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Growth and grazing responses of the mixotrophic dinoflagellate *Dinophysis acuminata* as functions of light intensity and prey concentration

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ABSTRACT: *Dinophysis acuminata*, a photosynthetic marine dinoflagellate, possesses plastids of cryptophyte origin and causes diarrhetic shellfish poisoning (DSP). Recent work has shown *D. acuminata* to be a mixotroph that grows well when feeding on the photosynthetic ciliate *Myrionecta rubra*. Using established cultures, we examined the effects of light intensity and prey (*M. rubra*) concentration on growth and ingestion rates of *D. acuminata*. Growth rates increased with increasing prey concentration under continuous illumination of 60 µE m^{-2} s^{-1}, with maximum mixotrophic growth (0.91 d^{-1}) almost 5 times higher than growth in the absence of prey (0.19 d^{-1}). The maximum ingestion rate of *D. acuminata* was 1296 pg C *Dinophysis*$^{-1}$ d^{-1} (3.2 *M. rubra* cells *Dinophysis*$^{-1}$ d^{-1}) for data fitted to a Michaelis-Menten equation. Growth rate also increased with increasing light intensity, an effect even stronger when prey was supplied. Increased growth with increasing irradiance was accompanied by a corresponding increase in ingestion. While *D. acuminata* continued to grow in semi-continuous food-replete cultures at high (200 µE m^{-2} s^{-1}) and low (10 µE m^{-2} s^{-1}) light intensity, it failed to grow in darkness, despite the presence of prey. Our results suggest that *D. acuminata* is an obligate mixotroph that requires both light and prey for long-term survival. Results indicate that *Dinophysis* species are typically prey-limited in the field.

KEY WORDS: *Dinophysis acuminata* · *Myrionecta rubra* · Growth · Grazing · Light · Mixotrophy · Phagotrophy

INTRODUCTION

The marine dinoflagellate genus *Dinophysis* Ehrenberg includes both photosynthetic and non-photosynthetic species. *Dinophysis* species are globally distributed in tropical and temperate marine environments over a broad range of salinities, although usually found in low abundances (mostly <100 cells l^{-1}; Nishitani et al. 2002, 2005). Photosynthetic species in this genus, however, have been reported to cause dense blooms (up to 10^7 cells l^{-1}) responsible for diarrhetic shellfish poisoning (DSP) in many parts of the world (Yasumoto et al. 1980, Hallegraeff & Lucas 1988, Lee et al. 1989). Due to the impact of DSP on aquaculture and human health, photosynthetic *Dinophysis* are recognized as a harmful algal bloom (HAB) species of socioeconomic importance. Nonetheless, the biology, ecophysiology, and toxicology of these species are not

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well understood, as photosynthetic *Dinophysis* have only recently been cultured and maintained in the laboratory (Park et al. 2006).

Dinophysis acuminata Claparède et Lachmann is a photosynthetic species that contains cryptophyte-like plastids (Schnepf & Elbrächter 1988). Despite the presence of plastids, long-term growth has never been sustained in any culture medium (e.g. f/2 medium) known to support growth of other phytoplankton species (e.g. Sampayo 1993, Granéli et al. 1997). Setälä et al. (2005) noted that plastids of *D. acuminata* incubated in the laboratory showed reduced yellow-orange fluorescence, became smaller, and gradually aggregated into 1 or 2 'packages' over time. Light and electron microscopic observations on *D. acuminata* have revealed the presence of feeding structures, including food vacuoles and a peduncle-like arrangement of microtubular ribbons (Lucas & Vesk 1990, Jacobsen & Anderson 1994, Koike et al. 2005). In addition, recent molecular reports have proven that the plastid 16S rRNA and *psbA* genes of *D. acuminata* are identical to those of the cryptophyte *Teleaulax amphioxeia* (Takishita et al. 2002, Janson & Graneli 2003, Janson 2004, Minnhagen & Janson 2006). These studies support the notion that plastids in *D. acuminata* are kleptoplastids derived from prey containing a plastid of cryptophyte origin. If true, then *D. acuminata* is likely mixotrophic, capable of utilizing phagotrophic and photosynthetic nutritional strategies.

Although a number of heterotrophic dinoflagellates are known to retain prey plastids (e.g. Larsen 1988, Fields & Rhodes 1991, Skovgaard 1998, Lewitus et al. 1999a, Jakobsen et al. 2000, Eriksen et al. 2002), the relative importance of phagotrophy and photosynthesis for growth and survival of kleptoplastidic dinoflagellates is not well understood. The few studies that have addressed this issue (e.g. Skovgaard 1998, Jakobsen et al. 2000) indicate that kleptoplastids enhance growth and survival by providing an alternative carbon source when food is limited.

Recently, Park et al. (2006) successfully established *Dinophysis acuminata* in laboratory culture and reported that it uses a peduncle to extract and ingest cell contents of its prey organism, a plastidic ciliate *Myrionecta rubra* (= *Mesodinium rubrum*), itself a consumer of cryptophytes. Using cultures of *D. acuminata*, we explored (1) the effects of prey (*M. rubra*) concentration on *D. acuminata* growth and ingestion, and (2) the effects of irradiance on the phototrophic growth, feeding, and mixotrophic growth of *D. acuminata*. Ecophysiological responses of this species, including species-specific feeding and growth characteristics, provide new insights into *Dinophysis* autecology, vertical distribution patterns, and environmental conditions that underline bloom dynamics. Expanding knowledge about the autecology of *Dinophysis* species may improve predictive capabilities necessary for optimal management of shellfish industries affected by DSP.

MATERIALS AND METHODS

Cultures. *Dinophysis acuminata* (strain DA-MAL01) was isolated from Masan Bay, Korea, in December 2005 and grown in 30 psu f/2-Si medium at 20°C under continuous illumination of 60 µE m^{-2} s^{-1} (Park et al. 2006). Stock cultures were maintained as ~200 ml volumes in 250 ml polycarbonate bottles and fed by adding 5 to 10 ml of the ciliate *Myrionecta rubra* (strain MR-MAL01) as prey every 2 to 3 d. Immediately after adding prey, densities of *D. acuminata* and *M. rubra* were approximately equal. Stock cultures were diluted 1:9 with fresh medium and transferred to clean polycarbonate bottles at weekly intervals. The ciliate prey were maintained under the above conditions and fed the cryptophyte *Teleaulax* sp. at approximately 5 d intervals as described by Yih et al. (2004).

Growth and grazing responses as functions of prey concentration. Growth and grazing responses of *Dinophysis acuminata* were determined over *Myrionecta rubra* densities ranging from 0 to 10 000 cells ml^{-1}. Stock cultures of *D. acuminata* and *M. rubra* were diluted using fresh f/2-Si medium and distributed to 500 ml polycarbonate bottles to achieve triplicate treatments with target predator/prey concentrations (cells ml^{-1}) of 5/10, 10/50, 20/100, 50/500, 100/1000, 300/3000, 500/5000, and 1000/10 000. In addition, triplicate control bottles were established for prey only at densities as above and for predator only at 100 cells ml^{-1}.

Aliquots (5 ml) withdrawn from the bottles at the start of the experiment (Day 0) and at 24 h intervals over 7 d were fixed with acid Lugol's solution (final concentration of 2%). Abundances of *Dinophysis acuminata* and/or *Myrionecta rubra* were estimated for each Lugol's-fixed sample by scanning triplicate Sedgwick-Rafter chambers at 100× magnification (Olympus BX 50). Cells present in optical transects of each chamber were summed until the entire chamber was examined, or until >200 cells were counted. Growth rates (µ, d^{-1}) for *D. acuminata* and *M. rubra* were calculated for each sampling interval using the following exponential growth equation:

$$\mu = \frac{\ln N_1 - \ln N_0}{t_1 - t_0} \quad (1)$$

where N_1 and N_0 are cell concentrations at time t and time 0, respectively, and $t_1 - t_0$ is the time interval between samplings. From these data, 0 to 48 h was

selected as the optimal interval for calculating predator growth, as *D. acuminata* abundance increased steadily and prey density was reduced by less than 50%. Data for growth rates during the optimal interval were fitted to a modified Michaelis-Menten model that included a positive *y*-axis intercept, as *D. acuminata* is photosynthetic and continues to grow for several days in the absence of prey. Parameters for the modified Michaelis-Menten model were estimated using SigmaPlot software (Version 9.01, MMIV Systat Software) and the equation:

$$\mu = \frac{\mu_{max}(x - x')}{K_m + (x - x')} \quad (2)$$

where μ is growth rate of *D. acuminata*, μ_{max} is maximum growth rate, x is prey (*M. rubra*) concentration (cells ml^{-1}), x' is compensation point of prey concentration where growth is 0 ($\mu = 0$), and K_m is prey concentration sustaining $\frac{1}{2}\mu_{max}$.

Ingestion rate (I, as prey predator^{-1} h^{-1}) for *Dinophysis acuminata* was calculated according to Frost (1972), with adjustment for predator growth following Heinbokel (1978) and refined by Jeong & Latz (1994). Specifically, ingestion rates were calculated as:

$$I = [C]\, F \times 24 \quad (3)$$

where $[C]$ is mean prey (*Myrionecta rubra*) concentration (cells ml^{-1}) averaged for incubation time for treatment bottles, and F is the clearance rate (volume predator^{-1} h^{-1}):

$$F = V\mathrm{g}\,[P]^{-1} \quad (4)$$

where V is the volume (ml) of cultures in experimental bottles, g is the grazing constant (h^{-1}), and $[P]$ is mean number of predators in treatment bottles averaged for incubation time:

$$[P] = \frac{P_0(e^{\mu t} - 1)}{\mu t} \quad (5)$$

where P_0 is initial concentration of *D. acuminata*.

Growth and ingestion rates were plotted against mean prey concentration calculated as:

$$[C] = \frac{C_0(e^{t(k-g)} - 1)}{t(k - g)} \quad (6)$$

where C_0 is initial prey concentration in the treatments, and k is the prey growth constant, calculated as $k = \ln(C_t/C_0)/t$, where C_t is final prey concentration in control bottles after incubation for time t (h). The grazing constant (g) was calculated as:

$$g = k - \frac{\ln([C_t]/[C_0])}{t} \quad (7)$$

where the final prey concentration $[C_t]$ in treatments reflects the effects of grazing and growth; $[C_t] = [C_0]e^{t(k-g)}$.

Short-term responses of growth and grazing as a function of light intensity. A 1 wk old stock culture of *Dinophysis acuminata* was acclimated at each of 3 different light intensities (10, 60, 120 μE m^{-2} s^{-1}) for 3 to 4 d. Each acclimated culture was diluted to 100 *D. acuminata* ml^{-1} using fresh medium and distributed to each of twelve 50 ml tissue culture flasks as 50 ml culture volumes. For each set of 12 flasks, 6 received *Myrionecta rubra* at saturating prey concentration (1000 ml^{-1}) and 6 received no prey, with 3 prey-replete and 3 prey-free treatments incubated at each of 2 light levels. Light levels used for incubation were 0 and 10 μE m^{-2} s^{-1} for the 10 μE m^{-2} s^{-1} acclimated culture, 30 and 60 μE m^{-2} s^{-1} for the 60 μE m^{-2} s^{-1} acclimated culture, and 120 and 200 μE m^{-2} s^{-1} for the 120 μE m^{-2} s^{-1} acclimated culture. Triplicate prey-only controls (1000 *M. rubra* ml^{-1}) were incubated at each light level. Light intensities were measured with a radiometer (Model QSL-2101, Biospherical Instruments) placed near the center of 50 ml tissue culture flasks filled with f/2-Si medium. Subsamples were taken every 24 h from each flask to estimate abundance of predator and prey. An optimal incubation time of 72 h was chosen for calculating growth and ingestion rates, as that interval gave the highest growth rate and showed no depletion of prey (i.e. less than 50% reduction in prey density). Growth and ingestion rates were calculated as explained above, with growth in the presence and absence of prey representing mixotrophic and phototrophic growth, respectively.

Growth efficiency (GE, %) was defined as predator carbon produced per prey carbon ingested. GE of *Dinophysis acuminata* at each light treatment was calculated according to:

$$GE = \frac{(\mu_{mix} \times C_{D\text{-mix}}) - (\mu_{ph} \times C_{D\text{-ph}})}{I \times C_M} \times 100 \quad (8)$$

where μ_{mix} and μ_{ph} represent mixotrophic and phototrophic growth rates of *D. acuminata* in food-replete and prey-free culture, respectively, I is ingestion rate (prey cells *Dinophysis*$^{-1}$ h^{-1}), and $C_{D\text{-mix}}$, $C_{D\text{-ph}}$, and C_M indicate cell carbon content in mixotrophic *D. acuminata*, phototrophic *D. acuminata*, and *Myrionecta rubra*, respectively. Cell carbon content was estimated from cell volume determined by measuring cell length and width using an ocular micrometer on an Olympus microscope at 200× magnification, with at least 30 cells measured per replicate for each light intensity. Cell volumes of *M. rubra* and *D. acuminata* were calculated using V (μm^3) = (π/6) $w^2 \times l$ (Johnson & Stoecker 2005) and (π/6) $w \times d \times l$ (Vadrucci et al. 2007), respectively, where w, d, and l are cell width, depth, and length, respectively. Carbon content was calculated from cell volume using conversion factors 0.19 pg C μm^{-3} for oligotrich ciliates (Putt & Stoecker 1989) and carbon

Table 1. *Dinophysis acuminata* feeding on *Myrionecta rubra*. Predator and prey concentrations, ratios, and growth rates for treatments assessing functional response. Initial concentration of prey [C_0] and predator [P_0]; ratio of [C_0]:[P_0], growth rate (μ) of predator, prey growth constant (k), grazing constant (g), mean concentrations of prey ([C]) and predator ([P]) averaged for 48 h incubations, and ratio of [C]:[P]. Data are shown as mean (SE in parentheses)

[C_0] (cells ml^{-1})	[P_0] (cells ml^{-1})	[C_0]:[P_0]	μ (h^{-1})	k (h^{-1})	g (h^{-1})	[C] (cells ml^{-1})	[P] (cells ml^{-1})	[C]:[P]
6 (1)	5 (1)	1.2 (0.3)	0.014 (0.001)	0.044 (0.003)	0.006 (0.004)	16 (1)	7 (2)	2.4 (0.5)
44 (2)	8 (1)	5.8 (0.3)	0.020 (0.001)	0.032 (0.000)	0.001 (0.001)	103 (6)	13 (1)	8.0 (0.3)
80 (2)	17 (3)	5.1 (1.2)	0.017 (0.000)	0.037 (0.002)	0.010 (0.004)	163 (16)	26 (5)	6.7 (1.5)
461 (22)	41 (6)	11.6 (1.7)	0.031 (0.001)	0.032 (0.002)	0.008 (0.001)	883 (77)	94 (12)	9.6 (1.3)
1005 (64)	89 (6)	11.4 (1.4)	0.035 (0.003)	0.032 (0.002)	0.013 (0.002)	1659 (50)	196 (28)	9.0 (1.8)
3265 (314)	290 (8)	11.4 (1.5)	0.035 (0.000)	0.026 (0.000)	0.027 (0.001)	3236 (337)	753 (30)	4.3 (0.6)
5937 (168)	588 (37)	10.1 (0.4)	0.030 (0.001)	0.020 (0.003)	0.030 (0.001)	4749 (79)	1302 (86)	3.7 (0.2)
11118 (200)	1020 (64)	11.0 (0.6)	0.021 (0.002)	0.018 (0.000)	0.025 (0.003)	9448 (610)	1776 (42)	5.3 (0.2)

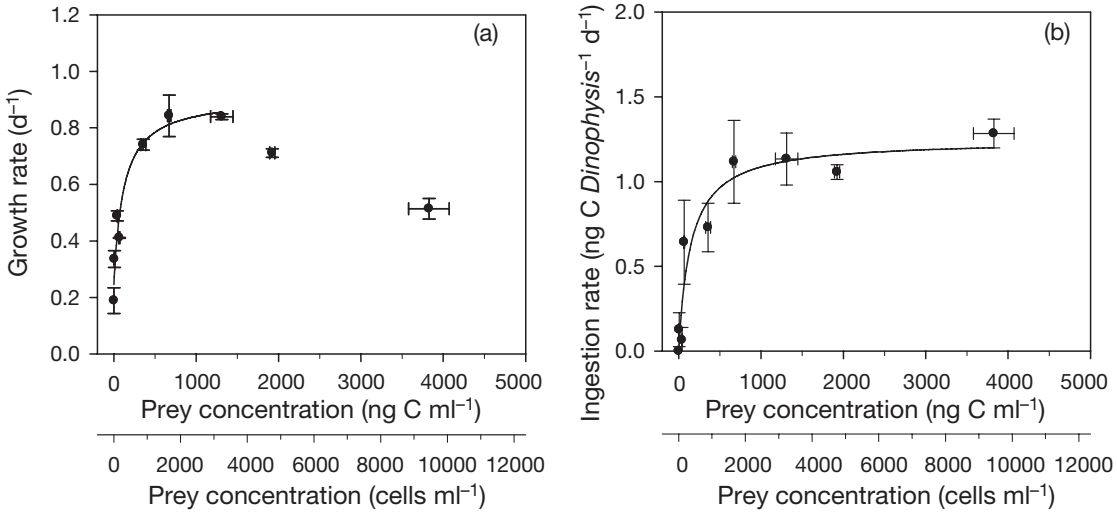

Fig. 1. *Dinophysis acuminata*. (a) Growth and (b) ingestion rates for the dinoflagellate as a function of mean prey density during 48 h incubations. Data points are shown as mean ± SE for 3 replicates. Data were fitted to a modified Michaelis-Menten equation (Eq. 2) for growth and a Michaelis-Menten equation for ingestion. Growth rate (d^{-1}) = $0.91(x + 77.4)/[210.3 + (x + 77.4)]$, $r^2 = 0.96$; ingestion rate (ng C *Dinophysis*$^{-1}$ d^{-1}) = $1.2x/(361.6 + x)$, $r^2 = 0.97$

(pg) = 0.216 × (volume, μm^3)$^{0.939}$ for dinoflagellates (Menden-Deuer & Lessard 2000).

Long-term effects of light intensity on growth kinetics. Growth kinetics of semi-continuous, food-replete *Dinophysis acuminata* cultures were determined under 3 different light regimens: continuous high light (200 μE m^{-2} s^{-1}), continuous low light (10 μE m^{-2} s^{-1}), and darkness (0 μE m^{-2} s^{-1}). Stock cultures (1 wk old) of *D. acuminata* and *Myrionecta rubra* were acclimated for 2 to 3 d at 2 different light levels: 200 μE m^{-2} s^{-1} and 10 μE m^{-2} s^{-1}. Each acclimated culture was diluted with fresh medium and distributed to eighteen 500 ml polycarbonate bottles to establish 9 prey only controls with 2000 *M. rubra* ml^{-1} and 9 predator–prey treatments with 200 *D. acuminata* ml^{-1} and 2000 *M. rubra* ml^{-1}. Bottles derived from the acclimated cultures were incubated in parallel with triplicate controls and triplicate treatments held at each of the 3 experimental light conditions. Samples taken from each bottle at the beginning of the experiment and at 24 h intervals over the following 21 d were fixed with acid Lugol's solution for determination of cell abundance as described above. At 4 to 5 d intervals, densities of *D. acuminata* and *M. rubra* were adjusted to initial predator and prey concentrations by addition of fresh medium and *M. rubra*. For the dark treatments, *D. acuminata* cultures were first concentrated by reverse filtration using 20 μm screening and then returned to densities existing prior to screening by addition of fresh medium and prey.

RESULTS

Effects of prey concentration on growth and grazing of *Dinophysis acuminata*

Mean abundances for *Myrionecta rubra* and *Dinophysis acuminata* at the start of incubations were very close to target densities and exhibited expected prey:predator ratios (Table 1). Growth and grazing rates for prey and predator resulted in changing densities and ratios over the following 48 h. *M. rubra* showed positive net growth in treatments with the 5 lower initial prey densities, while net growth in treatments with the higher initial prey densities was non-positive. In contrast, growth of *D. acuminata* was positive in all treatments. Prey:predator ratios calculated from mean abundances during 48 h incubation were similar (6.7 to 9.0) for treatments with intermediate prey densities (Table 1). Values at saturating prey densities (i.e. above ~2000 *M. rubra* ml^{-1} for growth and ingestion, see Fig. 1) were reduced by about half (3.7 to 5.3), while the mean prey:predator ratio for the lowest initial prey density was 2.4.

Mixotrophic growth rates of *Dinophysis acuminata* increased sharply with mean prey concentrations up to ~2000 cells ml^{-1}, remained constant between 2000 and ~3000 cells ml^{-1}, and decreased at prey densities above 3000 cells ml^{-1} (Fig. 1a). Maximum growth rate (μ_{max}) was 0.91 d^{-1} when data were fitted to a modified Michaelis-Menten equation, with prey concentration sustaining $\frac{1}{2}\mu_{max}$ being 210 cells ml^{-1}. By comparison, phototrophic growth rate of *D. acuminata* (i.e. growth in the absence of prey) was 0.19 d^{-1}.

Ingestion rates of *Dinphysis acuminata* feeding on *Myrionecta rubra* increased with increasing prey concentration, with saturation occurring above ~2000 cells ml^{-1} (Fig. 1b). Maximum ingestion rate (I_{max}) was 1296 pg C *Dinophysis*$^{-1}$ d^{-1} (3.2 cells *Dinophysis*$^{-1}$ d^{-1}) when fitted to a Michaelis-Menten equation, while prey concentration sustaining $\frac{1}{2}I_{max}$ was 362 cells ml^{-1}.

Short-term effects of light on growth and ingestion of *Dinophysis acuminata*

During short-term experiments, phototrophic and mixotrophic growth rates of *Dinophysis acuminata* increased with increasing light intensity, although the latter declined slightly at the highest light intensity (i.e. 200 µE m^{-2} s^{-1}) (Fig. 2a). Maximum mixotrophic and phototrophic growth rates were 0.78 and 0.34 d^{-1}, respectively, with light intensities sustaining $\frac{1}{2}\mu_{max}$ being 21.5 µE m^{-2} s^{-1} for mixotrophic and 24.2 µE m^{-2} s^{-1} for phototrophic *D. acuminata* cultures. *D. acuminata* biovolume in prey-replete cultures was positively related to light intensity, with a maximum of 12.5×10^3 µm^3 at 120 µE m^{-2} s^{-1} after 72 h (Table 2). By comparison, cell volume (mean ± SE) of *D. acuminata* in prey-free cultures showed no significant difference across light regimes, ranging from 8.1 ± 0.6 to 9.5 ± 0.6 × 10^3 µm^3.

Ingestion rates of *Dinophysis acuminata* on *Myrionecta rubra* increased sharply with irradiance up to 60 µE m^{-2} s^{-1} (Fig. 2b). I_{max} was 2265 pg C *Dinophysis*$^{-1}$ d^{-1} and light intensity sustaining $\frac{1}{2}I_{max}$ was 27.5 µE m^{-2} s^{-1}. Growth efficiency (GE, %) of mixotrophic *D. acuminata* cultures was variable over different light intensities, ranging from 14 to 54% (Table 2). GE was highest at a light intensity of 30 to 120 µE m^{-2} s^{-1}, with values on either side of that range reduced by 48 to 71%.

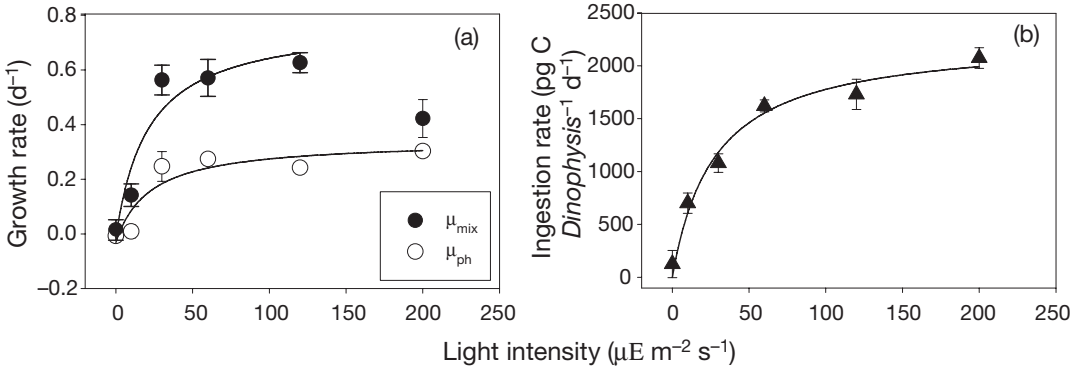

Fig. 2. *Dinophysis acuminata*. Short-term experiments showing (a) mixotrophic and phototrophic growth rates and (b) ingestion rate of the dinoflagellate as a function of light intensity. Data points are shown as mean ± SE for 3 replicates. Curves were fitted by a modified Michaelis-Menten equation (Eq. 2) for growth rates and a Michaelis-Menten equation for ingestion rates. Mixotrophic growth rate (μ_{mix}, d^{-1}) = $0.78(x - 0.38)/[21.50 + (x - 0.38)]$, $r^2 = 0.92$; phototrophic growth rate (μ_{ph}, d^{-1}) = $0.34(x - 2.02)/[24.23 + (x - 2.02)]$, $r^2 = 0.86$; ingestion rate (pg C *Dinophysis*$^{-1}$ d^{-1}) = $2265.46x/(27.54 + x)$, $r^2 = 0.98$

Table 2. Phototrophic growth (μ_{ph}) and mixotrophic growth (μ_{mix}) rates, ingestion rate (I), cell volume (V) and carbon content (C) of photo- and mixotrophic *Dinophysis acuminata* and of *Myrionecta rubra*, and growth efficiency (GE) of *D. acuminata* for cultures incubated at different light intensities. Data are shown as mean (SE in parentheses)

Light intensity (µmol m^{-2} s^{-1})	μ_{ph} (d^{-1})	μ_{mix} (d^{-1})	I (pg C d^{-1})	Cell volume (×10^3 µm^3)			Carbon content (pg C cell^{-1})			GE (%)
				$V_{D\text{-ph}}$	$V_{D\text{-mix}}$	V_M	$C_{D\text{-ph}}$	$C_{D\text{-mix}}$	C_M	
0	−0.01 (0.01)	0.01 (0.04)	126 (128)	8.2 (0.7)	6.4 (0.9)	1.6 (0.2)	1014 (85)	801 (107)	303 (36)	–
10	0.01 (0.02)	0.14 (0.04)	701 (96)	9.5 (0.6)	8.7 (0.9)	1.5 (0.2)	1171 (74)	1072 (104)	277 (33)	25 (9)
30	0.25 (0.05)	0.56 (0.05)	1080 (88)	8.1 (0.6)	10.5 (0.7)	1.8 (0.2)	1006 (70)	1292 (79)	337 (35)	54 (8)
60	0.27 (0.01)	0.57 (0.07)	1620 (57)	8.5 (0.2)	9.4 (1.0)	2.1 (0.2)	1052 (28)	1161 (118)	405 (41)	40 (7)
120	0.24 (0.00)	0.63 (0.04)	1730 (142)	9.0 (0.7)	12.5 (0.7)	3.0 (0.4)	1114 (76)	1517 (79)	568 (69)	50 (4)
200	0.30 (0.02)	0.40 (0.07)	2073 (97)	8.7 (0.6)	11.9 (0.8)	4.3 (0.6)	1073 (75)	1452 (86)	809 (117)	15 (5)

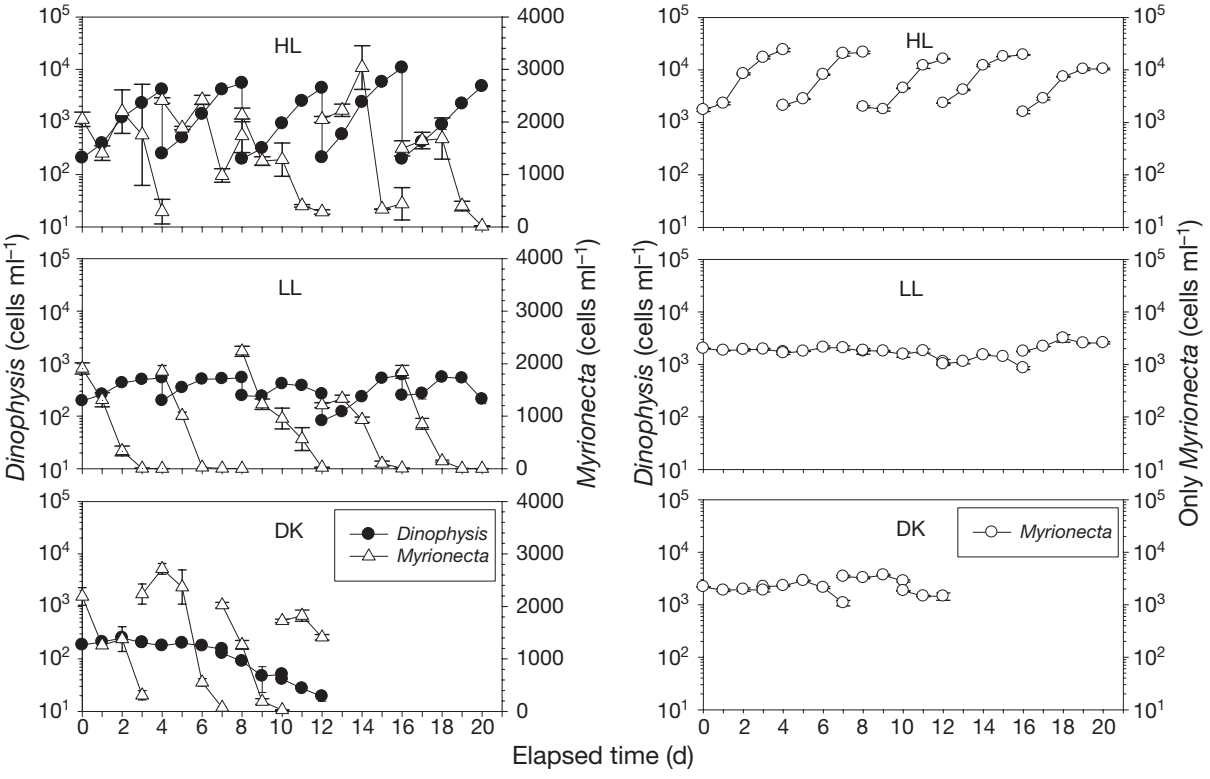

Fig. 3. *Dinophysis acuminata.* Long-term experiments showing growth kinetics of the dinoflagellate in semi-continuous food-replete cultures under high light (HL; 200 µE m^{-2}s^{-1}), low light (LL; 10 µE m^{-2} s^{-1}), and darkness (DK; 0 µE m^{-2} s^{-1}). Dilution with fresh f/2 medium and addition of prey *Myrionecta rubra* occurred periodically to return cultures to initial predator and prey concentrations. Data are shown as mean ±SE for 3 replicates

Long-term effects of light on growth kinetics of *Dinophysis acuminata*

During long-term experiments, semi-continuous cultures of *Dinophysis acuminata* transferred and fed at 4 to 5 d intervals maintained exponential growth for the first 2 to 3 d at 200 µE m^{-2} s^{-1}, even when prey were depleted (Fig. 3). By contrast, semi-continuous cultures in low light (10 µE m^{-2} s^{-1}) entered stationary growth shortly after depletion of prey. Growth rates (mean ± SE) of *D. acuminata* over the first 48 h of each feeding cycle averaged 0.90 ± 0.05 and 0.40 ± 0.03 d^{-1} in high and low light, respectively. Ingestion rates for that period were 1477 ± 55 and 1190 ± 65 pg C *Dinophysis*$^{-1}$ d^{-1} in high and low light, respectively. In darkness, *D. acuminata* abundance increased slightly over the first 2 d (μ = 0.16 ± 0.04 d^{-1}), remained stable over the following 5 d, and then declined (μ = −0.35 ± 0.16) even

though prey were being consumed (Fig. 3). When ingestion rates for the dark incubation were estimated over the first 48 to 72 h of each feeding cycle, the values for the first and second cycles were 791 ± 379 and 664 ± 143 pg C *Dinophysis*$^{-1}$ d^{-1}, respectively. The ingestion rate for the third period, however, increased dramatically to 2548 ± 556 pg C *Dinophysis*$^{-1}$ d^{-1}. There was no apparent ingestion during the last feeding cycle.

DISCUSSION

In reviewing protistan mixotrophy, Stoecker (1998) envisioned a spectrum of strategies ranging from primarily phototrophic organisms that feed to enhance growth when nutrients or light are limiting (Model II mixotrophs), to species that are primarily phagotrophic, but use photosynthesis to supplement carbon nutrition or enhance growth when prey are limiting (Model III mixotrophs). Ideal mixotrophs (Model I) grow equally well as phototrophs or as heterotrophs, but appear to be very rare. Several studies of dinoflagellate mixotrophy have considered species fitting Model II (e.g. *Akashiwo sanguinea*: Bockstahler & Coats 1993; *Ceratium furca*: Smalley et al. 2003; and *Prorocentrum minimum*: Stoecker et al. 1997), although species fitting Model III have been clearly documented (e.g. *Amphidinium poecilochroum*: Larsen 1988; *Cryptoperidinopsis* sp.: Eriksen et al. 2002; *Gymnodinium gracilentum*: Skovgaard 1998; and *Pfiesteria piscidia*: Lewitus et al. 1999b). Stoecker (1998) distinguished 2 types of Model III mixotrophs: those that are primarily phagotrophic, but that have their own plastids (Model IIIA) and those that harbor algal symbionts or sequestered prey plastids (Model IIIB). For Model IIIA organisms, carbon fixation decreases in the presence of prey. By contrast, carbon fixation by Model IIIB mixotrophs is positively related to ingestion and, thus, the availability of prey.

Dinophysis acuminata qualifies as a Model IIIB mixotroph, as ingestion of prey is required to sustain photosynthesis and growth (Park et al. 2006, this study). *D. acuminata* does not have algal symbionts, and sequestration of prey plastids has not been demonstrated conclusively. Recent work, however, provides strong indirect evidence supporting the notion that the cryptophye-like plastids of *Dinophysis* species are derived from their ciliate prey, *Myrionecta rubra* (Park et al. 2006, 2008).

Growth of *Dinophysis acuminata* increased with increasing prey concentrations, with maximum mixotrophic growth being almost 5 times higher than phototrophic growth. Mixotrophic growth rates, however, were somewhat reduced at very high prey concentrations, even though ingestion rates remained high. Why growth decreased when ingestion remained high is uncertain, but one possibility is that high density of photosynthetic prey (*Myrionecta rubra*) increased pH of the culture medium beyond the optimal range for *D. acuminata*. That suggestion is consistent with recent reports which showed that pH of culture medium strongly influences growth and survival of heterotrophic, as well as phototrophic dinoflagellates (Pedersen & Hansen 2003, Hansen et al. 2007).

Increasing light intensity had a positive effect on both phototrophic and mixotrophic growth of *Dinophysis acuminata*, but was more pronounced in the presence of prey, as indicated by slopes of the initial portion of fitted curves (Fig. 2). Ingestion rates also increased with light intensity, approaching saturation at irradiances giving maximum mixotrophic growth. Maximum growth in the presence of prey was roughly 3 times higher than in the absence of prey, although mixotrophic growth was reduced at the highest light intensity, showing rates similar to phototrophic growth, even though ingestion was high. There are at least 2 possible explanations for observed relationships of growth and ingestion rates with light level. Increased growth and feeding at higher irradiance may result from light-aided digestion of prey, as has been reported for other protists (Strom 2001). If true, then *D. acuminata* would be strongly heterotrophic, acquiring resources for growth through assimilation of prey biomass. Alternatively, *D. acuminata* may utilize essential growth factors and/or kleptoplastids from prey to support increased phototrophic growth at higher irradiances. One cannot, however, rule out a mixture of these 2 strategies. The cause for reduced mixotrophic growth at our highest irradiance, when phototrophic growth was unaffected, is more difficult to understand. If *D. acuminata* utilizes kleptoplastids, then rapid light-aided digestion at our highest irradiance may have stimulated catabolism of prey and inhibited incorporation of kleptoplastids. Conversely, rapid ingestion of prey at our highest light level may induce close packing of kleptoplastids, producing a 'self-shading' effect that limits photosynthesis and growth. In that case, reduced carbon fixation and growth should decrease the demand for resources obtained through feeding (i.e. essential nutrients and/or kleptoplastids from prey) and thus have negative feedback on ingestion rates. Our data, however, do not support that interpretation, as ingestion rates remained high, showing even a slight increase, at our highest irradiance.

Growth of prey and predator during 2 to 3 d incubations as used here can result in changes in the prey: predator ratio, possibly influencing estimates of growth and ingestion rates. Based on preliminary data,

we initiated incubations at prey to predator densities expected to generate similar mean ratios over the period used to calculate growth and ingestion. That attempt was partially successful in that the prey:predator ratio was comparable (6.7 to 9.6) at intermediate prey densities. At high prey densities (i.e. above 3000 cells ml^{-1}), however, mean prey and predator densities yielded lower ratios (3.7 to 5.3). Since *Dinophysis acuminata* growth and ingestion rates were saturated at those prey concentrations, prey:predator ratio should not have altered our estimates. Unfortunately, the prey:predator ratio at our lowest prey density (6 cells ml^{-1}) was also low (2.4) and may have affected our estimates for *D. acuminata* growth and ingestion. Nonetheless, estimates for growth and ingestion at the lowest prey density fall close to curves fitted to the data and thus appear to have little influence on observed functional responses.

Dinophysis acuminata is an obligate mixotroph as it cannot grow in the absence both of prey or of light. Obligate mixotrophy has been known for many years in ciliates (Stoecker et al. 1988), but has only recently been reported for dinoflagellates (Gast et al. 2007, this study). Ingestion rates of *D. acuminata* at saturating prey densities increased with irradiance, reaching maximum values (2073 ± 97 pg C d^{-1}) much higher than previously reported for mixotrophic dinoflagellates feeding on ciliates (710 to 888 pg C $grazer^{-1}$ d^{-1}; Bockstahler & Coats 1993, Smalley & Coats 2002, Smalley et al. 2003). When normalized to grazer biomass, maximum ingestion of *D. acuminata* (1.5 d^{-1}) is an order of magnitude higher than for *Akashiwo sanguinea* and *Ceratium furca* (Bockstahler & Coats 1993, Smalley & Coats 2002, Smalley et al. 2003). While ingestion rates increased to a maximum with increasing light level, utilization of prey resources, as indicated by our estimates for heterotrophic GE, did not. Rather, GE was highest (54%) at intermediate light levels. It is important to note that our estimates for GE are likely overestimates, as we were only able to adjust for phototrophic growth in the absence of prey. Any enhancement of phototrophic growth due to possible photosynthesis of ingested prey plastids would be incorporated in our estimates of GE. Estimates for heterotrophic GE in other mixotrophic dinoflagellates is limited, but our values are within the range (34 to 103%) reported for other species (Skovgaard 1998, Adolf et al. 2006, Jeong et al. 2006).

During semi-continuous incubation in the light, *Dinophysis acuminata* showed positive growth and ingestion for 21 d (23 and 12 generations under high and low light, respectively). By contrast, cultures held in the dark showed little to no growth for 6 to 7 d and then declined steadily. *D. acuminata* ingestion rates over the first 2 feeding cycles were moderate, increased sharply during the third cycle, and were undetectable in the 4th cycle. The fact that *D. acuminata* failed to ingest prey during senescence of the cultures (Days 10 to 12) suggests either the degradation of feeding structures, or loss of signals stimulating feeding (e.g. relative cell quotas for macronutrients) during long-term absence of photosynthesis. The dramatic increase in ingestion rates observed during early decline of cultures (i.e. Days 7 to 10) was unexpected and may reflect a 'starvation' response due to the inability to fix carbon in the dark.

Blooms of *Dinophysis* spp. in the Baltic Sea with densities of 1.8 to 15×10^4 cells l^{-1} typically occur near the thermocline (15 to 25 m for *D. norvergica*; Carpenter et al. 1995, Gisselson et al. 2002, Salomon et al. 2003), or at greater depth (80 m for *D. acuminata*; Setälä et al. 2005). Since the euphotic zone in that region does not exceed 20 to 30 m (Aarup 2002), blooms of *Dinophysis* spp. may often be light limited. Granéli et al. (1997) indicate that maximum photosynthesis of *D. norvegica* in the Baltic Sea (108 pg C cell h^{-1}) only support growth rates of 0.24 d^{-1}. Estimates for photosynthesis at depth (22 m) are much lower (13 pg C cell h^{-1}), supporting a growth rate of 0.03 d^{-1} (Gisselson et al. 2002). Using cell cycle analysis, Gisselson et al. (2002) estimated a growth rate of 0.4 d^{-1} for *D. norvegica* populations at 15 to 20 m, while extrapolation from photosynthesis–irradiance curves indicated that photosynthesis at that depth only accounted for growth of 0.05 to 0.1 d^{-1}. The authors thus suggested that heterotrophy likely accounted for 75% of population growth at depth, although food vacuoles were detected in only 10% of the cells. Our data for *D. acuminata* also indicated that phototrophic growth at low irradiance (10 µmol m^{-2} s^{-1}) is very slow (0.01 d^{-1}) and reaches a maximum of only 0.3 d^{-1} at 200 µmol m^{-2} s^{-1}. Mixotrophic growth at 10 µmol m^{-2} s^{-1}, however, was an order of magnitude higher (0.14 d^{-1}), increasing to 0.56 d^{-1} (about twice phototrophic growth) at 30 µmol m^{-2} s^{-1}. Whether enhanced growth in the presence of prey is the direct result of heterotrophy, as suggested by Gisselson et al. (2002), or the influence of increased photosynthesis due to acquisition of plastids or essential growth factors from prey is uncertain. Nonetheless, the generally low *in situ* growth rates reported for *Dinophysis* species (Reguera et al. 1996, Gisselson et al. 2002) suggest that these toxic dinoflagellates are typically prey limited in the natural environment. Further work is needed to fully understand the relative contribution of photosynthesis and heterotrophy to growth of *D. acuminata*.

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