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1 **Alkaline phosphatase activity of reef-building corals**

2

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4

5

6 **Running head:** Reef corals organic phosphate use

7

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8 **Abstract**

9 Extracellular alkaline phosphatase activity (APA) was measured in four tropical
10 (*Stylophora pistillata*, *Pocillopora damicornis*, *Pavona cactus*, *Galaxea fascicularis*) and two
11 Mediterranean (*Oculina patagonica*, *Cladocora caespitosa*) symbiotic coral species, both in
12 the coral host and associated symbionts, as well as in one tropical non-symbiotic coral
13 (*Tubastraea* sp.). The effects of light, feeding, and bleaching (loss of symbionts) were also
14 tested in *Stylophora pistillata*. Host APA increased both with long-term starvation, irradiance,
15 and bleaching suggesting that APA is linked to the metabolic activity of the host and
16 symbionts, and to their phosphate limitation or repletion status. The comparison of APA
17 between coral species containing different symbiont clades suggests that clade C is less
18 efficient than clades A and B, but this result remains to be confirmed. At environmental
19 phosphate concentrations, if the total amount of phosphate generated by APA is taken up by
20 the coral colony, it can supply 0.3 - 1.6 mmol P m⁻² d⁻¹. In comparison, dissolved inorganic
21 phosphorus (DIP) and particulate organic phosphorus (POP) have been shown supply 0.2 - 1.6
22 and 0.9 mmol P m⁻² d⁻¹, respectively, highlighting the importance of APA activity in
23 symbiotic corals.

24

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32

33 **Introduction**

34 Phosphorus is essential to living organisms because it enters into the composition of
35 many biological molecules as phosphate (e.g., deoxyribonucleic acids, ribonucleic acids,
36 phospholipids), and has a role in signaling pathways and metabolic reactions (mostly via the
37 role of adenosine triphosphate, ATP). Corals can fulfill their phosphate needs in several ways.
38 The first way is through the degradation of particulate organic phosphorus (POP), after the
39 capture of plankton caught by the coral host with its stinging tentacles (Johannes et al. 1970;
40 Wijgerde et al. 2011). POP in reef waters corresponds to a mean concentration of ca. 0.10
41 $\mu\text{mol P L}^{-1}$ (Furnas et al. 1988; Charpy 2001). Coral acquisition of phosphate from POP has
42 been seldom examined. A recent study estimated that extracoelenteric zooplankton feeding in
43 the species *Galaxea fascicularis* may provide up to $0.9 \pm 0.3 \text{ mmol P m}^{-2} \text{ d}^{-1}$ (Wijgerde et al.
44 2011), which was however obtained with a relatively high prey density (4100 nauplii L^{-1}).
45 Phosphate is also acquired from dissolved inorganic phosphorus (DIP), directly through an
46 active uptake mediated by the dinoflagellate symbionts (D'Elia 1977; Jackson and Yellowlees
47 1990; Godinot et al. 2009). This DIP presents concentrations between 0.05 and 0.50 $\mu\text{mol P}$
48 L^{-1} in reef waters (Atkinson 1987a; Charpy 2001), and contribute 0.2 to 1.1 $\text{mmol P m}^{-2} \text{ d}^{-1}$ to
49 reef benthos (Steven and Atkinson 2003; Cuet et al. 2011), and 1.6 $\text{mmol P m}^{-2} \text{ d}^{-1}$ to corals
50 (Godinot et al. 2009) at these concentrations. The last source of phosphate is represented by
51 dissolved organic phosphorus (DOP), which can be hydrolyzed into phosphate by
52 metalloenzymes called phosphatases. DOP concentrations in reef waters are in the same range
53 as DIP concentrations, i.e., generally lower than 0.5 $\mu\text{mol P L}^{-1}$ (Crossland and Barnes 1983;
54 Larned 1998). While the coral acquisition of phosphate from DIP has been frequently studied,
55 little research has been done on DOP degradation, and only isolated symbionts from
56 scleractinian corals were considered so far (Jackson et al. 1989; Annis and Cook 2002).
57 However, DOP may provide a significant alternative source of phosphate for corals, in

58 replacement or in addition to DIP and POP, because it has been shown that phosphate uptake
59 from DOP hydrolysis by reef benthos phosphatases could be as fast as DIP uptake (Atkinson
60 1987b).

61 In reef waters, DOP is present as phosphomonoesters, phosphodiester, and
62 phosphonates (molecules that contain a C-P bond). In this first study, we concentrated on the
63 use of phosphomonoesters. Phosphatases hydrolyzing phosphomonoesters are classified as acid
64 and alkaline depending on their pH optimum; and each type has different substrate
65 specificities, catalytic mechanisms, and inhibition pathways (Hollander 1971). Phosphatase
66 activity has been shown to be a good indicator of phosphate deficiency and low
67 environmental DIP in a large number of marine organisms, such as cyanobacteria (Yentsch et
68 al. 1972), dinoflagellates (Dyhrman and Palenik 1999), and symbiotic dinoflagellates (Annis
69 and Cook 2002). Coral hosts and their symbionts (commonly called zooxanthellae), either
70 freshly isolated (FIZ) or cultured (CZ), exhibit phosphatase activity. Only two studies have
71 investigated phosphatases in FIZ and CZ, showing both acid and alkaline phosphatases
72 activities (AcPA and APA, respectively) with intracellular (Jackson et al. 1989) and
73 extracellular (Annis and Cook 2002) activities reported. Some other studies only mentioned
74 the presence of intracellular (Goreau 1956) and extracellular (Hayes and Goreau 1977)
75 phosphatase activity in coral hosts.

76 This study therefore first aimed at examining the alkaline phosphatase activity in the
77 scleractinian coral *Stylophora pistillata* under different environmental conditions. We
78 therefore tested the effect of light, feeding, and bleaching (loss of symbionts) of the coral
79 host. The following hypotheses were tested: APA should increase with light as previously
80 observed with DIP (D'Elia 1977; Godinot et al. 2009), and APA should vary with the
81 availability of other phosphate sources. It should therefore increase with a loss of symbionts,
82 which leads to a decreased DIP uptake and a release of DIP (D'Elia 1977; Muller-Parker et al.

83 1990); and it should decrease following feeding of the host, which corresponds to a POP
84 supply. It was indeed previously reported that zooxanthellae (zoox) APA significantly
85 increased after 5 weeks of host starvation in FIZ or after cultivation in P-limited medium in
86 CZ (Jackson et al. 1989; Annis and Cook 2002). The second aim of this study was to compare
87 APA in coral species containing different symbiont clades. All these questions have never
88 been addressed in corals, but are essential and timely because phosphate uptake is related to
89 net carbon production (Atkinson 1992), and should therefore be one of the key factors
90 determining primary productivity of corals.

91

92 **Methods**

93 **Organisms**

94 APA was assessed in four tropical symbiotic species (*Stylophora pistillata*,
95 *Pocillopora damicornis*, *Pavona cactus*, and *Galaxea fascicularis*), two Mediterranean
96 symbiotic species (*Oculina patagonica* and *Cladocora caespitosa*), and one tropical non-
97 symbiotic species (*Tubastraea* sp.). APA was also assessed in bleached *S. pistillata*, prepared
98 by culturing nubbins for 6 weeks in the dark. Microscopy observations confirmed that
99 symbiont density was 3 orders of magnitude lower in bleached nubbins (6.7×10^3 zoox cm^{-2})
100 than in symbiotic ones (2.5×10^6 zoox cm^{-2}).

101 All colonies were maintained in aquaria supplied with natural seawater (exchange rate
102 2% h^{-1}) containing low nutrient concentrations (DOP $< 0.1 \mu\text{mol P L}^{-1}$, DIP $< 0.05 \mu\text{mol P L}^{-1}$,
103 nitrate $< 0.4 \mu\text{mol N L}^{-1}$ and ammonium $< 0.5 \mu\text{mol N L}^{-1}$). Seawater salinity was constant
104 at 38.2, pH was 8.1 ± 0.1 , and temperature was maintained at $25.0 \pm 0.5^\circ\text{C}$ for tropical corals
105 and $18.0 \pm 1.0^\circ\text{C}$ for Mediterranean ones. Irradiance was $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for
106 symbiotic corals, with a light:dark cycle of 12 h:12 h. Since corals were cultured at this light
107 intensity and adapted to it, it corresponded to a maximal rate of photosynthesis. The non-

108 symbiotic *Tubastraea* sp. and bleached *S. pistillata* were cultured in the dark. All corals were
109 fed twice a week with *Artemia salina* nauplii, but were used for the experiments three days
110 after the feeding (also for the experiments involving FIZ).

111 **Isolation of symbionts**

112 For each experiment with FIZ, symbionts were extracted from 5 coral nubbins using a
113 commercial-type Water-Pick. They were further purified by three centrifugations (2000
114 rotations per minutes for 5 min at 25°C) followed by re-suspension in seawater (Johannes and
115 Weibe 1970). Aliquots were frozen for subsequent cell counts, chlorophyll and protein
116 determinations. Zooxanthellae phylogeny was determined to the clade level according to
117 Santos et al. (2002), using chloroplastic ribosomal deoxyribonucleic acid sequences.

118 **APA assays**

119 In this study, all experiments were conducted in seawater (pH = 8.1) and should
120 therefore be considered as assays for APA. APA was measured using a colorimetric assay,
121 which is based on the non-biological substrate *p*-nitrophenyl phosphate (pNPP) to yield a
122 colored product, *p*-nitrophenolate (pNP), upon hydrolysis under alkaline conditions. Most
123 measurements were performed with the symbiotic coral *S. pistillata* and its FIZ, but APA was
124 also compared between different coral species cited above (*see* description below). In each
125 case, 5 nubbins or 5 FIZ samples were considered. All APA experiments were performed
126 using six beakers filled with 200-mL of filtered seawater (0.2 μ m membrane filters, pH of
127 8.1), maintained in a water bath at 26°C or 18°C (for tropical and Mediterranean species,
128 respectively). Light was supplied by 400 W HQI lights and measured using a spherical
129 quantum sensor (LiCor LI-193).

130 In experiments involving coral nubbins, individual nubbins suspended to a nylon wire
131 were introduced in five beakers. A beaker without coral served as a control for APA activity
132 in the filtered seawater and for any non-enzymatic hydrolysis of the pNPP substrate. Thirty

133 minutes after nubbins were placed in the beakers (acclimatization period), the water was
134 enriched with $250 \mu\text{mol P L}^{-1}$ of pNPP. Magnetic stirring bars ensured proper homogenization
135 inside the beakers. A 10-mL water sample was taken from each beaker immediately after
136 enrichment and then every 30 min for 120 min. Samples were immediately alkalized with
137 2 mL of 1 mol L^{-1} NaOH to stop the enzymatic reaction and yield the yellow colored *p*-
138 nitrophenolate (pNP). Absorbance was read at 400 nm after 30 minutes against a blank of
139 filtered seawater treated with NaOH. Concentrations of pNP were calculated from a standard
140 curve of pNP absorbance vs. concentration established during each experiment using known
141 concentrations of ACS reagent grade pNP in filtered seawater treated with NaOH. At the end
142 of the experiments, corals were frozen for subsequent normalization of the data.

143 For experiments involving FIZ, a concentrated zooxanthellae solution, obtained after
144 isolation of FIZ, was used to inoculate the five pNPP-enriched beakers, yielding
145 concentrations comprised between 2×10^4 and 10^5 FIZ mL^{-1} . Sampling protocol and sample
146 measurements were the same as above, except that samples were filtered ($0.45\text{-}\mu\text{m}$ membrane
147 filters) prior to alkalization to remove FIZ.

148 The working parameters for the APA assays were developed in preliminary tests. A
149 first preliminary test was performed to determine the optimal incubation length, using the
150 coral *S. pistillata* and its FIZ at various pNPP concentrations. During each assay, pNP
151 concentration increased linearly over time (independently of the pNPP concentration), during
152 ca. 180 min both for whole coral nubbins and FIZ. Thus, APA was considered linear over
153 these 180 min, and an incubation length of 120 min was chosen for the subsequent
154 experiments. We also examined the effect of pH on phosphatase activity in FIZ, since
155 previous studies suggested that pH optima is different for internal and external phosphatases,
156 and is likely related to the pH experienced by the zooxanthellae (Jackson et al. 1989; Annis
157 and Cook 2002). In the present study, external phosphatase activity of intact FIZ from *S.*

158 *pistillata* was predominantly alkaline, as phosphatase activity was 2.5 times higher at the pH
 159 of seawater (8.1) than at an acidic pH (5.8; Fig. 1). We therefore worked at a pH of 8.1 (in
 160 natural seawater, no buffer added) for the FIZ in the following experiments. We also
 161 examined the effect of initial pNPP concentration on APA to determine the kinetics
 162 characteristics of coral and zooxanthellae phosphatases. We measured host APA using pNPP
 163 concentrations of 0.05, 0.5, 1.0, 5.0, 25, 50, 250, 500, 1000, 2500, and 5000 $\mu\text{mol P L}^{-1}$, and
 164 FIZ APA using pNPP concentrations of 0.05, 5.0, 25, 50, 250, 1000, and 2500 $\mu\text{mol P L}^{-1}$.
 165 APA in the coral host was detectable at pNPP concentrations as low as 0.05, 0.5, and 1.0
 166 $\mu\text{mol P L}^{-1}$ and was equal to 0.8 ± 0.1 , 4.1 ± 0.4 , and 5.5 ± 0.7 $\text{nmol P mg prot}^{-1} \text{ h}^{-1}$,
 167 respectively, and was 1.2 ± 0.2 in FIZ at a pNPP concentration of 0.05 $\mu\text{mol P L}^{-1}$. FIZ and
 168 host extracellular APA were strongly dependent upon DOP concentration, as already reported
 169 for the intracellular phosphatases of FIZ (Jackson et al. 1989), and curves fitted with the
 170 Michaelis–Menten equation (Fig. 2; Eq. 1):

$$171 \quad \text{APA} = \frac{\text{APA}_{\text{max}} [\text{pNPP}]}{[\text{pNPP}] + K_m} \quad \text{APA} = \frac{\text{APA}_{\text{max}} [\text{pNPP}]}{[\text{pNPP}] + K}$$

172 (1) where [pNPP] is the initial concentration of p-nitrophenyl phosphate introduced in the
 173 beaker; K_m is the pNPP concentration at which activity is half-optimal, and corresponds to the
 174 inverse of the affinity of the alkaline phosphatase towards the pNPP substrate; and APA_{max} is
 175 the activity when the saturation concentration of pNPP has been reached. In both cases, APA
 176 saturated for pNPP concentrations between 250 and 500 $\mu\text{mol P L}^{-1}$, and in subsequent
 177 experiments, APA was measured at a pNPP concentration of 250 $\mu\text{mol P L}^{-1}$ (i.e., near the
 178 saturating concentration for both corals and FIZ). Other preliminary tests revealed that APA
 179 was strongly affected by light level and coral nutrition. These parameters were therefore
 180 tightly controlled in each of the subsequent experiments, to test their individual effect.
 181 Therefore, unless otherwise specified, light level was maintained at 150 ± 10 $\mu\text{mol photons m}^{-2}$
 182 s^{-1} , and corals were fed three days before the experiments. The set of experimental

183 conditions tested is summarized in Table 1. Moreover, for experiment 1 (Table 1), the dark
184 incubation was conducted at the end of the 12-hour dark period. For experiment 3 (Table 1),
185 APA measurements were also performed in the dark, at the end of the night, to prevent
186 metabolic changes associated with photosynthesis in the symbiotic nubbins.

187 **Data normalization and statistical analyses**

188 APA was expressed as units of pNPP hydrolyzed per hour, which corresponds to one
189 molecule of phosphate liberated by the enzyme per molecule of pNPP hydrolyzed. It was then
190 normalized for the coral host either to the chlorophyll (Chl) and zooxanthellae (zoox) content
191 (using the same methods as in Godinot et al. 2009), to the surface area of the nubbins (using
192 the wax-dipping method described in Stimson and Kinzie 1991), or to the protein (prot)
193 content (after extraction in 1 mol L⁻¹ NaOH for 30 min at 90°C, measured using the
194 commercially available BC Assay Interchim kit). Protein content is a good estimator of the
195 biomass, since there is a linear relationship between protein content and dry weight in *S.*
196 *pistillata* (unpublished results). Data for the FIZ were normalized to protein content, and to
197 cell density and chlorophyll content. All data are expressed as the mean ± standard error of 5
198 replicates. The software GraphPad Prism (version 5.0) was used to perform linear regressions
199 to examine the effect of light, and to study concentration-dependent APA using non-linear
200 regressions with Michaelis-Menten fittings. For the other experiments, StatView (version 5.0)
201 was used to perform *t*-tests, and one-way and two-ways analysis of variance (ANOVAs) on
202 the data, followed by Fisher's protected least significant difference (PLSD) tests.

203

204 **Results**

205 Since we used nubbins entirely covered with tissue and intact symbiont cells,
206 phosphatase activities should have been from external phosphatases only. In the following
207 results, we will refer to 'FIZ APA' for the activity related to the symbionts only, and to 'host

208 APA' for the activity related to the entire symbiotic association, but mainly coming from the
209 host because only the host tissue was exposed to the medium. Moreover, normalization of
210 APA per protein and skeletal surface area led to the same results, and only the normalization
211 per protein is presented. It is considered as the most appropriate to compensate for the
212 difference in biomass per skeletal surface area between the various coral species used, and
213 between coral hosts and FIZ. In the clade experiment, results are also presented with the
214 normalization to chlorophyll content.

215 **Effect of light and feeding on the APA of *S. pistillata***

216 APA increased linearly with light in both host ($r^2 = 0.61$, $F_{1,18} = 29$, $p < 0.0001$) and
217 FIZ ($r^2 = 0.87$, $F_{1,18} = 120$, $p < 0.0001$; Fig. 3), being c.a. 3.0 times higher at 350 μmol
218 photons $\text{m}^{-2} \text{s}^{-1}$ than in the dark. APA also significantly increased over time in both host and
219 FIZ when corals were starved (1-way ANOVA, both $p < 0.0001$, $F_{3,16} = 16.9$ for host, and
220 $F_{3,16} = 57.4$ for FIZ; Fig. 4). Host APA was the highest after 6 weeks of starvation ($165.5 \pm$
221 $20.3 \text{ nmol P mg prot}^{-1} \text{ h}^{-1}$). At this moment, activity was 4 times higher than in fed corals
222 ($40.0 \pm 2.4 \text{ nmol P mg prot}^{-1} \text{ h}^{-1}$), and remained 2.8 times higher until 9 weeks of starvation.
223 FIZ APA was the highest after 3 weeks of starvation ($154.6 \pm 4.3 \text{ nmol P mg prot}^{-1} \text{ h}^{-1}$), being
224 1.5 times higher than in FIZ from fed corals ($100.0 \pm 7.0 \text{ nmol P mg prot}^{-1} \text{ h}^{-1}$). After this
225 point, APA decreased back to the level of FIZ from fed corals.

226 **Effect of the symbiotic status**

227 In the non-symbiotic *Tubastraea* sp., light had no effect on APA, as APA was not
228 significantly different in the dark ($18.5 \pm 2.9 \text{ nmol P mg prot}^{-1} \text{ h}^{-1}$) and in the light (19.3 ± 2.2
229 $\text{ nmol P mg prot}^{-1} \text{ h}^{-1}$; t -test, $t = 0.27$, $df = 9$, $p = 0.76$). Host APA in the dark was not
230 significantly different between *Tubastraea* sp. and symbiotic *S. pistillata* (mean of 18.3 ± 1.6
231 $\text{ nmol P mg prot}^{-1} \text{ h}^{-1}$; 1-way ANOVA, $p = 0.89$, $F_{2,12} = 0.21$; Fig. 5), but increased by 6.4 fold

232 in bleached *S. pistillata* (115.1 ± 20.4 nmol P mg prot⁻¹ h⁻¹; 1-way ANOVA, $p = 0.0001$, $F_{2,12}$
233 = 21.85).

234 **Comparison of APA in different coral hosts and zooxanthellae clades**

235 When results obtained for the FIZ were normalized to the protein content, there was no
236 significant difference in APA between the clades (1-way ANOVA, $p = 0.18$, $F_{2,27} = 4.81$; data
237 not shown). Conversely, a significant difference in host APA was observed between species
238 (1-way ANOVA, $p < 0.0001$, $F_{5,24} = 21.7$; Fig. 6A). Differences were only related to
239 differences in the chlorophyll content normalized per protein content between coral species,
240 and were unrelated to the tropical or temperate status of the host, to the symbiont density, to
241 the chlorophyll content per surface area, or to the zooxanthellae clade (1-way ANOVAs, all
242 $p > 0.4$). For FIZ, results were also normalized to the chlorophyll content. With this
243 normalization, there was a significant difference in APA between species (1-way ANOVA,
244 $p < 0.0001$, 192.6; Fig. 6B), which was related to the zooxanthellae clade (1-way ANOVA,
245 $p < 0.0001$, 232.7, respectively).

246

247 **Discussion**

248 This study reports on the alkaline phosphatase activity in coral hosts and their isolated
249 symbionts under various environmental conditions. It is the first to examine APA in coral
250 hosts, and to highlight the importance of host metabolism, and phosphate limitation or
251 repletion, on this activity. It also emphasizes for the first time the effects of light and
252 zooxanthellae clade on APA, and confirms that host feeding has a negative effect on this
253 activity.

254 **Effects of metabolic activity on APA**

255 In symbiotic corals, APA was dependent upon symbiont activity, and likely upon
256 zooxanthellae photosynthetic activity, since APA was 3.0 times lower in the dark than at 350

257 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in a symbiotic coral (Fig. 3) while it did not increase with light in a non-
258 symbiotic one. APA was also 6.4 times higher in bleached than in symbiotic colonies (Fig. 5),
259 suggesting a phosphate limitation in bleached corals, as previously demonstrated (Annis and
260 Cook 2002). In symbiotic corals, most of the phosphate requirements are supplied by the
261 activity of the symbionts, which continuously take up DIP from the surrounding seawater
262 (D'Elia 1977; Godinot et al. 2009). The loss of symbionts in bleached colonies corresponds to
263 a reduction of DIP uptake, and even to a release of it (Muller-Parker et al. 1990), which may
264 both lead to a deficit of phosphate in the symbiotic association. Therefore, the host has to rely
265 on organic phosphate (and increased APA) to fulfill its high phosphate metabolic demand
266 (Godinot et al. 2011). Conversely, in the non-symbiotic *Tubastraea* sp., APA was 6.2 times
267 lower than in bleached *S. pistillata* (Fig. 5), suggesting that despite the fact that *Tubastraea*
268 also loses DIP (D'Elia 1977), its total phosphate demand may be lower. Alternatively,
269 although non-symbiotic, bleached, and symbiotic corals, were supplied the same zooplankton
270 regime before the experiments, their feeding rates remain to be examined in order to
271 determine if their phosphate inputs from POP were different.

272 In symbiotic *S. pistillata*, host APA was also affected by heterotrophy and starvation.
273 The observed increase in APA with long-term starvation of the host (Fig. 4) can be related to
274 a lack of phosphate availability in the symbiosis. Indeed, feeding provides the host with a
275 source of POP (Johannes et al. 1970; Wijgerde et al. 2011), and this source disappears with
276 starvation, which therefore might trigger the increase of host APA. The same feeding effect
277 was observed in FIZ (Fig. 4) and was also reported previously in FIZ (Jackson et al. 1989;
278 Annis and Cook 2002). After a prolonged starvation (more than 3 weeks in FIZ and more than
279 6 weeks in the host), APA decreased again, probably due to a reorganization and/or reduction
280 of the coral metabolic activity, including a possible shut-down of metabolic machinery and
281 the recycling of macromolecules. It is in fact well established that starvation lowers protein

282 and chlorophyll concentrations, symbionts density (Titlyanov et al. 2001; Ferrier-Pagès et al.
283 2003; Houlbrèque et al. 2003), and rates of respiration and photosynthesis (Szmant-Froelich
284 and Pilson 1984; Davy et al. 2006). Such reduction of the metabolism may either be due to the
285 catabolism of alkaline phosphatases or may have led to a lower phosphate demand explaining
286 the APA decrease.

287 Results obtained finally showed that light increased host and FIZ APA (Fig. 3), maybe
288 due to the higher metabolic activity of corals in terms of respiration rates in the light (Porter et
289 al. 1984). Another explanation can be a faster depletion of phosphate stocks of the host by
290 symbiont photosynthesis, as phosphate is a component of ATP and enters into the
291 composition of other compounds of the Calvin cycle (Hall and Rao 1987), and as DIP demand
292 has been shown to increase with light (Jackson and Yellowlees 1990; Godinot et al. 2009).
293 Moreover, light levels used in this study are not as high as those encountered naturally in
294 reefs, and APA might therefore be even higher under natural conditions, with a much faster
295 hydrolysis of organic phosphate into inorganic phosphate.

296 **Inter-species comparison and effect of zooxanthellae clade**

297 Results obtained in this experiment suggest for the first time a possible dependency of
298 APA upon zooxanthellae clade, with the cosmopolitan clade C (Stat et al. 2008) being
299 apparently less efficient than clades A and B (Fig. 6). Specific metabolic characteristics of the
300 different clades may influence APA, possibly through differences in the phosphate demand
301 and/or uptake. This theory remains however to be further investigated, because only one study
302 has examined up to date the relationship between eutrophication and zooxanthellae clades
303 (Lampert-Karako et al. 2008), and has found no differences. Since only one clade B coral was
304 available for the present study, the preliminary results from this experiment need to be
305 completed by expanding the number of samples within the same clade, as well as the number
306 of clades investigated.

307 **Contribution of DOP to the phosphate demand of corals**

308 APA measured at DOP concentrations of 0.05 and 0.5 $\mu\text{mol P L}^{-1}$, which corresponds
309 to the range of those occurring in situ (Crossland and Barnes 1983; Larned 1998), was equal
310 to 0.8 ± 0.1 and 4.1 ± 0.4 $\text{nmol P mg prot}^{-1} \text{ h}^{-1}$ in the light, respectively, and 0.4 ± 0.1 and 1.9
311 ± 0.3 $\text{nmol P mg prot}^{-1} \text{ h}^{-1}$ in the dark, respectively (2.2 times lower compared to the light
312 value). These values correspond to 0.3 to 1.6 $\text{mmol P m}^{-2} \text{ d}^{-1}$ (value $\times 2.2$ $\text{mg prot cm}^{-2} \times 12$ h
313 of day and night $\times 10,000$). If all phosphate provided by DOP hydrolysis through alkaline
314 phosphatase activity is entirely taken up by corals, DOP appears to be a significant source of
315 phosphate for corals, even at low environmental concentrations. Indeed, it has been shown
316 that DIP contributes 0.2 to 1.6 $\text{mmol P m}^{-2} \text{ d}^{-1}$ (Steven and Atkinson 2003; Godinot et al.
317 2009; Cuet et al. 2011) and POP brings of 0.9 ± 0.3 $\text{mmol P m}^{-2} \text{ d}^{-1}$ (Wijgerde et al. 2011).
318 DOP may have an even more significant contribution if sources like phosphodiesteres,
319 polyphosphates and phosphonates are taken into consideration. Coral species can benefit from
320 these sources using other phosphatases and phosphonatas. The question that remains to be
321 examined is how much of the phosphate recycled from DOP can effectively be absorbed by
322 coral colonies, since phosphate might be rapidly flushed out of the reef by water currents
323 (Atkinson 1987b). Some additional studies, using a ^{33}P -labeled DOP source, may help answer
324 this question, by tracing ^{33}P inside coral tissues.

325 In conclusion, this study showed that host and symbionts alkaline phosphatase
326 activities are dependent upon light and host feeding, and upon the metabolic activity of the
327 host and the symbionts (with a possible clade effect). We showed that DOP may represent a
328 significant source of phosphate for corals in oligotrophic reefs, since at similarly low
329 environmental concentrations (less than 0.5 $\mu\text{mol P L}^{-1}$), this source may bring as much
330 phosphate as DIP (1.6 $\text{mmol P m}^{-2} \text{ d}^{-1}$). The present study thus needs to be followed by
331 attempts at determining the amount of phosphate effectively recycled from various sources of

332 DOP and POP by corals, in order to complete our first calculation of the balance of phosphate
333 inputs from DIP, DOP, and POP in corals under oligotrophic conditions.

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426
427
428

429 **Table**

430

431 **Table 1**

432 Working parameters of the four experiments performed to measure APA in various

433 scleractinian corals.

Experiment	Species (clade)	Symbiotic status	Coral nubbin or FIZ	Light ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	Host feeding (measurements 3 days after feeding)	[pNPP] ($\mu\text{mol P L}^{-1}$)
1 - Effect of light	<i>Stylophora pistillata</i> (A)	symbiotic	coral FIZ	0 150 250 350	control	250
	<i>Tubastraea</i> sp.	asymbiotic	coral	0 150	control	250
2 - Effect of host feeding	<i>S. pistillata</i> (A)	symbiotic	coral FIZ	150	control starved 3 wks starved 6 wks starved 9 wks	250
3 - Effect of symbiotic status	<i>S. pistillata</i> (A)	symbiotic	coral	0	control	250
	<i>S. pistillata</i> (A)	bleached	coral			
	<i>Tubastraea</i> sp.	asymbiotic	coral			
4 - Effect of symbiont clade	<i>S. pistillata</i> (A) <i>Pocillopora damicornis</i> (C) <i>Pavona cactus</i> (C) <i>Galaxea fascicularis</i> (C) <i>Cladocora caespitosa</i> (A) <i>Oculina patagonica</i> (B)	symbiotic	coral FIZ	150	control	250

434 **Figure legends**

435

436 **Figure 1:** Effect of pH on APA of FIZ from *Stylophora pistillata* incubated with 250 $\mu\text{mol L}^{-1}$
437 pNPP. Data are presented as the mean \pm standard error of 5 measurements.

438

439 **Figure 2:** Concentration-dependent APA in host and FIZ from *S. pistillata*. Data are mean \pm
440 standard error of 5 replicates.

441

442 **Figure 3:** Light-dependent APA in *S. pistillata* nubbins and FIZ incubated with 250 $\mu\text{mol L}^{-1}$
443 pNPP. Data are mean \pm standard error of 5 replicates. Light was measured using a LiCor
444 instrument and is expressed as $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

445

446 **Figure 4:** Effect of starvation on APA in host and FIZ from the coral *S. pistillata* incubated with
447 250 $\mu\text{mol L}^{-1}$ pNPP. Data are mean \pm standard error of 5 replicates.

448

449 **Figure 5:** Dark APA in the non-symbiotic tropical coral *Tubastraea* sp., and in symbiotic and in
450 bleached *S. pistillata* incubated with 250 $\mu\text{mol L}^{-1}$ pNPP. Data are presented as the mean \pm
451 standard error of 5 measurements.

452

453 **Figure 6:** Comparison of APA in different (a) coral hosts (b) harboring symbionts from different
454 clades. Data are presented as the mean \pm standard error of 5 measurements, (a) normalized per
455 protein content or (b) per chlorophyll content.

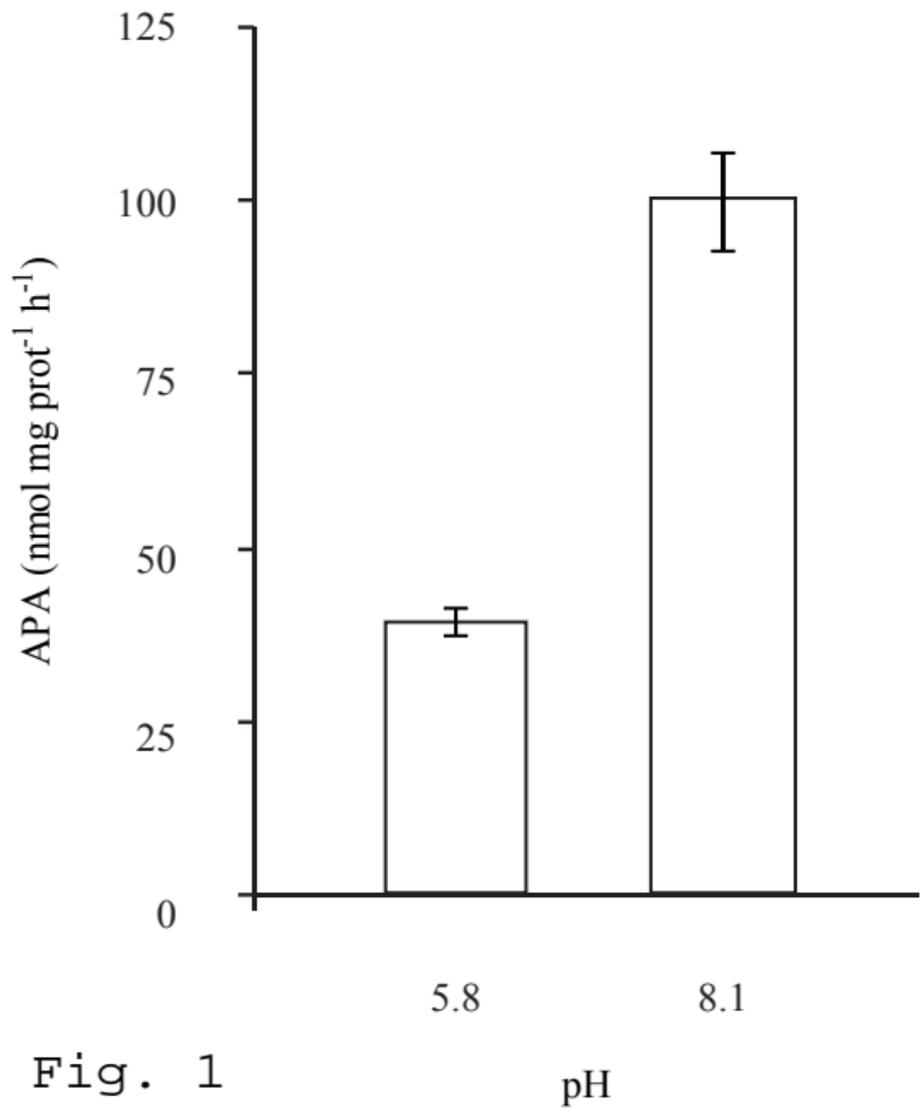


Fig. 1

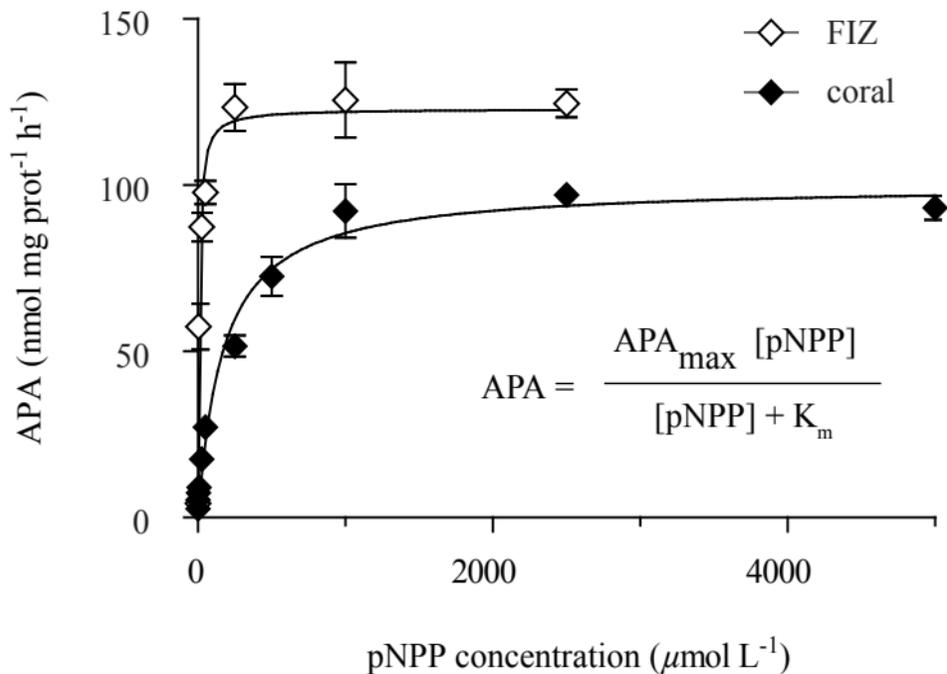


Fig. 2

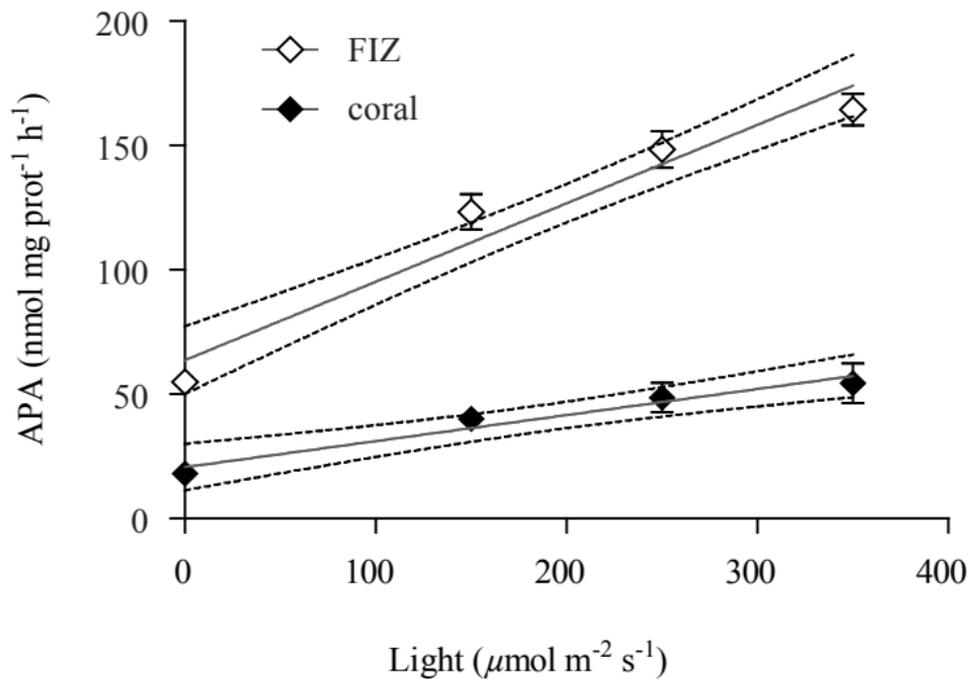


Fig. 3

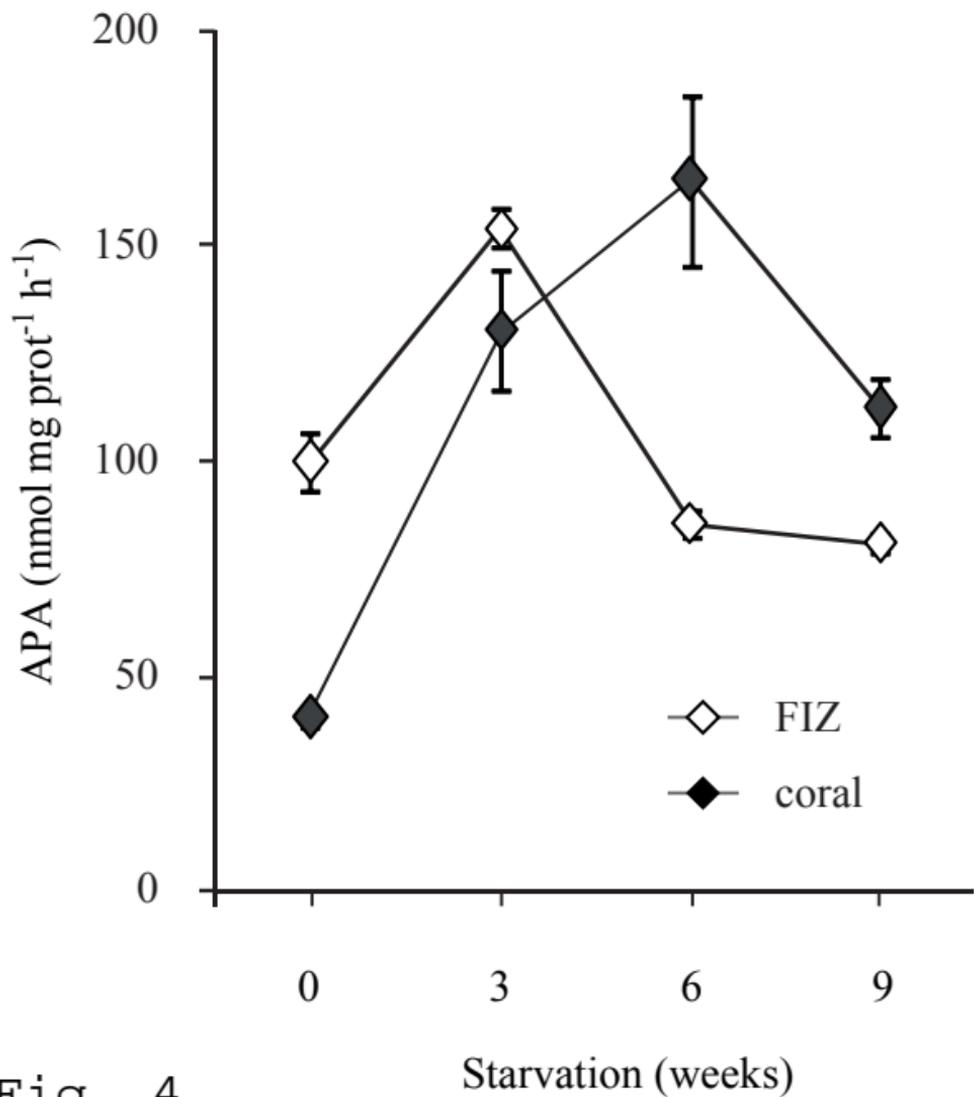


Fig. 4

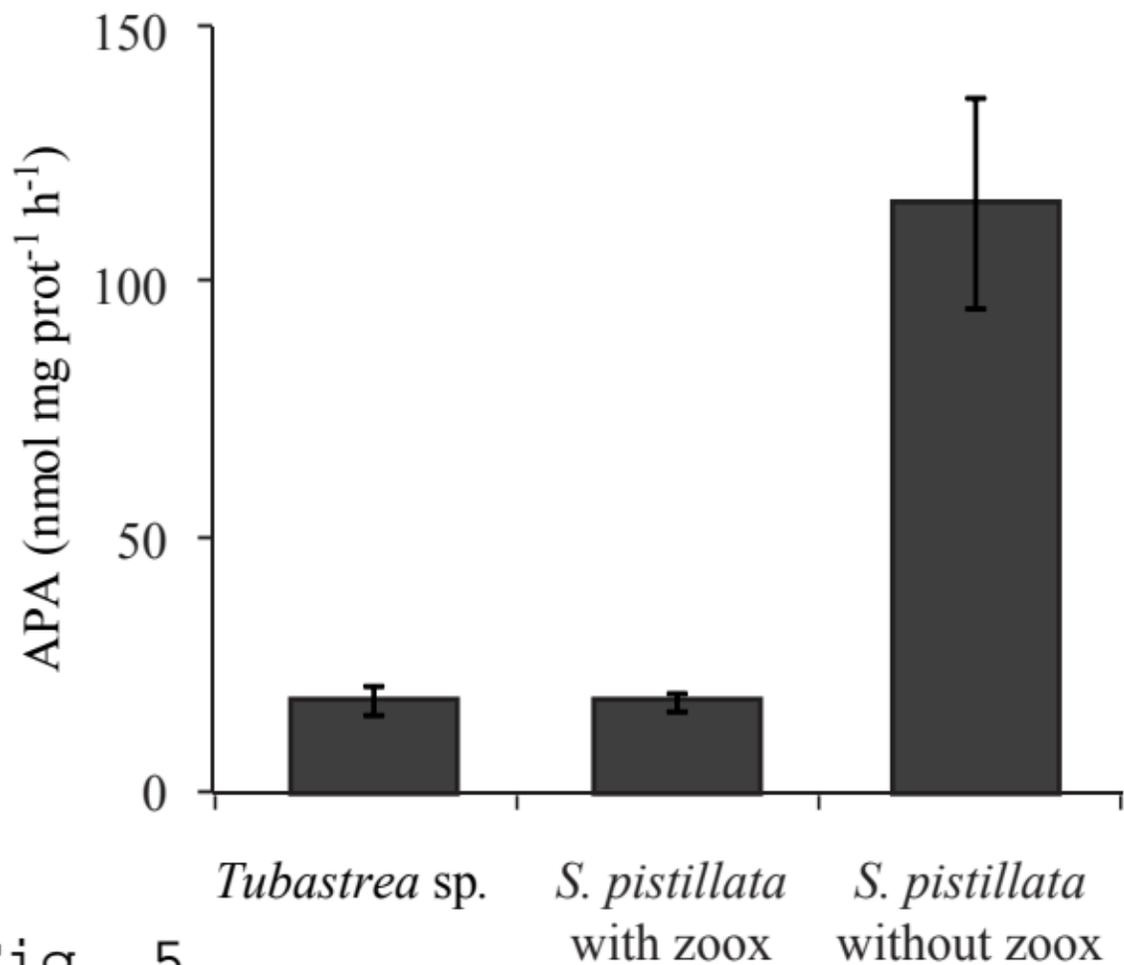


Fig. 5

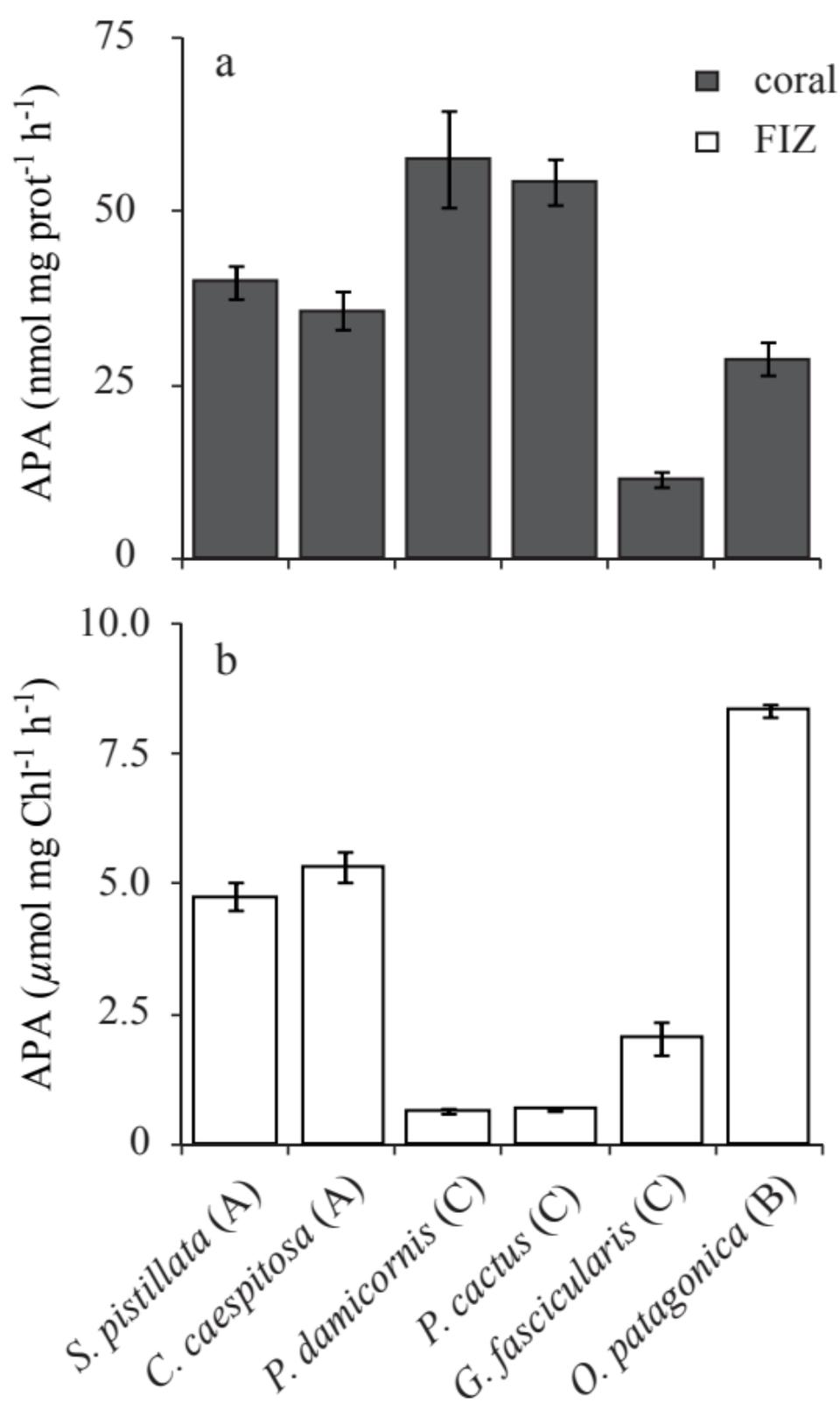


Fig. 6

Coral species