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RESEARCH ARTICLE

High phosphate uptake requirements of the scleractinian coral *Stylophora pistillata*

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SUMMARY

Several untested aspects of the regulation of inorganic nutrient uptake were examined using nutrient depletion experiments with the symbiotic coral *Stylophora pistillata*. The total inhibition of phosphate uptake in artificial seawater lacking sodium indicates the involvement of a sodium/phosphate symporter for the uptake of phosphate across host membranes. Addition of ammonium or nitrate (up to 6.0 μmol l^{-1}) did not enhance saturated phosphate uptake rates, thus indicating that corals, or their symbiotic algae, were not, or not sufficiently, nitrogen limited to modify their phosphate needs. Conversely, the saturated uptake rate of ammonium increased by 2.5-fold in the presence of 3.0 μmol l^{-1} of phosphate, thus indicating that the corals or their symbionts were lacking intracellular phosphate to take advantage of the inorganic nitrogen compounds dissolved in their surrounding medium. Overall, these results highlight some greater limitation in phosphate rather than in nitrogen. Finally, the rate of phosphate uptake decreased with particulate feeding of the host (organic phosphate source). Indeed, corals that were fed 1 and 3 days before the uptake experiment took up phosphate 42 and 19% slower, respectively, than corals that were fed 21 days before. This result provides additional evidence of phosphate limitation in *S. pistillata*. This study therefore brings new insights into the relationships between nutrients and symbiotic corals, and may provide a rapid and effective tool to investigate which nutrient is the most limiting for coral metabolism.

Key words: coral, nutrients, uptake, cotransporter, limitation.

INTRODUCTION

Phosphorus and nitrogen compounds are key nutrients for marine organisms (Benitez-Nelson, 2000; Elser et al., 2007) because they enter into the composition of many biological molecules (e.g. DNA, RNA, proteins and phospholipids) and have a role in biochemical mechanisms. In the oligotrophic surface reef waters, phosphate and inorganic forms of nitrogen (ammonium and nitrate) display a characteristic depletion, with concentrations often well below 0.5 μmol l^{-1} (Atkinson, 1987; Atkinson et al., 1995; Furnas, 1991). Zooxanthellae, the dinoflagellates living in symbiosis with corals, are known to take up from their host and concentrate, through active transport, dissolved nutrients, which are then transformed into organic molecules (Badgley et al., 2006; Bythell, 1990; D'Elia and Cook, 1988; D'Elia et al., 1983; Domotor and D'Elia, 1984; Godinot et al., 2009; Muscatine and D'Elia, 1978) and transferred back to the host for its metabolism (Muscatine and Porter, 1977). Such nutrient recycling within the symbiosis is often used to explain the success of corals in nutrient-poor environments. Knowledge of nutrient fluxes, and of their regulation, is therefore crucial for a better understanding of coral reef functioning. Although a lot of studies have investigated nutrient uptake by diverse scleractinian symbiotic coral species, most of them have focused on compounds of nitrogen rather than phosphate, and many questions remain unsolved.

The present study focused on three main questions regarding phosphate uptake that were untested in previous studies. First, even though phosphate uptake through the animal membranes is known to be mediated *via* active transport (phosphate is a charged species at seawater and physiological pHs and its uptake displays saturation kinetics in coral holobionts) (D'Elia, 1977; Godinot et al., 2009), the transporter involved at the host membrane has not been characterized for reef corals. Although phosphate uptake occurs *via* ATP-binding cassette transporters from the PhoT family in prokaryotes (Higgins, 1992) and *via* proton–phosphate cotransporters from the Pht family in plants (Raghothama, 1999; Smith et al., 2003), an active sodium-dependent phosphate co-transporter is more commonly found in animals (Markovich, 2010; Virkki et al., 2007), such as in the sea urchin *Strongylocentrotus purpuratus* (Schneider, 1985) or in the marine mollusc *Aplysia californica* (Gerencser et al., 2002). This sodium-dependent transport was demonstrated by a lack of phosphate uptake in sodium-free seawater (Gerencser et al., 2002; Schneider, 1985). Using similar techniques, the first goal of this study was to assess whether such a transporter was involved in the uptake of phosphate by the symbiotic coral *Stylophora pistillata*. The second goal of this study was to test whether phosphate uptake decreases with particulate feeding, as previously observed for this nutrient in sea anemones (Muller-Parker et al., 1990) and for ammonium and nitrate in corals (D'Elia and Cook, 1988; Grover et al., 2002). The third aim of this study was to test whether ammonium or nitrate enrichment could enhance the uptake of phosphate. This mechanism has never been investigated before, but might have great implications for coral metabolism if a limitation by one or both nutrients exists in the coral–zooxanthellae symbiosis, as was hypothesized in several studies (Falkowski et al., 1993; Miller and Yellowlees, 1989; Muscatine et al., 1989; Rees, 1991). Therefore, if the uptake mechanisms of phosphate and nitrogen compounds are competing for limited energy resources, it could lead to the preferential uptake of one of the two nutrients, as in some phytoplankton species (Terry,

1982a; Terry, 1982b). If, rather, there is a co-limitation between the two nutrients, it could lead to greater uptake rates of phosphate when nitrogen is fully available, as has also been shown in other phytoplankton studies (e.g. Suttle and Harrison, 1988).

If phosphate uptake can be limited by availability of nitrogen, the opposite is also true. However, the effect of phosphate limitation on ammonium or nitrate uptake has, to the best of our knowledge, never been investigated in corals. On the contrary, it is well-known, for example, that nitrate uptake is generally inhibited in the presence of ammonium (Badgley et al., 2006; Domotor and D'Elia, 1984; Grover et al., 2003), either through a repression of the nitrate reductase (Berges and Harrison, 1995; Guerrero et al., 1981; Syrett, 1981; Vergara et al., 1998) or a competition of ammonium and nitrate uptake mechanisms for energy resources, leading to the preferential uptake of ammonium (Terry, 1982a; Terry, 1982b). Therefore, we tested two additional questions. As phosphate has been suggested to be a limiting nutrient for zooxanthellae (Miller and Yellowlees, 1989), our fourth aim was to test whether the lack of phosphate is critical for ammonium uptake. Finally, we also tested whether the uptake of nitrate, the other form of inorganic nitrogen available for corals, was limited by phosphate availability in *S. pistillata*, as the two studies performed to date on *S. pistillata* have found no (Muscatine et al., 1984), or a very low (Grover et al., 2003), nitrate uptake rate by this species.

Answering these questions could give a better understanding of the regulation of nutrient uptake by corals. Such data could be used in future models of nutrient cycles within the coral–zooxanthellae symbiosis.

MATERIALS AND METHODS

Coral culture conditions

Colonies of the zooxanthellate coral *Stylophora pistillata* Esper 1797 were obtained from the Red Sea and maintained in open flow aquaria under controlled conditions (26°C, salinity of 38). Light was provided by hydrargyrum quartz iodide lights at a photosynthetic active radiation level of 220 μmol m^{-2} s^{-1} (measured using a spherical quantum sensor; LiCor LI-193, Lincoln, NE, USA), with a 12 h:12 h dark:light cycle. Two months before the experiments began, the apical branches of 10 parent colonies were cut using pliers to generate 200 nubbins (2.5±1.0 cm long and 0.6±0.3 cm in diameter). Nubbins were attached to nylon wires and suspended in aquaria until tissue fully covered the skeleton. They were lightly fed (once a week) with *Artemia salina* (Linnaeus 1758) nauplii during this maintenance period and were starved for 4 days before each experiment (unless otherwise specified). Seawater in the aquaria contained trace concentrations of phosphate (<0.05 μmol l^{-1}), nitrate (<0.4 μmol l^{-1}) and ammonium (<0.5 μmol l^{-1}), thus corals were considered to be maintained under oligotrophic conditions concerning inorganic nutrients.

Kinetic characteristics of nutrient transport in corals

As a preliminary step, we first examined the uptake kinetics of each inorganic nutrient in order to select the concentration that gave a maximal uptake rate of phosphate, nitrate or ammonium, which was used in subsequent uptake interaction experiments. For this purpose, we performed a series of three experiments, in which the depletion of phosphate, ammonium or nitrate was followed over time in stirred beakers enriched with the corresponding nutrient. Uptake rates were measured with six different concentrations of phosphate (0, 0.5, 1.0, 2.0, 3.0 and 4.0 μmol l^{-1}), ammonium (0, 0.5, 1.0, 2.0, 4.0 and 6.5 μmol l^{-1}) or nitrate (0, 0.5, 1.5, 3.5, 5.0 and 9.0 μmol l^{-1}). Five nubbins were used for each concentration tested. Uptake rates were performed as described in Godinot et al. (Godinot et al., 2009), using stock solutions of KH_2PO_4, NH_4Cl or KNO_3 (10 mmol l^{-1}) to reach the desired initial nutrient concentration, with water samplings every 15 min for 90 min. During each depletion experiment, nutrient concentrations decreased linearly over time for at least 60 min, as shown in Godinot et al. for phosphate (Godinot et al., 2009). Ammonium samples were treated with 1 ml of combined ammonium reagent immediately after sampling, and were kept in the dark for 3 h before manual determination by spectrofluorimetry according to Holmes et al. (Holmes et al., 1999). Phosphate and nitrate samples were frozen right away, and were measured no longer than 1 week after sampling. Phosphate concentrations were determined using the ascorbic acid method (Murphy and Riley, 1962). Nitrate concentrations were measured using an autoanalyzer (Axflow, Plaisir, France) and according to Aminot and Kérouel (Aminot and Kérouel, 2007). Initial uptake rates (V_0) were calculated from the quantity taken up by each nubbin in 60 min. Uptake rates were normalized to the total chlorophyll (chl) content, zooxanthellae concentration and surface area of the nubbins (see below).

Phosphate uptake: interaction with sodium, particulate food and nitrogen

In the second experiment, we characterized the phosphate transporter involved and assessed the effect of feeding and inorganic nitrogen enrichment on the uptake of phosphate.

Effect of sodium on phosphate uptake

To test for the presence of an active sodium-phosphate symporter, we incubated corals in sodium-free artificial seawater (ASW) containing 3.0 μmol l^{-1} P (the concentration giving the maximal uptake rate in the kinetic curves). Such an ASW protocol has often been used to test sodium-dependent transporters (Gerencser et al., 2002; Schneider, 1985), using various sodium osmotic equivalents in many models, including choline in scleractinian corals (Al-Moghrabi et al., 1993; Al-Moghrabi et al., 1996; Allemand et al., 1984). Phosphate uptake was therefore measured using ASW in which sodium had been replaced by its osmotic equivalent choline (0 Na ASW). Controls were performed with ASW in which sodium was present (control ASW). ASW was prepared freshly, following the simplified synthetic seawater recipe of Dickson et al. (Dickson et al., 2007), modified to achieve a salinity of 38.5 and to replace sodium with choline (using choline chloride and choline bicarbonate). The following final concentrations were achieved: 600 mmol kg^{-1} Cl^-, 31 mmol kg^{-1} SO_4^{2-}, 58 mmol kg^{-1} Mg^{2+}, 11 mmol kg^{-1} Ca^{2+}, 11 mmol kg^{-1} K^+, 2 mmol kg^{-1} HCO_3^- and 511 mmol kg^{-1} of either choline$^+$ or Na^+. Osmolarity was 1122±10 mOsm l^{-1}, alkalinity was 2233±16 mequiv. kg^{-1} and pH was 8.05±0.05. Coral coelenterons were rinsed during a 20 min acclimation in ASW before experiments started, and phosphate uptake rate was measured on five nubbins per ASW treatment (control and 0 Na ASW) under the same conditions as before, with water samplings every 15 min for 60 min. Choline chloride and choline bicarbonate have been widely used to prepare sodium-free seawater (e.g. Baker et al., 1969; Ettensohn et al., 2004; Hodgkin and Katz, 1949; Lee, 1984) and, as was observed previously in experiments involving corals (D.A., personal observation) (Al-Moghrabi et al., 1993; Al-Moghrabi et al., 1996), nubbins had their polyps fully expanded during the entire incubation period and mucus production was minimal.

Effect of feeding on phosphate uptake

To evaluate the impact of heterotrophy (organic phosphate supply) on phosphate uptake, phosphate uptake rates were measured over

2 h in experiments on five corals fed *A. salina* nauplii 1, 3 or 21 days before. Phosphate concentration was set to 3.0 μmol l^{-1} P, the concentration giving maximal uptake rates in our kinetic curves. Rates were measured under the same conditions as described before.

Effect of inorganic nitrogen enrichment on phosphate uptake

Results from the kinetics experiments were used to assess uptake rates of phosphate in the presence of ammonium or nitrate. The initial concentration of phosphate was chosen in order to match the maximal uptake rate (V_{max}) measured in the kinetics experiments, i.e. 3.0 μmol l^{-1}. Concentrations of ammonium or nitrate were chosen so as to have: (1) a low concentration close to levels relevant in the field, (2) a concentration representing eutrophic conditions in the field and (3) a high concentration (roughly matching V_{max}). A control was performed in which phosphate uptake was examined alone, without nitrogen compound enrichments. Thus, uptake rates of phosphate (3.0 μmol l^{-1}) were measured for five nubbins in the presence of 0, 0.5, 1.0 and 4.0 μmol l^{-1} ammonium or 0, 0.5, 2.5 and 6.0 μmol l^{-1} nitrate, under the same conditions as described above. One additional sample was taken at the beginning of each sampling period in order to verify the enrichment performed with the added nitrogen.

Inorganic nitrogen uptake: effect of phosphate enrichment

In this third set of experiments, results from the uptake kinetics were used to assess the impact of three phosphate enrichments on ammonium and nitrate uptake rates. Initial concentrations of nitrogen compounds were chosen in order to match the V_{max} measured in the previous experiments, i.e. 4.0 μmol l^{-1} for ammonium and 6.0 μmol l^{-1} for nitrate. Concentrations of phosphate were chosen using the same rationale as for testing the nitrogen impact on phosphate uptake. Controls were performed by measuring ammonium or nitrate uptake without phosphate enrichment. Thus, uptake rates of ammonium (4.0 μmol l^{-1}) or nitrate (6.0 μmol l^{-1}) were both measured in the presence of 0, 0.1, 0.5 and 3.0 μmol l^{-1} phosphate. Five nubbins were used for each experiment, under the same conditions as described above. One additional sample was taken at the beginning of each sampling period in order to verify the enrichment performed with the added nutrient.

Measurements of physiological parameters

At the end of the experiments, corals were frozen for subsequent normalization of the uptake rates. Data were normalized to the total chlorophyll and zooxanthellae content [using the methods described in Godinot et al. (Godinot et al., 2009)] and to the surface area of the nubbins [using the method described in Stimson and Kinzie (Stimson and Kinzie, 1991)].

Statistical analyses

Concentration-dependent nutrient uptake rates were studied using GraphPad Prism (version 5.0; San Diego, CA, USA) to obtain best-fit values for K (see Eqn 1) and V_{max}. StatView (version 5.0; Cary, NC, USA) was used to perform an unpaired t-test to test the sodium dependence of phosphate uptake, and to perform one-way ANOVAs, followed by Fisher's protected least significant difference (PLSD) tests, to identify the effect of feeding on phosphate uptake. Linear regressions were performed with GraphPad Prism to examine nutrient uptake under nutrient enrichment, and Fisher's PLSD tests were used to determine which uptake rates were significantly impacted by the presence of another nutrient (i.e. slope is significantly different from zero).

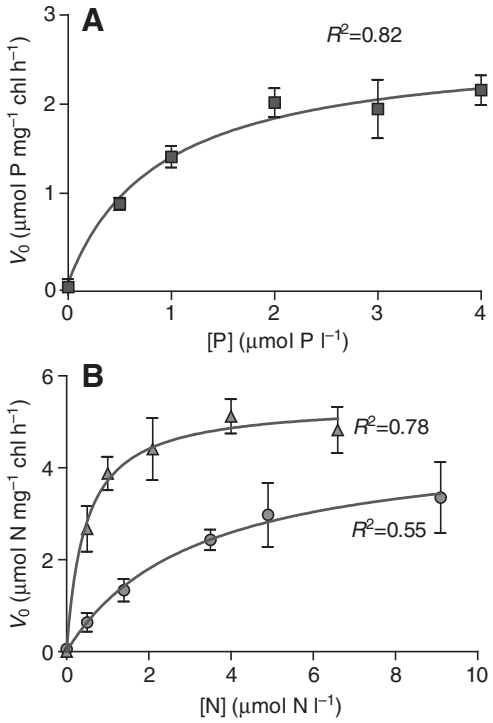

Fig. 1. Concentration-dependent uptake (V_0) of (A) phosphate (squares) and (B) ammonium (triangles) and nitrate (circles) in *Stylophora pistillata*.

RESULTS

Kinetic characteristics of nutrient transport in corals

For each nutrient when examined alone, initial uptake rates (V_0) *versus* nutrient concentration conformed to the Monod kinetics, based upon the Michaelis–Menten equation for enzymes (Fig. 1):

$$V_0 = \frac{V_{max}[X]}{[X] + K}, \quad (1)$$

where [X] is the initial concentration of either phosphate (P) or nitrogen (N, in the form of ammonium NH_4 or nitrate NO_3), K is the nutrient concentration at which uptake is half-optimal and V_{max} is the maximum uptake rate. For each nutrient considered, K corresponds to the inverse of the affinity of the carrier towards this nutrient. As a result, *S. pistillata* presented an affinity 2.0 and 6.8 times higher for ammonium than for phosphate or nitrate, respectively (Table 1). V_{max} was 1.7 to 2.0 times higher for nitrogen (for nitrate and ammonium, respectively) than for phosphate (Table 1).

Phosphate uptake: interaction with sodium, particulate food and nitrogen

In the control ASW, phosphate uptake rate (3.07±0.79 μmol P mg^{-1} chl h^{-1}) was in the same range as values

Table. 1. Calculated kinetics parameters for the uptake of PO_4, NH_4 and NO_3 by *Stylophora pistillata*

Nutrient	V_{max}	K
PO_4	2.69±0.30	0.94±0.32
NH_4	5.42±0.41	0.46±0.16
NO_3	4.63±1.17	3.14±1.97

Data are means ± s.e.m.
V_{max}, maximum uptake rate (μmol mg^{-1} chl h^{-1}); K, affinity constant (μmol l^{-1}).

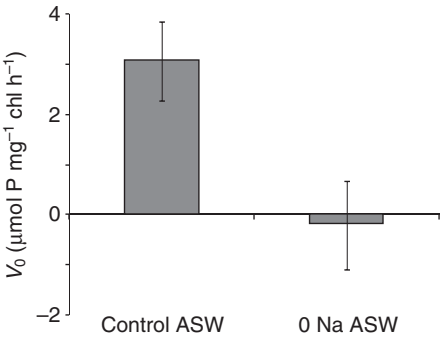

Fig. 2. Uptake of 3.0 µmol l^{-1} phosphate in artificial seawater (ASW) containing (control ASW) or lacking (0 Na ASW) sodium. In the latter case, sodium was replaced by choline. Data are mean ± s.e.m. uptake rates of five nubbins.

measured during the previous experiments in natural seawater, confirming that corals behaved normally in ASW (e.g. Figs 3 and 4). In 0 Na ASW, the phosphate uptake rate was close to zero (–0.20±1.46 µmol P mg^{-1} chl h^{-1}) and significantly different than in the presence of sodium (unpaired *t*-test, t=2.75, P=0.025; Fig. 2), suggesting strict sodium dependence.

The maximal uptake rate of phosphate was not significantly affected by any of the ammonium or nitrate enrichments tested (P=0.47 for ammonium, P=0.23 for nitrate; Fig. 3). Conversely, phosphate uptake decreased with proximity of feeding in time (Fisher's PLSD, F=8.232, P=0.006): corals that were fed 1 and 3 days before the uptake experiment took up phosphate 42 and 19% slower, respectively, than corals that were unfed for 21 days (Fig. 4).

Inorganic nitrogen uptake: effect of phosphate enrichment

The uptake rate of ammonium was significantly increased in the presence of 0.5 and 3.0 µmol l^{-1} phosphate (r^2=0.76, F=57.04, P<0.0001; Fisher's PLSD, both P<0.05; Fig. 5A). Linear regression followed by Fisher's PLSD revealed that, overall, nitrate uptake rate was not impacted by phosphate availability (P=0.93; Fig. 5B), although an unpaired *t*-test revealed that nitrate uptake was significantly enhanced by a low phosphate enrichment (0.5 µmol l^{-1}; unpaired *t*-test, t=–2.45, P=0.049).

DISCUSSION

First, this study tested the involvement of a sodium/phosphate symporter in the uptake of phosphate across the host membrane, an uptake that has previously been demonstrated to be active in corals (D'Elia, 1977; Godinot et al., 2009) but for which the transporter is unknown. Such cotransport is one of the most common in the animal kingdom. It has indeed been found in vertebrates, for which three families of Na/P_i symporters have been characterized (Markovich, 2010; Virkki et al., 2007), in marine invertebrates, such as the mollusc *Aplysia californica* (Gerencser et al., 2002) and the sea urchin *Strongylocentrotus purpuratus* (Schneider, 1985), and in the phycomycete *Thraustochytrium roseum* (Siegenthaler et al., 1967; Siegenthaler et al., 1966) and the cyanobacteria *Anabaena* (Valiente and Avendano, 1993). Although the use of 0 Na ASW can theoretically lead to the inhibition of all sodium-dependent co-transports, which includes some amino acids, glucose, lactate and protons (Preston, 1993; Scott, 1987), and therefore alter the cell physiology over the course of long-term experiments, it is likely that during short-term incubations, as used in the present study (less than 2 h), the primary effect of the absence of sodium in the external medium is the direct *cis*-inhibition of substrate transport. Such a short period of time did not allow, for example, significant changes in sodium-independent processes in the scleractinian coral *Galaxea fascicularis*, such as the photosynthesis and respiration of zooxanthellae freshly isolated from that coral (Al-Moghrabi et al., 1996), or the sodium-independent component of valine uptake (Al-Moghrabi et al., 1993). We thus conclude that the total inhibition of phosphate uptake in 0 Na ASW is a strong evidence for the involvement of a sodium/phosphate symporter at the host membrane of the symbiotic coral *S. pistillata*.

The main objective of this study was to determine whether ammonium or nitrate availability limited phosphate uptake, or the contrary, whether phosphate availability limited ammonium and nitrate uptake in the scleractinian coral *S. pistillata*. Such a limitation would suggest that one nutrient is essential to the use of the other and is, therefore, lacking within the symbiosis. Under the experimental conditions of this study (no heterotrophic input, no light stress or temperature stress), ammonium and nitrate availability did not change the uptake rates of phosphate, suggesting that corals, or their symbiotic algae, were not, or not sufficiently, nitrogen limited to change their needs in phosphate. This result does not exclude that the growth of the zooxanthellae was nitrogen limited, as previously demonstrated (Falkowski et al., 1993; Muscatine et al., 1989), but suggests that the zooxanthellae did not need more nitrogen to use all of the phosphate available in the incubation medium (with low to high concentrations of nitrogen). Conversely, we observed enhanced uptake rates of ammonium with phosphate enrichment (Fig. 5), suggesting in this case that the corals or their symbionts were lacking sufficient intracellular phosphate to take advantage of the inorganic nitrogen compounds dissolved in their surrounding medium. Overall, these results highlight some greater limitation in phosphate rather than in nitrogen compounds, and are in

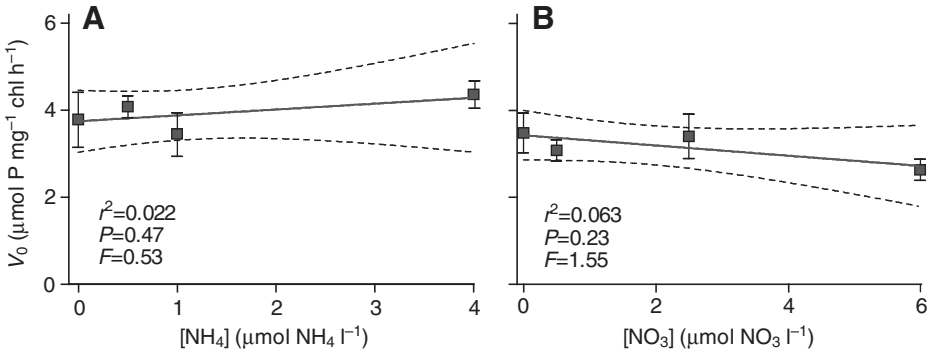

Fig. 3. Uptake rates of phosphate (3 µmol l^{-1}) with different enrichments of nitrogen: (A) nitrogen as ammonium, (B) nitrogen as nitrate. Best-fit linear regression lines (solid lines) are represented along with the 95% confidence intervals (dashed lines). Data are mean ± s.e.m. uptake rates of five nubbins.

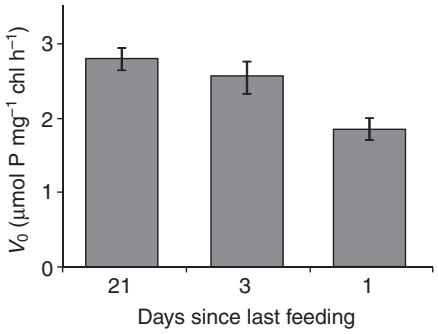

Fig. 4. Effect of heterotrophy on phosphate uptake by the coral *Stylophora pistillata*. Initial phosphate concentration was 3.0 µmol l^{-1} for each uptake measurement. Data are mean ± s.e.m. uptake rates of five nubbins.

agreement with (1) the high activity levels of the zooxanthellae alkaline phosphatase (Annis and Cook, 2002; Jackson et al., 1989) and (2) previous physiological data suggesting that algal growth within the symbiosis was more limited by phosphorus than by nitrogen (Jackson et al., 1989; Miller and Yellowlees, 1989).

The negative effect of feeding on inorganic phosphate uptake is additional evidence of phosphate limitation in the symbiotic association. When corals acquired organic phosphate from zooplankton, they lowered the uptake rates of phosphate compared with unfed corals because of a downregulation of phosphate uptake and/or a greater release of phosphate in the incubation medium. Both processes can occur in corals. Indeed, regulation of phosphate uptake was previously observed in cultured and isolated zooxanthellae, in which the activity of alkaline and acid phosphatases decreased in presence of dissolved organic phosphate in seawater (Annis and Cook, 2002; Jackson et al., 1989). Similarly, in the sea anemone *Aiptasia pallida*, phosphate uptake increased by 10 to 50 times in anemones starved for 1 month compared with those given a daily feeding (Muller-Parker et al., 1990). *Stylophora pistillata*, however, differs from *A. pallida* because it took up phosphate at a significant rate (1.84±0.14 µmol P mg^{-1} chl h^{-1} for a phosphate concentration of 3 µmol l^{-1}) even when it was heterotrophically starved just for 1 day. This suggests that *S. pistillata* requires a high concentration of phosphate to sustain its metabolism. Nonetheless, it is also possible that phosphate uptake rates decreased because of a release of phosphate. Indeed, D'Elia reported that symbiotic corals can release organic phosphate in the light, and both inorganic and organic phosphate in the dark (D'Elia, 1977). Although organic phosphate fluxes were not measured in the present study, and all rates reported were measured in the light, we cannot exclude the possibility that phosphate was released, or was released in greater quantities, in fed than in starved corals. This hypothesis remains to be investigated.

Finally, results obtained with the kinetics of nitrate uptake give insight into the capacity of *S. pistillata* to use this source of nitrogen for its metabolism. Despite its relatively high abundance in reef waters compared with ammonium (D'Elia et al., 1981; Lapointe and Clark, 1992), nitrate remains a much less significant source of nitrogen than ammonium for corals (D'Elia et al., 1983; Domotor and D'Elia, 1984; Grover et al., 2003; Wilkerson and Trench, 1986). Indeed, in the first study performed with zooxanthellae freshly isolated from field-collected corals (Muscatine et al., 1984), it was concluded that *S. pistillata* was not able to use nitrate as a nitrogen source because no activity of the nitrate reductase, which is the enzyme necessary for the assimilation of nitrate, could be detected in the samples. In a later study, Grover et al. (Grover et al., 2003) found that nitrate uptake rates of cultured *S. pistillata* nubbins were significant (0.1 and 0.4 nmol N cm^{-2} h^{-1} for 0.9 and 3.9 µmol l^{-1} NO_3 in seawater, respectively), although much lower (10 to 50 times lower) than those measured in the present study (e.g. 2.8 and 13 nmol N cm^{-2} h^{-1} for 0.5 and 3.5 µmol l^{-1} NO_3 in seawater, respectively) and from other coral species (from 2.5 to 20 nmol N cm^{-2} h^{-1} for NO_3 concentrations of 0.5 to 4 µmol l^{-1}) (reviewed in Badgley et al., 2006). Because the uptake of nitrate is clearly inhibited by the presence of ammonium in phytoplankton (Berges and Harrison, 1995; Guerrero et al., 1981; Syrett, 1981; Vergara et al., 1998) and corals (Grover et al., 2003), the discrepancies among the results might be linked to the ammonium supply of the colonies (which was relatively low in the present study, <0.5 µmol l^{-1}), as well as to the ammonium content of the cells, although this point remains to be further investigated. The discrepancies may also be linked to the availability of phosphate in the seawater and in the coral cells, if nitrate uptake is phosphate limited. Contrary to uptake of ammonium, nitrate uptake was not enhanced by a 3.0 µmol l^{-1} phosphate addition. However, in the presence of a lower phosphate concentration (0.5 µmol l^{-1}), nitrate uptake significantly increased, suggesting that nitrate uptake was not totally independent of phosphate presence. This increase in nitrate uptake at only the lowest phosphate concentrations needs more investigation.

In conclusion, measurements of phosphate or nitrogen compounds uptake in presence/absence of the other nutrient has proven to be a rapid and effective tool to investigate which of the two nutrients is the most limiting for coral metabolism. Such studies have never been performed with corals, but could be used in the field, perhaps in addition to alkaline phosphatase activity measurements (Annis

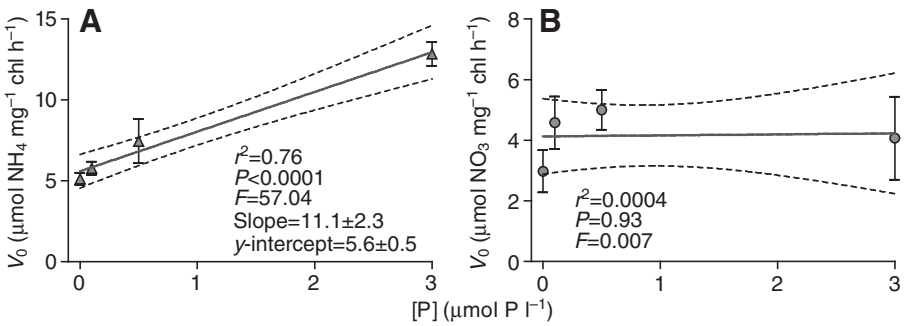

Fig. 5. Uptake rates of nitrogen with different enrichments of phosphate: (A) uptake of ammonium (4 µmol l^{-1}) in the presence of phosphate, and (B) uptake of nitrate (6 µmol l^{-1}) in the presence of phosphate. Best-fit linear regression lines (solid lines) are represented along with the 95% confidence intervals (dashed lines). Data are mean ± s.e.m. uptake rates of five nubbins.

and Cook, 2002), to assess the nutrient deficiency of corals in the natural environment. Results obtained here also highlight a regulation of the phosphate internal pools according to the trophic status of the colonies, or certainly, but this remains to be tested, by the external concentrations of organic phosphate. Finally, phosphate seems to be taken up *via* a sodium–phosphate transporter, as in the majority of animals, and it was demonstrated that *S. pistillata* can use nitrate at the same rate as some other coral species. The conditions for the uptake of nitrate by *S. pistillata*, or the lack thereof, remain to be determined.

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