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# Control of phosphate uptake by zooxanthellae and host cells in the scleractinian coral *Stylophora pistillata*

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1           **Control of phosphate uptake by zooxanthellae and host cells in the**  
2                           **scleractinian coral *Stylophora pistillata*.**

3

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5

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8 Running head: Phosphate uptake by corals.

9

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17 **Abstract**

18           Depletion experiments were used to assess the uptake rates of phosphate by the  
19 tropical coral *Stylophora pistillata* and its symbiotic zooxanthellae under different conditions.  
20 Our results showed the presence of active phosphate carriers both in the animal and the algal  
21 fractions, since uptake kinetics followed uniphasic saturation curves in both compartments  
22 (Michaelis-Menten shape). Transporters in the animal and the algae presented different  
23 affinities for phosphate, with higher half-saturation constants for the animal compartment than  
24 for the isolated zooxanthellae ( $K = 1.08 \pm 0.42$  vs.  $0.57 \pm 0.18 \mu\text{mol L}^{-1}$ ). The velocity of  
25 phosphate absorption increased in the light, suggesting a relationship with the photosynthetic  
26 activity of the zooxanthellae. A correlation was found between phosphate uptake rates and the  
27 organic or inorganic feeding history of the corals; rates were indeed 4.6 times higher in 8-  
28 week starved than in fed corals, and also depended on the repletion status of phosphorus  
29 stocks within the symbionts. We report evidence showing that zooxanthellae act as a sink of  
30 phosphate within the symbiosis.

## 31 **Introduction**

32

33 Coral reefs mainly thrive in shallow oligotrophic coastal areas of tropical and  
34 subtropical seas (Bythell 1990; Furnas 1991; Szmant 2002). Even though concentrations of  
35 nutrients such as nitrogen and phosphorus are usually below  $0.5 \mu\text{mol L}^{-1}$  in the surrounding  
36 waters (Atkinson 1987; Furnas 1991; Atkinson et al. 1995), reefs represent a large biomass,  
37 and are considered as oases in a blue desert. One of the explanations lies in the fact that corals  
38 harbor endosymbiotic dinoflagellates called zooxanthellae (zoox; Muscatine 1980), which  
39 actively take up dissolved nutrients (Pomeroy and Kuenzler 1967; Muscatine 1980), and  
40 transform them into organic molecules through their photosynthesis. Photosynthates are in  
41 turn transferred to the host for its own metabolism (Muscatine and Porter 1977). Knowledge  
42 of nutrient uptake rates by scleractinian corals is therefore crucial for a better understanding  
43 of the functioning of reefs.

44 Phosphorus is one of these key nutrients in marine environments (Benitez-Nelson  
45 2000; Elser et al. 2007), because it enters into the composition of many biological molecules  
46 (deoxyribonucleic acids, ribonucleic acids, phospholipids) and has a role in most biochemical  
47 mechanisms (e.g., via the energetic role of adenosine triphosphate (ATP)). It occurs in the  
48 form of dissolved orthophosphates ( $P_i$ ), condensed phosphates (pyro-, meta-, and other  
49 polyphosphates), and phosphorus bound to organic molecules (phosphate esters,  
50 phosphonates...), either in dissolved or particulate forms (detritus and in the body of aquatic  
51 organisms; Karl and Björkman 2002). Autotrophs can mainly use the ‘biologically available  
52 phosphorus’ (BAP), which consists in  $P_i$  and a small biolabile fraction of dissolved organic  
53 phosphorus (Karl and Björkman 2002).

54 In the oligotrophic waters surrounding coral reefs, inorganic forms of phosphorus  
55 display a characteristic depletion (Baturin 2003), and concentrations are well below  $0.5 \mu\text{mol}$

56 L<sup>-1</sup> (Furnas 1991). Several studies, performed at the community level (integrating multiple  
57 compartments such as coral species, macro and microalgae, invertebrates, sponges, and  
58 bottom sediment) have shown that phosphorus is a key nutrient (Atkinson 1987; Sorokin  
59 1992; Atkinson et al. 1995), since it is efficiently absorbed, generally in direct proportion to  
60 its ambient concentration in seawater (for phosphate concentrations of 1-7  $\mu\text{mol L}^{-1}$ ; Atkinson  
61 1987). Some studies have also assessed the uptake rates of phosphate in specific symbiotic  
62 organisms such as in the sea anemone *Aiptasia pallida* (Verril 1864; Muller-Parker et al.  
63 1990), the giant clam *Tridacna gigas* (Linné 1758; Belda and Yellowlees 1995), or the  
64 jellyfish *Cassiopea xamachana* (Bigelow 1892; Todd et al. 2006), all showing that  
65 phosphorus is taken up by the dinoflagellates living in symbiosis with those organisms.  
66 Uptake of phosphate by specific scleractinian corals has attracted much less attention than the  
67 whole reef community, and most studies focused on phosphate uptake by isolated  
68 zooxanthellae (cultured or freshly isolated; Deane and O'Brien 1981; Jackson and Yellowlees  
69 1990; R. Kelty unpubl.) rather than by the entire coral colony (D'Elia 1977). In the only  
70 studies on isolated corals, D'Elia (1977) and Sorokin (1992) showed that, at low ambient  
71 phosphorus concentrations, symbiotic corals took up and concomitantly released phosphorus.  
72 Uptake was light sensitive, suggesting that zooxanthellae influenced the phosphorus fluxes in  
73 their host. An active transport was also suggested, with a half-saturation constant K equal to  
74 0.375  $\mu\text{mol L}^{-1}$  (D'Elia 1977), but the location of the active transport sites could not be  
75 determined. The light enhancement of phosphorus uptake and the active transport of  
76 phosphorus were not confirmed in the other symbiotic association of *A. pallida* (Muller-  
77 Parker et al. 1990).

78         This study provides additional and new information concerning phosphate uptake by  
79 the scleractinian coral *Stylophora pistillata* (Esper 1797). Parallel experiments were  
80 performed with the entire coral association and with zooxanthellae freshly isolated from the

81 same coral colonies (FIZ) to understand the respective role of the host and symbionts in the  
82 uptake of phosphate, localize the active uptake sites, and compare the kinetics parameters  
83 between the two models. The K value, for example, differs widely from one study to another:  
84 for FIZ, it ranged from  $< 0.02 \mu\text{mol L}^{-1}$  (Deane and O'Brien 1981) to  $14 \mu\text{mol L}^{-1}$  (Jackson  
85 and Yellowlees 1990). We specified the effect of light on the phosphate uptake by FIZ, since  
86 both a dark (Deane and O'Brien 1981) and a light-enhancement (Jackson and Yellowlees  
87 1990) were observed. Finally, we highlighted some effects of inorganic and organic supply of  
88 phosphorus on the uptake rates of phosphate by *S. pistillata*. The results obtained bring new  
89 insights into the location of phosphorus pools in the colony and on the importance of feeding  
90 in getting phosphorus from the environment.

91

## 92 **Methods**

93

### 94 *Organisms and culture conditions*

95 Colonies of the zooxanthellate coral *S. pistillata* were obtained from the Red Sea, and  
96 maintained at the Centre Scientifique de Monaco (CSM) under controlled conditions in  
97 aquaria (26°C, Hydrargyrum Quartz Iodide lights (HQI), with a 12 h:12 h dark:light cycle,  
98 salinity of 38). Aquaria were continuously provided with unfiltered seawater pumped at 50 m  
99 depth. The 120 nubbins (i.e., branch tips) used during the experiments were prepared by  
100 cutting branches ( $2.5 \pm 1.0$  cm long and  $0.6 \pm 0.3$  cm in diameter) of about ten parent colonies  
101 with pliers (E. Tambutté unpubl.), 1.5 month before the experiments. Nubbins were attached  
102 on nylon wires and suspended in aquaria until tissue fully covered the skeleton. They were  
103 lightly fed (once a week) with *Artemia salina* (Linné 1758) nauplii.

104

### 105 *Phosphate uptake by coral nubbins*

106 Phosphate uptake by coral nubbins was studied with a series of 3 experiments, in  
107 which the depletion of phosphate was followed over time in phosphate-enriched beakers.  
108 Unless otherwise specified, all depletion experiments were performed under the following  
109 conditions. Six 200 mL beakers were maintained in a water bath at 26°C, under a  
110 photosynthetic active radiation (PAR) of 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$  supplied by 400W HQI lights and  
111 measured using a spherical quantum sensor (LiCor LI-193). Five coral nubbins, each  
112 suspended to a nylon wire, were introduced in separate beakers. A beaker without coral  
113 served as a control for changes in phosphate concentrations due to adsorption onto the beaker  
114 surface or to consumption by microbial activity. 30 min after nubbins were placed in the  
115 beakers (acclimatization period), a buffer solution of  $\text{KH}_2\text{PO}_4$  (10  $\text{mmol L}^{-1}$ ) was added to  
116 reach the desired initial phosphate concentration. Magnetic stirring bars ensured proper  
117 phosphate homogenization. A 10 mL water sample was taken from each beaker immediately  
118 after enrichment and then every 15 min for 90 min. Samples were frozen right away, and  
119 phosphate concentrations were measured no longer than a week after sampling. Levels of  
120 phosphate were determined by spectrophotometry on thawed samples, using the ascorbic acid  
121 technique (Murphy and Riley 1962).

122 Initial uptake rates of phosphate ( $V_0$ ) were calculated from the quantity absorbed by  
123 each nubbin in 60 min, after correction of phosphate concentrations for the diminution of the  
124 beaker volume. Uptake rates were normalized to the total chlorophyll (Chl) content, or the  
125 zooxanthellae concentration, or the surface area of the nubbins. Surface area was measured  
126 using the wax technique (Stimson and Kinzie 1991). Chlorophyll *a* and *c2* were extracted  
127 twice in 99% acetone (24 h at 4°C). The extracts were centrifuged at 11,000 x *g* for 10 min at  
128 4°C and the absorbances measured at 630, 663, and 750 nm. Chlorophyll contents were  
129 computed according to the 100% acetone equations of Jeffrey and Humphrey (1975).



130 Zooxanthellae were removed with a Water-Pik and counted using a counting chamber and an  
131 improved version of the Histolab v.5.2.3 image analysis software (Microvision).

132 *Concentration dependence of phosphate uptake:* Uptake rates of phosphate were  
133 measured at the following concentrations: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5  $\mu\text{mol L}^{-1}$ .  
134 Five nubbins were used for each concentration tested.

135 *Light effect on phosphate uptake rates:* Coral nubbins were incubated in 3.0  $\mu\text{mol L}^{-1}$   
136 phosphate either in light (200 or 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) or dark conditions. For each condition, 5  
137 nubbins were considered. During dark measurements, nubbins were collected shortly after the  
138 end of the dark cycle in the culture room.

139

#### 140 ***Phosphate uptake by freshly isolated zooxanthellae (FIZ)***

141 FIZ were extracted from coral nubbins maintained under the above regular conditions.  
142 Tissue was separated from the skeleton using a Water-Pik and zooxanthellae were further  
143 purified by repeated centrifugations (2000 rpm for 5 min at 25°C) and re-suspension in  
144 seawater (Johannes and Weibe 1970). Three washes were necessary to obtain clean  
145 zooxanthellae. Before each experiment, zooxanthellae were isolated from 3 nubbins, and  
146 pooled in order to minimize variability among nubbins. They were then re-suspended together  
147 in seawater and the mixture was used to inoculate 3 phosphate-enriched beakers. Subsamples  
148 of this solution were used to determine zooxanthellae concentrations (as described above).  
149 Initial uptake rates of phosphate were calculated as the quantity of phosphate absorbed by  
150 each zooxanthella in 60 min.

151 *Concentration dependence of phosphate uptake:* Uptake rates of phosphate by FIZ  
152 were measured at the following concentrations: 0.0, 0.5, 1.0, 2.0, 3.0, 5.0, and 8.0  $\mu\text{mol L}^{-1}$ . A  
153 beaker without FIZ served as a control for changes in phosphate levels due to microbial  
154 activity or adsorption. A water sample of 10 mL was taken from each beaker immediately

155 after enrichment and then every 20 min for 60 min. Samples were immediately filtered (0.45  
156  $\mu\text{m}$  membrane filters) in order to separate FIZ from the water, and filtrates frozen. Levels of  
157 phosphate were determined on thawed filtrates as described previously. Preliminary tests  
158 showed that filtration of the samples did not induce any contamination and that phosphate  
159 retention on filters was negligible.

160 *Light effect on phosphate uptake rates:* FIZ were incubated in  $3.0 \mu\text{mol L}^{-1}$  phosphate  
161 either in light ( $200$  and  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or dark conditions. During dark measurements, FIZ  
162 were isolated shortly after the end of the dark cycle in the culture room. Samples were filtered  
163 as described above.

164

#### 165 ***Effects of long-term phosphate exposure on corals***

166 Nubbins were cultured for 8 weeks in a separate experimental design of 6 tanks (3  
167 duplicates), in which concentrated buffer solutions of phosphate were continuously pumped  
168 with a peristaltic pump. Seawater with trace phosphate concentration ( $< 0.05 \mu\text{mol L}^{-1}$ ) was  
169 also continuously pumped, in order to obtain final phosphate concentrations of c.a. 0, 0.5, or  
170  $2.5 \mu\text{mol L}^{-1}$ . The seawater flow rate was ca.  $1.2 \text{ L h}^{-1}$ . Temperature, irradiance, and salinity  
171 were the same as previously described. Nubbins were not fed. Phosphate uptake rates were  
172 assessed after 8 weeks of incubation for 5 nubbins per condition (as described above), and  
173 zooxanthellae density as well as phosphorus contents within host tissue and algal cells were  
174 measured on 3 nubbins per condition. For this purpose, coral tissue was isolated from the  
175 skeleton using a Water-Pik, and was then homogenized using a Potter grinder. Subsamples of  
176 this solution were used to determine zooxanthellae concentrations (as described above).  
177 Homogenates were filtered on pre-heated GF/C filters ( $500^\circ\text{C}$  for 1 h) in order to separate  
178 zooxanthellae from animal tissue in solution. Filtrates and filters were then treated as follows,  
179 in order to transform organic phosphorus into dissolved phosphate. Filtrates were acidified to

180 a pH<2 with concentrated hydrochloric acid, and were then photo-oxidized (ultraviolet for 20  
181 min). Filters were ashed in a muffle furnace (500°C for 1 h), dissolved in boiling hydrochloric  
182 acid (105°C for 2 h), and the obtained solutions were then filtered using 0.45  $\mu$ m membrane  
183 filters. Levels of phosphate were determined on both filtrates as described previously. Results  
184 were normalized to the surface area of the nubbins and to the zooxanthellae concentrations,  
185 measured as described above.

186

### 187 *Statistical analyses*

188 Normality and homoscedasticity of data were tested using frequency histograms and  
189 Shapiro-Wilk tests (R version 2.2.0). Concentration dependence of phosphate uptake was  
190 studied using GraphPad Prism (version 5.0).  $K$  and  $V_{\max}$  were compared between nubbins and  
191 FIZ using  $t$ -tests (StatView version 5.0). 1-way analysis of variance (ANOVA) tests, followed  
192 by protected least significant difference (PLSD) Fisher tests, were performed to identify the  
193 effect of long-term phosphate acclimatization on its absorption by nubbins. Light dependence  
194 of phosphate uptake in FIZ and nubbins was tested using a 2-way ANOVA. As the normality  
195 assumption was not met for phosphorus and zooxanthellae concentrations inside the corals,  
196 those data were compared using non parametric Kruskal-Wallis tests, followed by Man-  
197 Whitney post hoc tests. Data from the 8-week enrichment did not differ among duplicate  
198 aquaria from the same treatment, and obtained results from those duplicates were pooled  
199 together for statistical analysis.

200

## 201 **Results**

202

### 203 *Phosphate uptake by nubbins and FIZ*

204 During each depletion experiment, phosphate uptake was linear during more than 60  
205 min both for whole colonies (nubbins; Fig. 1) and FIZ. Kinetics experiments (Fig. 2) revealed  
206 that uptake of phosphate occurred via an active carrier-mediated mechanism in both colonies  
207 and FIZ, since initial uptake rates ( $V_0$ ) followed a Michaelis-Menten model:

$$208 \quad V = \frac{V_{\max} [P]}{[P] + K} \quad (1)$$

209 where  $[P]$  is the initial phosphate concentration,  $V_{\max}$  the maximum phosphate uptake rate  
210 and  $K$  the concentration at which phosphate uptake is half-maximal.  $K$  represents the inverse  
211 of the affinity of the active carrier-mediated mechanism towards phosphate. From the  
212 Michaelis-Menten fitting, uptake rates of whole colonies ( $1.85 \pm 0.25 \times 10^{-15} \text{ mol zoox}^{-1} \text{ h}^{-1}$ )  
213 and isolated zooxanthellae ( $1.75 \pm 0.12 \times 10^{-15} \text{ mol zoox}^{-1} \text{ h}^{-1}$ ) were similar ( $t$ -test,  $p = 0.07$ ,  $df$   
214  $= 3$ ; Fig. 2). However, affinity constants differed significantly ( $t$ -test,  $p < 0.001$ ,  $df = 3$ ; Fig. 2),  
215 as the  $K$  calculated for FIZ ( $0.57 \pm 0.18 \mu\text{mol L}^{-1}$ ) was almost twice lower than that of  
216 nubbins ( $1.08 \pm 0.42 \mu\text{mol L}^{-1}$ ). In both cases, phosphate uptake saturated for concentrations  
217 of ca.  $2.0 \mu\text{mol L}^{-1}$ .

218 Phosphate uptake occurred both in the dark and in the light (Fig. 3), but with  
219 significantly higher rates in the light (2 way-ANOVA,  $p < 0.001$ ,  $F_2=84.9$ ), both in nubbins  
220 and FIZ. Dark uptake rates ( $0.19$  and  $0.26 \mu\text{mol mg Chl}^{-1} \text{ h}^{-1}$  for nubbins and FIZ respectively;  
221 Fig. 3) represented 46% and 53% of uptake rates measured at  $220 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  ( $0.42$   
222 and  $0.49 \mu\text{mol mg Chl}^{-1} \text{ h}^{-1}$  for nubbins and FIZ respectively). A 63.6% light increase between  
223  $220$  and  $360 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  led to a corresponding 64.3% and 69.0% increase in  $V_0$  for  
224 nubbins and FIZ respectively. For each light level, uptake rates of FIZ and nubbins were not  
225 significantly different (2 way-ANOVA,  $p = 0.054$ ,  $F_2=10.7$ ).

226

227 ***Effects of long-term phosphate exposure on corals***

228 Corals exposed 8 weeks to phosphate-enriched seawater significantly changed their  
229 uptake rates of phosphate. Indeed, a solution of  $3.0 \mu\text{mol L}^{-1}$  phosphate was taken up 5.3 and  
230 4.9 times faster by control corals cultured without phosphate addition than by corals cultured  
231 either with  $0.5$  or  $2.5 \mu\text{mol L}^{-1}$  phosphate (1-way ANOVA,  $p < 0.0001$ ,  $F_2 = 99.68$ ; Fig. 4).  
232 Uptake rates of enriched corals were not significantly different (PLSD Fisher test,  $p = 0.79$ ,  $df$   
233  $= 8$ ).

234 Corals exposed to phosphate-enriched seawater however presented the same  
235 phosphorus content per skeletal surface area in the animal tissue ( $0.15 \pm 0.01 \mu\text{mol P cm}^{-2}$ ;  
236 Kruskal-Wallis test,  $p = 0.30$ ,  $df = 2$ ). On the contrary, phosphorus content was significantly  
237 higher in zooxanthellae of the  $2.5 \mu\text{mol L}^{-1}$  phosphate-enriched corals, than in zooxanthellae  
238 from the control treatment (Mann-Whitney test,  $p < 0.05$ ,  $df = 5$ ), both when normalized per  
239 zooxanthellae cell (Fig. 5) and per skeletal surface area ( $0.24 \pm 0.02$  and  $0.34 \pm 0.05 \mu\text{mol P}$   
240  $\text{cm}^{-2}$  for zooxanthellae from the control and  $2.5 \mu\text{mol L}^{-1}$  P treatment respectively). The  
241 phosphorus content of the zooxanthellae from the lowest phosphate enrichment and the  
242 control were not significantly different (Mann-Whitney test,  $p = 0.51$ ; Fig. 5). Zooxanthellae  
243 levels were also not significantly different between the treatments ( $3.80 \pm 0.27 \times 10^6$  cells  $\text{cm}^{-2}$ ;  
244 Kruskal Wallis tests,  $p = 0.30$ ,  $df = 2$ ).

245

## 246 **Discussion**

247 This paper presents the kinetics of phosphate uptake by the entire symbiotic  
248 association and the isolated zooxanthellae of the scleractinian coral *S. pistillata*. Such  
249 comparison of the two systems under the same experimental conditions brings new insights  
250 into the functioning of the symbiosis, and confirmed the previous observations made  
251 separately on corals (D'Elia 1977) and isolated zooxanthellae (Deane and O'Brien 1981;  
252 Jackson and Yellowlees 1990), that phosphate uptake displays saturation kinetics, indicating

253 that it is mediated by carriers. Our results however showed that carriers were present both in  
254 the animal and the algal fractions, since uptake kinetics followed uniphasic saturation curves  
255 in both compartments (Michaelis-Menten shape). This result is different from the first  
256 conclusions of D'Elia (1977), who suggested that active transport uptake sites were located  
257 only in the zooxanthellae, with a diffusion of phosphate through coral tissue. However, the  
258 fact 1) that phosphate is an anion at seawater and physiological pH, 2) that membrane  
259 potential across the plasma membrane, the symbiosome, and the zooxanthellae membrane is  
260 negative, and that 3) phosphate concentrations in animal cells typically range from 1 to 10  
261 mmol L<sup>-1</sup> (Lehninger 1985; Miller and Yellowlees 1989), does not support a diffusion of  
262 phosphate through the animal membranes or a passive transport along an electrochemical  
263 gradient. Carrier-mediated uptake of phosphate should therefore be active both in animal cells  
264 and FIZ. Active uptake implies the presence of pumps or ionic exchange transporters allowing  
265 organisms to retrieve phosphate ions from the external medium and transport them across  
266 biological membranes. The nature of the transporters remains to be characterized, using either  
267 pharmacological or molecular approaches. If we refer to the observations made in marine  
268 invertebrates (Gerencser et al. 2002), an active sodium-stimulated phosphate transport across  
269 coral membranes might be involved.

270 Our study also showed that transporters in the animal and the algae presented different  
271 affinities for phosphate, since the half-saturation constants  $K$  of the two systems were  
272 significantly different. The animal compartment indeed presented a lower affinity than the  
273 isolated zooxanthellae ( $K = 1.08 \pm 0.42$  vs.  $0.57 \pm 0.18 \mu\text{mol L}^{-1}$ ). Though phosphate  
274 concentrations may be in the millimolar range in coral and zooxanthellae cells, concentrations  
275 adjacent to the zooxanthellae in hospite, (i.e., in perisymbiotic vesicles), are not known. If we  
276 draw an analogy with most transporter systems, which usually display half-saturation constant  
277 values close to substrate concentrations in their environment (Wright 1982), the very low  $K$  of

278 isolated zooxanthellae may indicate that they are subjected to micromolar phosphate levels.  
279 There might therefore be a limitation of phosphate fluxes toward zooxanthellae by the host  
280 via the activity of phosphate carriers present on perisymbiotic membranes; but this  
281 speculative suggestion needs to be verified, as FIZ may behave differently than in hospite  
282 zooxanthellae.

283         The half-saturation constant calculated in the present study for the coral colony ( $1.08 \pm$   
284  $0.42 \mu\text{mol L}^{-1}$ ) is slightly higher, but in the same range, as those measured for 3 other  
285 scleractinian corals ( $0.38 \mu\text{mol L}^{-1}$ ; D'Elia 1977). The  $V_{\text{max}}$  is similar in both studies, equal to  
286  $0.29$  (D'Elia 1977) and  $0.38$  (this study)  $\mu\text{mol P mg Chl}^{-1} \text{h}^{-1}$ . Overall, these parameters  
287 demonstrate that corals are indeed well adapted for a rapid acquisition of phosphate. This sub-  
288 optimal uptake rate measured at phosphate concentrations typically occurring in tropical  
289 oligotrophic waters ( $V_0(0,5 \mu\text{mol L}^{-1}) < V_{\text{max}}$ ) can be linked to the hypothesis expressed by  
290 Jackson and Yellowlees (1990), who suggested that zooxanthellae are adapted for rapid  
291 uptake in the presence of transient phosphate enrichment.

292         The  $K$  obtained with the FIZ ( $0.57 \pm 0.18 \mu\text{mol L}^{-1}$ ) is 10 times higher than the values  
293 measured by Deane and O'Brien (1981) on cultured zooxanthellae, but also 30 times lower  
294 than the value obtained by Jackson and Yellowlees (1990) with zooxanthellae freshly isolated  
295 from the coral *Acropora formosa* (Dana 1846). This large discrepancy can be due to different  
296 phosphorus pools in the zooxanthellae considered. Indeed, several authors already observed a  
297 decrease in uptake rates of isolated or cultured zooxanthellae following exposure to phosphate  
298 (Deane and O'Brien 1981; Jackson and Yellowlees 1990; R. Kelty unpubl.). Such  
299 'acclimatization' to phosphate was also observed in the entire coral colony (this work ; Fig. 4)  
300 and in two other symbioses, such as in the clam *T. gigas* (Belda and Yellowlees 1995) or the  
301 jellyfish *C. xamachana* (Todd et al. 2006). Those results suggest the adaptation of uptake  
302 rates to the repletion status of phosphorus pools inside the symbiotic association.

303           Measurements of the phosphorus content of nubbins after 8 weeks of exposure to  
304 phosphate showed that only zooxanthellae were phosphorus-enriched at the end of the  
305 experiment, thus demonstrating that zooxanthellae represent the only sink of inorganic  
306 phosphorus within the symbiotic association, while animal cells present a homeostatic  
307 regulation of phosphorus levels. Similar results were reported for dissolved inorganic forms  
308 of nitrogen in *S. pistillata*, with a rapid translocation of nitrate and ammonium toward the  
309 zooxanthellate fraction. This seems logical when we consider that animals in general, and  
310 non-symbiotic cnidarians in particular, are not able to effect net uptake of external inorganic  
311 nutrients (Muller-Parker et al. 1990) and generally excrete those nutrients into the  
312 surrounding water (D'Elia 1977; Muscatine and D'Elia 1978; Muscatine et al. 1979). In  
313 contrast, symbiotic cnidarians, owing to the presence of algae in their tissue, are able to take  
314 up, retain, and incorporate inorganic nutrients from seawater at micromolar or even  
315 nanomolar concentrations.

316           This paper also highlights the effects of two other parameters on phosphate uptake,  
317 light and particulate feeding. The velocity of phosphate absorption by *S. pistillata* nubbins  
318 and its FIZ increased in the light. Except two contrary results obtained by Muller-Parker et al.  
319 (1990) on the starved sea anemone *A. pallida* and Deane and O'Brien (1981) on cultured and  
320 freshly isolated zooxanthellae, light-enhancement is a general trend observed in several  
321 phytoplankton species (Reshkin and Knauer 1979; Nalewajko and Lee 1983), in  
322 zooxanthellae isolated from corals (Jackson and Yellowlees 1990), as well as in hermatypic  
323 corals (D'Elia 1977; Sorokin 1992). Stimulation of phosphate absorption by light in corals and  
324 FIZ confirms the major role of zooxanthellae in this process, via their photosynthetic activity.  
325 Concerning the feeding effect, even though this effect on phosphate uptake has not been  
326 directly tested in the present study, the comparison of results obtained for nubbins used during  
327 phosphate uptake experiments and those of control corals from the 8-week enrichment



328 indirectly demonstrates the influence of particulate feeding (zooplankton) on phosphate  
329 uptake by *S. pistillata*: the first corals were lightly fed (once a week) with *A. salina* nauplii,  
330 while the latter were starved during the 8 weeks of enrichment. Phosphate uptake rates were  
331 4.6 times higher for starved corals, indicating that those corals became phosphorus-depleted  
332 over 8 weeks. A similar trend was observed in another cnidarian, *A. pallida* (Muller-Parker et  
333 al. 1990), with uptake rates 10-50 times higher in 1 month-starved anemones than in daily-fed  
334 animals. Effects of light and particulate feeding underline the fact that phosphate acquisition  
335 from the surrounding seawater is controlled both by zooxanthellae and by their coral hosts.  
336 One remaining question is to know if organic forms of phosphorus are stocked in the tissue of  
337 the coral animal, or, like inorganic phosphorus, are transferred to the zooxanthellae.

338         Even if phosphate represents an important source of phosphorus for zooxanthellae, it  
339 is not the only form available in coral reefs. Several studies have shown the importance of  
340 organic phosphorus for the coral hosts (Johannes et al. 1970) and their symbionts (Jackson et  
341 al. 1989; Annis and Cook 2002). Organic phosphorus can be taken up by corals via particulate  
342 feeding (zooplankton and other forms of particulate organic matter; Johannes et al. 1970) or  
343 via the activity of excreted alkaline phosphatases (APA; Annis and Cook 2002) or acid  
344 phosphatases (Jackson et al. 1989), for which the existence was shown in isolated  
345 zooxanthellae. In order to better understand how the host can regulate its symbionts'  
346 phosphate uptake, and whether or not hosts can be responsible of a phosphorus limitation for  
347 their zooxanthellae, it seems important to further this study by a quantification of the  
348 importance of organic phosphorus uptake as compared to that of phosphate.

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412

413 **Figure legends**

414

415 **Figure 1:** Phosphate uptake ( $\mu\text{mol P mg total Chl}^{-1}$ ) for *S. pistillata* during a 7 h incubation in  
416  $3.0 \mu\text{mol L}^{-1}$  phosphate. The curve presents a linear portion between 0 and 90 min (white  
417 dots). Each point represents the mean  $\pm$  SE of 5 nubbins.

418

419 **Figure 2:** Concentration-dependent uptake of phosphate ( $10^{-15} \text{ mol P zoox}^{-1} \text{ h}^{-1}$ ) in freshly  
420 isolated zooxanthellae (FIZ, light grey dots) and whole *S. pistillata* nubbins (dark grey  
421 squares). Each point represents the mean  $\pm$  SE of 5 nubbins or 3 FIZ. Corresponding kinetics  
422 parameters are indicated in the table below.  $V_{\text{max}}$  is the maximum phosphate uptake rate ( $10^{-15}$   
423  $\text{mol P zoox}^{-1} \text{ h}^{-1}$ ) and K the solute concentration at which phosphate uptake is half-maximal  
424 ( $\mu\text{mol L}^{-1}$  phosphate).

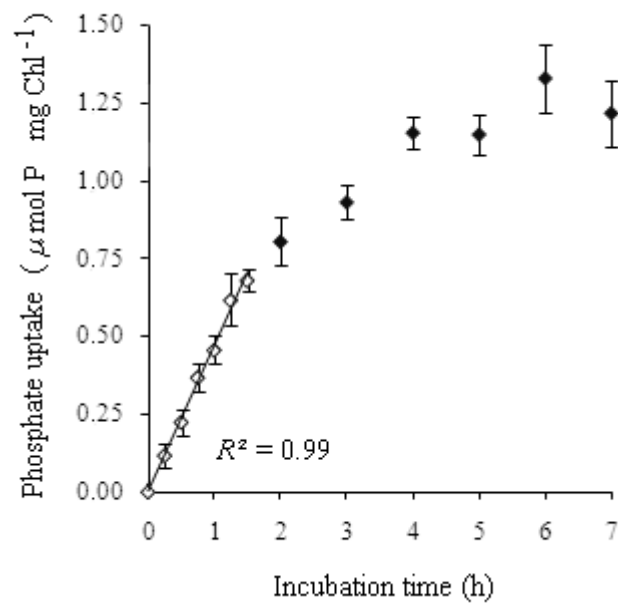
425

426 **Figure 3:** Light-dependent uptake of phosphate ( $\mu\text{mol P mg total Chl}^{-1}$ ) in *S. pistillata*  
427 nubbins and FIZ incubated in  $3.0 \mu\text{mol L}^{-1}$  phosphate. Each bar represents the mean  $\pm$  SE of 5  
428 nubbins or 3 FIZ. Light was measured using a Li-Cor instrument and is expressed as  $\mu\text{mol m}^{-2}$   
429  $\text{s}^{-1}$  PAR.

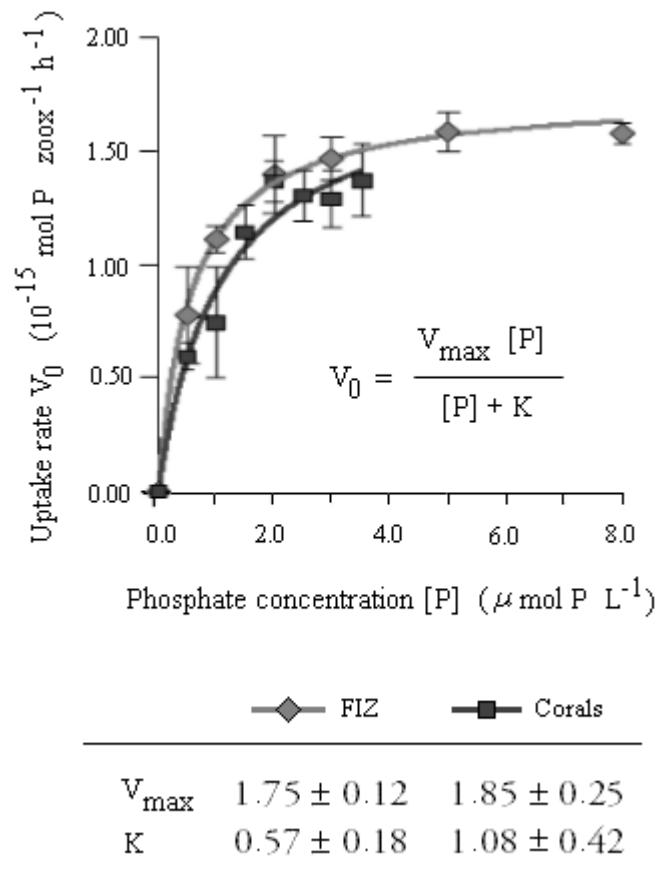
430

431 **Figure 4:** Effect of the phosphate past history on initial phosphate uptake rates ( $\mu\text{mol P mg}$   
432  $\text{total Chl}^{-1}$ ) in *S. pistillata*. Incubations were performed with  $3.0 \mu\text{mol L}^{-1}$  phosphate after a  
433 long-term acclimatization, performed as a continuous phosphate enrichment of 0, 0.5, or 2.5  
434  $\mu\text{mol L}^{-1}$  phosphate (Control, 0.5 P, and 2.5 P, respectively) during 8 weeks. Each bar  
435 represents the mean  $\pm$  SE of 5 nubbins.

436 **Figure 5:** Phosphorus content per zooxanthellae of *S. pistillata* nubbins maintained during 8  
437 weeks under continuous phosphate enrichments of 0, 0.5, or 2.5  $\mu\text{mol L}^{-1}$  (Control, 0.5 P, and  
438 2.5 P, respectively). Each bar represents the mean  $\pm$  SE of 3 nubbins.

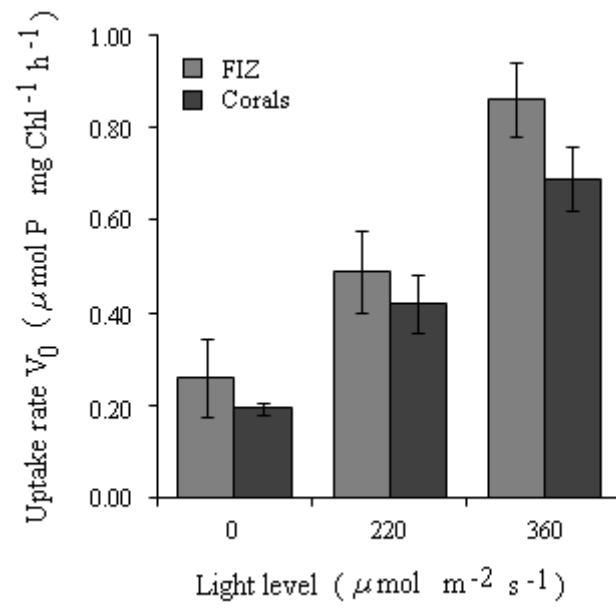


**Fig. 1**

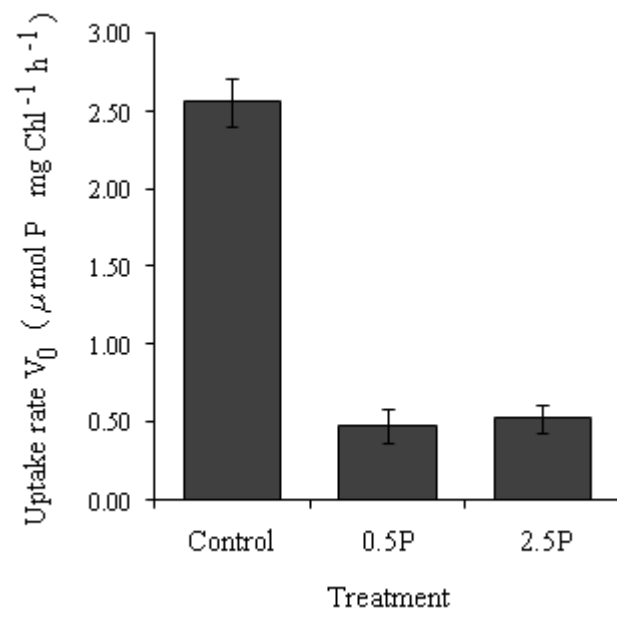


**Fig. 2**

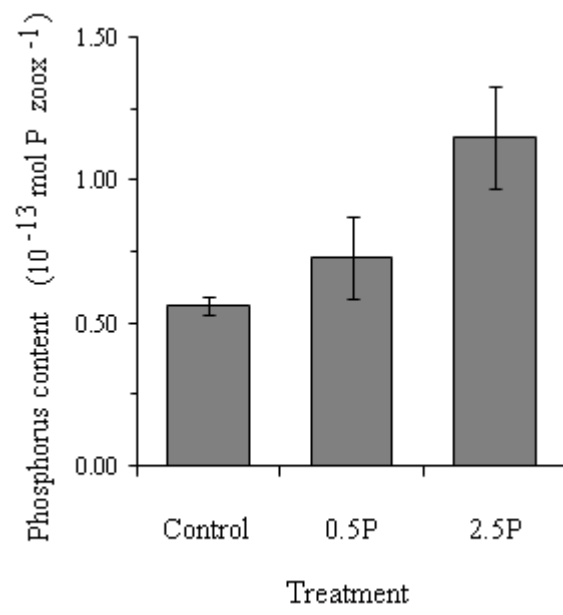




**Fig. 3**



**Fig. 4**



**Fig. 5**