Phosphate uptake by symbiotic zooxanthellae is dependent upon host control and internal phosphorus pools in the tropical coral *Stylophora pistillata*

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1 Introduction

Phosphorus is a key nutrient in marine environments because it enters into the composition of many biological molecules. It occurs under a wide variety of forms, but the fraction chiefly used by autotrophs mostly consists in dissolved orthophosphate (P_i). Coral reefs thrive in oligotrophic tropical seas, with P_i levels usually well < 0.5 μM. As such, P_i is a limiting nutrient for the production of corals (①), and has to be efficiently absorbed.

Corals harbor endosymbiotic algae, called zooxanthellae, which actively take up dissolved nutrients (②). The unique study performed 30 years ago by d'Elia (1977) on specific coral species showed that P_i uptake was light sensitive, and suggested an active transport, but could not conclude as to the location of P_i uptake sites and pools (②).

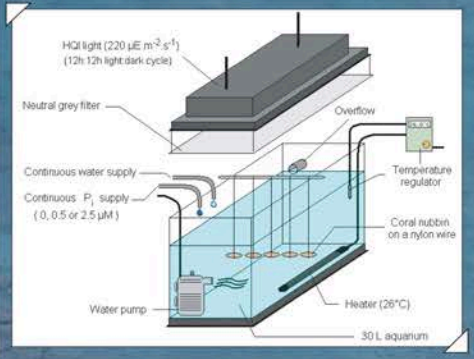
Our purpose was to precise current knowledge on how P_i is obtained from the water by corals and the respective roles of the host and the symbiont in this uptake.

2 Methods

Nubbins of the zooxanthellate coral *S. pistillata* (►) were maintained under controlled conditions and were lightly fed (*Artemia salina* nauplii, once a week).

Uptake was studied with a series of 3 experiments, in which the depletion of P_i was followed over 90 min in P_i-enriched beakers, for 5 replicates:

- concentration dependence of P_i uptake in freshly isolated zooxanthellae (FIZ) and corals
- effect of light intensity on P_i uptake by corals
- effect of long-term P_i exposure on uptake by corals, with nubbins maintained 8 weeks without food, and with either 0 (control), 0.5 or 2.5 μM P_i (see Fig. ▼).



Additionally, we measured phosphorus concentrations within host tissues and algal cells on 3 nubbins from each of the above P_i enrichments.

Results were tested with ≠ statistical tests not detailed here.

3 Results

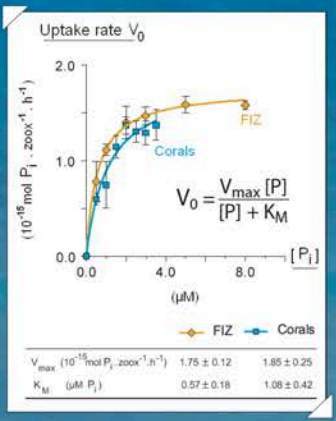
• P_i uptake by corals and FIZ

Kinetics experiments revealed that P_i uptake:

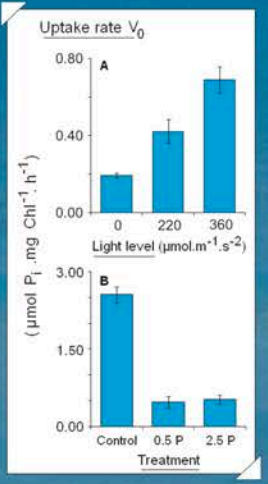
- was linear over 90 min
- was carrier-mediated, as V_0 displayed characteristics of saturation kinetics (Fig. 1)
- occured with ≠ affinities in nubbins and FIZ (K_M twice lower for FIZ, $p < 0.001$; Fig. 1)
- saturated at ca. 2.0 μM P_i in both cases, with similar maximum uptake rates ($p = 0.07$)

Uptake of P_i was light-enhanced ($p < 0.001$), but not completely light-dependent (net dark uptake, Fig. 2A).

Long-term P_i enrichment showed that there was an acclimatization to P_i. Indeed, a solution of 3.0 μM P_i was taken up 5 times faster by control corals than by enriched ones ($p < 0.0001$; Fig. 2B).



▲ **Fig. 1**: Concentration-dependant phosphate (P_i) uptake in FIZ and in *S. pistillata* nubbins (corals). Corresponding kinetics parameters are indicated in the table below. V_{max} is the maximum P_i uptake rate, K_M the solute concentration at which uptake is half- maximal, and V_0 is the initial uptake rate. Data = mean ± SE of 5 nubbins or 3 FIZ.

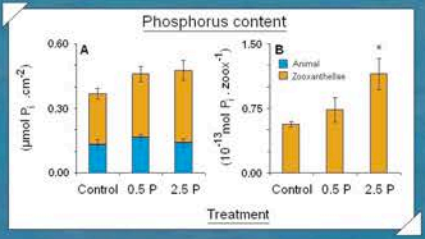


▲ **Fig. 2**: Effect of (**A**) light and (**B**) 8-week phosphate (P_i) exposure on P_i uptake by *S. pistillata*. Incubations performed with initial P_i = 3.0 μM. Data = mean ± SE of 5 nubbins.

• Phosphorus content of corals

Measurements of phosphorus content after 8 weeks of P_i enrichment revealed that:

- total or animal areal phosphorus content were the same in all treatments ($p = 0.30$; Fig. 3A)
- a phosphorus enrichment occurred in the zooxanthellae fraction only ($p < 0.05$; Fig. 3B).



► **Fig. 3**: Phosphorus content of *S. pistillata* individuals maintained during 8 weeks under continuous phosphate (P_i) enrichments of 0, 0.5 or 2.5 μM. Data = mean ± SE of 3 nubbins. * = significantly different from control. (**A**) Total areal phosphorus content, divided into animal and algal fractions. (**B**) Phosphorus content of zooxanthellae expressed on a per cell basis.

4 Discussion

• Adaptation of P_i uptake mechanisms of corals to their oligotrophic environment

Our results confirm previous observations of D'Elia (②) that P_i uptake is mediated by an active transport in corals.

The high affinity and capacity demonstrate that corals and their zooxanthellae are well adapted for a rapid acquisition of P_i in their oligotrophic environment.

• Role of the host in P_i uptake by symbiotic zooxanthellae

The decrease of P_i absorption in the dark suggests that zooxanthellae play a major role in this process *via* their photosynthetic activity, and was already observed in corals (②) and in isolated zooxanthellae (①). However, net dark uptake indicates that the host also plays a role. The lower affinity of corals compared to that of FIZ indicates that P_i uptake by zooxanthellae *in hospite* might be partly limited by the host, in agreement with the hypothesis of Jackson and Yellowlees (①).

• Phosphorus intern pools and regulation of P_i uptake in zooxanthellae

The decrease in uptake rates after a long-term (8 weeks) exposure to P_i demonstrates that P_i uptake is dependent upon the corals history. Measurements of the phosphorus content of enriched corals showed that only zooxanthellae were phosphorus-enriched at the end of the experiment, thus answering the question of D'Elia (②) as to the location of P_i uptake sites.

From our results, we hypothesize that zooxanthellae have the major role in regulating P_i uptake.

In order to better understand how the host can regulate P_i uptake by its symbionts, and whether or not hosts can be responsible of a phosphorus limitation for their zooxanthellae, it seems important to further this study by a quantification of the importance of organic phosphorus uptake as compared to that of P_i.

Litterature cited

① **Jackson, A. E., and D. Yellowlees. 1990**. Phosphate uptake by zooxanthellae isolated from corals. *Proc. Royal Society of London*, Ser. B 242: 201-204. - ② **D'Elia, C. F. 1977**. The uptake and release of dissolved phosphorus by reef corals. *Limnol. Oceanogr.* 22: 301-315.

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