

DIN, DON and PO₄ flux by a medusa with algal symbionts

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ABSTRACT: *Linuche unguiculata*, a coronate scyphozoan with symbiotic dinoflagellates (= zooxanthellae), was shown to be capable of taking up NH₄, NO₃, PO₄ and 3 amino acids from seawater. The algae constitute 32 % of the total organismal nitrogen. Both depletion and uptake experiments showed the flux of NH₄ to be more than that of NO₃. Rates of ¹⁵NH₄ uptake by freshly isolated zooxanthellae were comparable to those for free-living phytoplankton, but ¹⁵NO₃ uptake was much lower. Medusae held in the dark several days demonstrated a substantial decrease in their uptake (or depletion) of NH₄ and PO₄. The uptake of ¹⁵N-labelled glycine and leucine and ¹⁴C-labelled alanine was measured at a range of concentrations at rates similar to those measured for NH₄. Fractionation procedures after uptake experiments showed that both non-algal and zooxanthellae fractions were labelled within a few minutes but ¹⁵N from NH₄ appeared preferentially in the zooxanthellar fraction whereas nitrogen from amino acids went primarily to the non-algal (supernatant) fraction. Specific uptake rates of DIN, DON and PO₄ by intact *L. unguiculata* at ambient concentrations were calculated to be about 1 % d⁻¹ for nitrogen and 3.6 % for phosphorus. Although independent measurements of growth and reproductive demands for *L. unguiculata* imply that ingestion of particulate food is likely to be the major source of nutrients, the presence of zooxanthellae enables the medusae to conserve nutrients by preventing their loss from the association.

INTRODUCTION

Many algal-cnidarian symbioses are able to take up and sequester dissolved compounds from seawater (e.g. Muscatine 1980, Trench 1987). Recycling nutrients from the host to the endosymbiotic dinoflagellates (= zooxanthellae) apparently results from the close physical relationship of the invertebrate and algal partners. This apparent recycling conserves environmentally scarce nutrients within the association and therefore may confer a competitive advantage to symbiotic associations in nutrient-poor tropical habitats. Nutrient uptake by marine symbioses has most commonly been measured as the disappearance of dissolved inorganic nitrogen (DIN) or phosphate (PO₄) from seawater using wet chemistry techniques. Studies began with Yonge & Nicholls (1931) using PO₄ and Kawaguti (1953) measuring ammonium (NH₄) depletion by reef corals. Since the early studies both ammonium and nitrate (NO₃) depletion by a variety of corals, anemones and isolated zooxanthellae have

been described (e.g. Franzisket 1974, D'Elia & Webb 1977, Muscatine et al. 1979, Propp 1981, D'Elia et al. 1983). There is usually preferential removal of ammonium compared to nitrate, although the reverse was described for *Acropora palmata* (Bythell 1990). Not all symbioses have been shown to take up nitrate (see reviews by Wilkerson & Trench 1986, Miller & Yellowlees 1989); e.g. the anemone *Aiptasia pulchella* (Wilkerson & Muscatine 1984), the Red Sea coral *Stylophora pistillata* (Muscatine et al. 1984) and the jellyfish *Mastigias* sp. (Muscatine & Marian 1982). The lack of nitrate uptake may be due to repression of nitrate reductase in the zooxanthellae by host-produced ammonium (Wilkerson & Muscatine 1984, Wilkerson & Trench 1986), since zooxanthellae separated from the host and cultured showed nitrate uptake and nitrate reductase activity (Wilkerson & Trench 1985). An alternative explanation put forward by Miller & Yellowlees (1989) for corals is that nitrate depletion may be due to epilithic organisms associated with the coral skeleton rather than coral zooxanthellae. However, this seems

unlikely since Crossland & Barnes (1977) measured nitrate reductase activity in freshly isolated zooxanthellae from corals.

Incorporation of isotopes by marine symbioses has been used to measure DIN uptake directly. Gunnerson et al. (1988) and D'Elia & Cook (1988) incubated symbiotic anemones, corals, and isolated zooxanthellae with ^{14}C -methylamine (the radioactively labelled analogue of NH_4). Use of NH_4 and NO_3 labelled with the stable isotope ^{15}N gives more direct information on nitrogen incorporation that is not provided by nutrient perturbation experiments or those that use analogues, yet this method has not been commonly used. Wilkerson & Grunseich (1990) compared biomass specific (V) and volume specific (ρ) rates of ^{15}N uptake by a symbiotic ciliate *Mesodinium rubrum* with (1) depletion data and (2) other ^{15}N uptake studies from the phytoplankton literature. $^{15}\text{NH}_4$ uptake was measured in *Pocillopora damicornis* by Muscatine & D'Elia (1978) and in a number of corals and isolated zooxanthellae by Burris (1983). Both $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ were used in a single experiment with *Stylophora pistillata* by Muscatine et al. (1984). Summons & Osmond (1981) using gas chromatography-mass spectrometry methods determined the incorporation of ^{15}N -labelled NH_4 , NO_3 and urea into the amino acid pool of zooxanthellae isolated from giant clams.

Although less well studied, the uptake of dissolved organic nitrogen (DON, i.e. amino acids) by several species of cnidarians has also been documented (Shick 1975, Schlichter 1978, 1982, Ferguson 1988, Ferrier 1991). In addition, uptake of organic nitrogen has been shown for zooxanthellae isolated from symbiotic cnidarians (Carroll & Blanquet 1984a, b, Blanquet et al. 1988, Macon McDermott & Blanquet 1991).

Phosphate uptake by symbiotic cnidarians and zooxanthellae has been measured both as PO_4 disappearance (e.g. Yonge & Nicholls 1931, D'Elia 1977, Cates & McLaughlin 1979, Muller-Parker et al. 1990) and as $^{32}\text{PO}_4$ incorporation (e.g. Jackson & Yellowlees 1990). D'Elia (1977) and D'Elia et al. (1983) used $^{32}\text{PO}_4$ uptake data from isolated zooxanthellae and corals to propose the diffusion depletion hypothesis to explain how nutrients might enter invertebrate-algae symbioses.

The goal of this study was to quantify nutrient uptake by a non-calcareous pelagic symbiosis, *Linuche unguiculata* (Swartz), a coronate scyphozoan possessing zooxanthellae. This 'thimble jelly' occurs seasonally as medusae in the Bahamas. It is capable of carbon fixation at rates comparable to corals (Kremer et al. 1990) and maintains a ratio of production to respiration greater than 1 on a 24 h basis. Other essential elements, such as nitrogen and phosphorus, must be acquired from uptake of dissolved compounds from the seawater and heterotrophic ingestion of food particles.

NH_4 flux has been measured in 2 other symbiotic medusae: *Cassiopea* sp., a benthic mangrove medusa (Cates & McLaughlin 1976) and *Mastigias* sp. (Muscatine & Marian 1982), a scyphozoan in a tropical stratified saline lake with an ammonium-rich chemocline. As far as we are aware there are no published studies of phosphate depletion or uptake using pelagic cnidarians.

Our study was designed to meet 3 objectives: (1) quantify disappearance and uptake using ^{15}N - and ^{14}C -labelled nutrients for a variety of conditions and concentrations; (2) investigate the partitioning of incorporated DIN and amino acids between zooxanthellae and host tissue; and (3) put the measured rates of DIN and DON uptake into an ecological context by estimating the supply of nitrogen to *Linuche unguiculata* from ambient dissolved concentrations. In this paper, we first document depletion and uptake of ^{15}N labelled NH_4 and NO_3 by the association and its isolated zooxanthellae, and the effect of light and concentration on NH_4 uptake. Then PO_4 depletion by medusae is described. Additional experiments investigated the effect of holding medusae for several days (starved vs fed, light vs dark). Finally, the uptake of labelled (^{15}N and ^{14}C) amino acids by *L. unguiculata* is presented. We also examine the differential incorporation of ^{15}N -labelled DIN or DON by the animal and algal fractions.

MATERIALS AND METHODS

Collection and maintenance of medusae. Medusae of *Linuche unguiculata* were collected using dip nets and hand-held jars at several locations primarily in the Exuma Cays, Bahamas, during 2 cruises aboard the RV 'Calanus'. All medusae used to investigate the uptake of inorganic nitrogen and phosphorus were collected from 2 to 12 May 1988, while medusae for investigations of organic nitrogen uptake were collected approximately 1 yr later, 16 to 28 April 1989. On shipboard, the medusae were held in 20 l polycarbonate containers in a water bath at ambient surface light and temperature. Usually medusae were used for experiments within a few hours of collection but some were experimentally maintained for several days, either on deck at ambient surface light or darkened in the wet laboratory. Ambient temperature was approximately 26°C on deck and 24°C in the wet laboratory. Water was changed in the experimental tanks 1 to 2 times daily. Medusae used to investigate the uptake of ^{14}C -labelled alanine were transported to the University of Southern California, held unfed in an incubator at 26°C on a 12 h light:dark cycle and used within 2 wk of collection.

Biomass and composition measurements. Bell diameters of relaxed medusae suspended in water

were measured to the nearest mm using a ruler. The medusae were then blotted with paper towel to remove surface water and the displacement volume was measured to the nearest 0.1 ml in a graduated cylinder.

After obtaining the displacement volume, samples were homogenized with a hand-held tissue homogenizer in a known volume (5 to 20 ml, depending on number of medusae) of filtered sea water (FSW). An aliquot of homogenate was removed and diluted to measure *in vivo* fluorescence in a Turner Designs fluorometer. In order to calibrate these fluorescence results, some samples were also measured spectrophotometrically for chlorophyll *a* (chl *a*) (Jeffrey & Humphrey 1975). These calibration samples ($n = 8$) were filtered onto 25 mm GF/C filters, rinsed with 1 ml deionized water (DIW), extracted in 5 ml 100% acetone, pulverized with a motorized teflon pestle and read within 1 h on a Hitachi spectrophotometer at 665 nm and 630 nm. Calibration of fluorescence measurements to chl *a* used a range of medusae sizes (0.3 to 1.2 ml displacement volume) and gave an r^2 of 0.96. For some samples, an aliquot of the homogenate was preserved in Lugols solution and algal counts were made using a hemacytometer.

Medusae sampled for dry weight were blotted and dried at 60°C in preweighed aluminum pans. Aliquots of a subset of the dried samples were homogenized with a mortar and pestle, redried and analyzed for elemental phosphorus (Page et al. 1982).

Particulate nitrogen (PON) content of dried medusae tissues was measured using mass spectrometry for aliquots of whole medusae, zooxanthellae and some animal fractions. To separate zooxanthellae and non-algal tissue, homogenized samples of medusae were centrifuged, decanted, rinsed and resuspended 2 to 3 times in a bench top centrifuge. The pellets, containing the zooxanthellae fraction, were then resuspended in FSW and filtered on pre-ashed glass fiber filters which were dried and held at 60°C prior to analysis. The supernatant fractions were combined to give the 'animal' fraction including any associated microflora. *In vivo* fluorescence analysis of the supernatant showed there to be little or no chl *a* – i.e. zooxanthellae contamination.

DIN and phosphate depletion/uptake experiments: general protocol. Glassware used throughout these procedures was washed in 10% HCl and rinsed with DIW. Experimental incubations consisted of 5, 10 or 20 medusae in duplicate 125 ml Pyrex bottles filled with filtered surface seawater and held in an on-deck incubator at 26°C at ambient light or in the dark. Control incubations were carried out simultaneously with no medusae. After 5 to 10 min acclimation to the incubation vessels by the medusae, a spike of PO_4 or

^{15}N -labelled (99.3% enrichment) NH_4Cl or KNO_3 was added. Water samples (9 ml) were withdrawn from the bottles with a needleless syringe immediately after enrichment and at fixed time intervals for up to 2 h. Nutrient concentrations (NH_4 , NO_3 and PO_4) were measured immediately using a Technicon II Auto-Analyzer. Nitrate and phosphate determinations were made according to standard Technicon methods for seawater analysis. Ammonium was determined by a modification of Slawyk & MacIsaac (1972) as described in Szmant et al. (1990). At the end of the incubation the medusae were removed to measure bell diameter, displacement volume of medusae and the volume of remaining seawater. The medusae were then homogenized, an aliquot used for fluorescence measurements, and the remainder separated into 'animal' and algal fractions by centrifugation. The algal fraction was resuspended in FSW (usually 3 ml), filtered onto a precombusted 47 mm GF/C filter at low vacuum, dried at 60°C and stored with desiccant. Subsamples of the filters were obtained using a cork-borer and analyzed for atom % (at. %) N and PON using a Europa Scientific RoboPrep TracerMass mass spectrometer. Some animal fractions were frozen, then freeze-dried, ground with a spatula and aliquots used for mass spectrometry. Small samples (about 0.2 μM N) can be analyzed using the RoboPrep TracerMass due to the high stability, low noise characteristics of the system. For $(\text{NH}_4)_2\text{SO}_4$ standards run at the start and end of each batch, the mean at. % ^{15}N was 0.383 with a standard deviation of 0.023 ($n = 25$). At. % N excess values (i.e. the ratio of $^{15}\text{N}/^{14}\text{N}$ enrichment compared with natural abundance) were calculated.

DIN and phosphate depletion/uptake: rate calculations. The rate of depletion of nutrients over time was based on nutrient samples taken during the time series, correcting for the volume removed. Reported rates are actually means of rates derived from pairs of measurements in the time series. This approach minimized biases associated with any fixed time interval and enabled us to weigh all analyses equally. We also monitored whether depletion rates changed with time during the course of the experiment.

The nitrogen-specific depletion rate (V , h^{-1}) was calculated from the measured depletion and the measured displacement volume of medusae in the bottle and then converted to nitrogen equivalents using the relationship in Kremer et al. (1990). For ^{15}N experiments, the biomass specific uptake rate of the zooxanthellae (V_m , h^{-1}) was calculated according to Dugdale & Wilkerson (1986) from the at. % excess of ^{15}N and ambient nutrient concentrations. For free-living phytoplankton, uptake rates V_t were calculated and used to obtain estimates of transport rate (ρ , $\mu\text{mol l}^{-1} \text{h}^{-1}$) by multiplying by the PON of the sample.

DIN and phosphate depletion/uptake: experiments.

A number of different experiments were carried out in which either incubation conditions were changed or certain aspects of the overall depletion/uptake experiment design described above were manipulated.

Time series experiments: The first experiments were carried out with a series of 60 ml BOD (Biological Oxygen Demand) bottles in which 5 medusae were incubated with either $^{15}\text{NO}_3$ or $^{15}\text{NH}_4$ (approximately 7.5 μM). At fixed time intervals single incubation bottles were assayed for dissolved nutrients, medusae and mass spectrometry of the zooxanthellae fraction. In subsequent uptake/depletion experiments only depletion was sampled as a time series, and ^{15}N uptake values were obtained from a single end-point.

Uptake by isolated zooxanthellae: One experiment was carried out with freshly isolated zooxanthellae. Medusae were homogenized and fractionated into algal and animal fractions. An aliquot of the resuspended zooxanthellae was added to each incubation bottle prior to addition of the isotope. Samples were removed with a needleless syringe, filtered on a 25 mm precombusted GF/C filter, and dried for subsequent ^{15}N analysis.

Uptake by free-living phytoplankton: Surface water from 4 stations was incubated according to Dugdale & Wilkerson (1986) with 5 μM ^{15}N -labelled KNO_3 or NH_4Cl additions to 2 l polycarbonate bottles filled with surface water (obtained with a non-toxic plastic bucket) and screened with neutral density nickel screening to 50% of surface irradiance. Six-hour incubations were conducted around local noon on-deck in seawater-cooled incubators under ambient sunlight. Following incubation, the samples were filtered onto precombusted GF/F filters, placed in glassine envelopes and dried at 60°C using dessicant. Subsamples of the filters were obtained using a cork-borer and analyzed by mass spectrometry.

Pathway of uptake: A 'pulse' type experiment was carried out to trace the pathway of ^{15}N supplied to *Linuche unguiculata* as $^{15}\text{NH}_4$. Ten medusae were incubated in 30 μM $^{15}\text{NH}_4$ for 2 or 4 h, rinsed twice with FSW and held in FSW. Medusae were removed for analysis prior to ^{15}N addition, at the end of the initial ^{15}N incubation, and at intervals up to 33 h after the start of the experiment. Bell diameter was measured and the 'animal' and algal components separated and assayed for ^{15}N as described above.

Effect of varying concentration on uptake by medusae: One experiment was conducted to see how variations in initial concentration of ammonium affected the depletion/uptake rate of ammonium by *Linuche unguiculata* medusae. Duplicate series of 9 bottles, each containing 5 medusae, were spiked with $^{15}\text{NH}_4$ to give 9 initial concentrations. During the hour-long incubation, water was removed from each bottle every 20 min and

depletion of ammonium monitored. At the end of the incubation period, the medusae were removed and the zooxanthellae fraction separated and assayed for ^{15}N .

Effect of light versus dark on uptake by medusae: Medusae were incubated with $^{15}\text{NH}_4$ in dark bottles wrapped with black electrical tape for comparison with identical incubations carried out in ambient light.

Holding experiments: Holding experiments were carried out in which *Linuche unguiculata* were held in 20 l polycarbonate containers either at surface ambient light:dark cycle or in complete darkness without food for up to 4 d before being used in depletion or uptake incubations. Additional groups of medusae were held at ambient light with daily additions of freshly collected zooplankton obtained with a plankton net (150 μm mesh size). Medusae held at ambient light were subsequently incubated in the light while those held in the dark were incubated in darkness.

Amino acid uptake. Preliminary depletion experiments exposed medusae of *Linuche unguiculata* to a mixture of 16 amino acids, each at an initial concentration of 200 nM. Although the medusae were not axenic, they were rinsed in sterilized seawater prior to experimental incubation in sterilized seawater spiked with the amino acid mixture. Time series sampling and subsequent analysis by HPLC (High-Performance Liquid Chromatography) followed the protocol described in Manahan (1989). This analysis indicated that the intact *L. unguiculata* association ('animal' and algae) was capable of removing several amino acids from sea water. Uptake experiments using ^{15}N -labelled glycine and leucine (Isotec Inc., >99% enriched) and ^{14}C -labelled alanine (New England Nuclear, 168 mCi mmol^{-1}) were designed to quantify uptake rates for these 3 amino acids.

The ^{15}N -labelled glycine and leucine experiments were conducted at sea with freshly collected medusae. Groups of medusae were placed in BOD bottles in 300 ml FSW at a range of ^{15}N -labelled amino acid concentrations from 100 nM to 3 μM . Medusae were removed serially through time for analysis of ^{15}N incorporation. In one set of experiments, medusae were simply rinsed and dried whole, while in others, the medusae were homogenized and separated into algal and 'animal' components by centrifugation. Aliquots of the supernatant ('animal' fraction) were dried in aluminum pans, while the algal fraction was rinsed and recentrifuged before resuspension and filtration onto precombusted 47 mm GF/C filters. Dried samples from the aluminum pans were ground with a spatula, re-dried and aliquots analyzed. Aliquots of the filter were taken with a cork borer and analyzed in the mass spectrometer.

A second experimental approach involved transporting medusae to the University of Southern Califor-

nia where uptake of ^{14}C -labelled alanine was investigated. Stock ^{14}C -labelled alanine was diluted with nonradioactive alanine (Sigma Chemical Co.) in a ratio of 1.7 carrier: 1. Six time-series experiments of ca 1 h each were conducted to determine the uptake rate of alanine over the range of 160 to 800 nM. At each time increment (5 to 10 min), 1 to 3 medusae were removed, rinsed twice in unspiked sterilized seawater, blotted dry on a paper towel and homogenized in a glass tissue homogenizer. The homogenate was transferred to a small plastic microfuge tube, the homogenizer and pipette rinsed and the rinse water added to the homogenate until the total volume was 1.0 or 1.5 ml. An aliquot of 0.5 ml of the homogenate was combined with 5 ml scintillation cocktail (Fisher Bio-HP) and counted using a LKB Model 1211 scintillation counter.

In 2 alanine uptake time-series experiments, an aliquot of the homogenate was fractionated into algal and non-algal fractions by centrifugation. The homogenate was initially separated in a high-speed centrifuge (5 s at 12 000 to 15 000 g), then in 1 of the 2 experiments the pellet was resuspended and centrifuged in a small bench top centrifuge in a similar procedure as that used at sea in both the ^{15}N -labelled DIN and DON uptake experiments. Each of the supernatant fractions was counted separately, then combined to give a total estimate for the animal fraction.

In order to verify that the measurement of ^{14}C uptake actually represented a net flux of alanine into the medusae, a depletion experiment was conducted in which ^{14}C -alanine-spiked seawater was monitored simultaneously with sampling for HPLC analysis of alanine. This experiment showed that uptake of ^{14}C -alanine and alanine depletion as measured by HPLC were consistent with each other over the initial 35 min validating the interpretation of ^{14}C incorporation as a measurement of alanine uptake.

RESULTS

Biomass and composition results

The different parameters that were measured for *Linuche unguiculata* (number and nitrogen content of the zooxanthellae, concentrations of chl *a* and total phosphorus) are given in Table 1, with all units standardized to 1 ml displacement volume of medusa. Values from an earlier study of *L. unguiculata* (Kremer et al. 1990) are given for comparison. The results of the 2 studies compare very favorably. The mean zooxanthellae count, chl *a* content, and zooxanthellae N were not significantly different (Students *t*-test, $p > 0.05$) between the 2 studies. The chlorophyll concentration

Table 1 *Linuche unguiculata*. Conversion between biomass units based on calibration of displacement volume (dvol, ml) with other parameters. All means are normalized to 1 ml dvol, although derived with a size range of medusae

Unit	Mean	SD	Sample size (n)
Zooxanthellae count ($\times 10^6 \text{ ml}^{-1}$)			
This study	22.3	7.6	35
Kremer et al. (1990)	21.0	9.7	51
Chlorophyll <i>a</i> ($\mu\text{g ml}^{-1}$)			
This study, fresh	38.5	11.1	47
held > 1 d	26.2	7.7	48
Kremer et al. (1990)	37.1	13.9	22
Zooxanthellae N (mg ml^{-1})			
This study, fresh	0.35	0.15	115
held > 1 d	0.44	0.13	33
Kremer et al. (1990)	0.28	0.08	5
Phosphorus (mg ml^{-1})			
This study, fresh	0.16	0.01	5
held > 1 d	0.12	0.01	4

per algal cell ($2.0 \pm 0.5 \text{ pg cell}^{-1}$ ($n = 23$)) was not significantly different from Kremer et al. (1990), ($p > 0.1$). Both chlorophyll and phosphorus were significantly lower ($p < 0.001$) in medusae that had been maintained for longer than 1 d, while the zooxanthellae N did not change significantly ($p > 0.1$). Zooxanthellae nitrogen content was measured in this study using mass spectrometry, and had a mean value of $0.37 \pm 0.15 \text{ mg ml}^{-1}$ ($n = 148$), equivalent to 32 % of the total nitrogen biomass for the intact association. Zooxanthellae nitrogen per algal cell was calculated to be $15.7 \text{ pg zooxanthella}^{-1}$.

Ambient levels of dissolved inorganic nutrients

The ambient concentrations of NH_4 , NO_3 , and PO_4 in surface water samples were measured at 2 locations (Warderick Wells and Chub Cay) during the study. Ammonium was $0.16 \pm 0.06 \mu\text{M}$ (mean \pm SD, $n = 16$), NO_3 was $0.43 \pm 0.12 \mu\text{M}$ ($n = 16$) and PO_4 was $0.33 \pm 0.07 \mu\text{M}$ ($n = 16$). No measurements were made for dissolved organic nitrogen but alanine is typically 10 to 100 nM in the region (K. Mopper pers. comm.).

Time course of DIN depletion and ^{15}N uptake by association

Fig. 1 shows the results of a representative experiment in which 5 medusae per bottle were exposed to ^{15}N -labelled NH_4 or NO_3 , and incubated in the light.

Ammonium depletion by *Linuche unguiculata* was clear, whereas the disappearance of NO_3 was not measurable with this number of medusae (Fig. 1a). However, uptake of both ^{15}N -labelled NH_4 and NO_3 by *L. unguiculata* was measurable, with much more rapid uptake of NH_4 than NO_3 (Fig. 1b). Linear increase of at. % with time in the NH_4 uptake incubations indicated that there was no ammonification or ammonium dilution occurring that would result in a more curvilinear plot. Due to the large difference in uptake rates of NH_4 and NO_3 , we altered the protocol for subsequent experiments by using more medusae (i.e. > 5) for NO_3 incubations than for NH_4 incubations. This optimized the concentration changes, making NO_3 concentration differences measurable by wet chemistry as assayed by the Auto-Analyzer.

Uptake by freshly isolated zooxanthellae

Freshly isolated zooxanthellae were used in ^{15}N uptake experiments carried out in the light for comparison with rates for zooxanthellae in the intact association. As for intact medusae, uptake of NH_4 was much greater than for NO_3 (Fig. 2). Table 2 shows nitrogen-specific NH_4 uptake rates (VNH_4) by the isolated zooxanthellae and free-living phytoplankton sampled from the same waters were significantly more than for the intact association ($p < 0.02$). VNH_4 values for isolated zooxanthellae and free-living phytoplankton were not significantly different ($p > 0.1$). Nitrate uptake (VNO_3)

by the isolated zooxanthellae and the intact association were significantly lower than by the free-living phytoplankton ($p < 0.01$). Nitrate uptake by isolated zooxanthellae showed no significant difference from the intact association ($p > 0.1$).

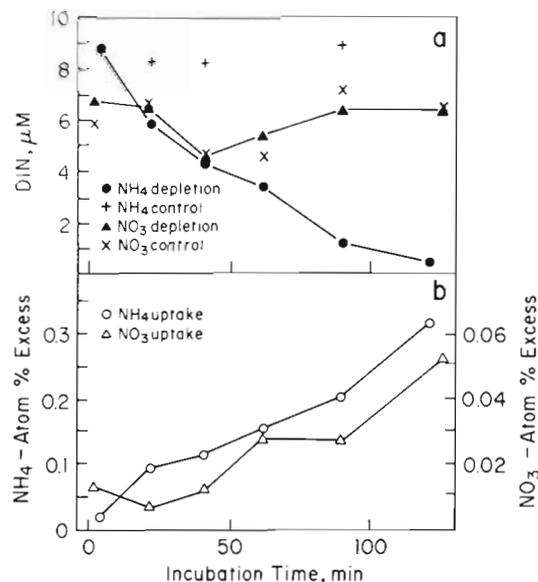


Fig. 1 *Linuche unguiculata*. Time course of (a) depletion and (b) uptake of ^{15}N -labelled NH_4 and NO_3 by intact medusae. Experimental bottles (60 ml) each had 5 medusae. Control bottles had no medusae. At. % excess values are for the zooxanthellae fraction

Table 2. Uptake of ^{15}N -labelled NH_4 and NO_3 (mean \pm SD) by freshly isolated zooxanthellae, intact *Linuche unguiculata* and free-living phytoplankton. V: algal PON-specific uptake rate; ρ : transport rate; f: % new production, $\rho\text{NO}_3/\rho\text{NO}_3 + \rho\text{NH}_4$. Additions of DIN of 10 μM for zooxanthellae and 5 μM for phytoplankton were used

	VNH_4 ($\text{h}^{-1} \times 10^{-3}$)	VNO_3 ($\text{h}^{-1} \times 10^{-3}$)	ρNH_4 ($\text{nmol l}^{-1} \text{h}^{-1}$)	ρNO_3 ($\text{nmol l}^{-1} \text{h}^{-1}$)	f (%)
Isolated zooxanthellae	9.30 ± 1.50 (n = 4)	0.20 ± 0.03 (n = 4)	--	--	--
Intact <i>L. unguiculata</i>	3.00 ± 1.00 (n = 4)	0.30 ± 0.10 (n = 3)	--	--	--
Free-living phytoplankton					
Stn 1 (25° 20.95' N, 77.0° 50.75' W)	18.40	2.40	10.50	2.10	10
Stn 2 (25° 08.50' N, 77.0° 26.80' W)	7.20	1.50	4.90	0.90	10
Stn 3 (25° 46.00' N, 78.0° 52.55' W)	21.90	3.10	16.40	2.50	10
Stn 4 (25° 30.70' N, 80.0° 00.02' W)	10.60	2.00	9.30	0.90	10
Mean	14.50 ± 6.80 (n = 4)	2.00 ± 0.67 (n = 4)	10.28 ± 4.74 (n = 4)	1.60 ± 0.83 (n = 4)	10

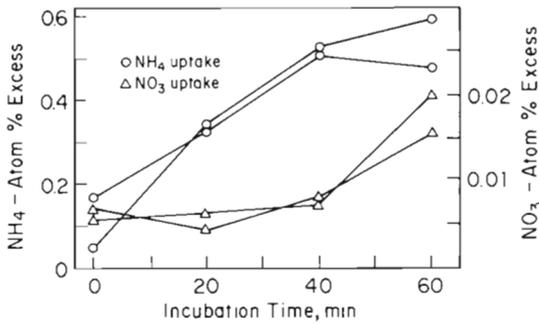


Fig. 2. *Linuche unguiculata*. Time course of ^{15}N -labelled NH_4 and NO_3 uptake by freshly isolated zooxanthellae. Initial nutrient concentration was $10\ \mu\text{M}$

DIN uptake by free-living phytoplankton vs zooxanthellae

Uptake by free-living phytoplankton was calculated as ρ (uptake on a per liter basis, Dugdale & Wilkerson 1986) as well as the nitrogen-specific uptake rate V . This value, obtained using $5\ \mu\text{M}$ NH_4 or NO_3 inoculations gives a measure of the potential new production that could be achieved if nutrients were available at saturating concentrations (Dugdale & Goering 1967) where $\rho\text{NO}_3/\rho\text{NO}_3 + \rho\text{NH}_4$ (the f -ratio) is the percent new production. The ρNO_3 values for free-living phytoplankton were 7-fold lower than those of ρNH_4 (Table 2) and a value for f of 10% was calculated (for optimal nutrient conditions) indicating that the productivity cycle is most likely regenerative (i.e. running on NH_4) and reliant on non-nitrate sources of nitrogen. This is typical of oligotrophic waters (e.g. Glibert et al. 1988). $V\text{NH}_4$ for free-living phytoplankton was comparable to rates measured for freshly isolated zooxanthellae. $V\text{NO}_3$ for free-living phytoplankton were about an order of magnitude greater than for isolated zooxanthellae ($p > 0.1$), suggesting that even with abundant nitrate, swarms or blooms of *Linuche unguiculata* would have a minimal contribution to new production in Bahamian waters.

Uptake of ^{15}N by 'animal' vs algal tissue

To establish whether the animal component of *Linuche unguiculata* became labelled with ^{15}N during $^{15}\text{NH}_4$ incubations, *L. unguiculata* were fractionated into 'animal' and algal fractions after a 'pulse' (2 or 4 h) of $^{15}\text{NH}_4$ ($30\ \mu\text{M}$). Fig. 3 shows the at. % excess in the 'animal' and algal fractions from the start of the experiment, with greater enrichment by medusae in the 4 h incubation. Interestingly, the 'animal' fraction showed appreciable ^{15}N incorporation, although on a mass-

specific basis this was only half the ^{15}N incorporated by the zooxanthellae fraction. During the subsequent 30 h of the experiment during which the medusae were placed in filtered water at ambient nutrients, there was no indication of net translocation of ^{15}N -label from one fraction to the other

Influence of environmental factors on NH_4 uptake and depletion

Substrate concentration

The effect of different initial NH_4 concentrations on rates of $^{15}\text{NH}_4$ depletion and uptake by *Linuche unguiculata* was tested using a range of 9 initial concentrations. Depletion of NH_4 was linear with time during the 1 h incubations for all concentrations used (data not shown). Nitrogen specific rates (V , h^{-1}) calculated from depletion results (entire medusa) and ^{15}N uptake (zooxanthellae fraction) increased at higher concentrations of NH_4 (Fig. 4a). A linear transformation was applied to the data by plotting S/V vs S where S = substrate concentration (Walter 1965, Segel 1975) and yielded a linear regression with $r = 0.92$ for the depletion data and 0.83 for the uptake data (Fig. 4b). According to Zimmerman et al. (1987) we used an iterative algorithm developed by Marquardt for a curvilinear fit to the data and a Monod formulation to calculate the kinetic constant K_s (concentration which results in half V_{max}) and

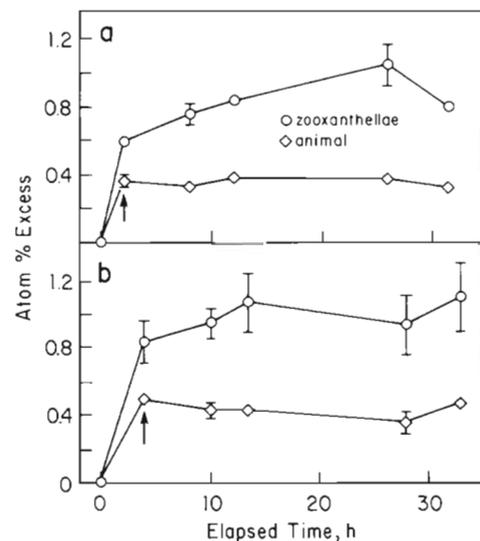


Fig. 3. *Linuche unguiculata*. ^{15}N enrichment of zooxanthellae and 'animal' fractions of *L. unguiculata* from incubations in $30\ \mu\text{M}$ $^{15}\text{NH}_4$ for (a) 2 or (b) 4 h. Arrows indicate transfer from spiked incubation water to filtered seawater with no added nutrients. Bars indicate range of duplicates; no bars are shown where the range fits within the symbol size

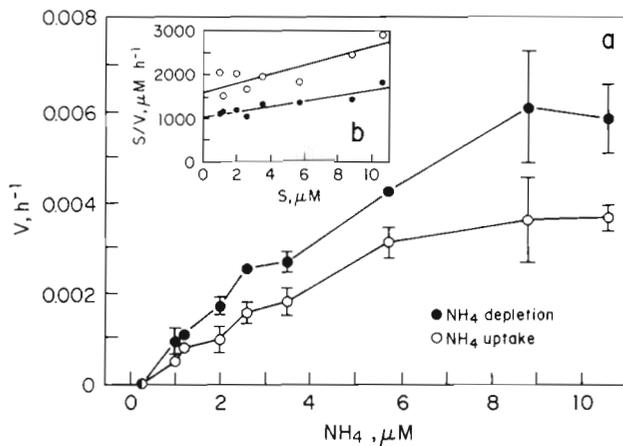


Fig. 4. *Linuche unguiculata*. (a) NH_4 depletion and uptake rates (biomass specific V) for medusae were measured for a range of concentrations of NH_4 (μM). Bars indicate range of duplicates; no bar shown where the range fits within the symbol size. (b) Hanes-Woolf transformation of data in (a) using substrate concentration ($S, \mu M$) and velocity (V, h^{-1})

V_{max} and their standard deviations. The apparent K_s was calculated as $14.1 \pm 4.3 \mu M$ for the depletion data and $10.9 \pm 3.3 \mu M$ for the uptake data. Apparent V_{max} values were $0.015 \pm 0.003 h^{-1}$ for the depletion data set and $0.008 \pm 0.0015 h^{-1}$ for the uptake data.

Irradiance

Medusae freshly collected from 2 different locations were incubated with ^{15}N -labelled NH_4 simultaneously in either ambient surface light or darkness. Although both depletion and uptake rates of NH_4 were generally greater in the light than dark (i.e. mean light:dark uptake was 1.3 and 1.4 for depletion and uptake respectively) there was no statistical difference in the means ($p > 0.1, n = 6$).

Holding experiments

Linuche unguiculata held for 4 d at ambient light and photoperiod with and without food and incubated with NH_4 in the light showed decreasing depletion rates (Fig. 5a) with time. Rates of similar magnitude were measured for $^{15}NH_4$ uptake in the light (Fig. 5b), but there was more variability between replicates. Depletion rates for medusae held in continuous darkness and incubated in the dark decreased steadily over time (Fig. 5a) while uptake rates (Fig. 5b) were roughly constant between Days 1 to 3. Even after 4 d in the dark there was no measurable net excretion (Fig. 5a) but the depletion and uptake data approached zero.

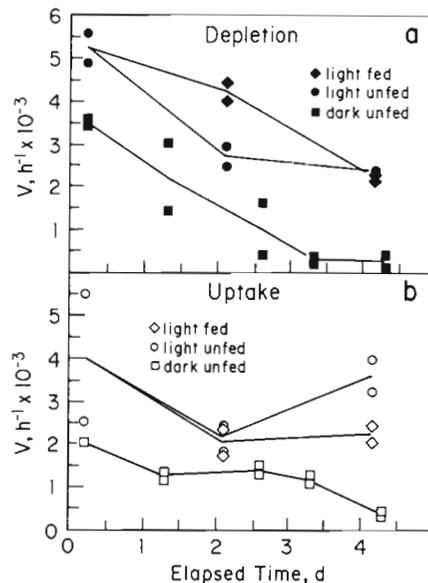


Fig. 5. *Linuche unguiculata*. Rates of ^{15}N -labelled NH_4 (a) depletion and (b) uptake for medusae held in the dark or at ambient light (either fed or unfed)

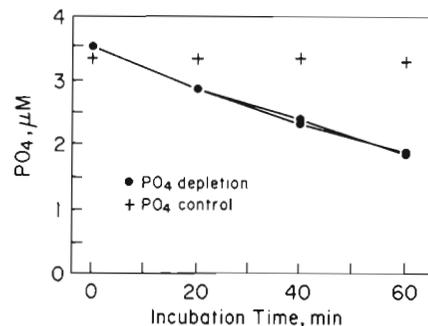


Fig. 6. *Linuche unguiculata*. Time course of depletion of PO_4 by 10 intact medusae in duplicate bottles (125 ml). Control bottles had no medusae

Phosphate depletion studies

Preliminary experiments showed that *Linuche unguiculata* medusae were able to deplete seawater of PO_4 in the light (Fig. 6). Two additional experiments showed that freshly collected medusae had similar depletion rates when incubated in the light or the dark (L:D = 0.9, $n = 4$). *L. unguiculata* held in the light (either starved or fed) showed only a slight decrease in depletion rates during 4 d (Fig. 7). By contrast, medusae held in the dark showed net release of PO_4 by Day 4.

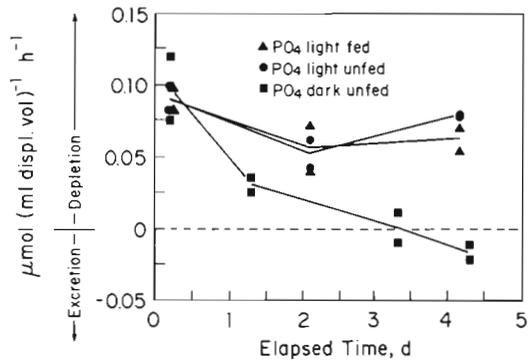


Fig. 7. *Linuche unguiculata*. PO_4 depletion rates of medusae held in the dark or ambient light (fed or unfed). Initial concentration was $4 \mu\text{M}$

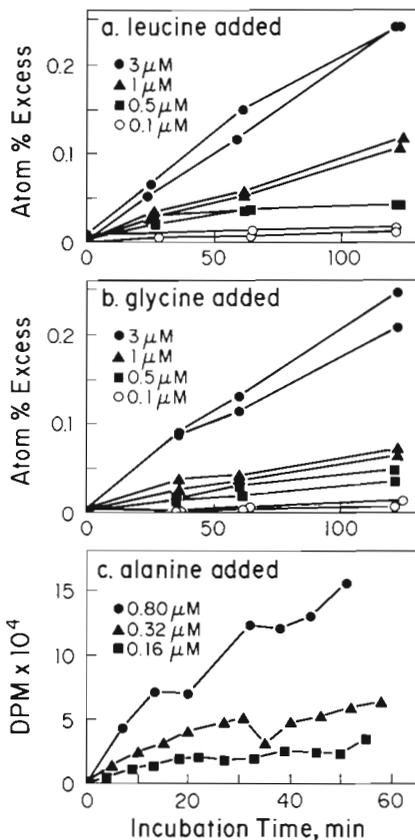


Fig. 8. *Linuche unguiculata*. Time course of uptake by intact medusae of (a) ^{15}N -leucine, (b) ^{15}N -glycine and (c) ^{14}C -alanine

Uptake of dissolved amino acids

Uptake by intact medusae of ^{15}N -labelled leucine and glycine and ^{14}C -labelled alanine was linear over time (Fig. 8). Therefore, the slopes of the linear regressions through these and additional uptake data were used to calculate uptake as a function of concentration for 3 amino acids (Fig. 9). A linear relationship between

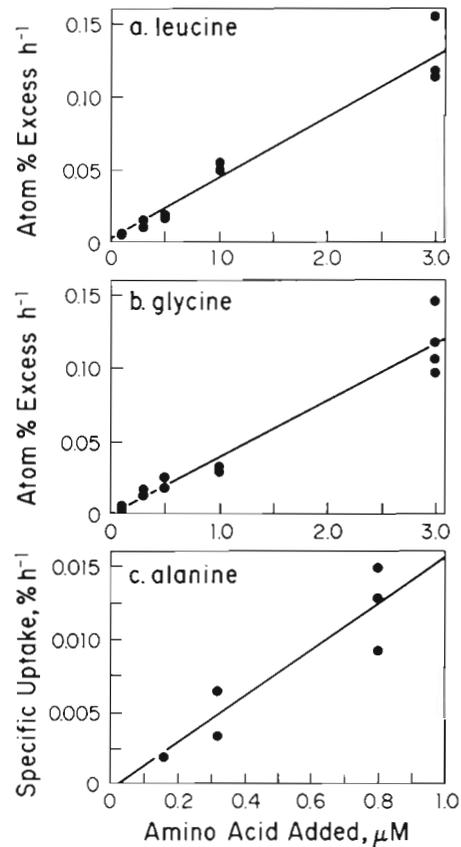


Fig. 9. *Linuche unguiculata*. Amino acid uptakes (biomass specific V) for medusae were measured for a range of concentrations of (a) ^{15}N -leucine, (b) ^{15}N -glycine and (c) ^{14}C -alanine supplied for 1 h

uptake rate and substrate concentration for both ^{15}N -labelled leucine and glycine was demonstrated for substrate concentrations ranging higher than ambient levels (K. Mopper pers. comm.). Therefore, the regression lines derived from at. % excess (Fig. 9) were used to estimate the nitrogen-specific uptake velocity ($\% \text{h}^{-1}$). For alanine, the nitrogen-specific uptake was calculated from the measured uptake of ^{14}C (dpm) knowing the specific activity of the ^{14}C -labelled alanine, the ratio of ^{14}C -alanine to unlabelled alanine in the nutrient spike, the amount of nitrogen in the medusae and the concentration of alanine in unspiked incubation water ($< 50 \text{ nM}$).

Fractionation of medusae into animal (supernatant) and algal components showed that both fractions took up ^{15}N from the amino acids, but in contrast to the results for $^{15}\text{NH}_4$ uptake (Fig. 3), uptake was greater in the animal fraction (Fig. 10). The animal:algal uptake ratio based on the slopes of the linear regressions from 9 time-series gave a measurement of the relative uptake rate by the 2 fractions. For leucine this animal:algal uptake ratio averaged 1.3 (range 1.2 to 1.7, $n = 6$),

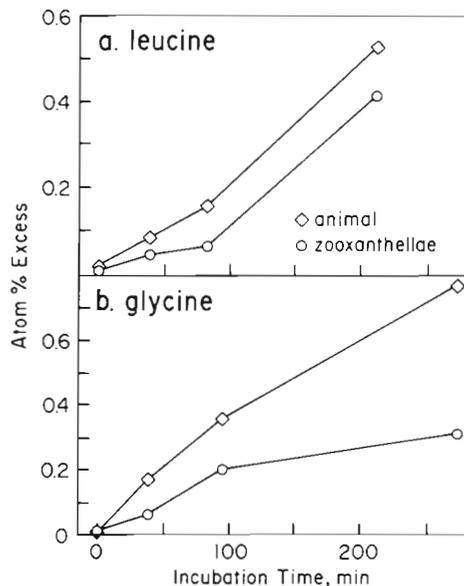


Fig. 10. *Linuche unguiculata*. Time course of ^{15}N enrichment of zooxanthellae and 'animal' fractions after incubation in (a) leucine and (b) glycine at $3\ \mu\text{M}$

glycine averaged 1.9 (range 1.1 to 2.6, $n = 3$), and alanine averaged 2.0 (ratios = 1.4 and 2.6, $n = 2$).

DISCUSSION

Uptake of DIN

This study documents the uptake of dissolved inorganic and organic nutrients by the medusa *Linuche unguiculata*, a pelagic non-calcareous symbiosis. The tropical waters where *L. unguiculata* occurs have concentrations of NO_3 , NH_4 and PO_4 of less than $1\ \mu\text{M}$; as low as reported for coral reef study sites (e.g. Muscatine 1980, Propp 1981, Ferrer & Szmant 1988, Bythell 1990) and near-shore waters where tropical symbiotic anemones have been studied (Muller-Parker et al. 1990). The upper estimate for percent new production ($f = 10\%$) obtained from substrate-saturated ^{15}N uptake by the free-living phytoplankton of Bahamian waters emphasizes their oligotrophic state. Primary production in these waters is most likely to result from recycled nutrients (e.g. ammonium and urea; Dugdale & Goering 1967) rather than being driven by new sources (i.e. nitrate). *L. unguiculata* can obtain dissolved inorganic and organic nutrients from surrounding waters, as well as from heterotrophic ingestion of food. In addition, the symbiotic association prevents catabolic losses of nutrients (excretion) into the surrounding waters.

Linuche unguiculata showed preference for

ammonium uptake over nitrate, with some evidence of spike uptake of ammonium (not shown), both of which are common in marine symbioses (e.g. Summons & Osmond 1981, Wilkerson & Trench 1986). We were able to measure nitrate uptake by *L. unguiculata*, the first study to demonstrate uptake of nitrate by a non-calcareous cnidarian. Earlier studies with different taxa which failed to show nitrate uptake relied solely on 'depletion' incubations (e.g. Muscatine & Marian 1982, Wilkerson & Muscatine 1984) and did not utilize ^{15}N . In this study the sensitivity of the ^{15}N approach documented rates of NO_3 uptake that were about an order of magnitude less than ammonium uptake rates. NO_3 uptake rates were not increased by holding medusae for 4 d in seawater enriched with 10 to $15\ \mu\text{M}$ NO_3 (not shown).

Typically, uptake vs concentration kinetics of intact symbioses do not follow the Michaelis-Menten pattern. Presumably the algal kinetics are masked by the animal physiology (D'Elia & Cook 1988). The high ratio of algal to animal tissue in *Linuche unguiculata* may diminish this. Zooxanthellae constitute 32% of the total organismal nitrogen in *L. unguiculata*, compared to corals: 5% in *Montastrea annularis* or 8% in *Acropora cervicornis* (Szmant et al. 1990). The atomic C:N:P ratio of 91:15:1 for intact *L. unguiculata* calculated from our particulate phosphorus measurements and the CHN data of Kremer et al. (1990) more closely resembles that for phytoplankton of 106:16:1 (Redfield 1958) than that typical of gelatinous zooplankton of 166:37:1 (Schneider 1990).

Most of the previous studies of ammonium uptake by cnidarian-algal symbioses have reported uptake (on a chl *a* basis) from experiments in which ammonium depletion is monitored, usually from an initial concentration of $10\ \mu\text{M}$. For this reason we have used this parameter (V^{10} - chl-*a*-specific uptake of ammonium from a $10\ \mu\text{M}$ spike) for comparing rates of uptake by *L. unguiculata* with other marine symbioses (Table 3). For the reports in which Michaelis-Menten constants are given we have calculated V^{10} from the expression $V = V_{\text{max}}S/(K_s + S)$. The value of V^{10} for intact medusae is intermediate between the low uptake rate for intact *Aiptasia pulchella* and the high ammonium uptake by intact *Mastigias* sp., and fits well with the coral data. There is no comparable value available for our V^{10} for the intact zooxanthellae fraction (i.e. that calculated from ^{15}N incorporation by the zooxanthellae fraction). This value is considerably lower than for the intact *L. unguiculata* association since there is uptake of $^{15}\text{NH}_4$ into the non-chlorophyll containing animal fraction (Fig. 3). The freshly isolated zooxanthellae from *L. unguiculata* show greater uptake per unit chl *a* (V^{10}) than the intact zooxanthellae within the association, with values that are similar to most of the estimates for

Table 3. Values of V^{10} (uptake from 10 μM) for NH_4 uptake in symbiotic organisms

Organism	V^{10} ($\mu\text{mol mg chl}^{-1} \text{h}^{-1}$)	Source
Medusae		
<i>Linuche unguiculata</i>		
Intact	12.2	Depletion data, this study
Zooxanthellae fraction	2.3	Uptake data, this study
Isolated zooxanthellae	5.8	Uptake data, this study
<i>Mastigias</i> sp.		
Intact	53.3	Muscatine & Marian (1982)
Isolated zooxanthellae ^a	35.2	Muscatine & Marian (1982)
Anemones		
<i>Aiptasia pulchella</i>		
Intact	3.7	Wilkerson & Muscatine (1984)
Isolated zooxanthellae	13.4	
Corals		
<i>Pocillopora damicornis</i> ^a		
	6.4	Muscatine & D'Elia (1978)
<i>Pocillopora meandrina</i> ^a		
	5.9	Muscatine & D'Elia (1978)
<i>Stylophora pistillata</i>		
	6.7	Rahav et al. (1989)
Freshly isolated zooxanthellae from^a:		
<i>Zoanthus sociatus</i>	7.8	D'Elia et al. (1983)
<i>Z. sociatus</i>	4.3	
<i>Zoanthus</i> sp.	12.8	
<i>Tridacna crocea</i>	7.6	
<i>T. crocea</i>	7.6	
<i>T. crocea</i>	10.0	
<i>T. crocea</i>	21.1	
<i>Seriatopora hystrix</i>	4.9	
<i>Montastrea annularis</i>	7.0	

^a V^{10} calculated using published Michaelis-Menten constants

freshly isolated zooxanthellae from a variety of hosts reported by D'Elia et al. (1983) but less than the higher values reported for zooxanthellae isolated from *A. pulchella* and *Mastigias* sp. The difference in uptake rates by intact *L. unguiculata* and the algae may be due to a combination of the acquisition rate by the animal, presumably diffusion and a low concentration made available to the algae.

Uptake of PO_4

In addition to DIN uptake *Linuche unguiculata* was able to remove PO_4 from incubation water. Uptake and retention of phosphate has been described for hermatypic corals (Pomeroy & Kuenzler 1969, D'Elia 1977), the anemones *Aiptasia pallida* (Muller-Parker et al. 1990) and *Anemonia sulcata* (Wilkerson 1980) and for isolated (Muller-Parker et al. 1988) and cultured (Deane & O'Brien 1981) zooxanthellae. Our short-term experiments showed no effect of darkness on PO_4 uptake by *Linuche unguiculata* in agreement with results for *Aip-*

tasia pallida (Muller-Parker et al. 1990) and in contrast to those for corals (D'Elia 1977). However, long-term dark experiments with *L. unguiculata* agreed with results from corals (D'Elia 1977) showing decreased uptake and eventually excretion. Phosphate excretion was also measured in the anemones *Condylactis* sp. (Cates & McLaughlin 1979) and *Aiptasia pallida* but in the latter, excretion was due to zooplanktonic feeding not dark treatment (Muller-Parker et al. 1990). *L. unguiculata* supplied with zooplanktonic food, depleted PO_4 at a similar rate to starved medusae. No excretion was measured in medusae exposed to an ambient light cycle.

Uptake of amino acids

This study demonstrated that *Linuche unguiculata* medusae can take up dissolved organic matter (DOM) in the form of amino acids. Both glycine and alanine (2 of the 3 amino acids used in this study) are among the most abundant forms of amino acids in seawater.

Therefore our results suggest that amino acid uptake could represent a major means of nitrogen acquisition. Several other studies have established DOM uptake by cnidarians with and without algal symbionts (Stephens 1962, Shick 1975, Schlichter 1982, Ferguson 1988) but few have attempted to quantify the uptake at ecologically reasonable concentrations and evaluate the results in terms of energetic or nitrogen needs. Schlichter (1982) calculated that the symbiotic soft coral *Heteroxenia fuscescens* could meet 80% of its energy demands of respiration from DON uptake with a total amino acid concentration of 2 μM . Shick (1975) calculated that a concentration of 0.8 μM glycine would supply scyphistomae of *Aurelia aurita* (no algal symbionts) with 0.4 to 1.3% of its body nitrogen per day. Shick's rates are essentially identical to our results (Table 4).

¹⁵N-incorporation into animal and algal fraction

Fractionation procedures showed that both 'animal' and algal fractions were labelled within a few minutes in ¹⁵N uptake experiments. When ¹⁵NH₄ was used, the label appeared preferentially in the zooxanthellae. The presence of ¹⁵N in 'animal' tissue after incubation in ¹⁵NH₄-spiked seawater is consistent with both the diffusion-depletion hypothesis (D'Elia 1977, D'Elia et al. 1983) and the possibility that ¹⁵N is held in regulatory pools being maintained and released by animal enzyme activity, according to the scenario described by Rees (1987) for green hydra. When ¹⁵N-labelled amino acids were used, the label appeared predominantly in the animal fraction. Fitzgerald & Szmant (1989) found a similar distribution after incubation with ³H-labelled amino acids; over 60% of ³H from added valine, glutamine and lysine was found in the supernatant (animal) fraction of corals. In our results, there was no

evidence of net translocation of ¹⁵N. The nature of our preliminary experiments precludes interpretation as to what may be occurring biochemically to the incorporated N but indicates that the system is more complicated than previously believed.

Nitrogen budgets

Different strategies are used by marine symbioses to meet their nitrogen requirement. Odum & Odum (1955) first indicated that zooxanthellae of corals rely to a great extent on animal-recycled NH₄ and Rahav et al. (1989) showed that 90% of the zooxanthellae requirement of *Stylophora pistillata* for nitrogen was provided in this way. Interestingly, ammonium uptake by *Mastigias* sp. in saline lakes with a strong nutricline was enough to satisfy its daily nitrogen requirement (Muscatine & Marian 1982), whereas DIN uptake by *Acropora palmata* contributed only 30% to the coral's average daily N requirement (Bythell 1988). In Table 4 the uptake rates for *Linuche unguiculata* for ammonium, nitrate, glycine, and phosphate are calculated for substrate concentrations of 1 μM and for ambient concentrations. If an upper limit for the ambient total dissolved amino acid pool is taken to be 500 nM and glycine uptake is assumed to be representative of all amino acid uptake, then nitrogen-specific uptake of amino acids (DON) by *L. unguiculata* may reach up to 0.5% d⁻¹. This is about the same as ammonium uptake (calculated from depletion data), whereas nitrate uptake is an order of magnitude lower. Therefore, *L. unguiculata* can obtain about 1% d⁻¹ of its standing stock of nitrogen from dissolved nitrogen uptake alone and 3.6% of its phosphorus from phosphate uptake. Although this is of the same magnitude as their nitrogen demands for growth (3% d⁻¹ for both animal and zooxanthellae growth; Kremer et al. 1990) and reproduction (about 2% for egg production; Kremer pers. comm.), it is likely that holozoic feeding provides the bulk of the nitrogen to the association. Taylor (1984) demonstrated 'back translocation' of ¹⁵N and ¹⁴C (derived from labelled zooplanktonic food) from a flatworm host (*Amphicolops* sp.) to its endosymbiotic algae. A single preliminary experiment recovered ¹⁵N from ingested food in the zooxanthellae of *L. unguiculata* (Wilkerson & Kremer unpubl.). A detailed accounting of nitrogen acquisition from ingestion to the anemone *Anthopleura elegantissima* was carried out by Zamer & Shick (1989). The next step is to accomplish this for *L. unguiculata* and complete our nitrogen and phosphorus budgets for this pelagic symbiosis.

Cnidarians without endosymbiotic algae show net excretion of nutrients (e.g. Szmant-Froelich & Pilson 1977, Muscatine et al. 1979, Muscatine & Marian 1982,

Table 4. *Linuche unguiculata*. Comparison of biomass-specific (either as PON or total P) uptake rates for 4 dissolved nutrients at 1 μM and the ambient concentration. Daily rates were calculated from hourly values $\times 24$

Nutrient	Specific uptake at 1 μM (% d ⁻¹)	Ambient conc. (μM)	Specific uptake at ambient conc. (% d ⁻¹)
NH ₄	2.4	0.2	0.48
NO ₃ ^a	0.1	0.4	0.05
Glycine	1.0	0.1	0.10
PO ₄ ^a	11.9	0.3	3.60

^a Calculated from rates at higher concentrations assuming linear relationship between concentration and rate

Wilkerson & Muscatine 1984, McAuley 1990). The large zooxanthellae component of *Linuche unguiculata* apparently enables these dissolved nutrients to be sequestered. This capability for dissolved nutrient uptake prevents loss of nitrogen and phosphorus from the association to the surrounding water and is of major significance for *L. unguiculata*. This adaptation (harboring zooxanthellae) helps support high growth and reproductive rates for *L. unguiculata* in oligotrophic waters.

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