillus apex, Caulerpa ramuli</i>	8,4871	***
<i>Penicillus apex, Ulva</i>	2,081	*
<i>Penicillus apex, Cystoseira</i>	7,9284	**
<i>Penicillus apex, Caulerpa stem</i>	17,872	***
<i>Penicillus stem, Caulerpa ramuli</i>	16,202	***
<i>Penicillus stem, Ulva</i>	6,922	**
<i>Penicillus stem, Cystoseira</i>	15,666	***
<i>Caulerpa stem, Caulerpa ramuli</i>	3,2011	*
<i>Caulerpa stem, Ulva</i>	5,2929	**
<i>Caulerpa stem, Cystoseira</i>	5,4286	**
<i>Caulerpa ramuli, Ulva</i>	4,5263	*
<i>Caulerpa ramuli, Cystoseira</i>	3,436	*
<i>Ulva, Cystoseira</i>	3,8046	*

684

685 **Figure 1.** Fresh (A) and dry (B) algal biomass daily consumption rates and time to reach the target  
686 ( $\pm$  standard error) by sea urchins exposed separately to the four algae. Lower case letters indicate the  
687 results of the post-hoc tests as summarized in Table 2.  
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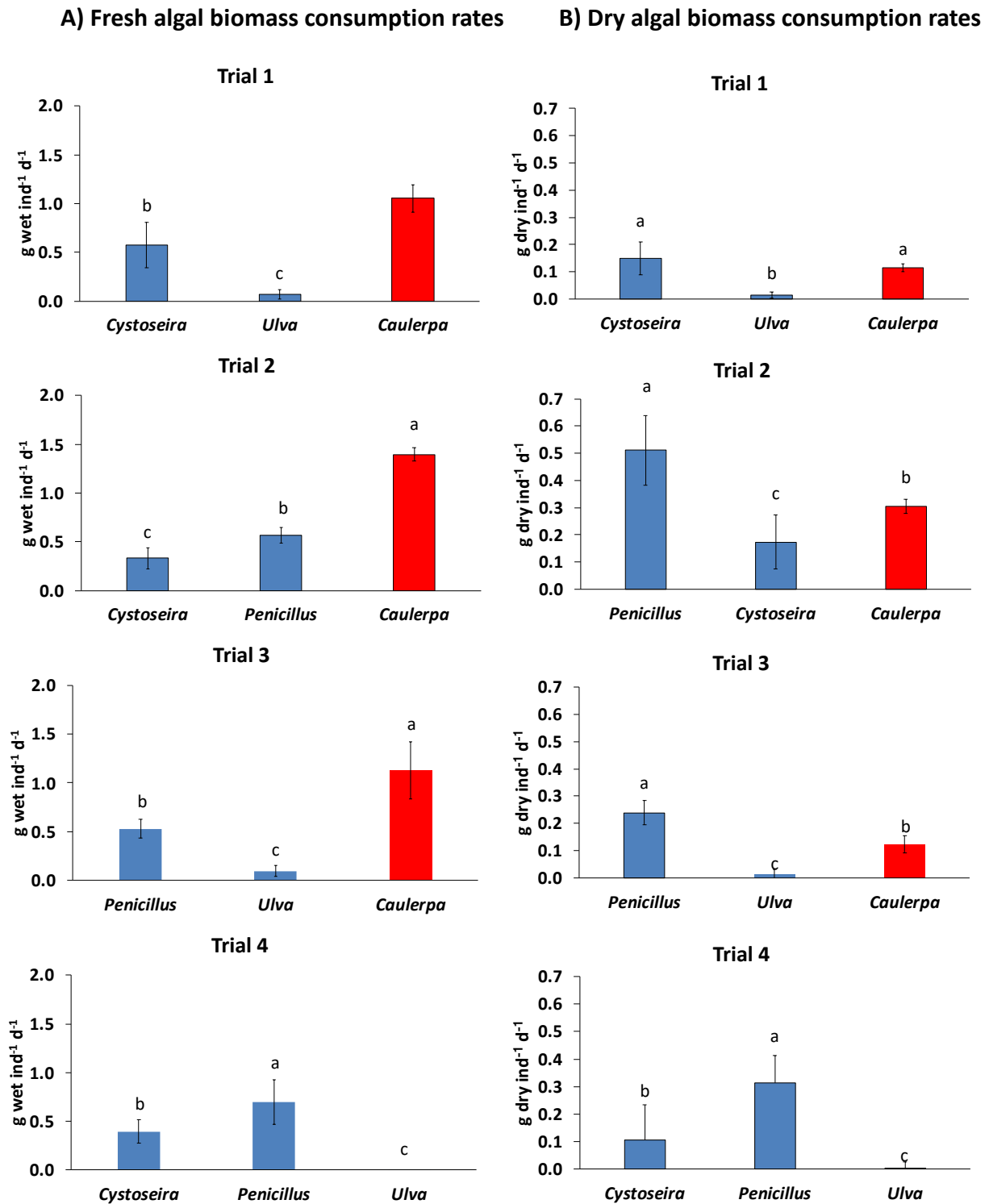


690 **Figure 2.** Bi-plot produced after a canonical analysis of the principal coordinates showing the  
691 multivariate dispersion of the sea urchin feeding behaviour on the four algae. The distance among  
692 clusters of different dots is proportional to the difference in the feeding behaviour of the sea urchins.  
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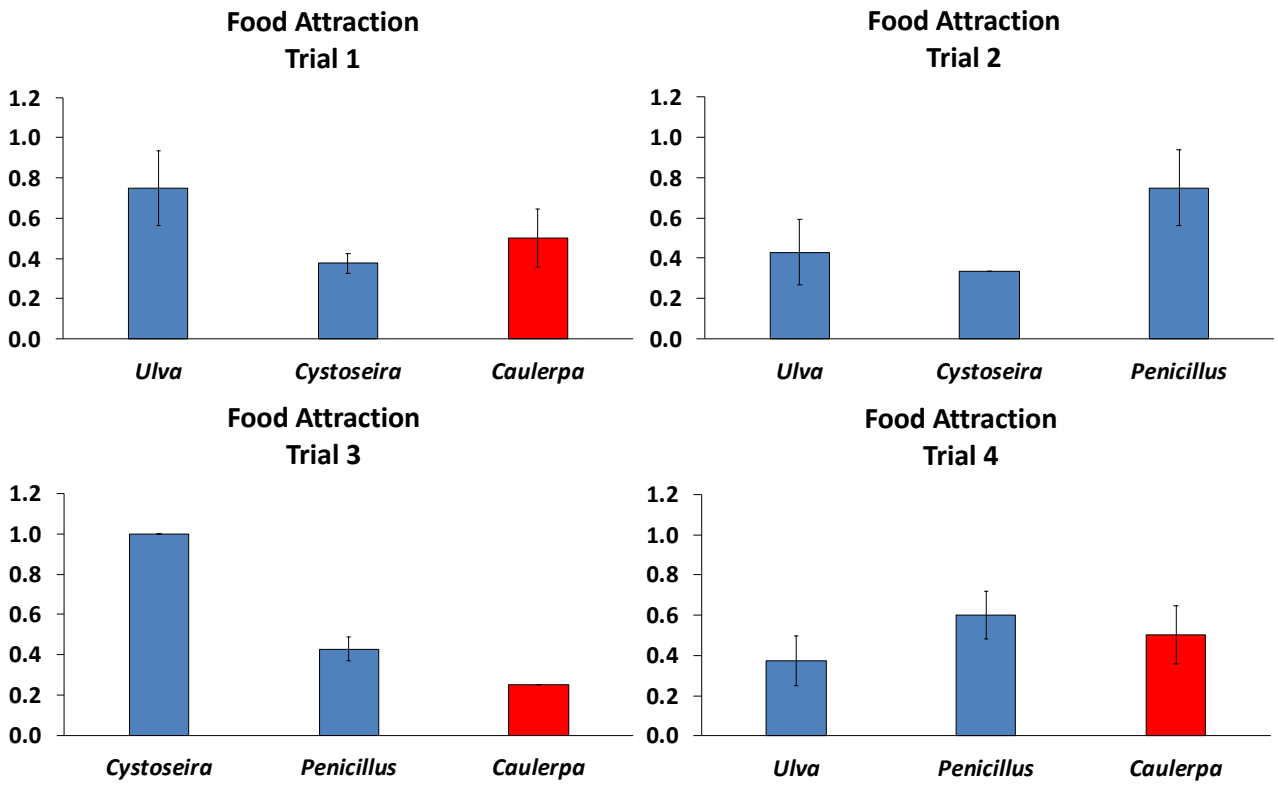
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695 **Figure 3.** Fresh (A) and dry (B) algal biomass daily consumption rates ( $\pm$  standard error) by sea  
 696 urchins exposed to the four algae in different ternary combinations. Lowercase letters indicate the  
 697 results of the post-hoc tests as from the results reported in Table 4.  
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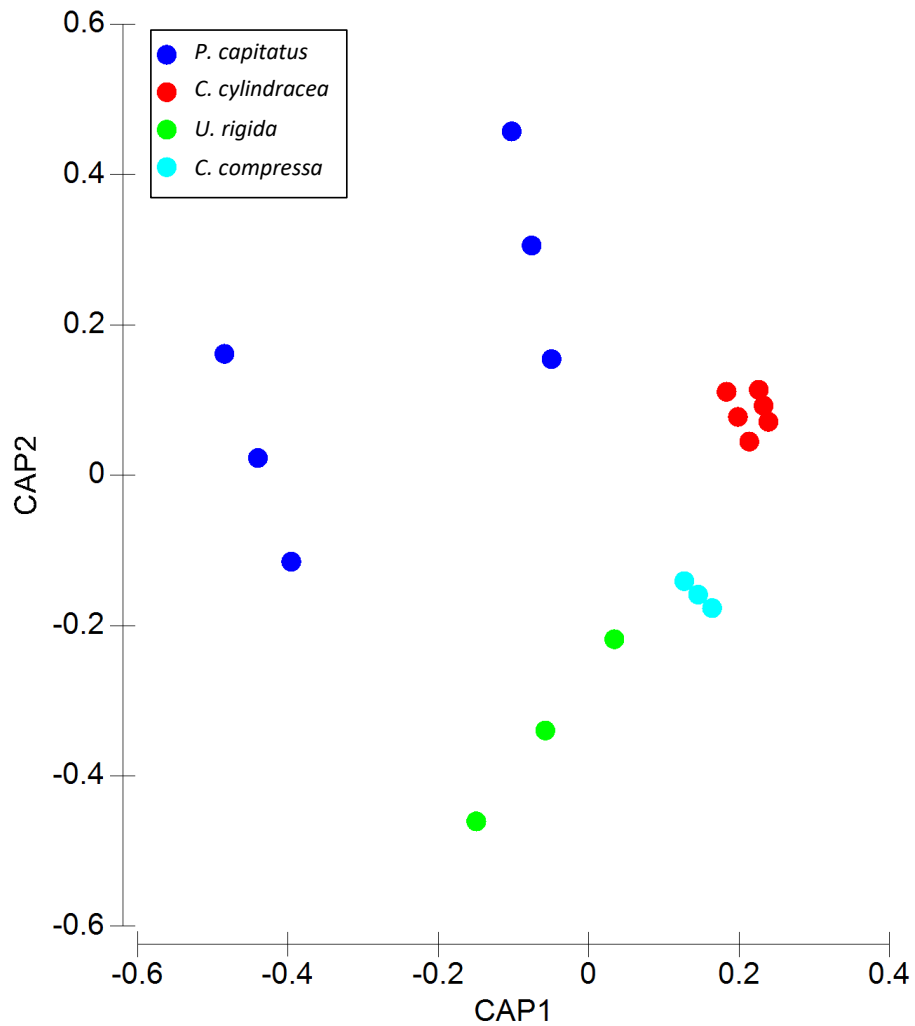
701 **Figure 4.** Food attraction of the four algae offered to *P. lividus* during experiments carried out using  
702 different combinations of algae.  
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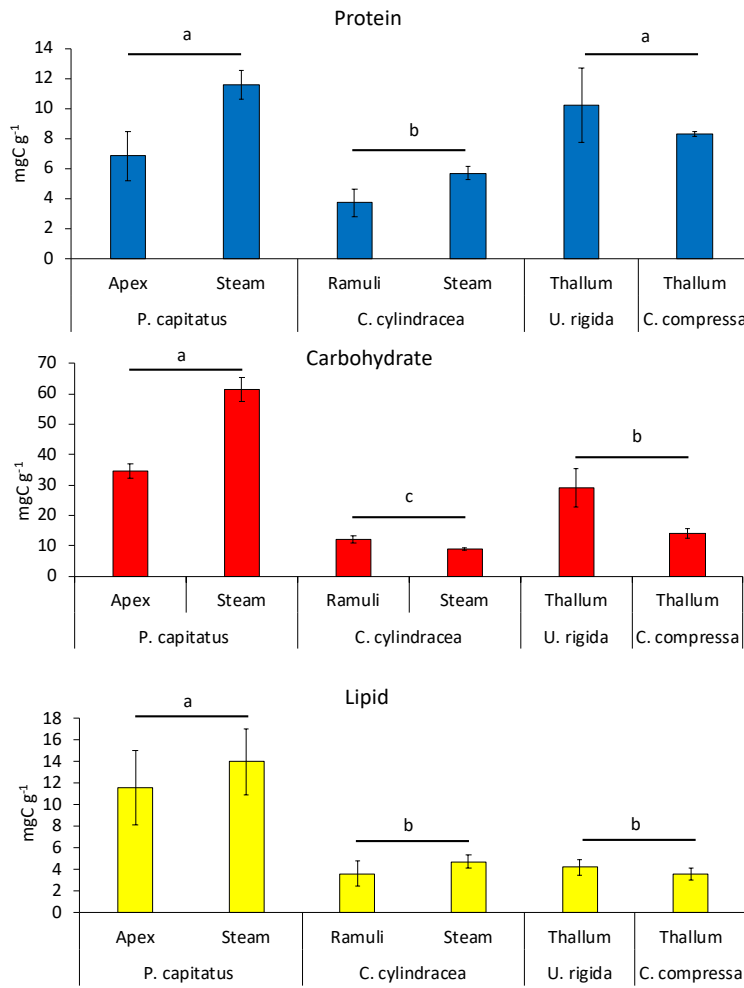


705 **Figure 5.** Bi-plot produced after the CAP analysis illustrating multivariate differences in the  
706 biochemical composition among the four algal species. For *P. capitatus* and *C. cylindracea*  
707 differences include both thallum and stem. The distance among dots is proportional to the differences  
708 in the biochemical composition among algae (dots of different colours) and among replicates of the  
709 same algae (dots of the same colour).  
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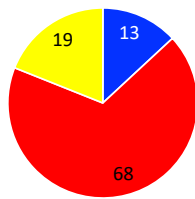
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713 **Figure 6.** Protein, carbohydrate, and lipid contents of the four algae and their biochemical  
 714 composition expressed as protein, carbohydrate, and lipid contribution to biopolymeric C.  
 715 Compositional data for *C. cylindracea* and *P. capitatus* are means of the stem and ramuli and stem  
 716 and apex, respectively.



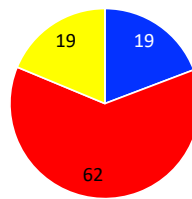
717

*Penicillus*



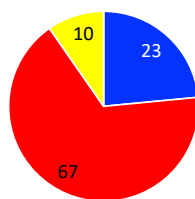
■ Protein ■ Carbohydrate ■ Lipid

*Caulerpa*



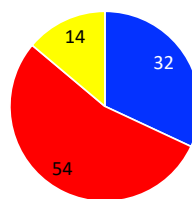
■ Protein ■ Carbohydrate ■ Lipid

*Ulva*



■ Protein ■ Carbohydrate ■ Lipid

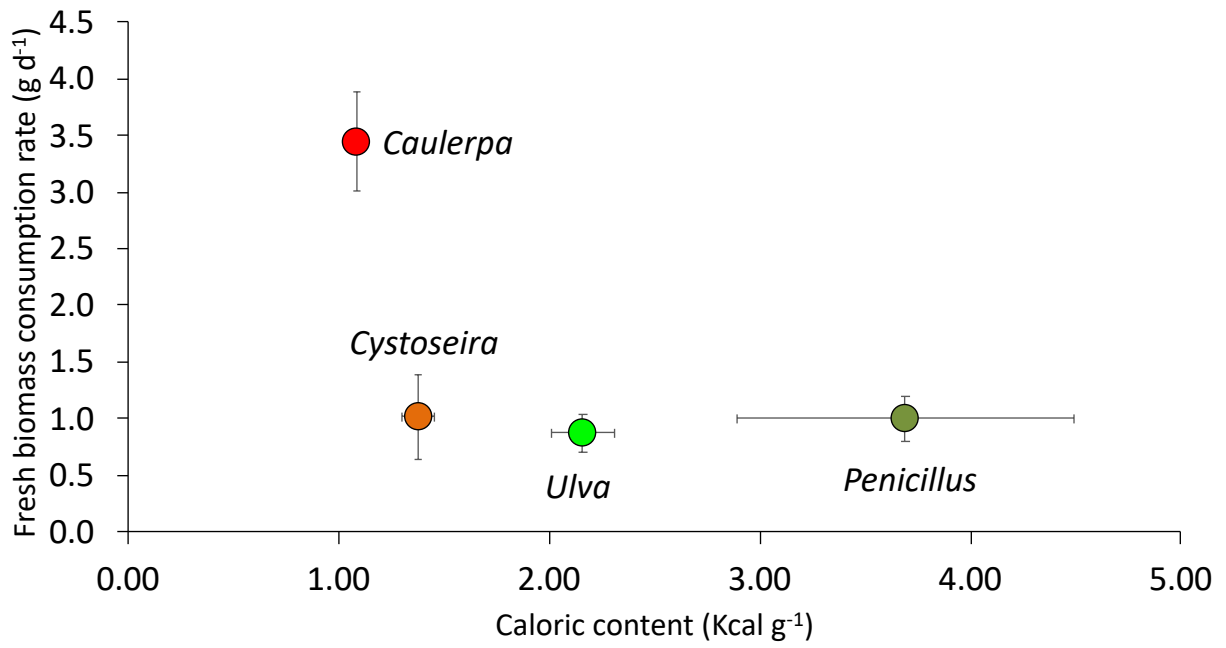
*Cystoseira*



■ Protein ■ Carbohydrate ■ Lipid

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721 **Figure 7.** Relationship between caloric content and consumption of fresh algal biomass. The error  
722 bars refer to the standard error.  
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