UTILIZATION OF DISSOLVED INORGANIC NUTRIENTS OF ZOOXANTHELLAE CELLS OF TRIDACNID CLAMS

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ABSTRACT

There was a net daily uptake of ammonium and nitrogen by the zooxanthellae cells of Tridacnid clams from the natural sea water. This uptake was modulated according to nutritional environment, light environment, ammonium concentration and possible biological rhythmicity. The zooxanthellae cells take up dissolved inorganic nitrogen in the form of ammonia / ammonium (NH_3) or nitrate (NO_3), as shown by depletion of these from the medium. Rates of uptake were 10 times higher in light than in darkness. The zooxanthellae cells were reported to take up NH_3 at a higher rate than NO_3. In addition, this study demonstrates reduced rates of NO_3 uptake in the presence of NH_3. Zooxanthellae cells supplied with additional NH_3 and NO_3 showed two to three times higher division rates than those of the control.

INTRODUCTION

Giant clams (Family Tridacnidae) live in symbiosis with symbiotic dinoflagellates of the genus *Symbiodinium* (hereafter also referred to as zooxanthellae), closely related to those living in reef-building corals. The zooxanthellae in clams live extracellularly at high densities in the mantle and are able to transfer excess carbon from photosynthesis to host tissues, possibly in quantities that are sufficient to satisfy respiratory requirements in the tridacnid clams (Trench *et al.* 1981; Fisher *et al.* 1985; Klumpp *et al.* 1992; Klumpp & Lucas 1994).

Zooxanthellae in tridacnids are thought to be nitrogen-limited, based on their ability to take up ammonia and nitrate (Wilkerson & Trench 1986), increased amounts of ammonia will increase the photosynthesis rate (Summons *et al.* 1986) and increase growth rate of clams with addition of dissolved inorganic nitrogen (DIN) (Hastie & Heslinga 1988; Onate & Naguit 1989; Hastie *et al.* 1992).

Wilkerson & Trench (1986) demonstrated that tridacnids take up DIN (as nitrate or ammonia) in low concentrations from sea water; and the zooxanthellae are thought to be responsible for this uptake (D'Elia *et al.* 1983; Summons *et al.* 1986). It has also been suggested that assimilation by zooxanthellae of ammonium-nitrogen excreted by the host, and subsequent recycling of that nitrogen when released as amino acids, utilized by host tissues, is of central importance for the rapid growth of tridacnid clams (Wilkerson & Trench 1986; Lucas 1994).

The purpose of this study was to document the rates of depletion of DIN by zooxanthellae from *Tridacna squamosa*, and monitor concurrent changes in zooxanthellae division rates.

MATERIAL AND METHODS

Zooxanthellae cells

A piece of the mantle tissue of giant clam, *T. squamosa*, was cut. Zooxanthellae cells were extracted from the mantle by maceration using a Teflon tube homogeniser. The homogenate was filtered through a 20 µm mesh nylon cloth to remove animal tissue debris. The filtrate was centrifuged repeatedly at about 280 G until the

supernatant was clear. The supernatant was discarded and the freshly isolated zooxanthellae cells were resuspended in 1 µm filtered sea water.

Depletion of DIN

Uptake rates of DIN were determined by measuring depletion of ammonia / ammonium or nitrate from sea water culturing the zooxanthellae cells. The rates were determined both in light and in darkness using the method of D'Elia & Cook (1988). A total of 5,000 zooxanthellae cells were placed in 1 L acid-washed glass containers (density: 5 cells / ml) with 1 µm filtered sea water. Ammonium chloride or sodium nitrate (or a combination of both) were added to the sea water to make a final concentration of 20 µM.

Duplicate samples of sea water were removed from each experimental vial every hour and analysed for DIN concentration. The controls consisted of vials containing only filtered sea water and zooxanthellae cells. Ammonium and nitrate concentrations were measured using a calibrated DREL 200 HACH nutrient analyser. Five 1 ml samples were counted with a haemocytometer under the microscope to determine the number of zooxanthellae during the initial and final

Table 1: Depletion of ammonium-nitrate from sea water culturing zooxanthellae cells in light.

Treatment	Concentration (µM) at different times (h)								
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
Ammonium	20.0	16.1	12.3	7.5	5.5	4.8	3.7	1.3	0.5
Nitrate	20.0	18.8	17.7	15.8	12.6	9.3	9.5	8.6	2.3
Ammonium + nitrate:									
Ammonium	20.0	15.8	13.7	7.9	5.9	4.9	1.8	1.5	0.7
Nitrate	20.0	18.1	17.5	17.9	17.3	13.8	6.9	3.1	2.5
Control I: Zooxanthellae only									
Ammonium	0.2	0.2	0.3	0.2	0.3	0.1	0.2	0.2	0.1
Nitrate	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Control II: Sea water only									
Ammonium	0.2	0.2	0.2	0.2	0.2	0.1	0.0	0.1	0.1
Nitrate	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1

Table 2: Depletion of ammonium-nitrate from sea water culturing zooxanthellae cells in darkness.

Treatment	Concentration (µM) at different times (h)								
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
Ammonium	20.0	19.8	19.2	18.8	18.1	16.0	14.8	14.1	12.0
Nitrate	20.0	19.9	19.8	18.8	18.0	18.2	17.3	17.5	14.8
Ammonium + nitrate:									
Ammonium	20.0	19.9	19.3	19.5	18.6	18.5	18.3	17.5	17.2
Nitrate	20.0	19.8	18.9	19.3	18.7	18.3	18.4	17.6	17.5
Control I: Zooxanthellae only									
Ammonium	0.2	0.2	0.1	0.2	0.2	0.1	0.0	0.0	0.1
Nitrate	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Control II: Sea water only									
Ammonium	0.2	0.1	0.1	0.2	0.0	0.1	0.0	0.1	0.1
Nitrate	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1

Table 3. Number of zooxanthellae cells in culture medium added with ammonium and/or nitrate in light.

Treatment	Zooxanthellae count at different times (h)						
	0 h	1 h	2 h	3 h	4 h	5	6 h
Ammonium	5015 ± 57	5057 ± 63	4897 ± 75	5099 ± 64	5197 ± 84	5206 ± 79	5264 ± 81
Nitrate	4957 ± 49	5017 ± 79	4989 ± 75	5151 ± 68	5077 ± 73	5108 ± 54	5223 ± 69
Ammonium + nitrate	5178 ± 51	5198 ± 49	5162 ± 68	5197 ± 58	5205 ± 53	5251 ± 67	5376 ± 51
Control I: Zooxanthellae	5195 ± 65	4912 ± 71	4857 ± 53	4934 ± 50	5199 ± 67	5152 ± 62	5165 ± 55

Treatment	Zooxanthellae count at different times (h)	
	7 h	8 h
Ammonium	5396 ± 65	5378 ± 94
Nitrate	5219 ± 72	5233 ± 86
Ammonium + nitrate	5398 ± 73	5576 ± 98
Control I: Zooxanthellae	5216 ± 69	5329 ± 75

stage of the experiment.

Statistical analyses

The significance of correlation between treatments was evaluated by the analyses of variance (ANOVA) hypothesis test in which a linear model and the overall response mean were compared at the confidence level of 5 % ($p < 0.05$).

RESULTS

Tables 1 and 2 show the depletion of ammonium and nitrate from sea water culturing zooxanthellae cells. The zooxanthellae cells seemed to deplete sea water with ammonia at a faster rate than with nitrate when these forms of DIN were added separately in light condition. The fastest depletion rate of ammonia occurred after 1 to 3 hours, while for nitrate it was most evident after 3 to 5 hours . However, when nitrate and ammonia were added together a significant depletion of nitrate occurred only when ammonia concentrations fell below 4.9 µM. The concentration of ammonia was reduced to almost 0 µM by the end of 8 hours of exposure to the zooxanthellae.

The depletion of ammonia and nitrate in sea water with zooxanthellae cells in darkness, either separately or together, was very minimal or almost non-existent. The uptake rates of DIN were 10 times higher in light than in darkness.

Zooxanthellae cells exposed to increased concentrations of ammonia and nitrate showed 2 to 3 times higher division rates compared to the zooxanthellae from the controls (Table 3). The number of zooxanthellae cells were approximately around 5,000 at the beginning of the exposure but increased after 8 hours to 5576 cells. There were no significant changes in the cell division of zooxanthellae when exposed in darkness (Table 4).

Table. 4. Number of zooxanthellae cells in culture medium added with ammonium and/or nitrate in darkness.

Treatment	Zooxanthellae count at different times (h)						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Ammonium	5177 ± 59	4951 ± 99	5067 ± 75	5161 ± 98	4876 ± 105	5187 ± 95	5174 ± 86
Nitrate	5201 ± 62	5179 ± 89	5067 ± 78	4987 ± 67	5121 ± 79	5098 ± 84	5005 ± 73
Ammonium + nitrate	5018 ± 51	4951 ± 73	5071 ± 85	4955 ± 69	4893 ± 83	5061 ± 75	5196 ± 79
Control I: Zooxanthellae	5198 ± 67	5201 ± 59	5056 ± 84	4911 ± 75	5014 ± 69	4995 ± 101	5087 ± 81

Treatment	Zooxanthellae count at different times (h)	
	7 h	8 h
Ammonium	4805 ± 116	5206 ± 108
Nitrate	4995 ± 85	5157 ± 94
Ammonium + nitrate	5043 ± 87	5103 ± 51
Control I: Zooxanthellae	5175 ± 94	5069 ± 97

DISCUSSION

Studies need to be carried out to assess the extent to which zooxanthellae may be responsible for primary assimilation of inorganic nitrogen from the sea water. Zooxanthellae have been assumed to be the component of giant clams, which are responsible for the assimilation of inorganic nitrogen (Wilkerson & Trench 1986). The DIN uptake capabilities of zooxanthellae are considered to be a central feature of nitrogen recycling and conservation in coral reef habitats, which are dominated by symbiotic systems (Summon *et al.* 1986).

The results of this study demonstrate: (a) zooxanthellae cells take up ammonia at a higher rate than nitrate, (b) rates of uptake were 10 times higher in light than in darkness, (c) reduced rates of nitrate uptake occurs in the presence of ammonia, and (d) added DIN increases division rates of zooxanthellae cells.

Ammonium-nitrogen uptake by zooxanthellae will be translocated to support the clam's nitrogen requirements (Hawkins & Klumpp 1995). Rates of nitrate uptake were consistently less than of ammonium uptake, when offered separately. This is similar to the results obtained in *T. derasa* (Fitt *et al.* 1993). This may be due to the energy which is required for the active uptake across the membrane as well as the subsequent reduction to ammonia (Wilkerson & Trench 1986). Ammonia on the other hand can be directly assimilated by zooxanthellae.

This study indicated that the higher uptake rate of ammonia is from 1 hour to 3 hours of exposure to added ammonia in sea water. This coincides with the findings by Hawkins & Klumpp (1995) on *T. gigas*, where after 3 hours incubation in ammonium-^{15}N chloride, more than 50 % of the ^{15}N retained by *T. gigas* was present in zooxanthellae.

The study demonstrates reduced rates of nitrate uptake in the presence of ammonia. Uptake of nitrate only occurred at ammonium concentration below 4.9 µM. This phenomenon is similar to that observed in phytoplankton (Syrett 1981; Wheeler 1983) and also in *T. derasa* (Fitt *et al.* 1993). A non-specific transport system may act to facilitate movement of nitrate into the animal cells, where presumably in the near absence of ammonia, it is actively transported into the zooxanthellae, reduced to ammonia and assimilated by zooxanthellae (Miller & Yellowlees 1989).

Ammonia and nitrate uptake is certainly dependent upon light, where the uptake in light is 10 times higher than in darkness. The data show a higher capacity for uptake of DIN by zooxanthellae in light than in darkness. Similar results were also reported in *T. derasa* (Fitt *et al.* 1993), and *T. gigas* (Wilkerson & Trench 1986; Hawkins & Klumpp 1995). Uptake of DIN by zooxanthellae cells was not significant in darkness. Kinetic studies indicate that sustained DIN uptake requires exposure to light (Domotor & D'Elia 1984; Wilkerson & Trench 1986) but the degree of light dependence may vary according to the DIN source.

The number of zooxanthellae cells in this study increased with addition of DIN in the culture medium in the light. Increased densities of zooxanthellae are characteristic for zooxanthellae symbiosis enriched with nitrogen (Cook *et al.* 1988; Hoegh-Guldbury & Smith 1989; Muscatine *et al.* 1989). Zooxanthellae cells do not show significant increase in darkness even though nutrients were added. In darkness, zooxanthellae do not take up much nitrogen, leading to a stable number of cells. This is different from the findings by Fitt *et al.* (1993) on *T. derasa*, where mitotic index peaked at dawn. The greater density of zooxanthellae in the clam will increase the photosynthetic rates, and also result in a greater amount of carbon translocated to the host. In addition, the increased availability of organic nitrogen, will then lead to increased growth rates of

the giant clams.

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