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Effects of host feeding and dissolved ammonium on cell division and nitrogen status of zooxanthellae in the hydroid *Myrionema amboinense*

Abstract There is a relationship between host feeding, nitrogen status and mitotic activity of zooxanthellae symbiotic with the marine hydroid *Myrionema amboinense*. Decreases in the mitotic index of zooxanthellae in starved *M. amboinense*, and in internal pool sizes of glutamine and glutamate, amino acids involved in ammonium assimilation via the glutamine synthetase–glutamate synthase (GS/GOGAT) pathway, were partially restored by addition of ammonium chloride to seawater in which hydroids were incubated. Levels of glutamine were more sensitive to host starvation than levels of glutamate, resulting in a decrease in the glutamine:glutamate molar ratio to that found in zooxanthellae cultured on nitrate. Hydroids starved for 5 d and then incubated in different concentrations of ammonium chloride showed a positive correlation between ammonium concentration and mitotic index of their symbiotic zooxanthellae. Host starvation caused a decrease in perturbation of levels of glutamine and glutamate during ammonium assimilation, as well as decreases in rates of assimilation of [^{14}C]-leucine into TCA-insoluble protein, and in photosynthetic incorporation of [^{14}C]-bicarbonate. These observations suggest that host starvation reduces nitrogen supply to the zooxanthellae, causing nitrogen stress to the symbionts and reduction in metabolic processes associated with nitrogen assimilation and photosynthesis as well as with cell division.

Introduction

Regulation of symbiont population size is important in sustaining a stable relationship in algal/invertebrate symbioses. In constant conditions, populations of symbionts are maintained at constant, predictable levels (Pardy 1974; Muscatine and Pool 1979), although when isolated in culture, growth rates of symbionts may be up to ten times higher than those of their hosts (Douglas and Smith 1984; Cook and D'Elia 1987). Expulsion of excess symbionts appears to be trivial in most symbioses (McAuley 1981; Hoegh-Guldberg et al. 1987), so that maintenance of a constant density of the symbiont population must depend upon regulation of the growth rate of the symbionts to that of their host.

It has been suggested that in symbioses between dinoflagellates (zooxanthellae) and marine invertebrates, cell division of symbiotic zooxanthellae may be limited by the supply of nitrogen (Cook and D'Elia 1987). Starvation of the sea anemone *Aiptasia pallida* resulted in nitrogen stress of the zooxanthellae, as shown by decreased growth rate, population density and chlorophyll *a* content, and increased C:N (Cook et al. 1988), changes in the uptake kinetics of methylamine (D'Elia and Cook 1988), and increased ammonium enhancement of dark carbon fixation (Cook et al. 1992). Conversely, nitrogen flux, and presumably the amount of nitrogen available to symbiotic zooxanthellae, was increased by feeding in the coral *Astrangia danae*, and fed corals supported a greater density of zooxanthellae (Szmant-Froelich and Pilson 1977, 1984). Muscatine and Marian (1982) observed that zooxanthellae symbiotic with jellyfish in a stratified lake in Palau showed a peak of mitotic activity associated with migration of their hosts to the ammonium-rich thermocline. Addition of ammonium to ambient seawater increased the population density of zooxanthellae in a number of coral species (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989, Dubinsky et al. 1990; Stambler et al. 1991; Hoegh-Guldberg 1994; Muller-Parker et al. 1994). Similarly, addition of phosphate and ammonium stimulated mitosis of zooxanthellae in unfed *Aiptasia pallida* (Cook et al. 1988). Clearly, it is of considerable interest to determine the nitrogen status of symbiotic zooxanthellae, and correlate changes in nitrogen status with changes in the mitotic index.

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In the marine hydroid *Myrionema amboinense*, zooxanthellae show an increase in mitotic activity associated with host feeding; when hosts are starved this peak is progressively reduced in size, but reduction may be slowed by supplementing the seawater with ammonium (Cook and Fitt 1990; Fitt and Cook 1990). The aim of this paper is to investigate the effect of feeding, starvation and ammonium supplementation on mitosis and the nitrogen status of the symbiotic zooxanthellae, as evaluated by measuring the molar ratio of internal pools of the amino acids glutamine and glutamate (Flynn et al. 1989; Flynn 1990) of zooxanthellae symbiotic with *M. amboinense*.

Materials and methods

Organisms

Colonies of the athecate hydroid *Myrionema amboinense* were collected from a mangrove pond, Coney Island, Bermuda (Thomas et al. 1992), and maintained in 0.45 μm-filtered low-nutrient Sargasso sea water (FSW) at 25°C in a 12 h light:12 h dark diurnal cycle [60 μmol photons m^{-2} s^{-1} photosynthetically available radiation (PAR)]. Colonies were fed daily at the beginning of the dark period on freshly hatched nauplii of *Artemia* sp., or starved in FSW±20 μ*M* ammonium chloride. Seawater was changed daily in all treatments, and all experiments were conducted in the same incubator as the stock colonies.

A cultured strain of *Symbiodinium* sp. zooxanthellae, originally isolated from the sea anemone *Anemonia pallida*, was maintained in ES medium with nitrate as the nitrogen source (Provasoli 1966) in an illuminated constant temperature incubator at 25°C±1C°, 12 h light:12 h dark photoperiod, 60 μmol photons m^{-2} s^{-1}.

Isolation of symbiotic zooxanthellae

Excised hydranths of hydroids of *Myrionema amboinense* were homogenised in FSW using glass micro-tissue homogenisers, and the resulting homogenate was centrifuged twice to remove animal debris from the pellet of zooxanthellae. The zooxanthellae were resuspended, passed through 60 μm-mesh plankton netting, and recentrifuged. This gave a suspension which microscopical examination revealed to consist of unclumped zooxanthellae cells free of animal contamination. Cell densities and mitotic indices (measured as percent cells in the doublet stage with a cell plate (Wilkerson et al. 1983) of zooxanthellae were determined using an improved Neubar brightfield haemocytometer. Mitotic indices were measured at 07.00 hrs, at the start of the light period; in each sample, at least 1000 cells were examined for the presence of doublets indicative of division.

Extraction and measurement of free amino acid pools

Samples of zooxanthellae, isolated from hydranths of fed, starved, or starved ammonium-treated colonies of *Myrionema amboinense*, were extracted for 24 h at 4°C in 80% ethanol (v:v). Extracts were dried at 70°C and resuspended in an appropriate volume of 12.5 μ*M* α-amino-butyric acid (AABA), which acted as internal standard. Drying in ethanol did not alter the content of a mixture of amino acids used as standard. Amino acid content of aliquots of extracts were determined using reverse-phase high-performance liquid chromatography (HPLC), as previously described (McAuley 1992). The glutamine peak was clearly distinguishable from that of an unknown non-protein amino acid which has been shown to co-elute with either glutamine or histidine in several other amino acid HPLC systems (Flynn and Flynn 1992).

Uptake of [^{14}C]-leucine by freshly isolated zooxanthellae, and incorporation into trichloracetic acid-soluble and insoluble fractions

Freshly isolated zooxanthellae, isolated from 20 freshly excised hydranths of *Myrionema amboinense* as described in above subsection "Isolation of symbiotic zooxanthellae", were suspended in FSW (0.7 to 1.0×10^6 cells ml^{-1}) containing 1.0 μ*M* U-[^{14}C]-leucine (specific activity 11.6 GBq $mmol^{-1}$, NEN Research Products). [^{14}C]-leucine has been shown to be rapidly taken up and incorporated into trichloracetic acid (TCA) insoluble (protein) fractions of free-living and symbiotic *Chlorella* sp. (Richards and Thurston 1980; McAuley 1988). Suspensions were incubated in glass centrifuge tubes under similar conditions of light and temperature to those in which hydroid colonies were maintained. Partition of [^{14}C]-leucine into protein (TCA-insoluble) and pool (TCA-soluble) fractions was determined from the total uptake and amount retained after extraction in 5% TCA (McAuley 1987). Radioactivity was determined by adding 10 ml Ecolume scintillant to filter papers, and counted using a United Technologies/Packard 4530 scintillation counter with corrections for quench and background using the external standard of the counter calibrated against a quenched ^{14}C series.

Photosynthesis of isolated symbionts

We used [^{14}C]-bicarbonate to measure photosynthesis by zooxanthellae isolated from colonies of *Myrionema amboinense* that had been fed, starved in FSW or starved in 20 μ*M* NH^+_4 for 6 d. Zooxanthellae were isolated from 20 hydroids as described earlier, and replicate samples of the resulting suspensions were adjusted to a volume of 1 ml in glass scintillation vials; cell densities were 5.31×10^5 (fed), 2.46×10^5 (starved), and 0.75×10^5 (ammonium) $cell^{-1}$ ml^{-1}. Following the addition of 1 μCi of NaH $^{14}CO_3$ (58 mCi $mmol^{-1}$), the vials were placed on a light table (180 μmol photons m^{-2} s^{-1}, determined with a Li-Cor 192a cosine-corrected quantum sensor fitted with a LI-1000 data logger) and incubated for 30 min. The suspensions were counted in vials following acidification and neutralization as described by Cook et al. (1992).

Results

Effect of host feeding and ammonium supply on mitotic index and amino acid pools of zooxanthellae

Colonies of *Myrionema amboinense* were fed or starved in FSW, or starved in FSW supplemented with 20 μ*M* ammonium chloride. After 5 d of treatment, zooxanthellae were isolated, and mitotic indices and the glutamine and glutamate content of free amino acid pools were determined (Table 1). As previously reported (Fitt and Cook 1990), starvation of *M. amboinense* caused a reduction in the mitotic index of the symbiotic zooxanthellae compared to those from fed hydroids, which was partially restored by addition of ammonium. Zooxanthellae from starved hydroids, but not zooxanthellae from starved hydroids treated with ammonium, also possessed significantly lower pools of glutamine (one-way ANOVA; F=5.95, p=0.050) and glutamate (one-way ANOVA; F=7.75, p=0.032). Levels of glutamine (Gln) were more sensitive to host starvation than those of glutamate (Glu), resulting in a fall in the Gln:Glu molar ratio. There was no significant difference between glutamine (one-way ANOVA; F=1.87, p=0.220) and glutamate (one-way ANOVA; F=2.92, p=0.138) pool sizes of

Table 1 *Symbiodinium* sp. Effect of 5 d feeding or starvation of *Myrionema amboinense* in presence or absence of 20 μ*M* ammonium on mitotic index and glutamine and glutamate content of freshly isolated symbiotic zooxanthellae. Values are means ±SD of four replicate experiments. Levels of glutamine and glutamate from three replicate culture tubes of cultured *Aiptasia pallida* zooxanthellae in late logarithmic phase (1.0×10^6 cells ml^{-1}), grown with nitrate as a nitrogen source, are given for comparison

Treatment	Mitotic index (%)	fmol amino acid $cell^{-1}$		
		glutamine (Gln)	glutamate (Glu)	Gln:Glu
Cultured	–	1.03±0.42	3.93±0.67	0.38±0.03
Fed	10.08±0.75	2.95±1.23	4.91±1.09	0.58±0.13
Starved	3.90±0.87	3.12±0.67**	1.32±0.53**	0.42±0.10*
Starved plus $NH4^+$	7.30±0.42	1.84±0.1.06	3.65±0.99	0.50±0.20

* Significantly different from fed values (ANOVA) at *$p< 0.10$ and ** $p< 0.05$, respectively

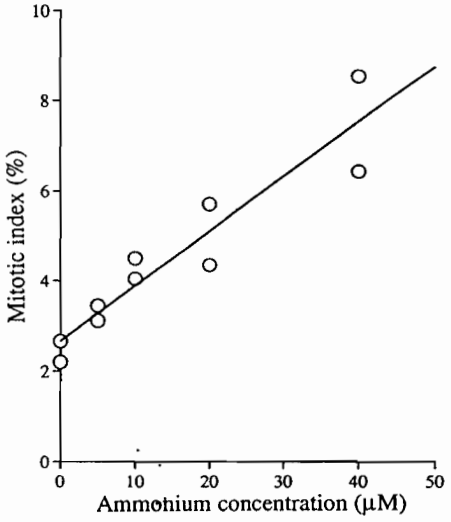

Fig. 1 *Symbiodinium* sp. Relationship between ammonium concentration in ambient seawater and mitotic index of freshly isolated zooxanthellae from *Myrionema amboinense* after 24 h treatment of colonies previously unfed for 5 d. $y=2.67+0.12x$; $r=0.94$; $p< 0.001$

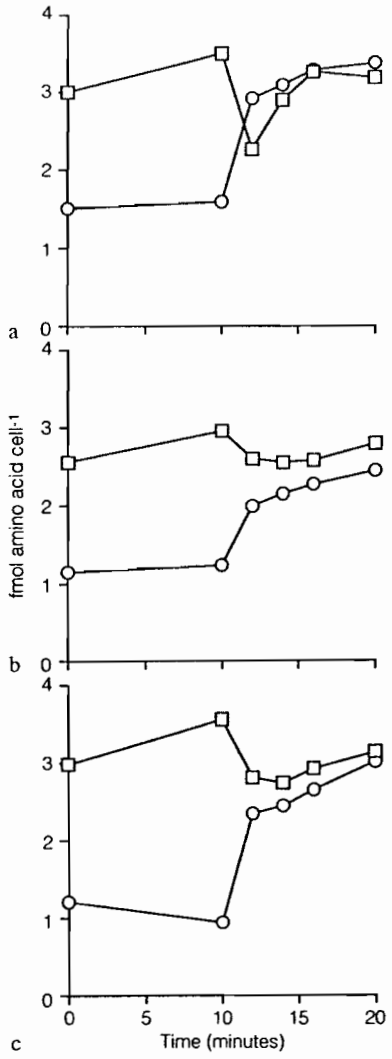

Fig. 2 *Symbiodinium* sp. Effect of addition of ammonium on glutamine (○) and glutamate (□) pools of zooxanthellae freshly isolated from colonies of *Myrionema amboinense* which had been fed (**a**), starved (**b**), or starved in filtered seawater (FSW) supplemented with 20 μ*M* ammonium chloride (**c**). Ammonium chloride was added to give a final concentration of 100 μ*M* 10 min after start of incubation

zooxanthellae isolated from fed and starved, ammonium-treated hydroids. The levels of these two amino acids were also measured in a cultured strain of zooxanthellae grown with nitrate as a nitrogen source, and were found to be similar to levels in zooxanthellae from starved colonies of *M. amboinense*.

To examine the relationship between the N status and mitotic index of zooxanthellae, colonies of *Myrionema amboinense* which had been starved for 5 d were incubated for 1 d in FSW containing different concentrations of ammonium, and mitotic indices of freshly isolated zooxanthellae were then determined. A positive correlation ($r=0.94$, $p< 0.001$) was found between the concentration of ammonium in which colonies were incubated and the mitotic indices of their symbiotic zooxanthellae (Fig. 1).

Effect of host starvation on assimilation of ammonium, amino acid incorporation and photosynthesis by zooxanthellae

There were differences in short-term ammonium assimilation, as shown by changes in glutamine and glutamate pools after addition of 100 μ*M* ammonium chloride, in zooxanthellae isolated from *Myrionema amboinense* colonies which had either been fed or starved, or starved in FSW supplemented with ammonium (Fig. 2). In zooxanthellae from all three treatments, addition of ammonium caused an increase in glutamine, initially at the expense of the glutamate pools, but initial rates of incorporation of ammonium into glutamine were highest in zooxanthellae from fed *M. amboinense* colonies and lowest in zooxanthellae

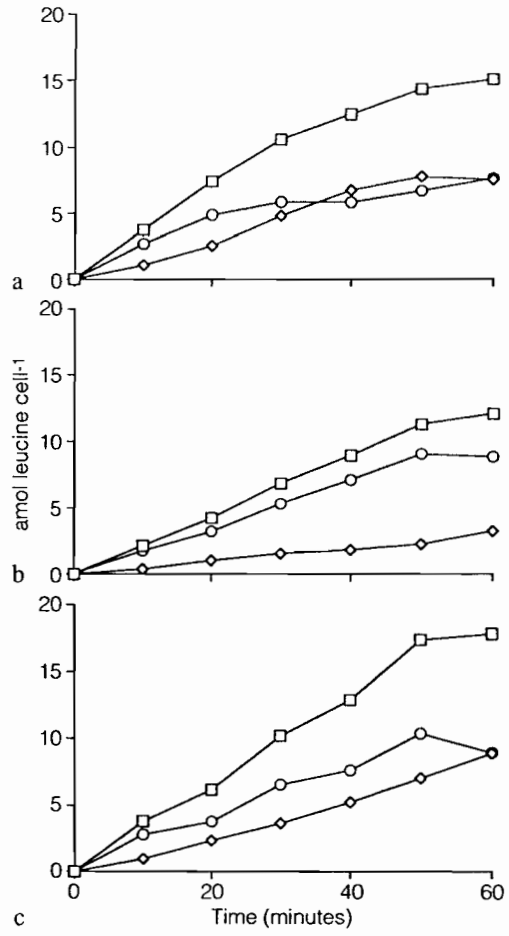

Fig. 3 *Symbiodinium* sp. Total uptake (□) and partitioning into TCA-insoluble fraction (protein) (◇) and TCA-soluble fraction (○) of [^{14}C]-leucine by zooxanthellae from *Myrionema amboinense* which had been fed (**a**), starved (**b**), or starved in FSW supplemented with 20 μ*M* ammonium chloride (**c**)

Table 2 *Symbiodinium* sp. Photosynthesis of zooxanthellae isolated from *Myrionema amboinense* after 6 d of feeding, or 6 d starvation in presence or absence of 20 μ*M* NH_4^+. Statistical probabilities from Tukey–Kramer between-groups ANOVA: fed vs starved = $p< 0.001$; NH_4^+ vs starved = $p< 0.001$; fed vs NH_4^+ = NS, $p>0.05$

Treatment	Photosynthetic rate (pg C cell^{-1} h^{-1})	(*N*)
Fed	1.83 ± 0.12	(5)
Starved	1.12 ± 0.14	(2)
Starved plus NH_4^+	1.62 ± 0.02	(2)

from unfed colonies. In all cases, glutamate pools were restored to initial levels within 10 min of ammonium addition.

Measurement of [^{14}C]-leucine uptake showed that incorporation of newly assimilated amino acid into the TCA-insoluble fraction (protein) was more rapid in zooxanthellae freshly isolated from fed colonies than from starved colonies (Fig. 3). Differences in the rates of incorporation may be due to differences in dilution of [^{14}C]-leucine by existing pools of [^{12}C]-leucine within the zooxanthellae; large internal [^{12}C]-leucine pools would cause a decrease in incorporation of [^{14}C]-leucine. However, HPLC analysis showed that leucine pools were slightly smaller in zooxanthellae from starved *Myrionema amboinense* (0.50±0.20 fmol cell^{-1}) compared to those from fed *M. amboinense* (0.86±0.52 fmol cell^{-1}). Initial rates of uptake of leucine (measured over the first 30 min, Fig. 3) were also highest in zooxanthellae from fed colonies (21.4 amol cell^{-1} h^{-1}) and lowest in those from starved colonies (13.5 amol cell^{-1} h^{-1}).

Zooxanthellae from *Myrionema amboinense* which had not been starved for 6 d had lower cell-specific photosynthetic rates than those from either daily-fed colonies or those which had been starved in 20 μ*M* NH_4^+ for 6 d (Table 2). Photosynthesis by zooxanthellae from starved colonies was 61% less than that of zooxanthellae from fed colonies and 68% of that of zooxanthellae from colonies kept in ammonium ($p< 0.001$ for photosynthetic rates; Tukey–Kramer post-hoc analysis of variance). There was no difference in the photosynthetic rates of zooxanthellae from fed colonies and those kept in ammonium ($p>0.05$).

Discussion

We found a clear relationship between host nitrogen supply and mitotic indices and the N status of zooxanthellae symbiotic with *Myrionema amboinense*. The mitotic index, and sizes of glutamine and glutamate pools, the amino acids primarily involved in ammonium assimilation via the GS/GOGAT pathway (Miflin and Lea 1976), were higher in zooxanthellae freshly isolated from fed or ammonium-supplemented colonies of *M. amboinense* than in colonies starved in seawater lacking dissolved inorganic nitrogen. In the case of ammonium addition, there was also a strong positive correlation between stimulation of the mitotic index and the concentration of ammonium in which *M. amboinense* colonies were incubated, suggesting that cell division of the zooxanthellae was directly influenced by availability of a source of nitrogen external to the symbiosis.

It is believed that zooxanthellae utilise ammonium produced by host catabolism (Yonge and Nicholls 1931; Cates and McLaughlin 1979; Trench 1979; Steen 1986). However, comparison of nitrogen budgets and cellular growth rates in corals indicates that although efficient uptake of ammonium by symbiotic zooxanthellae prevents loss of nitrogen from the symbiosis to the medium, this internal recycling of ammonium is insufficient to support continued growth of the zooxanthellae (Rahav et al. 1989). The results described here provide further evidence that cell division of zooxanthellae symbiotic with *Myrionema amboinense* is stimulated by host feeding (Fitt and Cook 1990), and that it is limited by nitrogen supply when the host is not fed. Similar findings have been reported for the sea anemone *Aiptasia pallida* (Cook et al. 1988), although in

this case the decline in symbiont growth rate was much more gradual. Host feeding has little effect on the growth rate of zooxanthellae symbiotic with the reef coral *Stylophora pistillata* (Muscatine et al. 1989). These differences perhaps reflect the level of reserves in host tissue, which may diminish rapidly when *M. amboinense* is not fed, or they may reflect the relative ratio of zooxanthellae:host biomass in different symbioses. Those in which density of zooxanthellae is relatively high may be more dependent on external sources of nitrogen, either derived from host feeding or from dissolved inorganic nitrogen in seawater, to maintain the N status of the symbionts. There is evidence that in corals, the N status of symbiotic zooxanthellae, measured as ammonium enhancement of dark carbon fixation (Cook et al. 1994) or as chlorophyll *a* content (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989), is inversely correlated with the density of symbionts in host tissue.

Starvation of *Myrionema amboinense* caused a fall in the molar ratio glutamine:glutamate (Gln:Glu) of its symbiotic zooxanthellae. These amino acids are involved in the GS/GOGAT pathway of ammonium assimilation, which Summons and Osmond (1981) have shown to be in operation in zooxanthellae in vivo and in vitro. The temporary decrease in glutamate and the increase in glutamine observed immediately after addition of ammonium are indicative of the operation of this pathway in zooxanthellae freshly isolated from *M. amboinense* (Fig. 2 a). It has been suggested that, since the first product of ammonium assimilation via GS/GOGAT is glutamine, Gln:Glu is a sensitive indicator of nitrogen status in micro-algae (Flynn et al. 1989; Flynn 1990). Flynn et al. (1993) found that a number of species of free-living dinoflagellates responded to a decreased availability of nitrogen with a decrease in Gln:Glu. Conversely, maintenance of the coral *Pocillopora damicornis* in 20 or 50 μM ammonium chloride for up to 8 wk increased Gln:Glu in its symbiotic zooxanthellae (McAuley 1994). Gln:Glu in zooxanthellae freshly isolated from starved *M. amboinense* colonies were similar to those in zooxanthellae cultured in a seawater-based medium with nitrate as the N source. Micro-algae may be N-replete only in the presence of excess ammonium (Flynn 1990); growth on nitrate causes a degree of N stress because assimilation requires a rate-limited enzymatic step (Levasseur et al. 1993), and the Gln:Glu of microalgae is generally lower when grown on nitrate than on ammonium (reviewed in Flynn 1990). Wilkerson and Trench (1985) found that induction of nitrate reductase is slow in cultured zooxanthellae, and addition of ammonium to zooxanthellae cultured on nitrate increases Gln:Glu (McAuley unpublished results). However, while a Gln:Glu of ~0.4, found in both zooxanthellae from starved *M. amboinense* and in zooxanthellae cultured on nitrate, may be indicative of N stress, the degree of N stress caused by host starvation is not yet known.

We observed a decrease in photosynthesis by zooxanthellae of unfed *Myrionema amboinense* colonies compared to those from fed colonies. Cook et al. (1992) reported a similar finding with the zooxanthellae of *Aiptasia pallida*. In the case of *A. pallida*, zooxanthellae from 1 wk-starved anemones had similar photosynthetic rates to those of fed anemones, whereas those from anemones starved for 3 wk had significantly reduced rates. As with cell division rates, the effects of starvation on the photophysiology of the zooxanthellae from *M. amboinense* occurred more rapidly than in sea anemones.

We tested two other physiological indicators of N stress: the effect of short-term ammonium assimilation on glutamine and glutamate pools; and uptake and incorporation of amino acid into protein. Addition of ammonium to N-stressed algae usually causes an immediate decrease in glutamate and an increase in glutamine (Turpin and Harrison 1978; Flynn et al. 1989). Here, it was found that ammonium assimilation caused smaller fluctuations in glutamine and glutamate pools in zooxanthellae freshly isolated from starved colonies of *Myrionema amboinense* than in zooxanthellae from fed or starved, ammonium-treated colonies (Fig. 2). N-stress generally causes an increase in both uptake of amino acids and their incorporation into protein by micro-algae (North and Stephens 1971; Wheeler and Stephens 1977; McAuley 1987). However, in our experiments, host starvation reduced both uptake and incorporation into protein of [^{14}C]-leucine by the symbiotic zooxanthellae of *M. amboinense* (Fig. 3).

Zooxanthellae from 5 and 6 d-starved colonies have drastically lower growth rates, as measured by mitotic indices (Fitt and Cook 1990; and present Table 1), and we suggest that all cellular processes in these algae have been reduced. Thus, it appears that nitrogen stress of zooxanthellae symbiotic with *Myrionema amboinense* may reduce metabolic processes associated with nitrogen assimilation and with photosynthesis. Photosynthetic rates in zooxanthellae from starved hydroids may be limited by either chlorophyll *a* content, or enzymes involved with photosynthesis such as ribulose 1,5 biphosphate carboxylase oxygenose (RuBisCO). Both could have resulted from nitrogen limitation of algae from starved colonies of *M. amboinense*. Measurement of these physiological parameters and measurement of how the changes observed affect productivity of zooxanthellae requires further study, not only using zooxanthellae freshly isolated from symbiosis, but also using zooxanthellae grown in nitrogen-limited culture.

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References

Cates N, McLaughlin JJA (1979) Nutrient availability for zooxanthellae derived from physiological activities of *Condylactus* spp. J exp mar Biol Ecol 37:31–41

Cook CB, D'Elia CF (1987) Are natural populations of zooxanthellae ever nutrient-limited? Symbiosis 4:199–212

Cook CB, D'Elia CF, Muller-Parker G (1988) Host feeding and nutrient sufficiency for zooxanthellae in the sea anemone *Aiptasia pallida*. Mar Biol 98:253–262

Cook CB, Fitt WK (1990) Some effects of dissolved inorganic nutrients on the growth of zooxanthellae in the hydroid *Myrionema amboinese*. In: Nardon P, Gianinazzi-Pearson V, Grenier AM, Margulis L, Smith DC (eds) Endocytobiology. IV. INRA, Paris, pp 285–288

Cook CB, Muller-Parker G, D'Elia CF (1992) Ammonium enhancement of dark carbon fixation and nitrogen limitation in symbiotic zooxanthellae: effects of feeding and starvation of the sea anemone *Aiptasia pallida*. Limnol Oceanogr 37:131–139

Cook CB, Muller-Parker G, Orlandini CD (1994) Ammonium enhancement of dark carbon fixation and nitrogen limitation in zooxanthellae symbiotic with the reef corals *Madracis mirabilis* and *Montastrea annularis*. Mar Biol 118:157–165

D'Elia CF, Cook CB (1988) Methylamine uptake by zooxanthellae–invertebrate symbioses: insights into host ammonium environment and nutrition. Limnol Oceanogr 33:1153–1165

Douglas AE, Smith DC (1984) The green hydra symbiosis. VIII. Mechanisms in symbiont regulation. Proc R Soc (Ser B) 221: 291–319

Dubinsky Z, Stambler N, Ben-Zion M, McCloskey LR, Muscatine L, Falkowski PG (1990) The effect of external nutrient resources on the optical properties and photosynthetic efficiency of *Stylophora pistillata*. Proc R Soc (Ser B) 239:231–246

Fitt WK, Cook CB (1990) Some effects of host feeding on growth of zooxanthellae in the marine hydroid *Myrionema amboinese* in the laboratory and in nature. In: Nardon P, Gianinazzi-Pearson V, Grenier AM, Margulis L, Smith DC (eds) Endocytobiology. IV. INRA, Paris, pp 281–284

Flynn KJ (1990) The determination of nitrogen status in microalgae. Mar Ecol Prog Ser 61:297–307

Flynn KJ, Dickson DMJ, Al-Amoudi OA (1989) The ratio of glutamine:glutamate in microalgae: a biomarker for N-status suitable for use at natural cell densities. J Plankton Res 11:165–170

Flynn KJ, Flynn K (1992) Non-protein free amines in microalgae: consequences for the measurement of intracellular amino acids and of the glutamine/glutamate ratio. Mar Ecol Prog Ser 89:73–79

Flynn K, Flynn KJ, Jones KJ (1993) Changes in dinoflagellate intracellular amino acids in response to diurnal changes in light and N supply. Mar Ecol Prog Ser 100:245–252

Hoegh-Guldberg O (1994) The population dynamics of symbiotic zooxanthellae in the coral *Pocillopora damicornis* exposed to elevated ammonium. Pacif Sci 48:263–272

Hoegh-Guldberg O, McCloskey LR, Muscatine L (1987) Expulsion of zooxanthellae from symbiotic cnidarians from the Red Sea. Coral Reefs 7:113–116

Hoegh-Guldberg O, Smith GJ (1989) Influence of the population density of zooxanthellae and supply of ammonium on the biomass and metabolic characteristics of the reef corals *Seriatopora hystrix* and *Stylophora pistillata*. Mar Ecol Prog Ser 57:173–186

Levasseur M, Thompson PA, Harrison PJ (1993) Physiological acclimation of marine phytoplankton to different nitrogen sources. J Phycol 29:587–595

McAuley PJ (1981) Ejection of algae in the green hydra symbiosis. J exp Zool 217:23–31

McAuley PJ (1987) Nitrogen limitation and amino-acid metabolism of *Chlorella* symbiotic with green hydra. Planta 171:532–538

McAuley PJ (1988) Uptake of leucine by *Chlorella* symbionts of green hydra. Proc R Soc (Ser B) 234:319–332

McAuley PJ (1992) The effect of maltose release on growth and nitrogen metabolism of symbiotic *Chlorella*. Br Phycol J 27:417–422

McAuley PJ (1994) Amino acid content of zooxanthellae freshly isolated from *Pocillopora damicornis*. Pacif Sci 48:247–253

Miflin BJ, Lea PJ (1976) Review: the pathway of nitrogen assimilation in plants. Phytochem 15:873–885

Muller-Parker G, McCloskey LR, Hoegh-Guldberg O, McAuley, PJ (1994) Response of animal–algal biomass of the coral *Pocillopora damicornis* to seawater ammonium enrichment. Pacif Sci 48:273–283

Muscatine L, Falkowski PG, Dubinsky Z, Cook PA, McCloskey LR (1989) The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc R Soc (Ser B) 236:311–324

Muscatine L, Marian RE (1982) Dissolved inorganic flux in symbiotic and nonsymbiotic medusae. Limnol Oceanogr 27:910–918

Muscatine L, Pool R (1979) Regulation of numbers of intracellular algae. Proc R Soc (Ser B) 219:131–139

North BB, Stephens GC (1971) Uptake and assimilation of amino acids by *Platymonas*. II. Increased uptake by nitrogen-deficient cells. Biol Bull mar biol Lab, Woods Hole 140:242–254

Pardy RL (1974) Some factors affecting the growth and distribution of the algal endosymbionts of *Hydra viridis*. Biol Bull mar biol Lab, Woods Hole 147:105–118

Provasoli L (1966) Media and prospects for the cultivation of marine algae. In: Watanbe A, Hattori R (eds) Cultures and collections of algae. Tokyo, pp 63–75

Rahav O, Falkowski P, Dubinsky Z, Achituv Y (1989) Ammonium metabolism in the zooxanthellate coral *Stylophora pistillata*. Proc R Soc (Ser B) 236:325–337

Richards L, Thurston CF (1980) Uptake of leucine and tyrosine and their intracellular pools in *Chlorella fusca* var. *vacuolata*. J gen Microbiol 121:39–47

Stambler N, Popper N, Dubinsky Z, Stimson J (1991) Effects of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. Pacif. Sci 45:299–307

Steen RG (1986) Evidence for heterotrophy by zooxanthellae in symbiosis with *Aiptasia pulchella*. Biol Bull mar biol Lab, Woods Hole 170:267–278

Summons RE, Osmond CB (1981) Nitrogen assimilation in the symbiotic marine alga *Gymnodinium microadriaticum*: direct analysis of ^{15}N incorporation by GCMS methods. Phytochem 20: 575–578

Szmant-Froelich A, Pilson MEQ (1977) Nitrogen excretion by colonies of the temperate coral *Astrangia danae* with and without zooxanthellae. Proc 3rd int coral Reef Symp 1:417–423

Szmant-Froelich A, Pilson MEQ (1984) Effects of feeding frequency and symbiosis with zooxanthellae on nitrogen metabolism and respiration of the coral *Astrangia danae*. Mar Biol 81:153–162

Thomas MT, Logan A, Cook SB, Cook CB (1992) A guide to the shoreline and shallow-water marine communities of Bermuda. W. C. Brown, Dubuque, Iowa

Trench RK (1979) The cell biology of plant–animal symbiosis. A Rev Pl Physiol 30:485–531

Turpin DH, Harrison PJ (1978) Fluctuations in free amino acid pools of *Gymnodinium simplex* (Dinophyceae) in response to ammonia perturbation: evidence for glutamine synthetase pathway. J Phycol 14:461–464

Wheeler PA, Stephens GC (1977) Metabolic segregation of intracellular free amino acids in *Platymonas* (Chlorophyta). J Phycol 13:193–197

Wilkerson FP, Muller-Parker G, Muscatine L (1983) Temporal patterns of cell division in natural populations of endosymbiotic algae. Limnol Oceanogr 28:1009–1014

Wilkerson FP, Trench RK (1985) Nitrate assimilation by zooxanthellae maintained in laboratory culture. Mar Chem 16:385–393

Yonge CM, Nicholls AG (1931) Studies on the physiology of corals. IV. The structure, distribution and physiology of the zooxanthellae. Scient Rep Gt Barrier Reef Exped 1:135–176