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Photoautotrophic growth of *Noctiluca scintillans* with the endosymbiont *Pedinomonas noctilucae*

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Abstract: Photosynthesis and respiration of a heterotrophic dinoflagellate *Noctiluca scintillans* that contained *Pedinomonas noctilucae* as an endosymbiont, were examined on cultures and natural populations in Manila Bay, Philippines, using a Clark-type oxygen electrode. The cultures isolated from the inner Gulf of Thailand were of two types: one required external supply of *Dunaliella tertiolecta* as food (feeding strains) and the other did not (non-feeding strains). The non-feeding strains grew photoautotrophically for generations, but they also fed on *D. tertiolecta*, indicating phagotrophy was facultative. Gross photosynthesis was at the same level in both types, but net photosynthesis was significantly higher in the non-feeding strains than the feeding ones. The difference was due to high respiration activity in the feeding strains. This was consistent with observations in the natural population of Manila Bay, where net photosynthesis was significantly higher in cells lacking food vacuoles than those with food vacuoles. The relationship of photosynthesis with irradiance was characterized by low intensity of light saturation and absence or weak photoinhibition, showing efficient utilization of a wide range of light intensities. *P. noctilucae* likely assures a supply of organic matter to the host, and facilitates survival of *N. scintillans* during shortages of food particles.

Key words: *Noctiluca scintillans*, *Pedinomonas noctilucae*, symbiosis, dark respiration, photosynthesis

Introduction

Noctiluca scintillans (Macartney) Ehrenberg is a cosmopolitan heterotrophic dinoflagellate, and forms distinct red tides in temperate waters (Elbrachter & Qi 1998). However this species causes greenish discoloration of surface seawater in tropical Southeast Asian waters (Sweeney 1971). This is due to the presence of a photosynthetic endosymbiont *Pedinomonas noctilucae* (Subrahmanyan) Sweeney in a vacuole in *N. scintillans*. Thus, it is called "green *Noctiluca*" due to the color. Taxonomically, the green *Noctiluca* is considered to be identical to *N. scintillans* in temperate waters (Sweeney 1978).

Sweeney (1971) was the first to grow the green *Noctiluca* under laboratory conditions, and demonstrated that the green *Noctiluca* survives in light without addition of food for at least four weeks, and that in darkness the symbiotic flagellate disappeared and the green *Noctiluca* died within a few days without a food supply. However, even under illumination the green *Noctiluca* stopped cell division with a prolonged fasting of over two weeks, and the numbers of green *Noctiluca* declined rapidly (Sweeney 1971). While photosynthetic carbon uptake is confirmed in the green *Noctiluca*, it does not survive beyond two weeks without a food supply (Hansen et al. 2004). These observations suggest that green *Noctiluca* is an obligatory phagotroph and that photosynthesis by the symbiont does not meet the organic matter demand of the host. However, recently, we have isolated strains of the green *Noctiluca* that survive for generations without a food supply (Furuya et al. in press). This implies the green *Noctiluca* can grow photoautotrophically. In this light, is phagotrophy obligatory or facultative in the green *Noctiluca*? Respiration is critical to answer this question. However, little is known about respiration in *N. scintillans*.

The present study aimed to quantify dark respiration and photosynthetic activity of the green *Noctiluca* and to clarify their relationships with external food supply using both cultures and a natural population.

Materials and Methods

A total of 37 clonal cultures of the green *Noctiluca* were obtained from Chon Buri coast in the upper Gulf of Thailand in 2000 and 2001, and 7 clonal cultures from coastal waters of Palawan Island, Philippines in May 2002 (Fig. 1).

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Among them, 14 strains isolated from the Gulf of Thailand were maintained with a supply of *Dunaliella tertiolecta* Butcher as food and the rest of the cultures were maintained without a food supply. These are referred to as feeding and non-feeding strains, respectively, hereafter. The cultures were kept in the Daigo IMK medium (Wako, Japan) under an illumination of approximately 150 μmol m^{-2} s^{-1} with 14:10 h L : D cycle at 28°C. The cultures were stable for more than three years under these conditions. All the cultures were transferred into fresh medium at an interval of two or three weeks. During the inoculation 1.6×10^5 cells (0.63 μg chlorophyll *a*) of *D. tertiolecta* were supplied to one *Noctiluca* cell of the feeding strains.

N. scintillans was also collected from Harima Nada, Seto Inland Sea, Japan in May 2002 (Fig. 1), and a culture was established. The culture lacked the symbiont, and is dubbed as red *Noctiluca* hereafter. The culture was kept under the same conditions as the feeding strains but at 20°C. Then, temperature was raised gradually to 26°C, to which the red *Noctiluca* was acclimated.

For photosynthesis and respiration, oxygen concentration was determined using a Clark-type oxygen electrode (Hansatech Oxygraph). A reaction chamber was set to 0.5 mL, filled with the fresh IMK medium, and one cell of the green *Noctiluca* was introduced. Before measurement, a *Noctiluca* cell was washed 3 times with autoclaved, 0.2-μm filtered seawater to remove any food organisms. Red *Noctiluca* cells were washed in the same manner and starved 24 h to eliminate food vacuoles. Changes in the oxygen concentrations were recorded. Temperature in the chamber was controlled by running water through a jacket covering the chamber. A slide projector with a halogen lamp was used as the light source. The chamber was covered with black cloth for the measurements of respiration rate.

Photosynthesis vs irradiance (P-E) curves of the green *Noctiluca* were measured on a one-cell basis using the non-feeding strains in their light period. The measurement was also done using cells in a natural population in Manila Bay in March 2002. After keeping for 15 minutes standing in the chamber without light, dark respiration was measured, then net photosynthesis was measured for 5 to 10 minutes at each light level from lower to higher light intensities. Because the decrease rate of oxygen concentration became constant immediately after the start of the measurement, it was considered that the oxygen concentration inside and outside the cell was balanced. Eight different light levels were set up from PAR intensities of 0, 5, 10, 15, 25, 50, 60, 75, 100, 125, 250, 500, 650, 1000, and 1500 μmol m^{-2} s^{-1}. In order to obtain a steady change of oxygen concentration with time during measurements, gentle but sufficient mixing of the water in the chamber was critical. Therefore, rotation of a magnetic stirrer in the chamber was controlled to avoid mechanical damage to *Noctiluca* cells from bursting but to obtain a uniform oxygen distribution. Photosynthetically active radiation (PAR) within the chamber was measured using a 2π sensor (Hansatech QPT-1). Light intensity

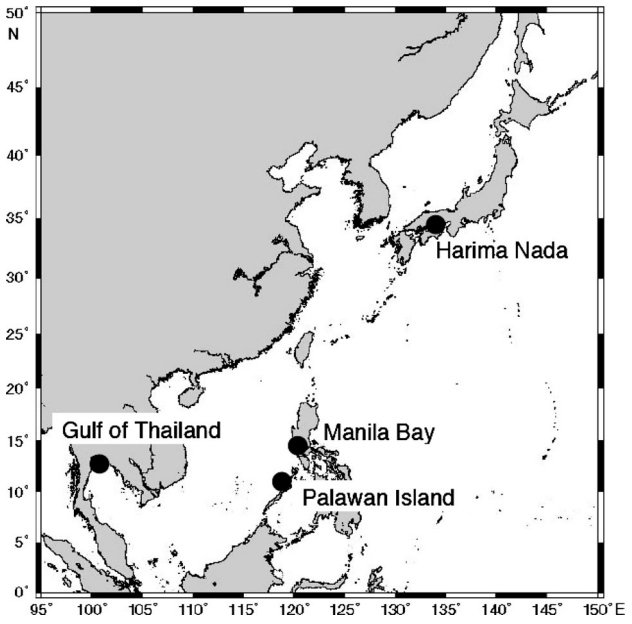

Fig. 1. The sampling locations in the Gulf of Thailand, Manila Bay and Harima Nada.

within the chamber was controlled by adjusting the distance between the projector and the chamber. Maximum rate of cellular photosynthesis (P_{max}), initial slope (α), photoinhibition index (β) and the onset of light saturation (E_k) were obtained from the following equation by the quasi-Newton fitting (Platt et al. 1980):

$$P=P_s\cdot(1-e^{-(\alpha E/P_s)})\cdot e^{-(\beta E/P_s)}-R$$

where P is photosynthetic rate at photosynthetically available radiation E (PAR) and P_s is potential P_{max} in the absence of photoinhibition (Platt et al. 1980). R is the dark Respiration at $E=0$. P_{max} was calculated from P_s, α and β (Platt et al. 1980). Then, each cell of the green *Noctiluca* was introduced into *N*,*N*-dimethylformamide and chlorophyll *a* was extracted for 24 h at 5°C (Suzuki & Ishimaru 1990). Chlorophyll *a* content was determined using a fluorometer (Turner Design 10R).

Dark respiration and photosynthesis at an irradiance of 150 μmol m^{-2} s^{-1} where photosynthesis was light saturated, were measured for 10 minutes using both feeding and non-feeding strains. Gross photosynthesis was calculated by summing the rate of respiration measured just before photosynthesis and the rate of net photosynthesis. Dark respiration of non-feeding strains and red *Noctiluca* was measured for 10 minutes. After measuring oxygen consumption or production, the diameter of the *Noctiluca* cell was measured under a light microscope. Then, chlorophyll *a* content was determined by fluorometry in the same manner as above.

The P-E curve of a green-*Noctiluca* cell was determined also on a natural population in Manila Bay in March 2002 (Fig. 1), when a large-scale bloom prevailed in the western part of the bay. The surface population was sampled around

Table 1. Parameters of photosynthesis vs irradiance curve of non-feeding strains of the green *Noctiluca*.

Origin of strains	Maximum photosynthesis*	Initial slope**	Photoinhibition index**	Onset of light saturation***	Dark respiration*
Palawan Island (n=7)	0.15±0.07	0.030±0.020	0.001±0.002	9.9±11.7	0.15±0.07
Gulf of Thailand (n=5)	0.25±0.18	0.025±0.007	0.000±0.000	10.8±9.1	0.15±0.15

* ($nmolO_2\,cell^{-1}\,min^{-1}$), ** ($[nmolO_2\,cell^{-1}\,min^{-1}]\,[\mu mol\,m^{-2}\,s^{-1}]^{-1}$), *** ($\mu mol\,m^{-2}\,s^{-1}$)

Table 2. Photosynthesis and respiration of the green *Noctiluca*. Mean rates are shown with standard deviations. Strains include those both from the Gulf of Thailand and Palawan Island. Photosynthesis was measured at 150 $\mu mol\,m^{-2}\,s^{-1}$ PAR. Significant differences are shown with marks.

	Dark respiration	Net photosynthesis	Gross photosynthesis	Chlorophyll *a*
		($nmolO_2\,cell^{-1}\,min^{-1}$)		(ng/cell)
Non-feeding (n=30)	0.15±0.10§	−0.02±0.11¶	0.14±0.10	9.14±14.0
Feeding (n=14)	0.36±0.32§	−0.24±0.33¶	0.12±0.15	5.53±4.95

§,¶ $p<0.05$ (*t*-test)

9:00 local time using a plastic bucket and immediately transferred to a land laboratory for measurement. Samples were kept in the dark at *in situ* temperature until oxygen measurement. Oxygen concentration was determined in the same manner as above, but up to 1500 $\mu mol\,m^{-2}\,s^{-1}$. After the measurement, *in vivo* fluorescence was measured using a palmtop fluorometer (Turner Design *Aqua*fluor) and the cells were observed under a light microscope to examine food vacuoles, and also to measure cell diameter.

Results

Respiration and photosynthesis of cultures

P-E curves of the non-feeding strains were characterized by a steep initial slope, low light intensity at the onset of saturation (E_k), and no apparent or weak photoinhibition (Table 1). Non-feeding strains collected from the Gulf of Thailand and Palawan Island showed similar tendencies in P-E curve parameters, and there was no significant difference ($p>0.05$) by *t*-test in P-E parameters such as; maximum photosynthesis (P_{max}), initial slope, photoinhibition index and onset of light saturation between the two original localities (Table 1). The most remarkable features were the low E_k and slight presence or complete absence of photoinhibition, indicating the capability for efficient use of a wide range of light intensities by the symbiont. Although statistically insignificant, P_{max} was higher in the strains from the Gulf of Thailand than those from Palawan Island. Consequently, together with a similar magnitude of dark respiration between both, net photosynthesis at light saturation tended to be higher in the former than in the latter (Table 1).

There was no significant difference in cellular gross photosynthetic rate between the feeding and non-feeding strains at light saturation (Table 2). Since chlorophyll *a* content did not differ between the feeding types, chlorophyll *a* specific gross photosynthetic rates were also similar. However, net photosynthesis was significantly higher in the non-feeding strains than the feeding ones. The difference was due to the cellular respiration rate; the feeding strains showed a significantly higher rate of respiration.

The higher level of dark respiration in the feeding strains (Table 2) indicates that feeding enhances respiration. We then compared dark respiration of the non-feeding strains with that for red *Noctiluca* starved for 24 h (Table 3). During the starvation period, food vacuoles disappeared and the possible influence of feeding on dark respiration was likely eliminated after 24-h starvation in the red *Noctiluca*. Although the cellular dark respiration rate of the red *Noctiluca* was significantly higher than that of the non-feeding strains (Table 3), this was primarily due to its larger cell size, and there was no difference in volume-specific respiration between both types of *N. scintillans*. Instead, the volume- specific respiration tended to be higher in the green *Noctiluca* compared with the red one, probably due to the additional contribution of dark respiration by the symbiont.

Natural population in Manila Bay

The low E_k and weak photoinhibition were confirmed in a natural population (Table 4). E_k was higher in the natural population than the cultures, and the highest E_k was 106 $\mu mol\,m^{-2}\,s^{-1}$ (n=9). The cellular net photosynthetic rate at light saturation was markedly different between cells with and without food vacuoles (Table 5). Those without

food vacuoles showed positive net photosynthesis, whereas those with food vacuoles consume oxygen even under saturating light intensity. This was due to the combined effects of higher dark respiration and lower gross photosynthesis of the green *Noctiluca* with food vacuoles, although the differences were statistically insignificant (Table 5). The lower gross photosynthesis was ascribed to lower cellular chlorophyll *a* content, and chlorophyll-*a* specific gross photosynthesis was similar between those with and without food vacuoles. Food vacuoles contained *Gymnodinium catenatum* Graham which was the secondary dominant species.

Discussion

Gross photosynthesis of the non-feeding strains under saturated light was, on average, balanced with dark respiration (Table 2). Hence there should be no substantial growth of the non-feeding strains. However, this was not the case and the strains grew photoautotrophically. This apparent contradiction likely arises from a variable balance between net photosynthesis of *Pedinomonas noctilucae* and respiration of *Noctiluca scintillans*. As evidenced by large variations in both dark respiration and gross photosynthesis of the non-feeding strains, the balance does not seem stable, but highly dynamic. Since the present study was based on instantaneous measurement of oxygen budget, the balance was considered to be short-term. Growth of the non-feeding strains for generations in the inorganic synthetic medium implies that the green *Noctiluca* achieves a surplus organic matter budget. In fact, net photosynthesis under saturating light intensity was positive in the strains from the Gulf of Thailand during the initial measurement (Table 1). However, the same strains showed neutral or slightly negative net photosynthesis after a lag of time. This dynamic balance is presumably manifested in part by variations in cellular abundance and photosynthetic activity of *P. noctilucae*. The cellular abundance of *P. noctilucae* fluctuates considerably according to temperature, salinity and nutrient conditions (Lirdwitayaprasit and Sriwoon, unpublished), and probably depends on consumption by the host, and growth of *P. noctilucae* itself. The feeding strains rarely exhibit cannibalism. In clonal cultures of the non-feeding strains, some cells occasionally contained another *Noctiluca* cell in a food vacuole. When supplies of organic matter produced by *P. noctilucae* become insufficient under some conditions, such as unfavorable light conditions, cannibalism may function as a means for satisfying organic matter demands.

Cellular gross photosynthesis of 0.14 nmol cell^{-1} min^{-1} in Table 2 is equivalent to 101 ngC cell^{-1} h^{-1} and 11.1 ngC ng(Chl *a*)$^{-1}$ h^{-1}, provided the photosynthetic quotient is unity. Hansen et al. (2004) reported the ^{14}C uptake rate of unfed green *Noctiluca* collected from Manila Bay to be 10 ngC cell^{-1} h^{-1} at 150 μmol m^{-2} s^{-1} PAR. Although no direct comparison of oxygen- and carbon tracer-based mea-

Table 3. Dark respiration of non-feeding strains of the green *Noctiluca* and red *Noctiluca*. Asterisks denote significant differences.

	Cellular respiration (nmolO_2 cell^{-1} min^{-1})	Volume specific respiration (nmolO_2 mm^{-3} min^{-1})	Cell diameter (mm)
Non-feeding strains of green *Noctiluca* (n=13)	0.18±0.09*	4.44±3.93	0.49±0.12
Red *Noctiluca* (n=13)	0.29±0.09*	3.31±1.36	0.56±0.07

* $p<0.05$ (*t*-test)

Table 4. Parameters of photosynthesis vs irradiance curves of a natural population of the green *Noctiluca* in Manila Bay.

Sample number	Maximum photosynthesis*	Initial slope**	Photoinhibition index**	Onset of light saturation***	Dark respiration*
9	1.05±0.71	0.11±0.15	0.000±0.000	27.9±32.4	1.21±0.70

* (nmolO_2 cell^{-1} min^{-1}), ** ([nmolO_2 cell^{-1} min^{-1}] [μmol m^{-2} s^{-1}]$^{-1}$), *** (μmol m^{-2} s^{-1})

Table 5. Photosynthesis and respiration of the green *Noctiluca* in Manila Bay. Mean rates are shown with standard deviations. Photosynthesis was measured at 150 μmol m^{-2} s^{-1} PAR. Asterisks denote significant differences.

	Dark respiration	Net photosynthesis	Gross photosynthesis	Chlorophyll *a*
	(nmolO_2 cell^{-1} min^{-1})			(ng cell^{-1})
Without food vacuole (n=9)	0.75±0.57	0.38±0.29§	1.19±0.82	12.6±5.03¶
With food vacuole (n=8)	0.92±0.81	−0.20±0.30§	0.73±0.86	6.05±3.55¶

§, ¶ $p<0.05$ (*t*-test)

surements is warranted (Williams 1993), a large difference between the gross production and the carbon uptake rate suggests that the respiration load occupies a major portion of gross production. This inference is consistent with our observation (Table 2).

The P-E curves of the green *Noctiluca* both under laboratory conditions and in the natural population were characterized by a low E_k and the minor influence of photoinhibition, indicating the capability for efficient use of a wide range of light intensities by the green *Noctiluca*. The low E_k allows for efficient photosynthetic performance even within a dense bloom, where self shading reduces light availability. Hansen et al. (2004) conducted a P-E experiment in a PAR range of 0 to 300 μmol m^{-2} s^{-1} and observed no saturation of photosynthesis up to 250 μmol m^{-2} s^{-1}. This is not supported by our observations (Table 1). Freshly collected green *Noctiluca* from Manila Bay, where Hansen et al. (2004) obtained their samples, showed far lower E_k (Table 4). Experimental details are not provided fully in Hansen et al. (2004), e.g., how light intensity was measured, why variable incubation times between 1 to 3 h were applied to different light levels. As for the incubation time, light acclimation might occur during incubation for a long time duration, and the variable incubation time might induce different degrees of acclimation among P-E response (Sakshaug et al. 1997). Therefore, it is difficult to explain this apparent discrepancy.

The dark respiration rate per unit cell volume of both green and red *Noctiluca* did not differ significantly. Since the recorded respiration rate of the green *Noctiluca* included that of *P. noctilucae*, the present study failed to characterize the respiration of the host alone. The cellular density of *P. noctilucae* declines with prolonged starvation (Sweeney 1971). Thus, green *Noctiluca* without the symbiont can be produced. However, these cells likely suffer physiological stress due to the deficiency of organic matter. In the ciliate *Paramesium bursaria* Ehrenberg and *Chlorella* sp. symbiosis, respiration of the symbiotic consortium is lower than that of aposymbiotic *P. bursaria* (Reisser 1980), that is, the respiration of the consortium is not a simple sum of that of the host and the symbiont. On the other hand, the *Hydra viridis* and *Chlorella* sp. symbiosis shows the same cellular respiration rates as aposymbiotic *H. viridis* (Cantor & Rahat 1982). Thus, the contribution of symibionts to the respiration of the symbiotic consortium appears complex and there are interactive controlling mechanisms in the symbiosis (Cantor & Rahat 1982).

The non-feeding strains of green *Noctiluca* grew photoautotrophically for generations, and they also fed on *D. tertiolecta*, indicating that phagotrophy is facultative in the green *Noctiluca*. The low net photosynthesis (Table 2) suggests that photosynthesis of *P. noctilucae* alone does not support active growth of the green *Noctiluca*. Instead, the competitive advantage of the symbiosis is that *P. noctilucae* assures a supply of organic matter to the host, and facilitates survival of the green *Noctiluca* during shortages of food particles. Since P. *noctiluca* do not grow in seawater (Sweeney 1971, Okaichi et al. 1991), the survival of the host is in turn crucial to *P. noctilucae*. This is in good agreement with the observation that the non-feeding strains without food grow significantly slower than the feeding strains (Furuya et al. in press).

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