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Biomass and nutrient flux measurements on Holothuria atra populations on windward reef flats at Enewetak, Marshall Islands

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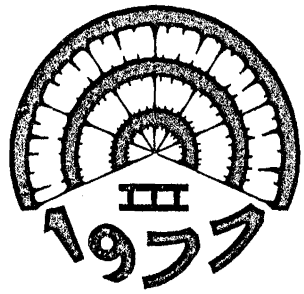

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THE DISSOLVED NITROGEN FLUX OF REEF CORALS

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Introduction

Although it is reasonably well established that corals symbiotic with endozoic algae (the zooxanthellae) are capable of "multitrophic" (1) existence, we are still a long way away from evaluating the relative importance of the various trophic pathways (2). There is no question that zooplankton provide a source of materials and energy to corals, yet there is doubt whether zooplankton are plentiful enough to supply all the needs for these essentials (3). There appear to be alternate sources of supply. Sufficient energy is likely to be available to the symbiotic association by virtue of the photosynthetic capacity of the zooxanthellae (4); thus, energy-wise, the effects of the limited quantities of available zooplankton may be obviated. But what of the balance of nutrient elements such as N and P? Recent evidence for P (5) and N (6, 7) suggests that the zooxanthellae help recycle and conserve these elements, and in fact enable the symbiotic association to remove net quantities of inorganic forms from solution even at the low concentrations characteristic of tropical seas. Thus phosphate (5), nitrate (6, 7), and ammonium (8) are all apparently assimilated from solution in seawater. To produce mathematical models necessary for assessment of the importance of this N and P uptake in coral nutrition, an understanding of the kinetics of uptake of the dissolved inorganic forms is necessary. Here we report data on the kinetics of nitrate uptake and on the flux of dissolved total nitrogen between symbiotic corals and seawater. We use these data to estimate the relative importance of the uptake of dissolved N in the nutrition of the corals studied.

Materials and Methods

This study was conducted at the Mid Pacific Marine Laboratory (MPML), Enewetak Atoll, Marshall Islands. The coral used, *Pocillopora elegans*, was selected because specimens virtually free of infauna could be found easily in the quarry near MPML. Corals were collected within two meters of the surface; they were dislodged from the substratum with a diving knife, care being taken not to injure the coral tissue during that process. Experimental organisms were normally used immediately after collection, on one occasion they were stored in flowing seawater at MPML for three days, as required by experimental protocol. Before use, corals were examined carefully for the presence of commensal crabs which, if found, were removed with forceps. Experimental incubations were performed in a 6.4 liter capacity cylindrical plexiglas chamber (Figure 1). The chamber was provided with an external submersible pump for vigorous mixing. By immersing the pump in a cold water bath, temperature was maintained at 27 ± 1^{o} C. Light was provided by an incandescent floodlight delivering approximately 12,000 lux illuminance. Seawater for incubations was collected fresh from the lagoon and used directly, without filtration, as it contained little biological activity.

Analysis for ammonium, scaled down to 5 ml samples, was the modified Solórzano technique (9) of Liddicoat *et al.* (10). Color development was in the dark (11), but a poor source of deionized water prevented our obtaining blank values of less than 0.150 absorbance units in a 10 cm cell. Replication was generally more erratic than we have experienced in the past. Semi-automated methodology was used for nitrate and nitrite, a peristaltic pump being used to sample water continuously from the incubation chamber, to control flow rate through the Cd column, and to add the reagents (12). Samples were collected manually and read in a 5 ml capacity 10 cm cuvette. Nitrite was determined on samples not passed through the Cd reduction column, but treated with the same reagents. 10 ml samples for total nitrogen (TN) were passed through pre-rinsed Gelman AE filters in a syringe filter holder. Digestion for TN was according to D'Elia *et al.* (13). The digests were neutralized with 0.3N HCl and nitrate and nitrite determined as above to obtain a TN value. Dissolved organic nitrogen (DON) is thus determined as the difference between TN and dissolved inorganic nitrogen (DIN, i.e., ammonium + nitrate + nitrite). Chlorophyll *a* was determined either a) by "blasting" coral tissue off the corallum with a Water Pik (14) and extracting 10 parts of a sample of "blastate" with 90 parts absolute acetone, or b) by extracting the whole coral in 90% acetone; thereafter all samples were centrifuged at high speed for 10 minutes in a clinical centrifuge and read at the wavelengths used in the Parsons and Strickland equations (15). A Beckman DU spectrophotometer was used for all spectrophotometric readings. Tissue TN was determined by

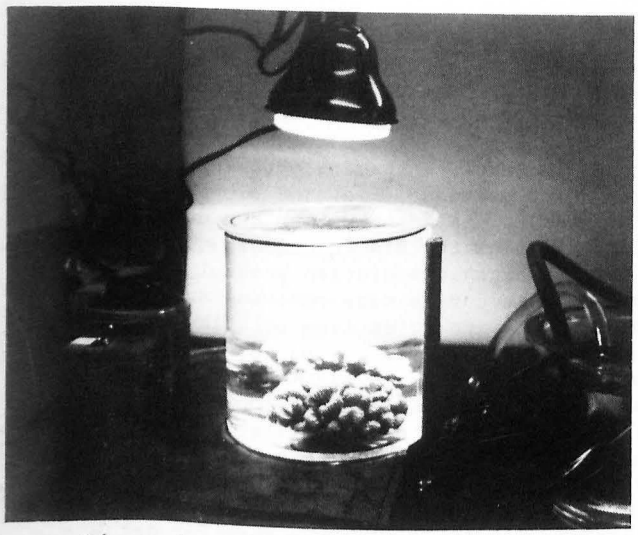

Figure 1. The incubation chamber.

CHN analysis using a Perkin-Elmer Model 240 Elemental Analyzer on lyophilized samples of coral blastate.

Uptake constants, K (half-saturation constant) and V_{max} (maximum uptake rate), for use in the calculation of the uptake rate (V) as a function of substrate concentration (S) from the "Michaelis-Menten" equation

$$V = V_{max}(S/(K+S)) \qquad (1)$$

were determined by a computer program (16) that uses as input the time course of substrate depletion from the medium (i.e. S vs. time, T). This program fits the integrated form of the Michaelis-Menten equation to the uptake data, and can only be used when volume changes as a result of sampling are negligible during experiments (as in the present case).

Results and Discussion

Nitrate Uptake

When incubated in the light in seawater enriched with nitrate (+1.5 µM) heads of *P. elegans* rapidly depleted the medium of nitrate (Figure 2A). On the following day, the same corals, incubated as before, but in the dark, again depleted the medium of nitrate at a nearly identical rate (Figure 2B). This is consistent with previous observations (6). To determine whether the uptake activity was localized in the coral tissue and zooxanthellae or in the small amounts of inflora (e.g. *Ostreobium*, a green alga, and bacterioflora) in the corallum, the subsequent day the following experiment was performed. All living tissue was "blasted" (14) off of the surface of the corallum, which was then rinsed in several changes of fresh seawater and incubated as before in the light. No significant change in nitrate concentration was observed in the seawater (Figure 2C); this indicates that the ability to take up nitrate was present in the living

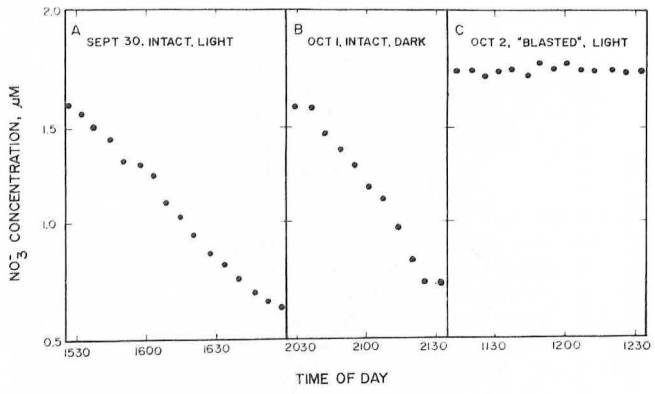

Figure 2. Nitrate concentration in the medium during incubations of P. elegans.

coral tissue or zooxanthellae or both. It is also conceivable, although unlikely, that the bacterioflora on the surface of the coral tissue exhibited the uptake ability. We performed no incubations with ^{15}N tracer, nor did we design other experiments to test for the site of accumulation of the N removed from the medium. Since Franzisket (6) found evidence for nitrate and nitrite accumulation in *P. damicornis* tissue in experiments similar to ours, we presume that it is the zooxanthellae that effect the uptake of nitrate and make it available in reduced form for translocation to coral tissue as amino acids (17). Conceivably, the nitrate uptake by the coral is effected by the zooxanthellae creating a concentration gradient between the seawater and the coral tissue in which they are contained; thus nitrate would diffuse down this gradient from the seawater through coral tissue to the algae. We cannot, however, discount the possibility that assimilatory nitrate reductase (ANR) is present in the coral tissue itself, as there are reports of finding ANR in animal tissues (18, 19).

Additional nitrate depletion experiments were performed to obtain kinetic constants. A representative experiment is shown in Figure 3. In these experiments, nitrite was also monitored and was found to change little in concentration, not exceeding a value of approximately 150 nM. Thus there was no appreciable efflux of nitrite accompanying nitrate uptake. A slight, barely measurable amount of nitrate was present in the medium when uptake ceased in other cases (not shown). Caperon and Meyer (16) and Rhee (20) made a similar observation when studying nitrate uptake by phytoplankton. Our approach, as Caperon and Meyer's, was to treat this as a threshold concentration, S_o, below which no uptake could occur; the small S_o obtained in experiments was subtracted from all S values before S and T values were entered in the computer program. This S_o may be due to "leaking" of internal pools of nitrate at rates equal to those of uptake at low concentrations (as in the case of phosphate flux of corals--see reference 6), nitrification by bacterioflora within the corallum, or an inhibition of uptake at low

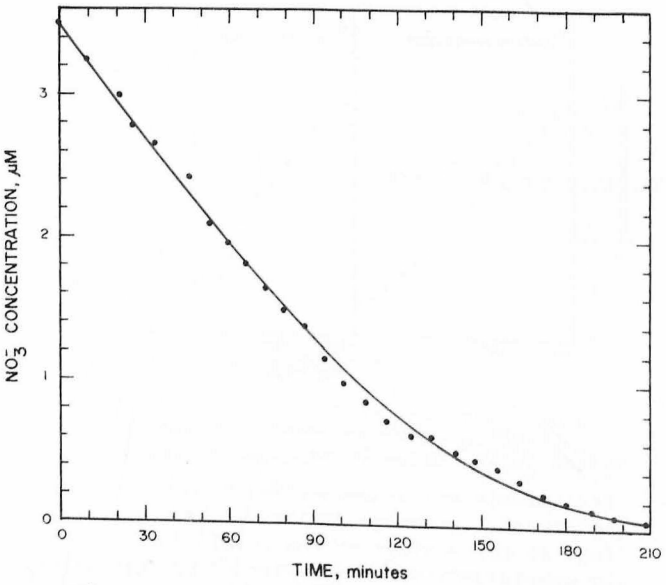

Figure 3. Depletion of nitrate from medium during an incubation of P. elegans *in the light. Curve fitted by computer program described in text.*

concentrations.

Kinetic parameters obtained in this series of experiments are shown in Table 1. The mean value of K was 649 nM and for S_o of 57 nM. V_{max} values, when expressed in terms of chlorophyll *a* (a conveniently obtained measure of biomass), had a mean of 29.9 ng-atoms N.mg chlorophyll a^{-1}.min^{-1}. When expressed in terms of total tissue N, the value for V_{max} was 5.69 ng-atoms.mg-atom N^{-1}.min^{-1}.

Dissolved Total Nitrogen Flux

While it has been shown here and elsewhere that symbiotic corals remove dissolved inorganic nitrogen (DIN) from solution in seawater, we cannot estimate the importance of this N source without first determining what and how great are the concomitant losses of N by corals. That is, DIN sources may be inconsequential if organic N losses are great and vice versa. Quantification of particulate losses (mucus, planulation, etc.) is beyond the scope of this study; we have, however, made a limited number of determinations to assess the flux of dissolved organic nitrogen (DON). Figure 4 shows the result of an incubation of *P. elegans* in the light in which we measured the concentrations of total nitrogen (TN), ammonium, nitrate, and nitrite at various intervals. Initial concentrations of ammonium and nitrate were elevated to two to three times above typical ambient levels for Enewetak waters (for purposes not germane to this discussion). To simplify data analysis, linear regressions were fitted to the time courses of concentration of the various N species, in lieu of more complicated kinetic expressions; this enables the easy calculation of DON change (DON = TN - DIN). Nitrite (not shown separately in Figure 4) remained virtually undetectable during the experiment. Both ammonium and nitrate concentrations declined at a fairly steady rate, the scatter in the points of the former reflecting the poor analytical precision we encountered rather than actual variation in concentration. TN concentration reflected the decrease of its inorganic components, but the rate of TN decrease (i.e. the slope of the fitted line) was not as great as the sum of the rates of decrease of the DIN fractions. This indicates that some organic excretion probably occurred. Thus, if the corals were removing substantial amounts of amino acids from solution as has been suggested (21), the N gained in doing so was apparently more than offset by other DON losses. As the rates of DIN accumulation do more than offset DON losses, DIN accumulation could conceivably play a significant role in coral nutrition. D'Elia (5) found a net loss of organic P for symbiotic corals at typical environmental phosphate levels; it appears that for net P acquisition and growth to occur, some particulate P would be required. When particulate organic N losses by such corals are accounted for, the analogous situation may prove true for N as well.

Relative Rates of Inorganic Nutrient Uptake

The Michaelis-Menten expression (equation 1) has been used to model the uptake of dissolved inorganic nutrients by corals after modifying the expression to provide for certain experimental anomalies. Muscatine and D'Elia (22) have found a strong diffusion type component in the uptake of ammonium by corals; this uptake was well described by the modified expression:

$$V = \left(\frac{V_{max}}{K+S} + K_d\right) S \qquad (2)$$

where V, V_{max}, K, and S are as in equation 1, and K_d is a diffusion constant. Similar uptake models have been shown elsewhere, e.g. amino acid uptake by invertebrate gill tissue (23).

Net uptake of nitrate (7) and phosphate (5) by corals has been shown during uptake experiments to reach zero at some low concentration within the range of 50 to 300 nM substrate concentration. This phenomenon is similar to that reported by Caperon and Meyer (16) and Rhee (20) for nitrate uptake by phytoplankton and by Paasche (24) for silica uptake by diatoms. Uptake hyperbolae (i.e. V vs. S) in such cases contrast with that expected for strict Michaelis-Menten type uptake: in the latter case the curve passes through the origin and in the former it intercepts the abscissa at some positive S value. Caperon and Meyer considered the non-zero intercept to indicate a threshold concentration below which uptake did not take place. Accordingly, they used the expression:

$$V = V_{max}((S-S_o)/(K+(S-S_o))) \qquad (3)$$

where all values are as defined above. This approach effectively moves the origin to the intercept on the abscissa. D'Elia (5) interpreted the non-zero intercept to be the phosphate con-

Table 1. Values of kinetic parameters for nitrate uptake by P. elegans.

	K*	S_o*	V_{max}+	V_{max}‡
	976	0	7.25	41.3
	164	20	6.27	31.5
	806	150	3.54	16.8
mean	649	57	5.69	29.9
std. err.	247	47	1.11	7.1

*nM

+ng-atoms N.mg-atom $N^{-1}.min^{-1}$

‡ng-atoms N.mg chlorophyll $a^{-1}.min^{-1}$

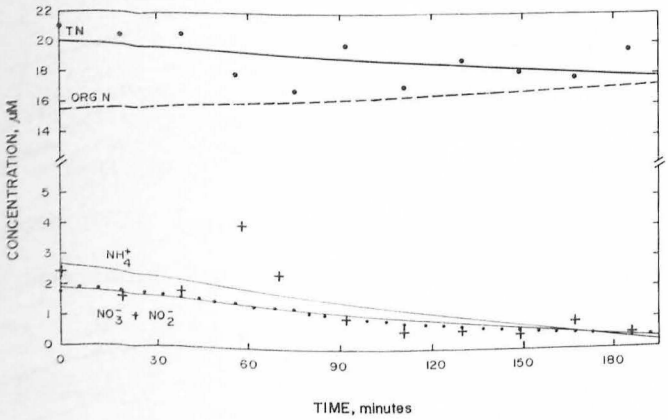

Figure 4. Changes in concentration of N fractions during an incubation of P. elegans *in the light. (•) TN, (+) ammonium, (·) nitrate + nitrite.*

Table 2. Values of kinetic parameters for Pocillopora *spp.*

	K*	S_o*	V_{max}+	V'+	K_d‡
Phosphate	365	—	4.77	0.477	—
Ammonium	642	—	55.97	—	0.0081
Nitrate	649	57	29.87	—	—

*nM

+ng-atoms (N or P).mg chlorophyll $a^{-1}.min^{-1}$

‡liter.mg chlorophyll $a^{-1}.min^{-1}$

centration at which substrate concentration independent leakage of internal phosphate and substrate concentration dependent uptake rates were equal. He was able, using a radioisotopic tracer, to perform experiments supporting this interpretation. Accordingly, the following expression was derived to model phosphate flux:

$$V = V_{max}(S/(K+S)) - V' \qquad (4)$$

where V, V_{max}, K, and S are as above and V' is a term for phosphate efflux.

In the absence of a positive indication of nitrate efflux or of nitrification during the present experiments, we chose equation 3 as an appropriate model to produce the values for nitrate uptake parameters (Table 1).

Table 2 summarizes mean values for kinetic parameters determined for various species of the genus *Pocillopora* (phosphate--*P. danae* (5), ammonium--*P. damicornis* (22), and nitrate--*P. elegans* (this study)). Note that while values for K are similar and are consistent with values reported for free-living phytoplankton (25), the differences in kinetic expressions and V_{max} values greatly influence the magnitude of uptake at different concentrations and thus the shapes of the uptake (V vs. S) curves. To demonstrate this, uptake curves, shown in Figure 5, were calculated based on the values in Table 2 and the appropriate mathematical expression. For Enewetak, typical reef water ammonium concentrations range from about 200 to 300 nM (26), nitrate concentrations from about 200 to 300 nM (26), and phosphate from about 120 to 260 nM (27). These ranges of concentration are indicated on the appropriate curves to show the regions of interest in terms of realistic, natural uptake rates. If one assumes that the mean concentration within the range of concentrations shown above is typical, and that a general model can be applied collectively to the three *Pocillopora* species from which the curves in Figure 5 were derived, then ammonium uptake normally occurs at a rate slightly greater than twice that of nitrate. Thus ammonium is probably the more significant source of dissolved N for *Pocillopora*.

Note that the ratio of DIN:phosphate assimilation extrapolated from uptake rates at mean ambient concentrations shown in Figure 5 would be approximately 20:1, a value consistent with the Redfield (28) ratio of 16:1. As ratios of N:P in particulate sources of nutrition are unknown,

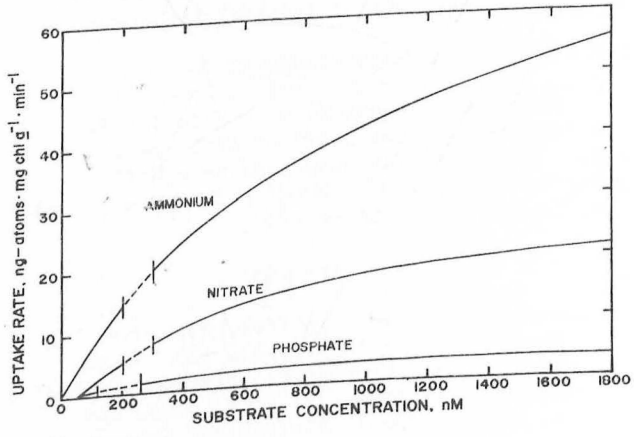

Figure 5. Idealized uptake curves for phosphate, ammonium, and nitrate for Pocillopora *spp.*

not to mention the ratios of those elements in coral tissues, further speculation about this will be left until such time as those determinations can be made.

Significance of DIN Uptake

Even without knowing the flux of particulate N into and out of the corals studied, we can draw some general conclusions about the significance of DIN uptake to them. It is apparent from the results reported above that the influx of DIN is not substantially different from the efflux of DON, and may in fact represent a "break even" situation. Hence, in order for coral tissue to grow (i.e. increase the amount of tissue biomass and not merely the size of the corallum through calcification) some particulate N would appear to be required. A simple calculation serves to reinforce this notion. Assuming a net DON flux of zero (i.e. no loss, no gain), at typical ambient levels of ammonium and nitrate at Enewetak, accumulation of N from those sources by *Pocillopora* would amount to approximately 25 ng-atoms N.mg chlorophyll a^{-1}.min^{-1}. We found an approximate relationship between tissue N and chlorophyll *a* of about 0.19 mg-atoms N to 1.0 mg chlorophyll *a*. Thus DIN accumulation would amount to a growth rate of about 4.8 ng-atoms N.mg-atom N^{-1}.min^{-1}. This represents a biomass doubling time (doubling time = 0.693 ÷ growth rate) of 1.44 x 10^5 min or 100 days--an estimate *assuming no loss of particulate or dissolved organic N*. So, DIN uptake can be viewed as supplementing, not supplanting particulate N supplies such as zooplankton, particularly if high growth rates are necessary to the corals. Further research, especially regarding the flux of particulate and dissolved organic N will be needed to establish the relationship between particulate food and coral growth. In addition, it must be borne in mind that the data presented here represent a limited number of observations on a single genus of symbiotic corals. Generalizations about the nutrition of other genera are tenuous at best without further experimentation.

Acknowledgments

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