Green *Noctiluca scintillans*: a dinoflagellate with its own greenhouse

Per J. Hansen[1,*], Lilibeth Miranda[2,3], Rhodora Azanza[2]

[1]Marine Biological Laboratory, University of Copenhagen, Strandpromaden 5, 3000 Helsingør, Denmark
[2]The Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines

[3]*Present address:* University of Connecticut, Department of Marine Sciences, 1080 Shennecossette Rd, Groton, Connecticut 06340, USA

ABSTRACT: The effect of irradiance on photosynthesis of the green form of the dinoflagellate *Noctiluca scintillans* was studied. Photosynthesis, measured in cells collected from the field and without the addition of prey, increased with irradiance to ca. 200 ng C cell^{-1} d^{-1} at an irradiance of ~250 to 300 µmol photons m^{-2} s^{-1}. *N. scintillans* cells were observed to prey and grow actively on a number of different algae, including the dinoflagellate *Pyrodinium bahamense* var*. compressum* which produces paralytic shellfish toxin. However, in all cases, *N. scintillans* lost its endosymbionts when grown in the laboratory for more than 3 wk and became colourless, irrespective of food item, concentration and irradiance. A factor necessary for endosymbiont growth was apparently missing, which was not provided to them by *N. scintillans* when fed the selected prey types. Thus, further experiments were carried out with freshly collected organisms. The growth rate of *N. scintillans* when grown without prey was 0.058 and 0.14 d^{-1} at irradiances of 45 and 150 µmol photons m^{-2} s^{-1} at a light:dark cycle of 12:12 h and a temperature of 26°C. When supplied with *P. bahamense* as food, *N. scintillans* increased its growth rate to 0.09 and 0.24 d^{-1}, at irradiances of 45 and 150 µmol photons m^{-2} s^{-1} and prey concentrations of 1610 and 2740 µg C l^{-1}, respectively. Ingestion rates were only measured at 150 µmol photons m^{-2} s^{-1}. At this irradiance, the ingestion rate increased linearly with prey concentration and showed no signs of satiation at a prey concentration of ~2700 µg C l^{-1}. A comparison of the contribution of photosynthesis and phagotrophy to the carbon metabolism revealed that phagotrophy only contributed significantly (30%) to the direct growth of the green *N. scintillans* at an irradiance of 150 µmol photons m^{-2} s^{-1}, when the prey concentration was very high. Clearance decreased with prey concentration, from ~0.06 ml *N. scintillans*$^{-1}$ d^{-1} at 25 µg C l^{-1} to ~0.03 ml *N. scintillans*$^{-1}$ d^{-1} at a prey concentration of 2740 µg C l^{-1}. This indicates that *N. scintillans*, when it occurs at bloom concentrations in nature (1 to 10 cells ml^{-1}), may have a significant impact on the bloom dynamics of *P. bahamense* var*. compressum*.

KEY WORDS: Green *Noctiluca scintillans* · *Pyrodinium bahamense* · Photosynthesis · Food uptake

INTRODUCTION

Noctiluca scintillans (Macartney) is a large and conspicuous dinoflagellate, which is common in coastal areas worldwide (Elbrächter & Qi 1998). The most widely spread form of *N. scintillans* is completely hetertrophic and is an omnivorous feeder that ingests a wide range of prey, including phytoplankton, microzooplankton, mesozooplankton and their eggs (Enomoto 1956, Prasad 1958, Chen & Qi 1991, Buskey 1995). However, in the tropical to sub-tropical areas of Southeast Asia (e.g. Thailand, India, Philippines, New

Guinea), a green form of *N. scintillans* is found. This variety harbours thousands of free-swimming cells of the prasinophyte *Pedinomonas noctilucae* inside itself (Sweeney 1976; Fig. 1).

Despite the fact that the green form of *Noctiluca scintillans* regularly forms massive blooms in many areas of Southeast Asia (see review by Elbrächter & Qi 1998), our knowledge of its biology is very sparse; it is, in fact, restricted to a single paper published by Sweeney in 1971. In laboratory studies she demonstrated that the green *N. scintillans* could survive and divide in the light without the addition of food for at

least 1 mo. In darkness, however, the symbiotic fla-
gellate disappeared and the *N. scintillans* cells died
within a few days unless a food organism, *Dunaliella
tertiolecta* (Butcher), was added. Thus, her results
indicate that *N. scintillans* either incorporates endo-
symbionts into food vacuoles or utilizes their photo-
synthetic products as nutrition. Long-continued growth
of *N. scintillans* in the light seems to require the
presence of prey, since the symbiotic flagellates were
gradually lost under all culture conditions assayed by
Sweeney (1971). Thus, the instability of the symbiosis
between *Pedinomonas noctilucae* and *N. scintillans*
suggests that the symbiotic relationship represents a
primitive stage in the establishment of symbiosis.

While the study by Sweeney (1971) gave some in-
sight into the symbiotic relationship between *Noctiluca
scintillans* and *Pedinomonas noctilucae*, it provided no
rates on either photosynthesis of the endosymbionts or
ingestion of prey by *N. scintillans*. Thus, whether the
green *N. scintillans* primarily functions as a phototroph
or as a heterotroph is unknown. Also, the importance
of *N. scintillans* as a grazer in the areas where it
blooms is unknown.

In the Philippines, the only organism that has caused
major toxic red tides is the dinoflagellate *Pyrodinium
bahamense* var. *compressum* (Böhm). In Manila Bay,
P. bahamense blooms and associated paralytic shellfish
poisonings (PSPs) were first experienced in 1988, and
since 1991 blooms occur almost yearly. It has caused
vast losses in the shellfish industry, amounting to
US\$ 300 000 d^{-1} during the height of the red tide scare
(Corrales & Maclean 1995). Recently, it has been
observed that dense populations of the green
Noctiluca (up to 1 to 10 cells ml^{-1}) often succeed
blooms of *P. bahamense* var. *compressum* in Manila
Bay (Azanza & Miranda 2001). Thus, *N. scintillans*
might play a significant role in the termination of
P. bahamense blooms in this area.

The aim of the present investigation was to quantify
photosynthesis, food uptake and growth of the green
Noctiluca scintillans in monocultures and in mixed
cultures with other algae, with special emphasis on
Pyrodinium bahamense var. *compressum*.

MATERIALS AND METHODS

**Collection, culture and carbon content of the
organisms.** The dinoflagellates *Pyrodinium baha-
mense* var. *compressum* and *Akashiwo sanguinea*
(Hirasaka) (=*Gymnodinium sanguineum*) with cell
dimensions of 50 to 65 and 40 to 75 µm, respectively,
were isolated from Manila Bay, Philippines (Corrales &
Hall 1993). The prasinophyte *Tetraselmis tetrahele* (G. S.
West), with a cell dimension of 10 to 16 µm, was collected

from Bolinao, Pangasina, Northern Philippines and pro-
vided by Ms. Helen Bangi of the Marine Invertebrate
Research Laboratory (UP-MSI). These algae were grown
in f/2 medium based on 36 psu seawater at a
temperature of 24 ± 2°C following a light:dark cycle of
12:12 h. Illumination was provided by cool white
fluorescent lamps at an irradiance of 150 µmol photons
m^{-2} s^{-1}. On several occasions during the years 2000 and
2001 green *Noctiluca scintillans* cells were collected
from the western part of Manila Bay with a plankton net
(mesh size 20 µm), poured into 2 l polycarbonate bottles
and immediately brought back to the laboratory. At the
laboratory, *N. scintillans* cells gathered at the
periphery of the containers close to the surface, from
where they were collected using a Pasteur pipette and
transferred to glass beakers containing f/2 medium.
N. scintillans cells were subsequently transferred 4 to
5 times to fresh f/2 medium, which in the end resulted in
a suspension containing only *N. scintillans* cells that was
used for the experimental work. Cellular carbon content
of *N. scintillans* and *P. bahamense* var. *compressum* was
determined by filtering cell suspensions onto pre-
combusted GF/C filters. These filters were dried at 65°C
for 48 h and analyzed for particulate organic carbon
(POC) using a CHN elemental analyzer (Model NA
1500, Fisons Instruments).

Attempts were made to grow the green form of
Noctiluca scintillans in laboratory cultures in mono-
cultures and mixed cultures with selected algal
species. These experiments were carried out in 72 ml
tissue culture flasks at an irradiance of 150 µmol photons
m^{-2} s^{-1}. A stock suspension of *N. scintillans* was prepared
and mixed with unialgal cultures of different prey
species. This resulted in experimental flasks with a final
concentration of ~1 *N. scintillans* ml^{-1}, and 100, 100
and 760 cells ml^{-1} of *Pyrodinium bahamense*, *Akashiwo
sanguinea* and *Tetraselmis tetrahele*, respectively.

The experimental flasks were placed on a plankton
wheel rotating at 1 rpm to keep the algae in suspension
and homogeneously distributed. *Noctiluca scintillans*
cells were counted directly from the bottles every 2nd
day under a dissecting microscope for the duration
of the experiments (up to 10 d). This was possible
because *N. scintillans* cells were positively buoyant. For
the enumeration of prey cells, subsamples (4 ml) were
taken every 2nd day. The culture flasks were gently
shaken before withdrawing samples to disrupt any
aggregates of cells that had formed. Fresh f/2 medium
was added to replace the sampled volume. The samples
were fixed in Lugol's solution (final concentration: 1%)
and counted in an inverted microscope.

Because our attempts to grow *Noctiluca scintillans*
failed (see 'Results'), we decided to carry out the
remaining part of this study using freshly collected
N. scintillans cells.

Experiments on *Noctiluca scintillans* photosynthesis. Photosynthesis of *N. scintillans* cells was measured using a modification of the 'single cell' method (Stoecker et al. 1988, Skovgaard et al. 2000). A suspension containing 10 *N. scintillans* cells ml^{-1} was prepared from dilution of a stock suspension and poured into scintillation vials (22 ml). A NaH^{14}CO$_3^-$ stock solution (specific activity 100 μCi ml^{-1}) was added to each vial, resulting in a specific activity of ~1.0 μCi ml^{-1}, and afterwards the vials were incubated on a plankton wheel (1 rpm) for 1 to 6 h (see below), starting 1 to 3 h after the onset of the light period. The vials were always accompanied by parallel dark vials, which were treated likewise, but wrapped in aluminum foil. After incubation, specific activity of the medium was measured by transferring 100 μl from each vial to new vials containing 200 μl phenylethylamine. The amount of fixed ^{14}C was measured by transferring 3 ml to each of 4 to 6 vials, which received 3.0 ml of 10% acetic acid in methanol to remove all inorganic C. These vials were dried overnight at 60°C, and afterwards the residue was re-dissolved with 2 ml of distilled water. Finally, 10 ml of Packard Insta-Gel Plus scintillation flour were added to all vials (including those for specific activity), and activity was measured using a Packard 1500 Tri-Carb liquid scintillation counter analyzer with automatic quench correction. Calculations of photosynthetic rates were based on Parsons et al. (1984). Dissolved inorganic carbon content was measured with a 225-Mk3 infrared gas analyzer (Analytic Development).

In the first set of experiments using this technique, the linearity of ^{14}C uptake for freshly collected *Noctiluca scintillans* cells over time were tested by incubating triplicate vials for 1, 2, 3, 4, 5 and 6 h. After this test, we carried out a second set of experiments using this technique to determine photosynthesis at 6 different irradiances using incubation times of 1 to 3 h. All experiments were carried out in triplicate.

The functional and numerical response of *Noctiluca scintillans* when fed *Pyrodinium bahamense*. The functional and numerical response of *N. scintillans* was studied when fed *P. bahamense* var. *compressum* at 2 irradiances. In the first set of experiments, *N. scintillans* (1 cell ml^{-1}) was incubated with different initial prey concentrations (0, 5, 10, 25, 50, 75, 100, 250 and 500 cells ml^{-1}) at an irradiance of 150 μmol photons m^{-2} s^{-1}. Based on the results obtained in this set of experiments, a second set of experiments was carried out, this time at a lower irradiance of 45 μmol photons m^{-2} s^{-1} and initial prey concentrations of 0, 5, 25, 75 and 250 cells ml^{-1}. Prior to the experiments, *N. scintillans* was acclimated to the prey concentrations and irradiances for 2 d. The initial concentrations of *P. bahamense* and *N. scintillans* in these experiments

Fig. 1. *Noctiluca scintillans* from Manila Bay, Philippines. Note the large number of free-swimming cells of the endosymbiotic prasinophyte *Pedinomonas noctilucae* inside the *N. scintillans* cell

were made by dilution of stock suspension with known cell densities. Control experiments with unialgal cultures of *P. bahamense* were carried out for all test concentrations. All experiments were carried out in 72 ml tissue culture flasks with 4 replicates. *N. scintillans* and *P. bahamense* cells were quantified every 2nd day as previously described in the section entitled 'Collection, culture and carbon content of the organisms'.

Growth (μ) of *Noctiluca scintillans*, measured as the change in cell number over time, was computed using the formula:

$$\mu = [\ln(N_{t_1}/N_{t_0})]\,t^{-1} \tag{1}$$

where N_{t_0} = concentration of cells at time 0 (cells ml^{-1}), N_{t_1} = concentration of cells at time 1 (cells ml^{-1}), and t = the duration of each experiment (d). Ingestion rate of *Noctiluca* was determined from the reduction in prey concentrations over 48 h periods as compared to the control cultures, according to Jakobsen & Hansen (1997). The ingestion rate U was estimated using the following 2 equations:

$$dx/dt = \mu_x x - Uy \tag{2}$$
$$dy/dt = \mu_y y \tag{3}$$

where (x) is ingested by grazer (y). It was assumed that the grazer (y) grows exponentially with the rate constant of μ_y and that the prey (x) grows with the rate constant of μ_x. The mortality due to grazing is Uy,

where U (dimension $x/y/t$) is the per capita ingestion rate, which is independent of x. As the solution to establish the value of μ is intractable, the ingestion rate (U) was iteratively calculated with time on a computer, allowing steps of 1 h. Clearance rate, the volume of water swept clear of *Pyrodinium bahamense* cells, was calculated as: $C = U(x)/x$.

RESULTS

Attempts to grow the green form of *Noctiluca scintillans*

We where able to keep and grow the green *Noctiluca scintillans* in monoculture using the f/2 growth medium for at least 2 wk (Fig. 2). The cultures increased typically in cell concentration from 1 to 2 cells ml^{-1} over a 2 wk period at an irradiance of 150 μmol photons m^{-2} s^{-1}, and the *N. scintillans* cells contained many endosymbionts. However, if incubated for longer period, the *N. scintillans* cells lost their endosymbionts and slowly died off (data not shown).

When supplied with prey the growth/survival response of *Noctiluca scintillans* depended on the prey species offered (Fig. 2). *N. scintillans* died out between Days 4 and 6 when fed *Tetraselmis tetrahele*, coinciding

with very high cell concentrations of this alga. *N. scintillans* did better when *Pyrodinium bahamense* or *Akashiwo sanguinea* were offered as food, and it grew from an initial concentration of 1 cell ml^{-1} to reach cell densities of 3.8 and 6 cells ml^{-1}, respectively, after 10 d of incubation. However, in the following days the growth of the *N. scintillans* stopped, and in some cases cell numbers even declined. This coincided with the loss of endosymbionts. We observed long-term growth (several months) of the *N. scintillans* itself using *P. bahamense* as prey, but the endosymbionts were always lost (data not shown).

Photosynthesis and carbon content of *Noctiluca scintillans* grown without prey

The ^{14}C uptake by *Noctiluca scintillans* was linear within the studied timeframe of 5 h (Fig. 3A), and a maximum incubation time of 3 h was selected for further experiments. Photosynthesis (P) of *N. scintillans* increased with increasing irradiance (Fig. 3B), reaching ~17 ng C cell^{-1} h^{-1} at an irradiance of 300 μmol photons m^{-2} s^{-1}, which corresponds to 200 ng C cell^{-1} d^{-1} (light:dark 12:12 h). No saturation of photosynthesis was observed. The carbon content of *N. scintillans* was 410 ng C cell^{-1} (Table 1).
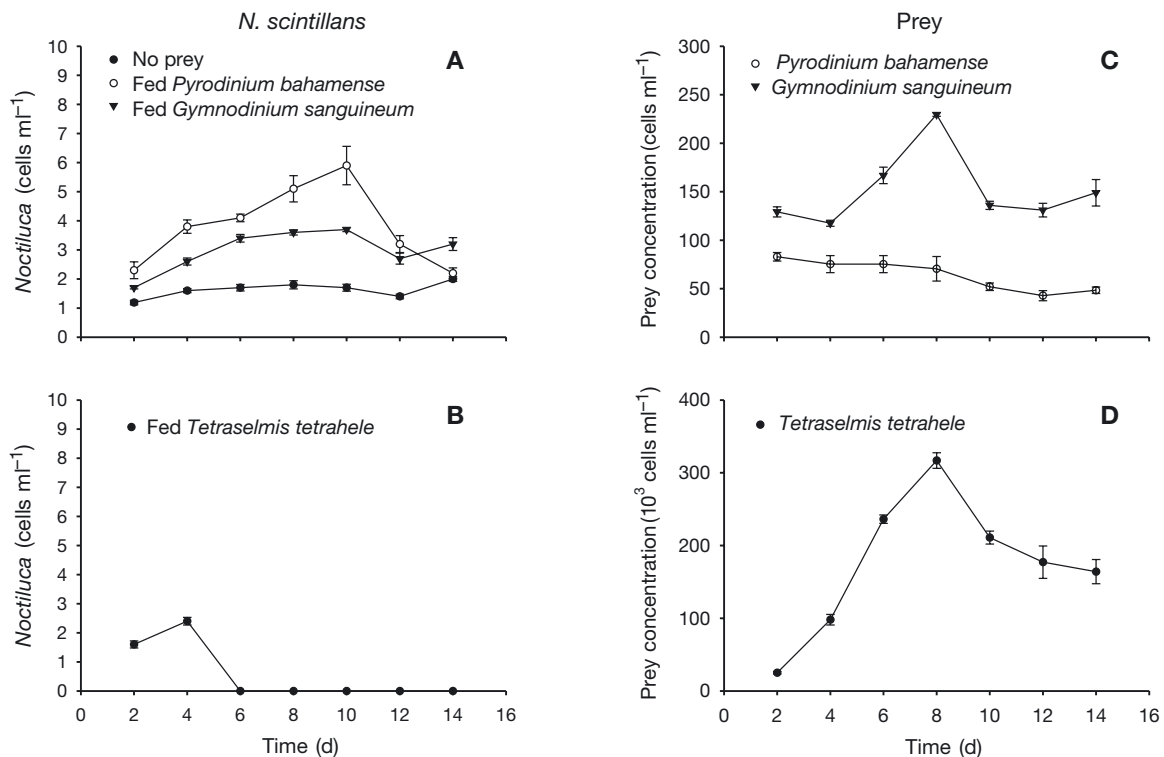

Fig. 2. *Noctiluca scintillans.* (A,B) Growth/survival responses when grown alone or with prey in mixed cultures during a 2 wk incubation in the laboratory. (C,D) Growth/survival responses of the prey in the mixed cultures. Data points represent means ± 1 SE (n = 3)

Table 1. Carbon contents of *Pyrodinium bahamense* var. *compressum* and the green *Noctiluca scintillans* used in the experiments

Species	Carbon content (ng C cell^{-1}) mean ($\pm$SE; n)
Pyrodinium bahamense var. *compressum*	5.17 (0.06; 4)
Noctiluca scintillans	410 (12; 5)

Growth and ingestion of *Noctiluca scintillans* when fed *Pyrodinium bahamense*

The growth of the green *Noctiluca scintillans* was studied at 2 irradiances, 45 and 150 µmol photons m^{-2} s^{-1} (Figs. 4, 5 & 6). In all cases, populations of *N. scintillans* grew exponentially for the first 10 to 14 d of incubation, regardless of whether or not food was supplied. However, growth subsequently stopped and the population declined in many cases for the re-

mainder of the experimental period, irrespective of irradiance. Taking only data from the first 10 d of incubation into consideration, the growth rate of *Noctiluca scintillans* was 0.058 and 0.14 d^{-1} at irradiances of 45 and 150 µmol photon m^{-2} s^{-1}, respectively, when grown without prey. When supplied with *Pyrodinium bahamense* as food, the growth of *N. scintillans* increased with prey concentration without showing any signs of saturation (Fig. 6). At 45 µmol photons m^{-2} s^{-1}, the growth rate increased to 0.09 d^{-1} at an average prey concentration of 315 cells ml^{-1} (~1550 µg C l^{-1}), while the growth rate at an irradiance of 150 µmol photons m^{-2} s^{-1} reached 0.25 d^{-1} at a prey concentration of 550 cells ml^{-1} (~2750 µg C l^{-1}). A linear regression analysis of the data sets revealed a correlation coefficient (R^2) of 0.90 and 0.59, at irradiances of 150 and 45 µmol photons m^{-2} s^{-1}, respectively. A paired *t*-test revealed no significant difference in the slopes of the 2 regression lines ($p > 0.05$), and thus indicates that growth increase due to food uptake was not related to irradiance (*t*-test, $p >$
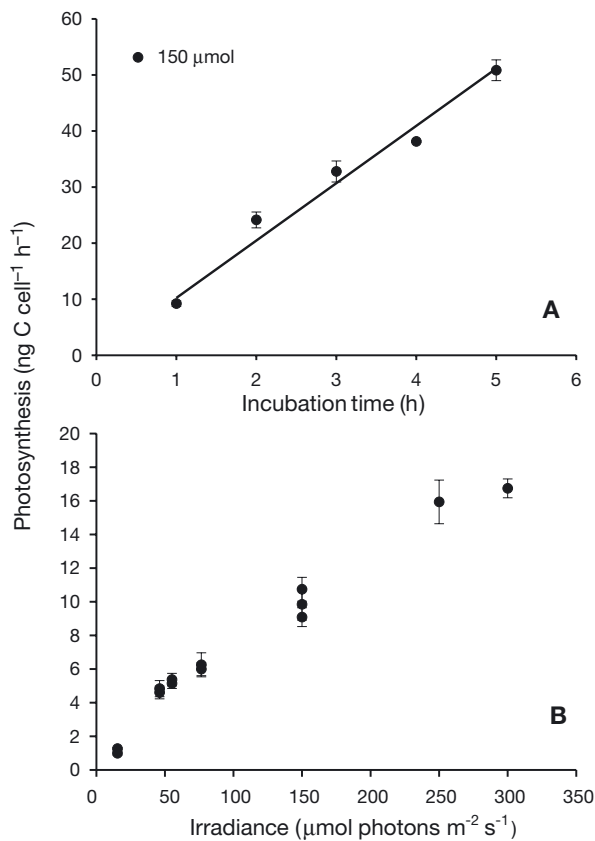

Fig. 3. *Noctiluca scintillans*. Photosynthetic performance of unfed individuals as a function of (A) incubation time at an irradiance of 150 µmol photons m^{-2} s^{-1}, (B) irradiance using <3 h incubation time. Data points represent means ± 1 SE (n = 4). The line in (A) represents a linear regression of all data points ($R^2 = 0.98$)
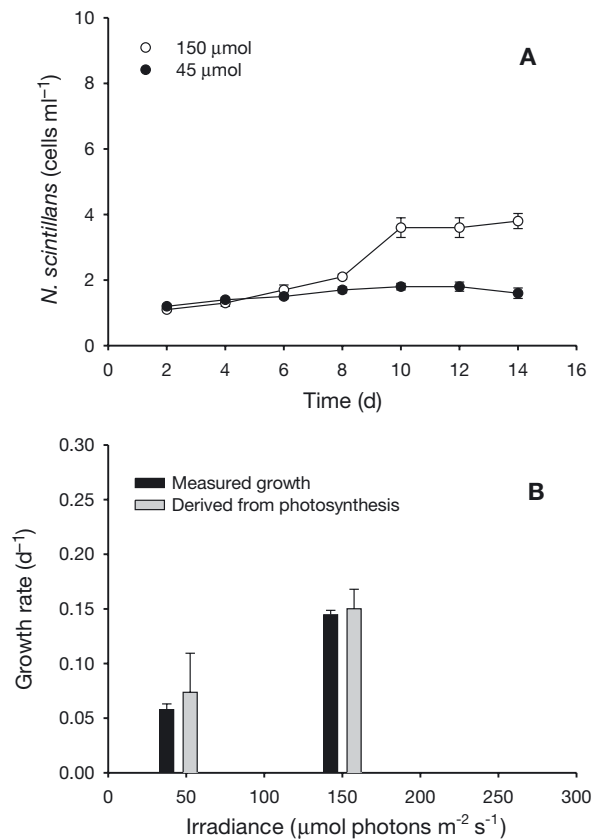

Fig. 4. *Noctiluca scintillans.* Unfed individuals. (A) Growth response as a function of incubation time at 2 irradiances: 45 and 150 µmol photons m^{-2} s^{-1}. (B) A Comparison of direct growth measurements, with growth rate estimates derived from measurements of photosynthesis assuming a photosynthetic growth yield of 50%
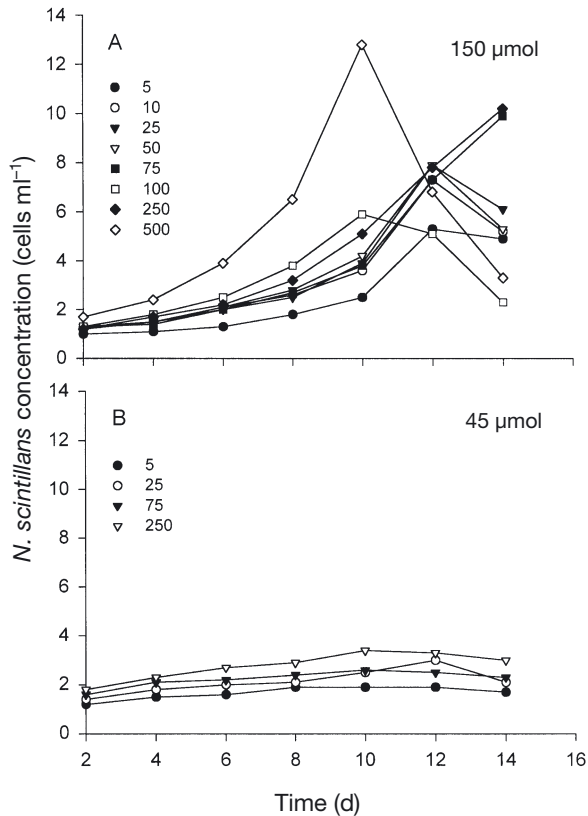
Fig. 5. *Noctiluca scintillans*. Growth/survival responses when fed different initial concentrations of *Pyrodinium bahamense* var. *compressum* at 2 irradiances: (A) 150 and (B) 45 µmol photons m^{-2} s^{-1}. Key shows concentrations of *P. bahamense* var. *compressum* in cells ml^{-1}
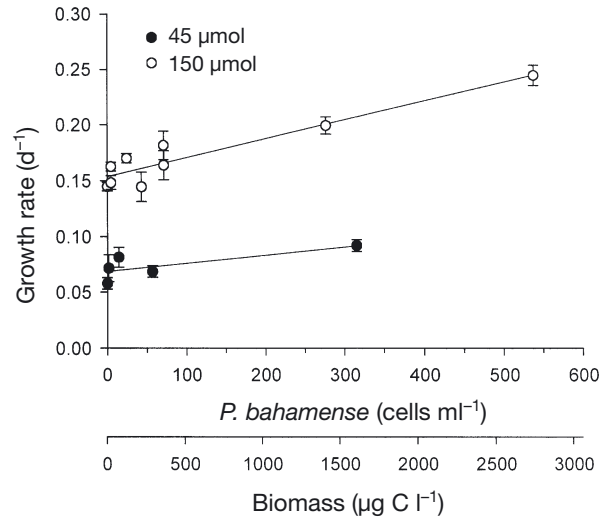

Fig. 6. *Noctiluca scintillans*. Growth rates as a function of the actual mean density in terms of both cell concentration (cells ml^{-1}) and biomass (µg C l^{-1}) at 2 irradiances: 150 and 45 µmol photons m^{-2} s^{-1}

observed growth, while a prey concentration as high as 2750 µg C l^{-1} was required for ingestion to explain 40% of the observed growth (Fig. 9).

The clearance of *Noctiluca scintillans* when fed *Pyrodinium bahamense* decreased only slightly with prey concentration, from 0.06 ml cell^{-1} d^{-1} at lowest prey concentrations to 0.03 ml cell^{-1} d^{-1} at very high prey concentrations (Fig. 10).

0.05). Ingestion rates were only measured at 150 µmol photons m^{-2} s^{-1} (Fig. 7). At this irradiance, the ingestion rate increased with prey concentration and showed no signs of saturation at prey concentrations below 540 *P. bahamense* cells ml^{-1}.

The experimental data on ingestion, growth and photosynthesis carried out at an irradiance of 150 µmol photons m^{-2} s^{-1} allow for a comparison of actually measured growth rates of *Noctiluca scintillans* with those that could be estimated from the photosynthesis and ingestion experiments. Assuming growth yields for photosynthesis and ingestion of 50 and 33%, respectively (e.g. Hansen & Nielsen 1997, Hansen et al. 2000), the measured rates of photosynthesis and ingestion can match the observed growth rates (Fig. 8). This makes it possible to compare the contribution of photosynthesis and ingestion to the growth the green *N. scintillans*. From this comparison, it is clear that the green *N. scintillans* gains most of its growth from the photosynthesis of its endosymbionts at an irradiance of 150 µmol photons m^{-2} s^{-1}. At a prey concentration of 1450 µg C l^{-1}, ingestion only explained 20% of the
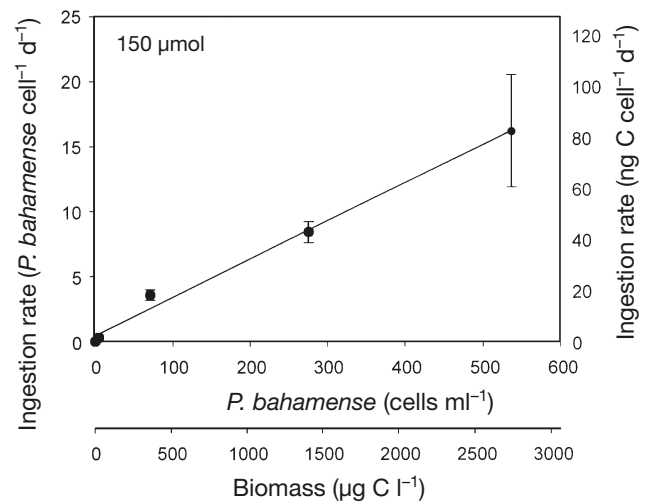

Fig. 7. *Noctiluca scintillans*. Ingestion rates as a function of *Pyrodinium bahamense* var. *compressum* concentration. Ingestion rates are given in both units of cells *Noctiluca*$^{-1}$ d^{-1} (left *y*-axis) and biomass cell^{-1} d^{-1} (right *y*-axis). Values of prey concentration are given in both terms of cell concentration (cells ml^{-1}) and biomass (µg C l^{-1})
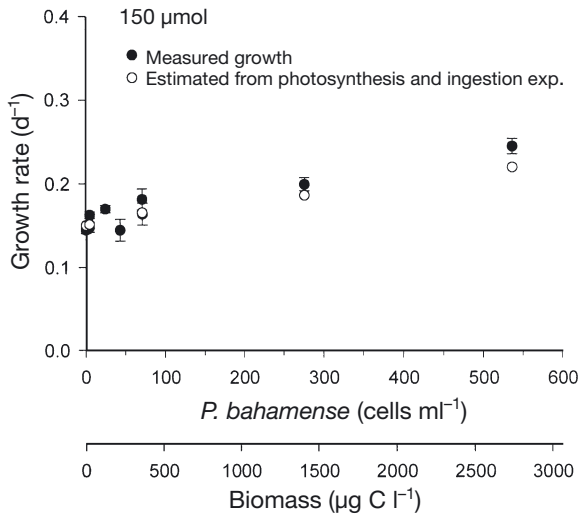
Fig. 8. *Noctiluca scintillans.* Growth rate (d^{-1}) as a function of prey concentration in cells ml^{-1} and biomass (µg C l^{-1}) when fed *Pyrodinium bahamense* var. *compressum* at an irradiance of 150 µmol photons m^{-2} s^{-1}. Growth rates derived from direct measurements of population growth (●) and from measurements of photosynthesis and ingestion rates (○), assuming a 50 and 33% growth yield for photosynthesis and ingestion, respectively

DISCUSSION

Role of photosynthesis and food uptake on the growth of green *Noctiluca scintillans*

In our experiments, the green *Noctiluca scintillans* grew in the light without the addition of prey, at least for a couple of weeks (Figs. 2 & 5). This is in accordance with experiments carried out by Sweeny (1971), also on freshly collected green *N. scintillans* cells. Here, we demonstrated that the growth of green *N. scintillans* in monoculture increases with irradiance,
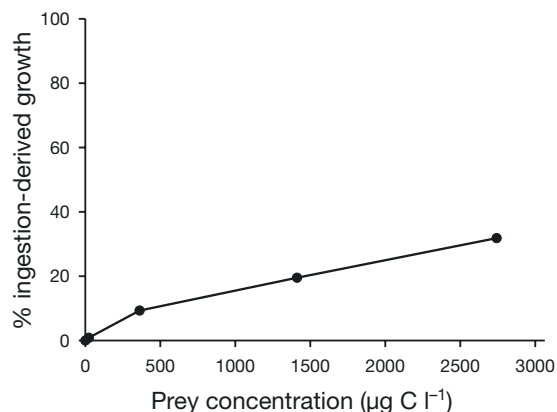

Fig. 9. *Noctiluca scintillans.* Percentage of growth obtained from ingestion as a function of *Pyrodinium bahamense* var. *compressum* concentration (µg C l^{-1}) assuming a 33% growth yield

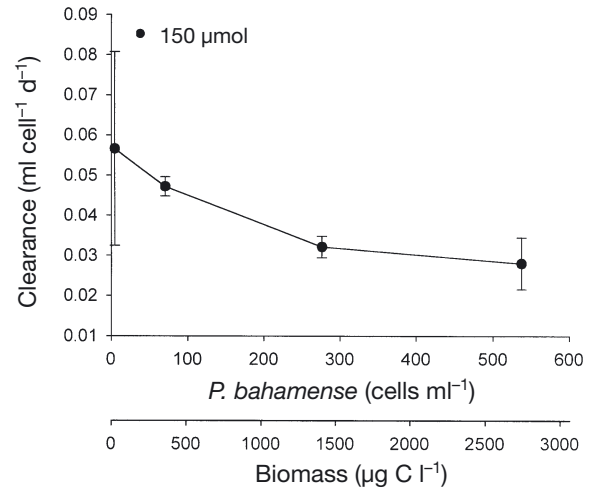Fig. 10. *Noctiluca scintillans.* Clearance (ml cell^{-1} d^{-1}) as a function of *Pyrodinium bahamense* var. *compressum* concentration in terms of cell concentration (cells ml^{-1}) and biomass (µg C l^{-1})

and that the increased growth could be explained by increased photosynthesis of its endosymbionts as irradiance increased (Fig. 4).

Sweeney (1971) observed the occasional presence of flagellates within small vacuoles in the cytoplasm of *Noctiluca scintillans*, suggesting the possibility of digestion of the endosymbionts by the host. However, according to her, food vacuoles were rarely seen. We looked for food vacuoles, but we observed no food vacuoles in *N. scintillans* cells when they were grown without the addition of prey. Thus, it seems most likely that the green *N. scintillans* largely depends on exudates from its endosymbionts, when grown in monoculture.

We fed 3 different prey items to the green *Noctiluca scintillans* and observed that it fed on all of them. The fastest growth rate of *N. scintillans* was achieved with the dinoflagellate *Pyrodinium bahamense* as prey, even though this species and isolate is known to produce saxitoxins (Oshima 1989). The addition of the other 2 algae, *Akashiwo sanguinea* (= *Gymnodinium sanguineum*) and *Tetraselmis tetrahele*, did lead to better growth compared to unfed controls, at least initially, but the rates were lower than with *P. bahamense* as prey. In fact, in the experiments in which *T. tetrahele* was used as prey, *N. scintillans* died out after 6 d of incubation, at which time the *T. tetrahele* population had become very dense (Fig. 2). *T. tetrahele* is not known to be toxic, and *N. scintillans* probably died out due to the development of very high pH in the dense mixed culture, caused by photosynthetic uptake of inorganic carbon (see Hansen 2002).

Even though *Pyrodinium bahamense* was the best of the 3 prey items tested, it only increased growth of the green *Noctiluca scintillans* by ~0.1 d^{-1} at high prey

concentrations. This might indicate that the green *N. scintillans* in nature primarily depends on the photosynthetic products from its endosymbionts. However, the growth rate of the red form of *N. scintillans* without endosymbionts has been shown to depend very much on the concentration, size and quality of the prey (Buskey 1995, Kiørboe & Titelman 1998, Nakamura 1998). The highest growth rates (>0.6 d^{-1}) have been obtained with diatoms and cryptophytes as prey, while intermediate growth rates (0.1 to 0.4 d^{-1}) typically have been obtained with dinoflagellates and other phytoflagellates as food. Algae, which do not support growth, or if they do it is only slight growth, are either very small phytoplankton species (<4 μm) or species of a poor food quality. Thus, the maximum growth increase of the green *N. scintillans* due to food uptake of ~0.1 d^{-1} found in the present study does not necessarily indicate that the green *N. scintillans* in nature will always primarily depend upon its endosymbionts. It is possible that higher growth rates could be obtained with prey other than those that we tested. The fact that food uptake in the green *N. scintillans* did not saturate even at very high concentrations of *P. bahamense* (2750 μg C l^{-1}) in our study supports this possibility. The best prey types for the red form of *N. scintillans* support maximum growth rates already at prey concentrations of between 500 and 1000 μg C l^{-1} (Buskey 1995, Kiørboe & Titelman 1998).

Impact of the green *Noctiluca scintillans* on natural cell concentrations of *Pyrodinium bahamense*

Gonzales (1989) was the first to observe that blooms of the green *Noctiluca scintillans* succeeded *Pyrodinium bahamense* blooms in Philippines waters. However, Azanza & Miranda (2001) gave the first indication that the green *N. scintillans* might be an important grazer on *P. bahamense* blooms. According to their study, *P. bahamense* blooms in Manila Bay, Philippines usually takes place during the southwest monsoon (May to October), characterized by the onset of the rainy season after a warm, dry period. However, in 1998 *P. bahamense* did not bloom. The reason for this is unknown, but the authors discovered that the green *N. scintillans* co-occurred with *P. bahamense* in May until about December of that year. This was unusual because the green *N. scintillans* is usually found in plankton samples in the dry Northeast monsoon from November to early March (Azanza & Miranda 2001). Thus, they hypothesized that the reason why the *P. bahamense* failed to bloom that year at least partly was due to the success of *N. scintillans*.

Our grazing experiments revealed that the green *Noctiluca scintillans* can remove *Pyrodinium beha-mense* cells from the water column with a clearance rate of between 0.03 and 0.06 ml *N. scintillans* d^{-1}, depending upon the cell concentration of *P. bahamense*. This means that between 3 and 60% of the *P. bahamense* population potentially will be removed from the water column every day by the green *N. scintillans* when it occurs in cell concentrations of 1 and 10 cells ml^{-1}, respectively. Therefore, even though grazing by *N. scintillans* on *P. bahamense* in most cases only contributes to a small fraction of the total carbon uptake of *N. scintillans*, it may still have a significant impact on dinoflagellate blooms in the area, such as *P. bahamense* blooms.

Why does *Noctiluca scintillans* lose its endosymbionts in laboratory cultures?

The green *Noctiluca scintillans* lost its endosymbionts after some time in our laboratory cultures under all culture conditions—an observation also made by Sweeney (1971). The fact that *Pedinomonas noctilucae* only has been found inside *N. scintillans* cells and that released cells do not survive for long in seawater or fresh water, not even at the low pH which is found inside *N. scintillans* cells (Sweeney 1971), indicates that the prasinophyte only can thrive inside *N. scintillans* cells. Therefore, an apparent unknown factor is lacking, which is essential for the growth of *P. noctilucae* and for a successful symbiosis between this species and *N. scintillans*. Most likely this growth factor derives from food ingested by *N. scintillans*.

Although many photosynthetic dinoflagellates are known to depend on external organic compounds (reviewed by Gaines & Taylor 1987), only *Gymnodinium gracilentum* has so far been shown to acquire essential growth factors through ingestion of food (Skovgaard 2000). However, other examples of photosynthetic algae, which depend on growth factors derived from their prey, have been found among mixotrophic chrysophytes (Kimura & Ishida 1985, 1989, Caron et al. 1993). Future work on *N. scintillans* is required to determine the nature of the growth factor and to study its role for the success of the green *N. scintillans* in nature.

CONCLUSION

The green *Noctiluca scintillans* is photosynthetically active and can grow for up to 2 wk in the laboratory without the ingestion of food. We failed to grow the green *N. scintillans* with its endosymbionts for longer time periods, despite the fact that we fed it different prey types. Thus, symbiosis between the green

N. scintillans and its endosymbionts seems to rely on a growth factor, which probably derives from the food it ingests. Future research is required to study the nature and role of the growth factor. We discovered that the green *N. scintillans* can feed on the toxic dinoflagellate *Pyrodinium bahamense* and utilize it as food for growth. Ingestion rates were, however, quite low, except at very high concentrations of *P. bahamense*. Despite this, our results suggest that the green *N. scintillans*, when it occurs in bloom concentrations, will have a substantial grazing impact on natural *P. bahamense* populations.

LITERATURE CITED

Azanza RV, Miranda LN (2001) Phytoplankton composition and *Pyrodinium bahamense* toxic blooms in Manila Bay, Philippines. J Shellfish Res 20:1251–1255

Buskey EJ (1995) Growth and bioluminescence of *Noctiluca scintillans* on varying algal diets. J Plankton Res 17:29–40

Caron DA, Sanders RW, Lim EL, Marrasé C, Amaral LA, Whitney S, Aoki RB, Porter KG (1993) Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. Microb Ecol 25:93–111

Chen H, Qi S (1991) The feeding and vegetative reproduction diurnal rhythms of *Noctiluca scintillans*. J Jinan Univ China 12:104–107

Corrales RA, Hall S (1993) Isolation and culture of *Pyrodinium bahamense* var. *compressum* from the Philippines. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, p 725–730

Corrales RA, Maclean JL (1995) Impacts of harmful algae on sea farming in the Asia-Pacific areas. J Appl Phycol 7: 151–162

Elbrächter M, Qi Y (1998) Aspects of Noctiluca (Dinophyceae) population dynamics. In: Anderson DM, Cembella A, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. NATO ASI Series, 41, Berlin, p 315–335

Enomoto Y (1956) On the occurrence and the food of *Noctiluca scintillans* (Macartney) in the waters adjacent to the west coast of Kyushu, with special reference to the possibility of the damage caused to the fish eggs by that plankton. Bull Jpn Soc Sci Fish 22:82–89

Gaines G, Taylor FJR (1987) Heterotrophic nutrition. In: Taylor FJR (ed) The biology of dinoflagellates. Botanical Monographs 21, Blackwell Scientific Publications, Oxford, p 224–268

Gonzales CL (1989) *Pyrodinium* blooms and paralytic shellfish poisoning in the Philippines. In: Hallegraeff GM, Maclean JL (eds) Biology, epdemiology and management of Pyrodinium red tides. ICLARM Conf Proc 21, Fisheries Department, Ministry of Development, Bandar Seri Begawan, and International Center for Aquatic Resources Management, Manila, p 39–47

Hansen PJ (2002) The effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. Aquat Microb Ecol 28:279–288

Hansen PJ, Nielsen TG (1997) Mixotrophic feeding of *Fragilidium subglobosum* (Dinophyceae) on three species of *Ceratium*: effects of prey concentration, prey species and light intensity. Mar Ecol Prog Ser 147:187–196

Hansen PJ, Bjørnsen PK, Hansen B (1997) Zooplankton grazing and growth: scaling within the size range 2 µm to 2000 µm. Limnol Oceanogr 42:687–704

Hansen PJ, Skovgaard A, Glud RN, Stoecker DK (2000) Physiology of the mixotrophic dinoflagellate *Fragilidium subglobosum*. II. Effects of time scale and prey concentration on photosynthetic performance. Mar Ecol Prog Ser 201:137–146

Jakobsen HH, Hansen PJ, Larsen J (2000) Growth and grazing responses of two chloroplast-retaining dinoflagellates: effect of irradiance and prey species. Mar Ecol Prog Ser 201:121–128

Kimura B, Ishida Y (1985) Photophagotrophy in *Uroglena americana*, Chrysophyceae. Jpn J Limnol 46:315–318

Kimura B, Ishida Y (1989) Phospholipid as a growth factor of *Uroglena americana*, a red tide Chrysophyceae in Lake Biwa. Nippon Suisan Gakkaishi 55:799–804

Kiørboe T, Titelman J (1998) Feeding, prey selection and prey encounter mechanisms in the heterotrophic dinoflagellate *Noctiluca scintillans.* J Plankton Res 20:1615–1636

Nakamura Y (1998) Growth and grazing of a large heterotrophic dinoflagellate, *Noctiluca scintillans* in laboratory cultures. J Plankton Res 20:1711–1720

Oshima Y (1989) Toxins in *Pyrodinium bahamense* var *compressum* and infested marine organisms. In: Hallegraeff GM, Maclean JL (eds) Biology, epidemiology and management of *Pyrodinium* red tides. ICLARM Conf Proc 21, Fisheries Department, Ministry of Development, Bandar Seri Begawan, and International Center for Aquatic Resources Management, Manila, p 73–79

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Prasad R (1958) A note on the occurrence and feeding habits of *Noctiluca* and their effects on the plankton community and fisheries. Proc Indian Acad Sci B 47:331–337

Skovgaard A (2000) A phagotrophically derivable growth factor in the plastidic dinoflagellate *Gyrodinium resplendens* (Dinophyceae). J Phycol 36:1069–1078

Skovgaard A, Hansen PJ, Stoecker DK (2000) Physiology of the mixotrophic dinoflagellate *Fragilidium subglobosum*. I. Effects of phagotrophy and irradiance on photosynthesis and carbon content. Mar Ecol Prog Ser 201:129–136

Stoecker DK, Silver MW, Michaels AE, Davis LH (1988) Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. Mar Biol 99:415–423

Sweeney BM (1971) Laboratory studies of a green *Noctiluca* from New Guinea. J Phycol 7:53–58

Sweeney BM (1976) *Pedinomonas noctilucae* (Prasinophyceae), the flagellate symbiotic in *Noctiluca* (Dinophyceae) in Southeast Asia. J Phycol 12:460–464