Growth and photosynthesis of the 'brown tide' microalga *Aureococcus anophagefferens* in subsaturating constant and fluctuating irradiance

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ABSTRACT During the summer of 1985, a small (2 to 3 µm) chrysophyte, *Aureococcus anophageffer-ens*, bloomed and dominated the phytoplankton assemblage, and recurred each summer for the following 12 yr in bays of Long Island, New York, USA. Macronutrients remained at high levels throughout the years but Secchi disc depths were as low as 30 cm in some areas, corresponding to a 1% light depth of about 80 cm. In batch culture, *A. anophagefferens* maintained high growth rates at low irradiances. The initial slope of the growth versus irradiance curve, α_g, was 0.021 ± 0.003 divisions d^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$ which is similar to that for bloom formers such as *Skeletonema costatum*. In continuous culture, constant and fluctuating irradiance regimes resulted in equivalent steady state growth rates at 0.46 divisions d^{-1}. Short-term carbon fixation per unit chlorophyll *a* was about twice as high in the fluctuating irradiance regime compared with constant irradiance. Differences between short-term carbon fixation and cell growth could not be accounted for by dark fixation or exudation and are assumed to be due to photorespiration. The photosynthesis versus irradiance parameter, P_{max} (light saturated photosynthetic rate), was similar in both irradiance regimes; however, α_p (initial slope) was consistently higher in the fluctuating regime, indicating that photosynthetic efficiency is higher in fluctuating irradiance.

KEY WORDS: *Aureococcus anophagefferens* · Photoacclimation · Brown tide Fluctuating irradiance

INTRODUCTION

Great South Bay and the Peconic Bay system of Long Island, New York (USA) are both shallow (1 to 3 m average depth) well-mixed estuaries. The summer phytoplankton assemblage is typically dominated by nanoplankton (2 to 20 µm) which can contribute greater than 80% of the total biomass in terms of chlorophyll *a* (chl *a*) (Bruno et al. 1983, Lively et al. 1983) and up to 95% of the total production (Lonsdale et al. 1996). This phytoplankton assemblage supports scallop *Argopecten irradians* and hard clam *Mercenaria mercenaria* populations. Primary production for the Great South Bay is high, and has been estimated to be around 450 g C m^{-2} yr^{-1} (Lively et al. 1983). Shallow

benthic communities are dominated by eelgrass *Zostera marina* which provides a nursery ground for many larval and juvenile finfish species and a substratum for the attachment of juvenile scallops (Dennison et al. 1989).

During the summer of 1985, a small (2 to 3 µm), previously undescribed, chrysophyte bloomed and dominated the phytoplankton assemblage of the Long Island bays. Its co-occurrence in both Narraganset Bay, Rhode Island, and Barnegat Bay, New Jersey, suggests involvement of regional meteorological conditions (Cosper et al. 1987, 1989). While the bloom never fully returned to Rhode Island or New Jersey, it recurred sporadically in Long Island bays each summer for the following 12 yr.

The alga was identified and described by Seiburth et al. (1988) as the chrysophyte *Aureococcus anophagef-*

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ferens gen. et sp. nov. Examination of its absorption and excitation-emission properties as well as the presence of 19'butanoyloxyfucoxanthin pigment have indicated that it is particularly suited to a blue light environment and this has led some researchers to hypothesize that A. anophagefferens may be an expatriate oceanic species (Bidigare 1989, Yentsch et al. 1989). In laboratory growth experiments A. anophagefferens has a high salinity preference and a wide temperature tolerance which allows it to overwinter in a vegetative state at low growth rates (Cosper et al. 1989). Laboratory and field experiments demonstrated that A. anophagefferens has a requirement for selenium and high concentrations (μM) of iron (Gobler 1995).

The bloom was first recognized by a golden-brown coloration of the water and high turbidity. Secchi disc depths were as low as 30 cm in some areas corresponding to a 1% light depth of about 80 cm. Cell concentrations were as high as 2×10^6 cells ml^{-1}, and estimates of carbon turnover times for the <5 μm size fraction were on the order of 3 h (Cosper et al. 1987, 1989). Chl a concentration and water column primary production rates were similar to those for non-bloom years, however. The high turbidity has been attributed to increased cell concentrations as well as increased scattering due to the small size of the cell (Cosper et al. 1987, 1989). The severe light attenuation contributed to the mass die-off of eelgrass Zostera marina and a reduction in its maximum depth range, from 3–4 m pre-1985 to 1.6–2.2 m in 1988 (Dennison et al. 1989). For reasons probably relating to chronic toxicity, scallops in affected areas experienced complete recruitment failure which resulted in a total loss of the populations (Bricelj & Kuenstner 1989).

Light availability is a potentially important factor in the blooms of Aureococcus anophagefferens. Macronutrient surveys during bloom periods have shown that nitrogen species (NH_4^+, NO_2^-, NO_3^-) and inorganic phosphorus were in excess at all times, and that nutrient concentrations were not correlated either with variations in biomass or productivity of A. anophagefferens (Cosper et al. 1987, 1989). Lively et al. (1983), in a study of Great South Bay, concluded that mean photic zone irradiance is limiting to primary production at intensities below 300 langley d^{-1} (57 mol quanta m^{-2} d^{-1}). Similar results were obtained by Bruno et al. (1983) working in Great Peconic Bay and Flander's Bay (New York) with 280 langley d^{-1} (53 mol quanta m^{-2} d^{-1}) being limiting.

Efficient light utilization during nutrient saturated conditions could be an important factor in determining plankton species composition. We therefore hypothesize that the ability of Aureococcus anophageffer-

ens to maintain high growth rates under low irradiance could be an important contributing factor to its dominance. In addition, the shallow (80 cm) photic zone observed during periods of maximum biomass combined with wind mixing allows for large fluctuations in the light field experienced by phytoplankton. Fluctuating light influences carbon assimilation and could potentially affect growth rates (Marra 1978). Therefore, carbon assimilation and growth rates of A. anophagefferens are compared in both fluctuating and constant irradiance.

METHODS AND MATERIALS

Culture methods. Cultures of Aureococcus anophagefferens isolated from Peconic Bay by E.M.C. were grown either in batch or continuous culture with modified f/2 enriched seawater (Guillard & Ryther 1962). Surface seawater (1000 l; salinity 28) was collected from Peconic Bay at New Suffolk, New York, and stored at 5°C in a polyethylene container. Batches (10 l) of seawater were prefiltered through a Gelman 0.2 μm cartridge filter and autoclaved for 50 min at 110°C with Tris (hydroxymethyl) aminomethane (THAM) buffer, with vitamins and $NaNO_3$ added. All other nutrients were autoclaved separately and added aseptically after the seawater was cooled in a 10°C water bath. Glycerophosphate was used at f/2 concentrations in place of orthophosphate and iron was reduced to f/20 concentration. Nitrilotriacetic acid (NTA, 5.0 μM) was used as the chelator in place of Disodium (Ethylenedinitrilo) tetraacetate (EDTA) (Cosper et al. 1993).

The sub-saturating irradiance used for continuous cultures was determined by a growth versus irradiance curve generated using batch cultures. Cultures were grown aseptically in triplicate 6 ml test tubes and were maintained at 23°C on a 12 h light:12 h dark cycle in the photosynthetron described below. Growth during acclimation to each irradiance was monitored daily using in vivo fluorescence (Brand et al. 1981) over a period of 2 wk or longer. After the acclimation period, cultures were transferred into fresh medium and growth was followed through the exponential growth phase. All cultures were preserved with Lugol's iodine for microscopic enumeration. Growth rates were calculated using cell number.

Continuous cultures of 5 l each were grown in light-limited cyclostat mode (Rhee et al. 1981) at 18 ± 2°C in a walk-in incubator. A constant average biomass was maintained using a peristaltic pump adjusted to the growth rate of the culture. Light was provided by a GE PAR 500 full spectrum tungsten bulb and filtered through 0.2% $CuSO_4$ solution with a 27 cm path length

to remove infra-red energy and to better simulate the natural light spectrum. Subsaturating irradiance with a 12 h light:12 h dark photoperiod was provided in 2 ways: as constant irradiance at 70 µmol quanta m^{-2} s^{-1} and as fluctuating irradiance, where irradiance was varied as a sine function with an amplitude between 10 and 180 µmol quanta m^{-2} s^{-1} and a period of 1 h. Fluctuating irradiance was attenuated with a disk of neutral density nickel screens rotated at 1 cycle per hour, consistent with possible Langmuir circulation rates (Cosper 1982b). Both irradiance treatments provided an equal daily quantum flux of 3.1 mol quanta m^{-2} d^{-1}. Irradiance was measured using a quantum meter (Biospherical Instruments QSL-100) fitted with a 4π sensor.

Once the cultures reached steady state, cells were sampled for photosynthetic rate, organic carbon excretion rate, and chl a specific absorption cross-section ($\bar{a}^*$) on 3 consecutive days at 2 and 6 h after the onset of the light period. These time points were chosen to include maximum photosynthetic rates (6 h) and submaximum rates (2 h). Photosynthesis versus irradiance (P vs I) curves were also generated for both time points. Samples were also collected and stored frozen for determination of chl a, carbon and nitrogen per cell, and fixed with Lugol's iodine for cell number.

Cell division. Specific growth rates in the continuous culture were calculated at steady state using the equation

$$\mu = v \cdot V_r^{-1} \qquad (1)$$

where v is the volume in liters of medium added per day and V_r is the volume of the culture ($V_r = 5$ l). Cell counts were performed microscopically at the same time each day, using a hemacytometer to determine when steady state was reached, and at each time point during the experimental period. At least 8 counts were performed for each time point.

Photosynthesis and excretion. Photosynthesis versus irradiance curves were obtained using the photosynthetron protocol of Lewis & Smith (1983). Incubations were run for 20 min. Rates at 70 µmol quanta m^{-2} s^{-1} are reported as short-term carbon fixation. Ten irradiances were used and the data fit to Eq. (2) (from Platt & Gallegos 1980) using non-linear regression.

$$P_B = P_s(1 - e^{-a})e^{-b} \qquad (2)$$

where P_B is the photosynthetic rate normalized to chl a, $a = \alpha E/P_s$ and $b = \beta E/P_s$; P_s is the maximum photosynthetic rate that would be achieved if no photoinhibition occurred; and α is the initial slope of the curve, β is the slope of the descending branch of the curve, and E is the scalar irradiance. Eq. (2) was also used in modified form to calculate the initial slope of the growth versus

irradiance curve and the light saturated growth rate, where α_g (the initial slope of the growth vs irradiance curve) is substituted for α, G_{max} (maximum growth rate) is substituted for P_s and the growth rate (divisions d^{-1}) is substituted for P_B.

Reported values for long-term light and dark carbon fixation rates were estimated using 2 h incubations with ^{14}C bicarbonate. Approximately 15 µCi NaH^{14}CO$_3$ was added to two 60 ml aliquots of culture and incubated for 2 h in a light bottle and a dark bottle adjacent to the continuous culture. Excretion of organic carbon in each bottle was estimated following Cosper (1982a). The filtrate from 0.45 µm nitrocellulose filters (Millipore) was acidified with HCl to a pH of 2 and aerated for 2 h. Activity of all ^{14}C samples was measured using scintillation counting.

Chlorophyll a. Triplicate 10 ml aliquots were filtered onto 13 mm Gelman AE glass fiber filters and stored frozen at $-20°C$ in 15 ml polycarbonate centrifuge tubes. Pigments were extracted in 10 ml 90% acetone for 24 h at 5°C. Samples were centrifuged for 5 min at $920 \times g$ in a bench top centrifuge to remove any filter debris and were measured fluorometrically on a Turner Designs fluorometer (Strickland & Parsons 1972). Chl a was calculated using fluorescence values before and after acidification with a few drops of 10% HCl. The fluorometer was calibrated against a Spectronic 1201 spectrophotometer using 90% acetone extracted chl a from an actively growing culture of Thalassiosira weissflogii (Jeffrey & Humphrey 1975).

CHN analysis. Triplicate 10 ml aliquots of culture were filtered onto precombusted 13 mm Gelman AE glass fiber filters and analyzed by the Nutrient Analytical Laboratory of the Chesapeake Bay Laboratory using standard gas chromatographic methods.

Absorption cross-section ($\bar{a}^*$). In vivo attenuation (absorption not corrected for scattering) was obtained on whole cell suspensions in a 10 cm cuvette using a Spectronic 1201 spectrophotometer. The spectrally averaged, chl a specific absorption cross-section was calculated according to Falkowski et al. (1985):

$$\bar{a}^* = \sum(a_\lambda^* \cdot I_\lambda \cdot \Delta\lambda_n) \cdot [\sum(I_\lambda \cdot \Delta\lambda)]^{-1} \qquad (3)$$

where $\bar{a}^*$ is the spectrally averaged, chl a absorption cross-section (m^2 mg^{-1} chl a), a_λ^* is the wavelength specific absorption cross-section, $\Delta\lambda$ is the wavelength interval of the spectral band n and I_λ is the irradiance in the spectral band $\Delta\lambda_n$.

Statistical analyses. Results were analyzed using 1-way and 2-way analysis of variance (ANOVA) after determining homogeneous variance using the methods of Sokal & Rohlf (1981). Samples taken from continuous cultures on consecutive days were considered independent.
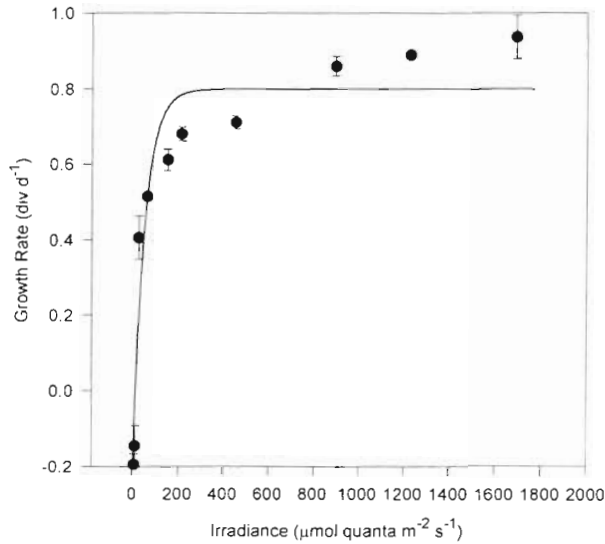
Fig. 1. *Aureococcus anophagefferens*. Growth rate at various irradiances for batch cultures shown with model fit to data (Eq. 2). α_g = 0.021 divisions d^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$. G_{max} = 0.8 divisions d^{-1}. Error bars show ±1 SD (n = 3)

RESULTS

Growth versus irradiance

The growth rate versus irradiance curve generated for batch cultures of *Aureococcus anophagefferens* shows that the growth efficiency (α_g) was 0.021 ± 0.003 divisions d^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$ when fitting the data to Eq. (2) (r^2 = 0.89). The half saturating growth irradiance I_k was 69 μmol quanta m^{-2} s^{-1} (Fig. 1). Negative growth rates at the lowest irradiances (8 and 11.5 μmol quanta m^{-2} s^{-1}) were probably not representative of the compensation irradiance considering that *in vivo* fluorescence showed that these cells were actively producing pigment.

Light treatments

Steady state growth rates of continuous cultures grown under constant and fluctuating irradiance were equivalent at 0.456 divisions d^{-1} ± 0 and 0.461 divisions d^{-1} ± 0.047 respectively. Long-term carbon fixation rates (2 h incubation) were also similar, but short-term carbon fixation rates (20 min incubation) in fluctuating irradiance were about twice as high as in constant irradiance (p < 0.01, Fig. 2A). Higher short-term carbon fixation rates cannot be accounted for either in long-term excretion of organic carbon, which was significantly higher (p < 0.05) in constant irradiance, or in long-term dark carbon fixation, which was not significantly different in either treatment.
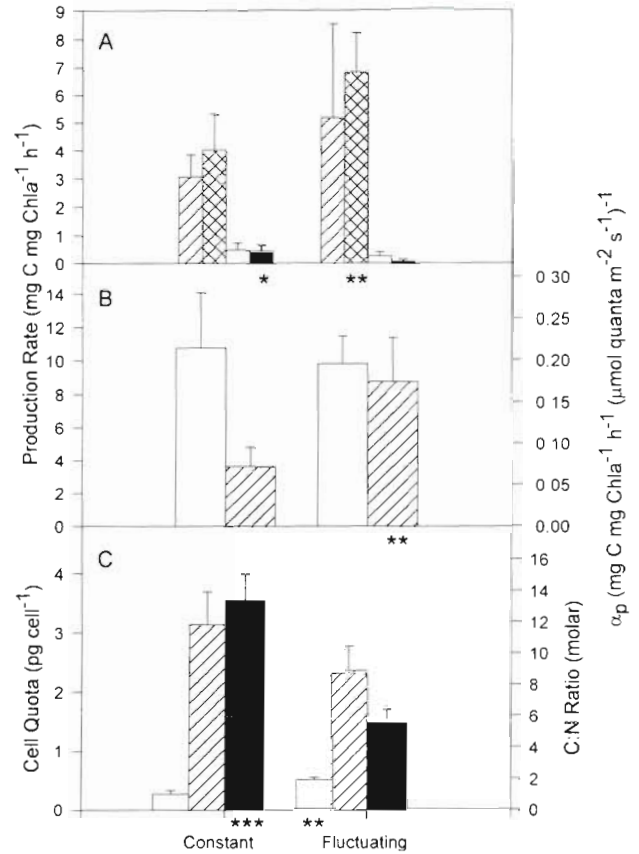

Fig. 2. *Aureococcus anophagefferens*. Comparison of photosynthetic rates and cell quotas for constant and fluctuating irradiance regimes. Morning and noon values pooled for illustration only. Significance values based on 2-way ANOVA of unpooled data: *0.05, **0.01, ***0.001. (A) Long-term carbon fixation rate (2 h, ▨), short-term carbon fixation rate (20 min, ▨), dark carbon fixation rate (2 h, ▢), carbon excretion rate (2 h, ■). (B) *P* vs *I* parameters. Maximum photosynthetic rate, P_{max} (left axis, ▢), initial slope α_p (right axis, ▨). (C) Nitrogen cell^{-1} (left axis, ▢), carbon cell^{-1} (left axis, ▨), C:N ratio (right axis, ■). Error bars are +1 SD

The initial slope of the *P* versus *I* curve (α_p) was significantly higher (p < 0.01) under fluctuating irradiance indicating a higher photosynthetic efficiency, while the light saturated photosynthetic rate (P_{max}) was not significantly different in either treatment (Fig. 2B). Photoinhibition was induced during incubation for cells grown in the fluctuating irradiance treatment (β = 3.10×10^{-3} ± 9.32×10^{-4}) but not in cells grown in constant irradiance (β = 6.37×10^{-4} ± 6.49×10^{-4}; Fig. 3).

Carbon per cell in constant irradiance was higher but was not significantly different from the fluctuating irradiance treatment (Fig. 2C). Nitrogen per cell was significantly higher (p < 0.01) in fluctuating irradiance and this leads to a significantly lower (p < 0.001) C:N ratio in fluctuating irradiance (Fig 2C). The spectrally
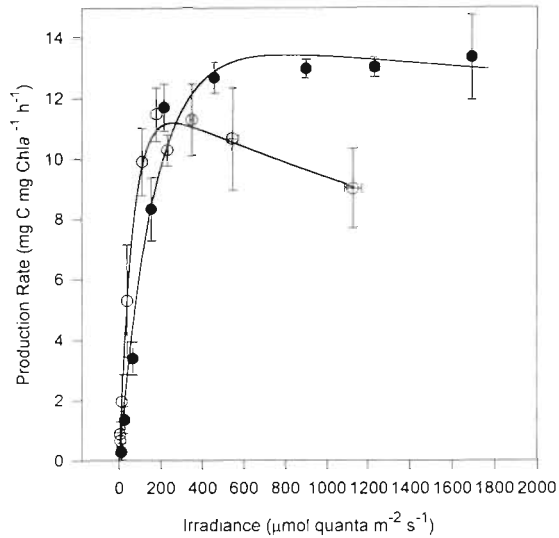
Fig. 3. *Aureococcus anophagefferens*. Noon time photosynthetic rates at various irradiances for constant (●) and fluctuating (O) irradiance regimes and model fit to data (Eq. 2). Error bars show ±1 SD (n = 6)
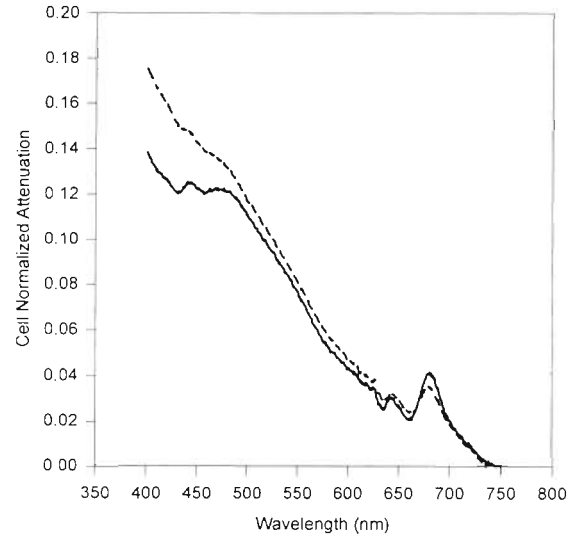

Fig. 4. *Aureococcus anophagefferens*. *In vivo* attenuation spectra normalized to cell number grown under constant (solid line) and fluctuating (broken line) irradiance

averaged chlorophyll a absorption cross-section ($\bar{a}^*$) was lower in fluctuating irradiance (Table 1); however, chl a per cell was similar (insignificant differences) in both treatments. Differences in absorption cross section were also reflected in the *in vivo* attenuation spectra which was significantly higher (p < 0.001) in the Soret region of the spectra in fluctuating irradiance (Fig. 4). The differences in attenuation spectra were determined from a 2-way ANOVA performed on the absorbance at 450 nm, which is near the peak absorbance for chl c, fucoxanthin and 19'butanoyloxy-fucoxanthin (Bidigare 1989).

Table 1. Parameters used in calculation of quantum efficiency of growth (ϕ_μ). See text for definitions of variables

Variable	Value (units)
Constant irradiance (12 h light:12 h dark)	
μ	0.32 (d⁻¹)
$\bar{a}^*$	0.0069 (m² mg chl a⁻¹)
Chl a	0.037 (pg cell⁻¹)
Carbon	3.145 (pg cell⁻¹)
Calculated ϕ_μ	0.104 (carbon-atoms photon⁻¹)
Calculated 1/ϕ_μ	9.46 (photons carbon-atom⁻¹)
Fluctuating irradiance (12 h flucuating light:12 h dark)	
μ	0.32 (d⁻¹)
$\bar{a}^*$	0.0054 (m² mg chl a⁻¹)
Chl a	0.029 (pg cell⁻¹)
Carbon	2.33 (pg cell⁻¹)
Calculated ϕ_μ	0.125 (carbon-atoms photon⁻¹)
Calculated 1/ϕ_μ	7.95 (photons carbon-atom⁻¹)

DISCUSSION

Light

While several authors have concluded that primary production in the bays of Long Island is often light limited, the question remains whether all macro and micronutrients were saturating during the blooms of *Aureococcus anophagefferens* (Bruno et al. 1983, Lively et al. 1983). In an effort to objectively assign light limiting or nutrient saturating conditions, Bannister (1974a) introduced 2 equations which predict maximum areal biomass in terms of chl a and maximum areal production under nutrient saturating conditions and, in a companion paper (Bannister 1974b), used them to define nutrient saturation in several lakes. Both equations require the assumption of steady state phytoplankton production and grazing pressure as well as the assumption of a homogeneously mixed water column which absorbs most of the incident light. The latter assumption is satisfied in bays of Long Island. It is unknown whether the former assumption is satisfied. The driving variables in both equations are the incident irradiance, I_0, the extinction coefficient of water, k_w, the concentration of chl a, the extinction coefficient of the phytoplankton, k_c, and the efficiency of the conversion of absorbed quanta to photosynthetic products, ϕ_{max}.

The maximum biomass of a totally absorbing, well-mixed water column can be calculated from the equation (Bannister 1974b):

$$\gamma = \frac{\Psi}{\theta \cdot (R_p + \mu)} - k_w \ d/k_c \qquad (4)$$

where γ is the maximum attainable biomass in mg chl a m^{-2}, θ is the weight ratio of carbon to chl a (dimensionless), R_p is the rate constant for carbon lost to respiration and excretion in d^{-1}, μ is the specific growth rate in d^{-1}, k_w is the extinction coefficient in m^{-1} for water and non-phytoplankton components (estimated from Secchi disk depth where $k_w = 1.7/$Secchi depth (m) – k_c), k_c is the extinction coefficient for phytoplankton, d is the depth of the mixed layer, and Ψ is the upper limit to production which is given by (Bannister 1974b):

$$\Psi = 12 \cdot \phi_{max} \cdot I_{0.7} \cdot \lambda \int_{-1/2}^{+1/2} \sinh^{-1} (I_0 + I_{0.7}) \cdot dt' \qquad (5)$$

where λ is the time in days from sunrise to sunset, t' is the rationalized time of day such that $-\frac{1}{2}$ is sunrise and $+\frac{1}{2}$ is sunset, ϕ_{max} is the maximum carbon yield in moles of carbon per mole quanta of visible light absorbed, $I_{0.7}$ is the irradiance in moles quanta m^{-2} d^{-1} at which photosynthesis is 70% of maximum. The factor 12 converts moles of carbon to grams. I_0 is irradiance in mol quanta m^{-2} d^{-1} just under the surface and is a function of time t' such that (Bannister 1974b):

$$I_0 = 0.5 \cdot I_{0\,max} \cdot (1 + \cos 2\Pi t') \qquad (6)$$

The above equations were used to calculate the maximum attainable biomass for West Neck Bay, Shelter Island, New York. This site has been a traditional field station due to the continuous presence of *Aureococcus anophagefferens*. Model parameters are given in Table 2.

Of field data from the *Aureococcus anophagefferens* bloom of 1991 in West Neck Bay, 4 sets fall into the region of the graph which predicts nutrient saturated conditions, while 1 set from 1991 and all sets from 1988 and 1987 fall within the region of nutrient limitation (Fig. 5). Measured nutrient (PO$_4^-$, NH$_4^+$, NO$_3^-$, NO$_2^-$) concentrations in West Neck Bay were in excess in the 1988 and 1987 samples (1991 not analyzed). Cell concentrations of *A. anophagefferens* were about 1×10^5 cells ml^{-1} in 1988 while in 1987 concentrations were on the order of 2.5×10^5 cells ml^{-1} and never rose above 1×10^6 cells ml^{-1}. The lower biomass of 1988 and 1987 samples could either be attributed to limitation by some unmeasured micronutrient such as iron, or more likely, by a non-steady state between production and consumers. This violates an assumption of this model. Nevertheless, nutrient saturating or light limiting conditions can be identified during the height of the 1991 bloom when cell concentrations were often above 1×10^6 cells ml^{-1} Based on this model steady state cell concentrations on the order of 10^5 cells ml^{-1} would be expected to be controlled by nutrient limitation and/or grazing pressure.

Table 2. Parameters used in calculation of maximum areal biomass under nutrient saturating conditions. Except where noted, all parameters from Bannister (1974b). See text for definitions of variables

Variables	Values (units)
ϕ_{max}	0.06 (mol carbon mol quanta^{-1})
$I_{0.7}$	8.64 (mol quanta m^{-2} d^{-1}) [a]
Equinox (40° N)	
$I_{0\,max}$	100 (µmol quanta m^{-2} s^{-1})
λ	0.5 (d)
Calculated Ψ	6.46 (g C m^{-2} d^{-1})
Summer solstice (40° N)	
$I_{0\,max}$	137.5 (µmol quanta m^{-2} s^{-1})
λ	0.626 (d)
Calculated Ψ	9.14 (g C m^{-2} d^{-1})
Secchi depth	0.21–1.0 (m) [b]
Chl a	10 and 60 (mg chl a m^{-2}) [b]
k_c	0.016 (m^{-2} mg chl a^{-1})
θ	0.0114 (g C mg chl a^{-1})
μ	1 (d^{-1})
R_p	0.186 (d^{-1})
d	2 (m) [c]

[a]Data for *Aureococcus anophagefferens* from this study
[b]Ranges of field data from Long Island bays
[c]Average depth of Long Island bays

The mean photic zone irradiance for light limited 1991 data is 5.2 ± 1.3 mol quanta m^{-2} d^{-1} and is much lower than the average of 55 mol quanta m^{-2} d^{-1} given by Lively et al. (1983) and Bruno et al. (1983). It
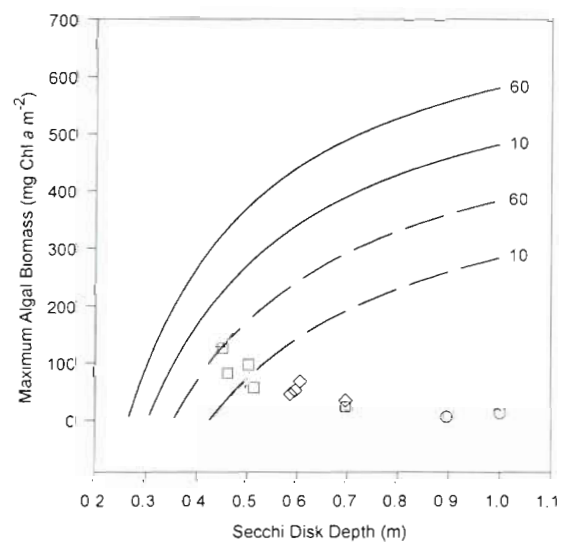

Fig. 5. Results from Bannister's (1974b) model predicting maximum biomass under nutrient saturating conditions. Summer solstice (solid lines) and equinox (broken lines) irradiances with k_w calculated from Secchi disk data and assuming a chlorophyll concentration of 10 and 60 mg chl a l^{-1} Field data from West Neck Bay, New York in 1991 (O), 1988 (◇), 1987 (□)
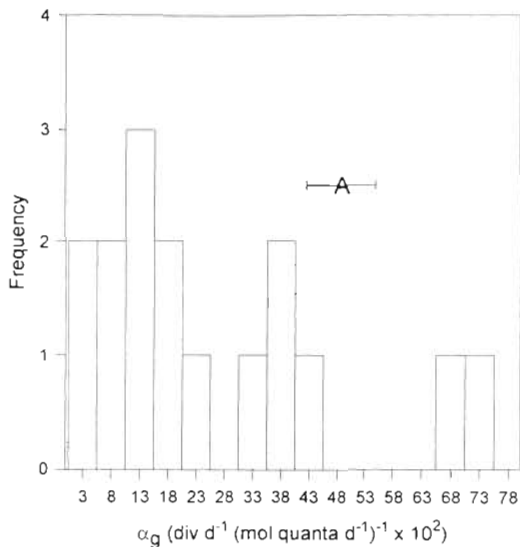
Fig. 6. Frequency distribution of 16 growth efficiencies (α_g) as reviewed by Langdon (1988) with the value for *Aureococcus anophagefferens* (A) plotted with 95% confidence limits. All growth efficiencies were normalized to daylength

appears unlikely that irradiances as high as 55 mol quanta m^{-2} d^{-1} would result in light limitation as this corresponds to a mean instantaneous irradiance of 1100 µmol quanta m^{-2} s^{-1}

The growth versus irradiance curve for *Aureococcus anophagefferens* shows that this species has a high growth efficiency. The α_g (the initial slope) value was 0.021 ± 0.003 divisions d^{-1} (µmol quanta m^{-2} s^{-1})$^{-1}$ or 0.49 ± 0.065 divisions d^{-1} (mol quanta m^{-2} d^{-1})$^{-1}$ when normalized to daylength and falls into the high end of measured growth efficiencies (Fig. 6). For comparison, each growth efficiency, as reviewed by Langdon (1988), has been normalized to daylength by using light periods from original manuscripts. Typical values range from 0.097 to 0.73 divisions d^{-1} (mol quanta m^{-2} d^{-1})$^{-1}$ In general *A. anophagefferens* is similar in its growth efficiency to diatoms such as the 'bloom' former *Skeletonema costatum* [α_g = 0.49 divisions d^{-1} (mol quanta m^{-2} d^{-1})$^{-1}$, Langdon 1987] and *Leptocylindrus danicus* [α_g = 0.39 divisions d^{-1} (mol quanta m^{-2} d^{-1})$^{-1}$, Verity 1981]. *A. anophagefferens* would therefore be expected to maintain a growth rate under severe light limitation which would be competitive with other known bloom formers. However, during the period in which blooms of *A. anophagefferens* form, usually mid-summer, large diatoms such as *S. costatum* and *L. danicus* are not present in Long Island bays.

Light utilization efficiency can also be assessed by calculating the quantum efficiency of growth ϕ_μ, which is the efficiency of the conversion of absorbed quanta to carbon which is retained for growth (excludes carbon lost to respiration, photorespiration and excretion) and is

given in units of moles of carbon (mol quanta)$^{-1}$. ϕ_μ can be calculated from the equation (Falkowski et al. 1985):

$$\phi_\mu = \mu \cdot [\bar{a}^* \cdot (\text{chl:C}) \cdot I_0 \cdot 12\,000]^{-1} \qquad (7)$$

where μ is the specific growth rate of carbon d^{-1}, chl:C is the weight ratio (in g) of chl *a* to carbon per cell, I_0 is the incident irradiance in mol quanta m^{-2} d^{-1}, and $\bar{a}^*$ is the spectrally averaged, chl *a* specific absorption cross-section calculated according to Eq. (3). The constant 12000 converts mg carbon to moles carbon. All parameters used are given in Table 1.

The calculated quantum requirements (1/ϕ_μ) for *Aureococcus anophagefferens* grown under constant and fluctuating irradiance are 9.5 photons carbon-atom^{-1} and 7.9 photons carbon-atom^{-1} respectively. These values appear unlikely as they are close to the theoretical limit for photosynthesis (8 photons carbon-atom^{-1}) and well below reported values which range from 20 to 180 (Falkowski et al. 1985). It is possible that *A. anophagefferens* could be supplementing its carbon demand by using dissolved organic carbon (DOC). This would violate the implicit assumption of photolithotrophy in the above model and explain low quantum requirements. Dzurica (1988) has shown that *A. anophagefferens* can rapidly assimilate glutamic acid, and this source could supply up to 200% of the carbon and nitrogen demands of *A. anophagefferens* over short-term incubations (3 h). Using the photosynthetic rates for constant irradiance from morning (3 h) and noon (6 h), the carbon excretion rate (Table 2) and an estimated respiration rate of 12%, we calculate an additional carbon requirement of 0.57 pg C cell^{-1} d^{-1} Based on mass balance of carbon under cyclostat conditions, a total level of 110 µM DOC would be required to support observed growth rates. The glycerophosphate added to culture media would provide 108 µM DOC. Glycerol has been shown to be an effective carbon source for mixotrophic phytoplankton (Lewitus & Caron 1991). Also, DOC in coastal waters is several orders of magnitude above this level (Sugimura & Suzuki 1988).

Fluctuating light has been considered important to algal photosynthesis and is also considered a possible source of error in estimates of primary production (Cullen & Lewis 1988). Fluctuating irradiance appears to have no influence on the growth rate of *Aureococcus anophagefferens* but does influence carbon fixation. The higher carbon fixation rates observed in the fluctuating irradiance regime were most likely due to the higher photosynthetic efficiency as observed in the α_p values, suggesting that electron transport has become more efficient under fluctuating irradiance. The excess carbon was not contributing to growth, however, and may be lost as CO_2 through photorespiration, especially considering that long-term excretion rates were

actually lower in fluctuating irradiance. The presence of photoinhibition in fluctuating irradiance is also consistent with higher photorespiratory rates; however, cells are not exposed to photoinhibitory levels during culturing (Fig 3) The findings presented here agree with Cospers' (1982a) conclusions that photosynthetic rates of *Skeletonema costatum* were enhanced in fluctuating irradiance but that growth rate remained the same under equal daily quantum flux and excess carbon fixed under fluctuating irradiance was found to be lost through photorespiration.

The higher nitrogen content of cells exposed to fluctuating irradiance may in part be due to photorespiratory recycling of internal NH_4^+ Alternatively, higher nitrogen quotas may be reflecting an accumulation of pigment-protein. Although accessory pigments were not measured directly, the higher *in vivo* absorbance in the Soret band of cells grown in fluctuating irradiance indicate that there were differences in accessory pigment content (Fig 4). The increase in photosynthetic efficiency (α_p) also supports this idea. This evidence along with the lower $\bar{a}^*$ value for fluctuating irradiance indicates that *Aureococcus anophagefferens* was acclimating to an irradiance somewhat below the average instantaneous irradiance of 70 µmol quanta m^{-2} s^{-1} used in the fluctuating irradiance treatment. A decrease in $\bar{a}^*$ is a common response to acclimation to low light (Falkowski et al. 1985).

While high light-utilization efficiency of *Aureococcus anophagefferens* may explain its persistence once the photic zone is reduced to 80–100 cm, it does not explain the initiation of a bloom. In summer, at the onset of a bloom, irradiance and nutrients are saturating and phytoplankton biomass (95 % < 5 µm) is most likely grazer controlled by microzooplankton (D. J. Lonsdale, M. A. Frey, R. Mehran, G. T. Taylor & R. M. Waters unpubl.). Mehran (1996) has found that protozooplankton may discriminate against *A. anophagefferens* when cell concentrations are > 2.5×10^5 cells ml^{-1}, although discrimination below 2.5×10^5 cells ml^{-1} has not been observed. Based on the above model results, cell concentrations on the order of 10^5 cells ml^{-1} ought to be controlled by nutrient concentrations or grazing Further work is required to elucidate the interactions between grazers and *A. anophagefferens* at the early stages of bloom initiation. Clearly higher grazing rates on phytoplankton other than *A. anophagefferens* could give this species a competitive advantage at the early stage of a bloom before light levels become limiting.

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