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Feeding mechanism, prey specificity and growth in light and dark of the plastidic dinoflagellate *Karlodinium armiger*

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ABSTRACT: The food uptake mechanism and prey specificity of the most recently described member of the ichthyotoxic photosynthetic dinoflagellate genus *Karlodinium* (*K. armiger*) was studied. *K. armiger* extracts the contents of prey through an inconspicuous feeding tube (peduncle), but may also ingest whole prey cells. This species is omnivorous, ingesting prey from all major groups of marine protists. *K. armiger* displays a searching pre-capture behavior with attraction to prey cells and formation of feeding aggregates. In batch cultures, growth rates in the light without food were low (0.01 to 0.10 d^{-1}), but when the culture medium was enriched with soil extract, initial growth rate increased (0.19 d^{-1}); it further increased (0.60 d^{-1}) when fed the cryptophyte *Rhodomonas marina* in the light (170 µmol photons m^2 s^{-1}). *R. marina* was also ingested in the dark, but did not support positive growth rates and survival. Thus, *K. armiger* is an omnivorous obligate phototrophic mixotroph which seems to obtain a growth-essential substance or growth factor through phagotrophy.

KEY WORDS: Food uptake mechanism · Prey specificity · Mixotrophy · Dinoflagellate · Feeding tube

INTRODUCTION

Species belonging to the dinoflagellate genus *Karlodinium* form toxic blooms in protected areas around the world and have often been associated with fish kills (Garcés et al. 1999, 2006, Deeds et al. 2002, De Salas et al. 2005). The genus contains 4 described species: *K. armiger*, *K. australe*, *K. veneficum* and *K. vitiligo* (Bergholtz et al. 2006), and new species are currently being discovered. Toxins called karlotoxins have been chemically identified from *K. veneficum*. They affect the permeability of cell membranes, leading to osmotic lysis (Place et al. 2006). No toxins have been chemically described in any of the other species, but *K. armiger* has been shown in the laboratory to induce mortality of finfish, mussels, rotifers and copepods (Delgado & Alcaraz 1999, Fernandez-Tejedor et al. 2004, Garcés et al. 2006).

All described species of *Karlodinium* contain chloroplasts, but so far only *K. veneficum* and *K. australe* are known to take up particulate food in food vacuoles, i.e. they are mixotrophic (Li et al. 1999, De Salas et al. 2005). The best known species is *K. veneficum* (synonym: *K. micrum*), which has been extensively studied in the field and the laboratory (Nielsen 1996, Li et al. 1999, 2000a, 2000b, Adolf et al. 2006a). Without addition of food, *K. veneficum* may grow in the light at fairly low rates (0.25 to 0.5 d^{-1}), while higher growth rates occur in mixotrophic cultures (0.75 d^{-1}) with cryptophytes as food. *K. australe* and *K. armiger* grow poorly in the light without food (De Salas et al. 2005, Bergholtz et al. 2006, T. Berge & Ø Moestrup pers. obs.).

Food uptake has not been reported in *Karlodinium armiger*, but the species was suggested by Bergholtz et al. (2006) to be mixotrophic. They reported the presence of a peduncular microtubular strand in both *K. armiger* and *K. veneficum*. This finding indicates that mixotrophic members of this genus are tube feeders. However, Li et al. (1999) reported that ingestion of

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the cryptophyte *Storeatula major* by *K. veneficum* apparently was by direct engulfment of whole prey cells (phagocytosis sensu stricto). In the present study of *K. armiger*, we investigated the feeding mechanism, prey specificity, feeding and growth in light and in dark with and without addition of food or soil extract.

MATERIALS AND METHODS

Cultures and experimental conditions. Cultures were obtained from the Scandinavian Culture Centre for Algae and Protozoa (SCCAP; www.sccap.bot.ku.dk) and the Provasoli-Guillard Center for Culture of Marine Phytoplankton (CCMP; Maine, USA). The culture of *Karlodinium armiger* was a direct clone of the strain from which the species was described (Bergholtz et al. 2006). It was isolated from Alfacs Bay (Spain) during a bloom in 2000. All stock cultures were grown in L-medium (L1 in Guillard & Hargraves 1993) at 15°C, 32 PSU at an irradiance of 50 to 100 µmol photons m^{-2} s^{-1} in a 14:10 h light:dark cycle using cool-white lamps. The cells were acclimatized to the experimental light intensity at least 5 d before incubation. The growth medium (L) was based on autoclaved filtered seawater. Cell concentrations were determined from counts of at least 300 cells fixed in acid Lugol's and placed in a Sedgwick rafter counting chamber. The biovolume of the different species (µm^3 $cell^{-1}$) was estimated by measuring the length and width of the first 20 cells encountered using an inverted microscope (40× objective), and calculated assuming simple geometric shapes. All growth experiments were conducted in triplicate. Growth rates, based on cell counts, were calculated assuming exponential growth and only linear parts of semi-log plots between cell concentration and time were included. Differences in mean growth rates between treatments were tested using a 1-factor ANOVA test.

Food uptake and prey specificity. To test whether *Karlodinium armiger* feeds on different types of prey and to determine the food uptake mechanism, the dinoflagellate was offered 32 different species of phytoplankton, 2 ciliates and 1 heterotrophic dinoflagellate. Phototrophically grown cells of *K. armiger* in the stationary phase of growth at a cell concentration of 1500 cells ml^{-1} were mixed with exponentially growing prey cells in 2.7 ml wells in multidishes (24 wells). The multidishes were wrapped in parafilm to reduce evaporation and kept at 15°C. Irradiance was 50 µmol photons m^{-2} s^{-1}. After 30 min, 24 h, 48 h and 72 h, the multidishes were screened for feeding events and/or food vacuoles with an inverted microscope using 10× and 40× objectives. A Sony color video camera (3CCD model DXC-390P) connected to a digital video recorder (Sony DVCAM model DXC-390P) was used for recording food uptake.

For transmission electron microscopy (TEM), a culture of *Karlodinium armiger* (pH = 8) was fed *Rhodomonas salina* and fixed for 60 min in 2% glutaraldehyde in 0.1 M cacodylate buffer with 0.3 M sucrose added to minimize osmotic shock during fixation. Following a rinse in buffer, the cells were post-fixed in osmium tetroxide and dehydrated in an alcohol series. The cells were embedded in Spurr's resin mixture and the sections were examined in a JEM-1010 electron microscope (Jeol).

Phototrophic growth rate in batch cultures with and without soil extract. Phototrophic growth of *Karlodinium armiger* in batch cultures was monitored in L-medium and in L-medium + soil extract (0.2% final concentration). Triplicate 500 ml glass bottles containing 245 ml of the respective medium (pH = 8.0) were incubated under an irradiance of 170 µmol photons m^{-2} s^{-1} in a 14:10 h light:dark cycle. For a period of up to 23 d, samples (1.5 ml) were withdrawn every 1 to 5 d for determination of cell concentration and biovolume. The total volume of subsamples represented <10% of the initial volume.

Feeding experiments (light and dark). Feeding and growth experiments were conducted in light and in dark. In the first experiment, *Karlodinium armiger* was fed the cryptophyte *Rhodomonas marina* at an initial predator:prey concentration ratio of 1:10. A predator and a prey control were set up in parallel, consisting of only *R. marina* cells and *K. armiger* cells, respectively. Irradiance was 170 µmol photons m^{-2} s^{-1} in a 14:10 h light:dark cycle. The experimental cultures were allowed to grow for 3 d, prior to subsampling daily for 9 d. At each sampling, cell concentrations and cell dimensions of the predator.

The aim of the dark experiment was to examine whether *Karlodinium armiger* is an obligate phototroph, i.e. dependent on light or capable of purely heterotrophic growth (facultative mixotrophy). A culture was fed *Rhodomonas marina* cells every 3 d and incubated in the dark for up to 27 d. Initial predator:prey cell concentration ratio was 1:15, and additional prey cells were added during sampling to maintain high concentrations of prey (range 5000 to 15 000 cells ml^{-1}). Cell concentrations and biovolume of *K. armiger* were measured as described above.

RESULTS

Food uptake and prey specificity

Food uptake and food vacuoles were observed when *Karlodinium armiger* was offered nearly all the different protists, the exceptions being the majority of diatoms (Table 1). The frequency of feeding and the num-

Table 1. Organisms offered as potential prey for *Karlodinium armiger* in the food uptake experiment. ESD = equivalent spherical diameter, y = yes, n = no, nd = no data, na = not available

Class	Species	Prey size (Biovolume, µm^3)	Prey size (ESD, µm)	Ingestion y/n	Strain no.
Bacillariophyceae	*Nitzschia navis-varingica*	3500	18.2	n	na
	Nitzschia sp.	nd	nd	n	na
	Pseudo-nitzschia sp.	nd	nd	n	na
	Thalassiosira pseudonana	nd	nd	n	CCMP 1010
	Thalassiosira weissflogii	930	11.8	y	na
	Leprocylindrus danicus	nd	nd	n	CCMP 0469
	Chaetoceros affinis	nd	nd	n	CCMP 0158
	Chaetoceros decipiens	nd	nd	n	CCMP 0173
	Chaetoceros sp.	nd	nd	n	na
Cryptophyceae	*Rhodomonas baltica*	690	10.7	y	K-0332
	Rhodomonas marina	640	10.1	y	K-0435
	Rhodomonas salina	400	9.0	y	K-0294
	Hemiselmis sp.	70	5.0	y	K-0513
	Teleaulax amphioxeia	220	7.3	y	K-0434
Dinophyceae	*Gyrodinium instriatum*	17 980	31.2	y	K-0273
	Akashiwo sanguinea	26 000	35.5	y	K-0405
	Alexandrium ostenfeldii	25 000	35.0	y	K-0287
	Prorocentrum minimum	970	12.0	y	K-0336
	Prorocentrum micans	10 000	25.8	y	K-0335
	Heterocapsa triquetra	1350	13.3	y	K-0335
	Heterocapsa rotundata	140	6.3	y	K-0447
	Oxyrrhis marina	3000	17.0	y	na
Prasinophyceae	*Pyramimonas propulsa*	700	10.7	y	K-0293
	Pyramimonas orientalis	100	5.6	y	K-0003
	Tetraselmis suecica	320	8.3	y	na
Raphidophyceae	*Fibrocapsa japonica*	5350	21.1	y	K-0542
	Heterosigma akashiwo	990	12.0	y	K-0541
Prymnesiophyceae	*Prymnesium nemamethecum*	150	6.4	y	K-0394
	Prymnesium parvum	140	6	y	K-0544
	Chrysochromulina simplex	40	4.2	y	K-0272
	Pavlova lutheri	120	6.0	y	na
	Isochrysis galbana	35	4.0	y	na
	Phaeocystis sp. (colony)	nd	nd	y	na
Spirotrichae	*Euplotes* sp.	2 050 000	150	y	na
Oligohymenophorea	*Pseudocohnilembus* sp.	1900	15	y	na

ber of cells containing food vacuoles differed between prey species. They were low when *Karlodinium* sp. was fed the thecate dinoflagellate *Alexandrium ostenfeldii* and high when members of the cryptophyte genus *Rhodomonas* and the raphidophytes *Fibrocapsa japonica* and *Heterosigma akashiwo* were offered. Despite long observation times and addition of several different species of diatoms (Table 1), ingestion and subsequent formation of food vacuoles was only observed twice (on *Thalassiosira weissflogii*). Cells of *K. armiger* were often observed on the surface of diatoms, but after some time (10 s to 2 min) the predator cells swam away without any traces of food vacuole formation.

When feeding took place, ingestion started within minutes after addition of prey. *Karlodinium armiger* displayed a distinct and intense swimming behavior with increased swimming speeds and frequent changes of direction prior to ingestion (Video 1, available as AME Supplementary Material at: www.int-res.com/articles/suppl/a050p279_videos/). A complete feeding sequence of *K. armiger* ingesting an intact *Rhodomonas salina* (Fig. 1a–f) revealed that the predator cell encountered a prey cell with its apical part (Fig. 1a; Video 2, available as AME Supplementary Material at: www.int-res.com/articles/suppl/a050p279_videos/). After contact with a prey cell, the swimming speed of the predator slowed down. During this stage, >50% of the prey cells (*R. salina*) escaped. Occasionally, the prey was attached to the predator by an up to 10 µm long structure, but still seeking to escape (Video 1). This structure was only observed in the video and it was probably thinner than a flagellum.

Rhodomonas spp. were able to jump at extremely high speed, which most often resulted in release from the connection (Video 1). When capture was successful, the predator revolved around its anterior-posterior axis, placing its ventral side facing the prey (Fig. 1b, Video 2). During this stage, a small protrusion was sometimes observed, which very rapidly (<1 s) reached a length of approximately 2 to 10 µm and a thickness of

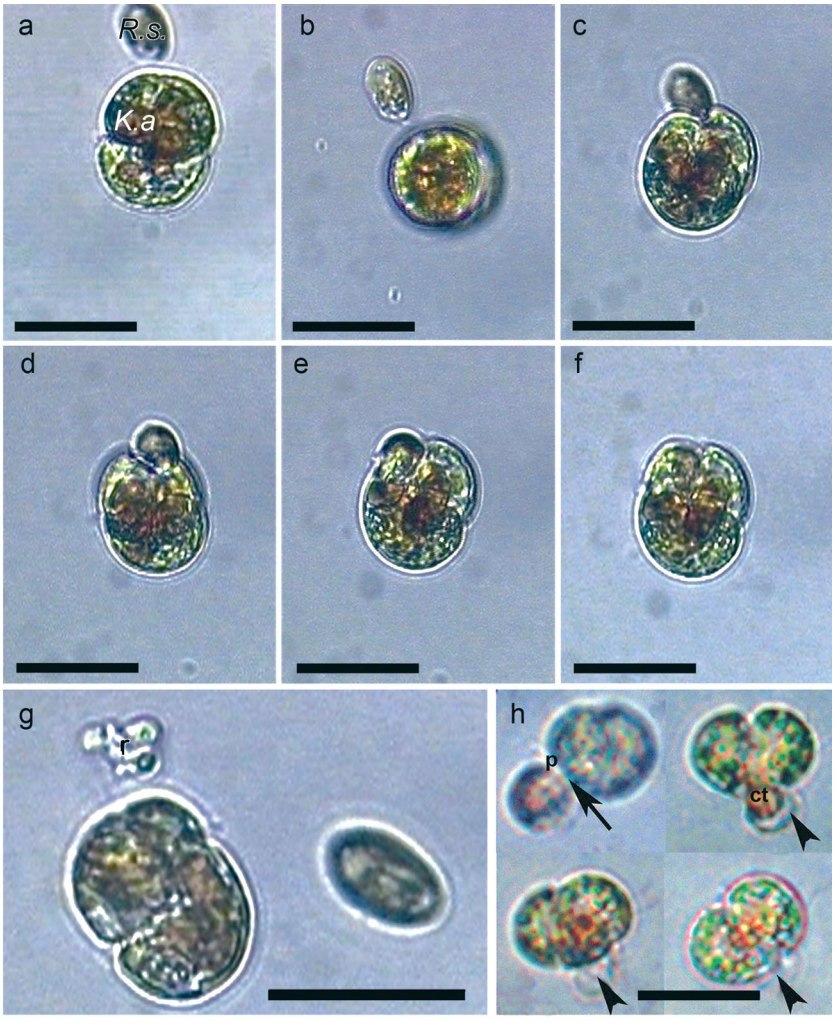

Fig. 1. *Karlodinium armiger* (*K. a.*) ingesting the cryptophyte *Rhodomonas salina* (*R. s.*). (a–f) Food uptake of an intact cell of *R. salina*, including (a) encounter, (b) capture and (c–e) phagocytosis. (g) Remnants (r) of the prey after ingestion of cell contents. (h) Ingestion by myzocytosis or tube feeding. Note the feeding tube (p) indicated by the arrow, and the separation of cytoplasm (ct) from the cryptophyte periplast (arrowheads). Scale bars = 20 µm

2 µm (indicated by 'p' in Fig. 1h), before the prey cell came into close contact with the ventral part of the predator. However, most often close contact between the prey and predator cell was established immediately after capture without any signs of the protrusion. Phagocytosis took place in the sulcal area (Fig. 1c). Often, the whole intact *Rhodomonas* cell was apparently dragged or sucked into a food vacuole (Fig. 1a–f, Video 2). Occasionally, cytoplasm ('ct' in Fig. 1h) was separated from the cryptophyte periplast (arrowheads in Fig. 1h), and observed flowing through the sulcus (arrow in Fig. 1h). The periplast was then left behind ('r' in Fig. 1g). Similar observations of prey remnants were observed when *Karlodinium armiger* fed on other small prey species (<10 µm equivalent spherical diameter [ESD]), including the dinoflagellate *Heterocapsa rotundata*, the haptophyte *Prymnesium parvum* and the prasinophyte *Tetraselmis suecica*. Ingestion of intact prey was also observed during feeding on *H. rotundata*, the haptophytes *Isochrysis galbana* and *Chrysochromulina simplex* and a small (~2 µm) hetero-

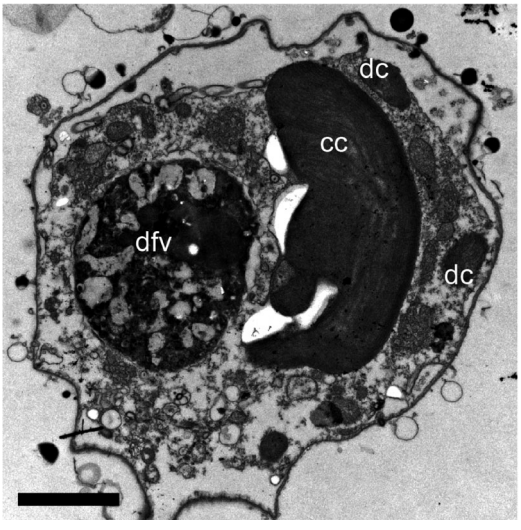

Fig. 2. *Karlodinium armiger*. TEM section showing ingested cryptophyte plastid (cc) and a food vacuole containing digested material (dfv). *K. armiger*'s own chloroplasts (dc) are small. Scale bar = 2 µm

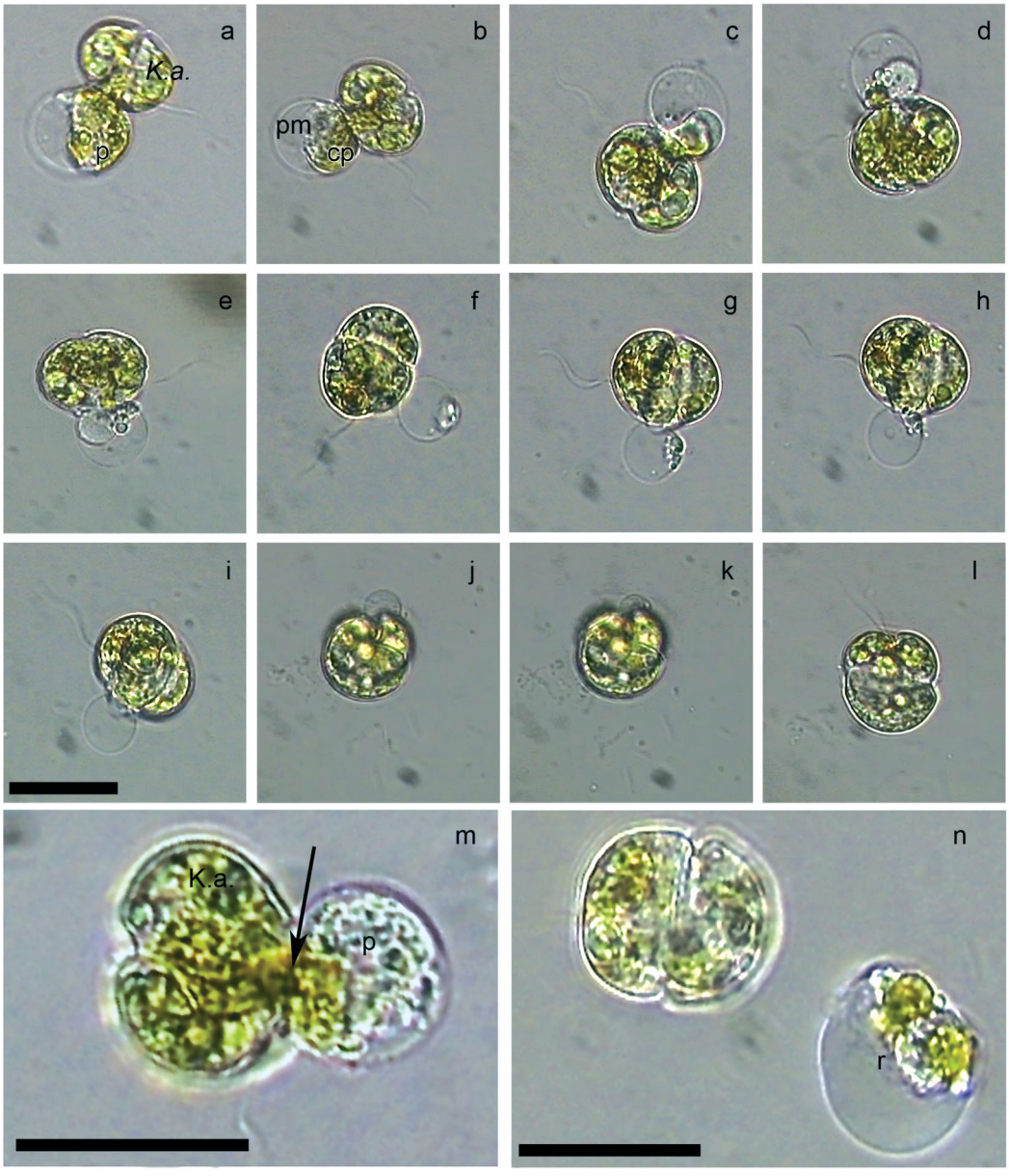

Fig. 3. *Karlodinium armiger* (*K. a.*) ingesting the raphidophyte *Fibrocapsa japonica* (p) by myzocytosis. (a–l) Note the separation of cytoplasm (cp) from the plasma membrane (pm) and (i–l) the complete ingestion of the prey plasma membrane. (m) Peduncle shown by the arrow. (n) Prey remnants (r). Scale bars = 20 µm

trophic flagellate contaminating some of the cultures. When ingestion was complete (Fig. 1f,g), the predator cell resumed the pre-capture searching type of swimming behavior and was able to ingest another prey immediately afterwards. Cryptophyte plastids were observed inside the cells of *K. armiger* investigated with TEM, but periplasts were apparently lacking inside the food vacuole (Fig. 2).

Ingestion of relatively large prey (>10 µm) differed somewhat from the feeding sequence of intact cells of *Rhodomonas salina* (Fig. 1a–f, Video 2). During feeding on the raphidophyte *Fibrocapsa japonica* (Fig. 3), the cytoplasm of the prey separated from the cell membrane and flowed through a narrow part (3 to 4 µm thickness) of the sulcal region of the predator (Fig. 3m) into food vacuoles. This resembled typical myzocytosis or tube feeding (Fig. 3a–h; Video 3, available as AME Supplementary Material at: www.int-res.com/articles/suppl/a050p279_videos/). Sometimes the predator also ingested the plasma membrane (Fig. 3i–l, Video 3). However, most often, pieces of the prey cell were left behind (Fig. 3n), and, with larger prey such as *Alexan-*

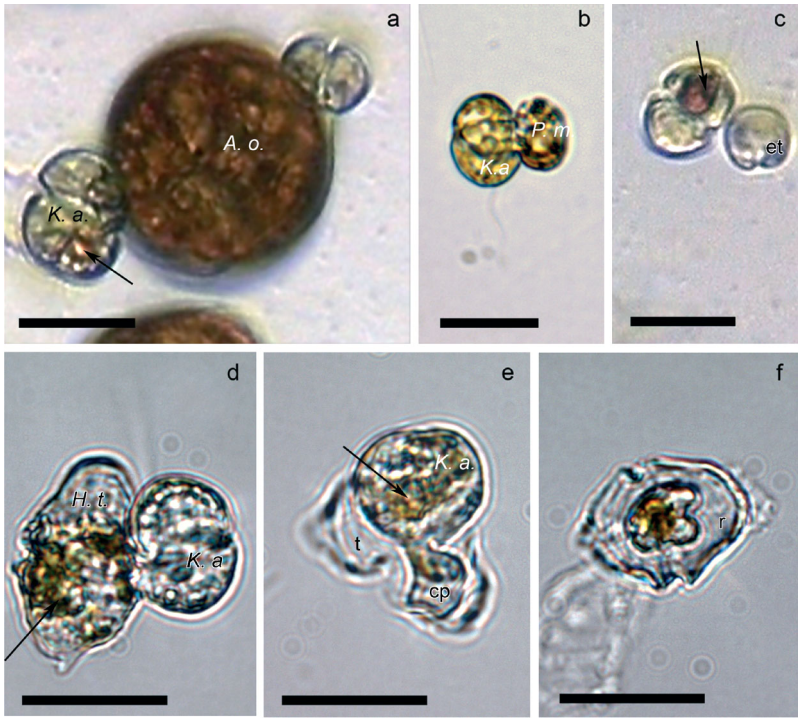

Fig. 4. *Karlodinium armiger* (*K. a.*). Tube feeding on thecate dinoflagellates: (a) *Alexandrium ostenfeldii* (*A. o.*); (b,c) *Prorocentrum minimum* (*P. m.*), its empty theca (et); (d–f) *Heterocapsa triquetra* (*H. t.*), its theca (t), cytoplasm (cp) and prey remnants (r). Arrows indicate food vacuoles. Scale bars = 20 µm

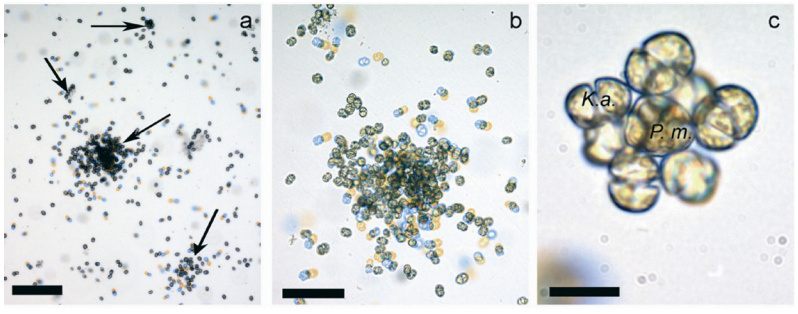

Fig. 5. *Karlodinium armiger*. (a–c) Aggregations (arrows) of *K. armiger* (*K. a.*) cells in cultures fed the thecate dinoflagellate *Prorocentrum minimum* (*P. m.*). Scale bars (a) = 200, (b) = 100 and (c) = 20 µm

drium ostenfeldii (ESD 35 µm) or *Gyrodinium instriatum* (ESD 31 µm), only a small part was ingested into a food vacuole (Fig. 4a). The predator might leave the prey cell at any stage during the food uptake. When feeding on thecate dinoflagellates, e.g. *Prorocentrum minimum* (Fig. 4b,c), *Heterocapsa triquetra* (Fig. 4d–f) and *P. micans,* the theca was pierced (Fig. 4a,b,d,e) and always left behind (Fig. 4c,f). Frequently, when the predator left its prey remnants, the thin connection described during the capture of cryptophytes was observed while the predator attempted to move away from the remnants (Video 4, available as AME Supplementary Material at: www.int-res.com/articles/suppl/a050p279_videos/).

Capture and manipulation of prey prior to phagocytosis (myzocytosis) lasted from 5 s to several minutes, and was the most critical part in the food uptake process. Both immobile and mobile cells were captured and ingested. Phagocytosis of larger prey such as *Fibrocapsa japonica* and *Heterocapsa triquetra* lasted for up to 35 min, while the complete uptake of *Rhodomonas salina* could last as little as 15 s. The biovolume of *F. japonica* is 3 to 5 times larger than that of the predator (see Table 1). A single feeding event on such a large prey led to a volume increase of the predator by up to 5 times (Fig. 3n, Video 3). The mean biovolume of *Karlodinium armiger* in non-fed cultures was ~1300 µm^3 cell^{-1}. However, in cultures with plenty of food, cells as large as 9000 µm^3 cell^{-1} were often observed, and the mean biovolume was approximately twice the size (2500 to 3000 µm^3 cell^{-1}) of non-fed cultures (1200 to 1500 µm^3 cell^{-1}).

Prey cells were often attacked and fed upon simultaneously by several cells of *Karlodinium armiger* (Fig. 5). This even applied to small prey such as *Heterocapsa rotundata*, *Rhodomonas* spp. and *Heterosigma akashiwo*. Larger prey cells such as *Gyrodinium instriatum* were sometimes completely covered by feeding predator cells. In cultures containing high concentrations of *K. armiger*, aggregates of predator cells were readily recognized swarming intensely around prey cells (Fig. 5a,b). Such aggregates resulted in very high swimming speeds of other predator cells in the culture as these were obviously attracted to the prey. The number of cells swarming around the aggregates sometimes exceeded 100 to 200 (Fig. 5b). The prey was pierced and cell contents released into the water before aggregations formed (Fig. 5c), but details of this process were not examined.

Phototrophic growth in batch culture with and without soil extract

Average phototrophic growth rate (non-fed cultures) in standard laboratory L-medium was ~0.05 d^{-1} during

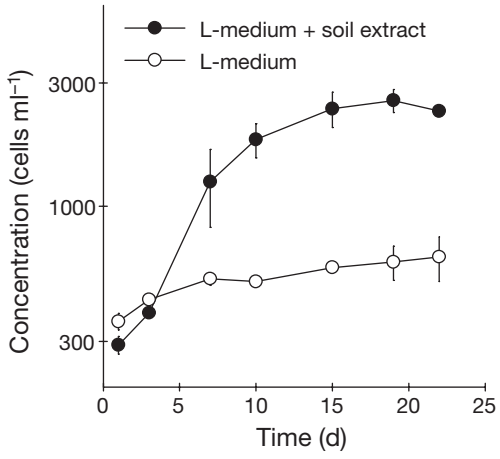

Fig. 6. *Karlodinium armiger*. Growth curve in phototrophic batch cultures grown in standard L-medium and in L-medium enriched with soil extract (0.2%). Data are mean ± 1 SE

the first 10 d of incubation in the batch culture experiment, when grown in an irradiance of 170 µmol photons m^{-2} s^{-1} (Fig. 6). In L-medium enriched with soil extract, growth rate (0.19 d^{-1}) was significantly higher ($p < 0.05$), and the culture reached stationary growth phase after 15 d.

Feeding experiments

When *Rhodomonas marina* was presented as food in concentrations of 100 to 3000 cells ml^{-1} (Days 1 to 6), the growth rate of *Karlodinium armiger* increased to ~0.6 d^{-1} (Fig. 7a), which was significantly different from the growth rates without food ($p < 0.001$). After depletion of prey in the mixed cultures (Day 7), growth again decreased and reached the rate ($p = 0.842$) in the non-fed cultures (Fig. 7a).

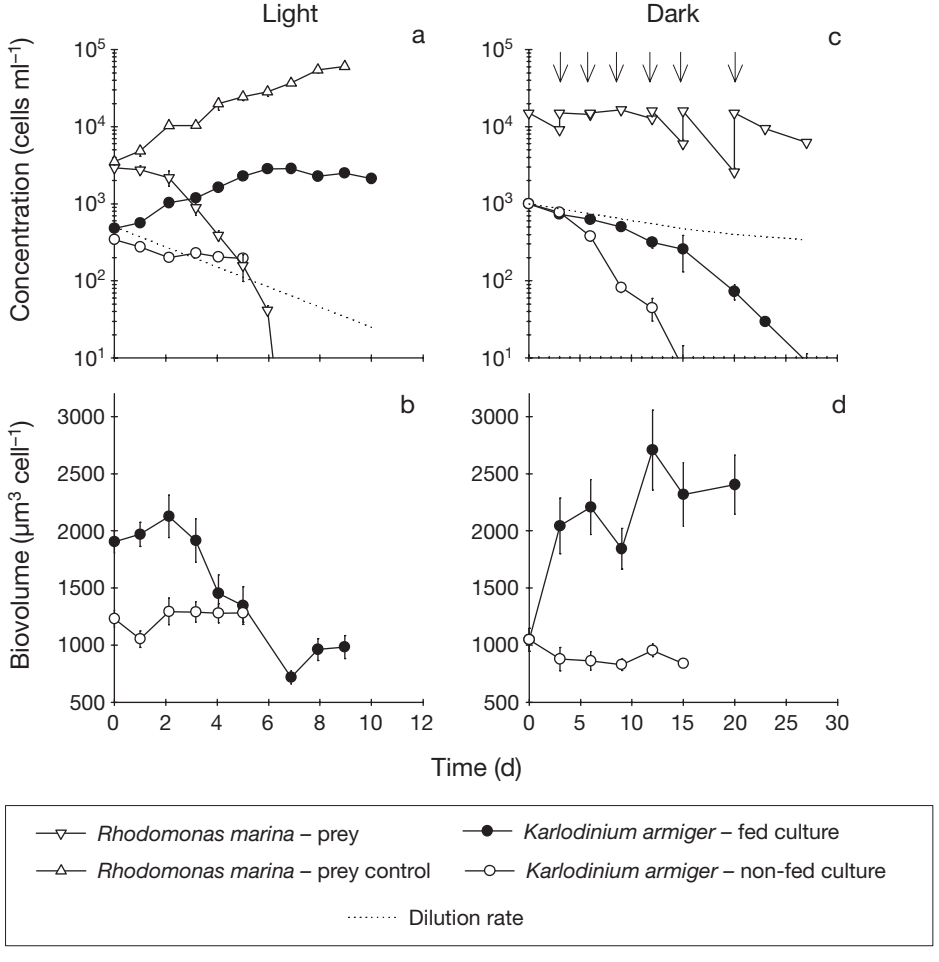

Fig. 7. *Karlodinium armiger*. Mixotrophic cultures fed the cryptophyte *Rhodomonas marina* under an irradiance of 170 µmol photons m^{-2} s^{-1} in (a,b) a 14:10 h light:dark cycle and (c,d) in the dark. (a,c) Cell concentrations (arrows indicate addition of prey cells) and (b,d) mean biovolumes. Data are mean ± 1 SE

The mean biovolume of *Karlodinium armiger* cells was ~1300 μm^3 $cell^{-1}$ in non-fed cultures, but was larger (~2000 μm^3 $cell^{-1}$) when prey concentrations exceeded ~1000 cells ml^{-1}(Fig. 7b) ($p < 0.001$). When the food was depleted, the biovolume of the predator cells decreased to a significantly lower ($p < 0.001$) level (800 μm^3 $cell^{-1}$) compared to the size of cells grown without food.

When *Karlodinium armiger* was fed cryptophytes in the dark, the cells survived for relatively longer periods than cells without food, but the growth rate was negative (Fig. 7c). All cells in the non-fed and fed cultures disappeared after 12 and 22 d, respectively. When fed in the dark, the biovolume of *K. armiger* cells was more than twice the biovolume of non-fed cells (Fig. 7d) ($p < 0.001$), and food vacuoles were observed.

DISCUSSION

Feeding mechanism and prey specificity

We observed that the plastid-containing dinoflagellate *Karlodinium armiger* may ingest several different types of prey (Table 1). Food was taken up using a feeding tube (peduncle). Occasionally, small prey (e.g. *Heterocapsa rotundata*, *Isochrysis galbana, Rhodomonas* spp. and *Pyramimonas orientalis*) appeared to be ingested whole, while larger prey or prey with a hard surface were only partly ingested with various fractions left behind. Under light microscopy, the feeding mechanism of *K. armiger* occasionally resembled direct engulfment (phagocytosis sensu stricto) when the cells were feeding on small prey cells, and tube feeding (myzocytosis) when feeding on larger or thecate prey. Similar observations have been reported in the tube-feeding dinoflagellate *Prosoaulax* (= *Amphidinium*) *lacustre* (Calado et al. 1998). The peduncle is not extended during myzocytosis, but the prey is quickly dragged into close association with the predator, leaving observations of the peduncle rare.

Prey specificity of *Karlodinium* spp. has not been studied before, and in previous studies on mixotrophy, *K. veneficum* was offered cryptophytes only (Li et al. 1999, Adolf et al. 2006a). Our investigation shows that *K. armiger* is omnivorous and able to ingest a large selection of prey sizes and types (Table 1). The smallest cell ingested was 2 to 3 µm (a heterotrophic flagellate) but no upper size limit was detected. However, we did not observe feeding on diatoms (with the exception of *Thalassiosira weissflogii*), suggesting that hard surface structures may provide an effective barrier for grazing, simply because of the inability or difficulty of the feeding tube to penetrate into the cells. In this respect *K. armiger* differs from *Paulsenella* spp., which are known to feed on a range of diatoms using a feeding tube (Schnepf et al. 1985, Schnepf & Drebes 1986).

Omnivory is not the general trend in mixotrophic dinoflagellates (Hansen & Calado 1999). Selective feeding has been reported in several of the ~50 known mixotrophic dinoflagellates. Thus, species of *Ceratium* and *Dinophysis* feed exclusively on ciliates, while *Fragilidium subglobosum* seems to feed exclusively on *Ceratium* spp. (Skovgaard 1996, Hansen & Nielsen 1997). An extreme case of specificity was seen in *Gyrodinium resplendens*, which fed only on *Prorocentrum minimum* and rejected the similar but larger *P. micans* (Skovgaard 2000). Results on prey selection including maximum ingestion and growth rates of *Karlodinium armiger* feeding on different prey species is reported separately (Berge et al. 2008, this issue).

Prey capture

Prior to ingestion, *Karlodinium armiger* displayed a characteristic pre-capture swimming behavior with increased swimming speed and frequency of turns. Aggregation of predator cells around prey indicates a chemical attraction (Fig. 5), and this has also been observed in other phagotrophic dinoflagellates (Spero & Moree 1981, Spero 1985, Schnepf & Drebes 1986, Calado & Moestrup 1997). It is unclear whether chemical attraction takes place when healthy prey cells are captured, but it certainly plays a role when a prey cell has been damaged and punctured. Increased swimming speed and attraction towards the prey increase the rate of predator-prey encounters. This may significantly enhance clearance rates compared to random collision. Chemical attraction by dinoflagellates was discussed by Hansen & Calado (1999) but further studies are needed to evaluate the significance of a chemosensory system in *Karlodinium* spp.

In *Karlodinium armiger*, prey capture was apparently by a thin thread, a capture filament, which has been reported also in other tube-feeding dinoflagellates, e.g. *Peridiniopsis berolinensis* (Calado et al. 1998) and *K. veneficum* (Li et al. 1999, Adolf et al. 2006b). In the present study, strong indications of its presence were observed both in capture when prey cells escaped and during the final stages of food uptake when the predator left behind remnants of the prey (Videos 1 & 4). However, capture filaments, being delicate and fast in action, are difficult to observe under a light microscope. In *P. berolinensis*, emission of the capture filament lasted <0.1 s (Calado & Moestrup 1997). Electron microscopy may provide

insight into the nature of the capture filament in *Karlodinium* spp.

Recently, toxins have been shown to be involved in prey capture by phagotrophic protists. Thus the haptophyte *Prymnesium parvum* excretes allelochemicals into the water which immobilize and lyse prey cells, which are subsequently ingested by phagocytosis (Skovgaard & Hansen 2003, Tillmann 2003). It has been suggested that toxins are involved in prey capture in *Karlodinium veneficum* (Adolf et al. 2006b). The toxins isolated from cultures of *K. veneficum* (karlotoxins) have been found to possess hemolytic and cytotoxic properties, and allelopathic growth inhibition of other algae has been reported (Adolf et al. 2006b). The growth curve of *Rhodomonas marina* obtained in the feeding experiment in light (Fig. 7a) may be explained by lysis of the cryptophytes. However, direct observation of the cultures showed no swelling of the prey cells, which appeared healthy and swam normally. In *K. veneficum,* allelopathic growth inhibition of other species of algae requires that the entire toxin content of 50 000 to 500 000 *K. veneficum* cells ml^{-1} is released into the water (Adolf et al. 2006b). These concentrations are far beyond those used in the present study. Moreover, Adolf et al. (2006b) stressed that >90% of the toxins are intracellular and not released into the medium. Swimming prey cells were often captured by *K. armiger*, but escapes of the captured prey were also common. Thus, allelopathic immobilization and/or lysis of prey cells was not required for food uptake in *K. armiger*. Toxins may be involved in food uptake in a more direct way, e.g. in the process of puncturing the cell to establish contact with the prey cytoplasm. Another possible role of toxins may be injection of paralyzing chemicals to immobilize the prey cell once contact and capture has been established (Calado et al. 1998, Adolf et al. 2006b).

Food uptake and growth

Attempts to grow *Karlodinium armiger* in different standard media (L, TL and F/2, at different salinities and nutrient ratios) did not result in growth rates >0.1 d^{-1} (the present study; Bergholtz 2004). When grown in L-medium enriched with soil extract, the initial growth in the batch culture was higher (~0.2 d^{-1}), indicating a growth factor in the soil extract that may stimulate photosynthetic growth. *K. armiger* requires light and cannot survive heterotrophically in the dark. Similar observations have been made for the obligatory phototrophic chrysophytes *Uroglena americana* and *Dinobryon cylindricum* (Kimura & Ishida 1985, Caron et al. 1993), which both obtain growth factors through feeding. *K. veneficum* also requires light (Li et al. 1999), while another mixotrophic dinoflagellate, *Gyrodinium resplendens*, depends on phagotrophy and may survive and grow heterotrophically in darkness. Cultures of this species can not be maintained in the light without the addition of food, but depend on a phagotrophically derived growth factor (Skovgaard 2000). Another mixotrophic dinoflagellate, *Fragilidium subglobosum,* is able to grow heterotrophically in the dark when offered *Ceratium* spp. as prey, and phototrophically in non-fed cultures, thus displaying a facultative mixotrophic strategy (Skovgaard 1996).

Although the growth rate in the fed culture of *Karlodinium armiger* in the dark was negative, the biovolume of the predator was approximately twice the size in non-fed cultures. Food vacuoles were present. Feeding thus occurred in the dark, in contrast to *K. veneficum,* where food uptake in the dark has not been reported (Li et al. 1999) and in which light stimulates feeding (Li et al. 2000a). The difference may be related to growth factors in *K. armiger*, a requirement that seems to be lacking in *K. veneficum*. *K. australe* probably resembles *K. armiger* in its requirements for phototrophic growth (De Salas et al. 2005).

When *Karlodinium armiger* was fed the cryptophyte *Rhodomonas marina* in light, mixotrophic growth rates were 6 to 10 times higher than the phototrophic growth in standard L-medium. Phagotrophy therefore provides the substances needed for growth in this species. Although food uptake in *K. veneficum* stimulates photosynthesis by providing nutrients and growth factors, heterotrophy has recently been found to dominate during mixotrophic growth (Li et al. 1999, Adolf et al. 2006a). Investigation of the functional and numerical responses of *K. armiger* and other roles of phagotrophy are addressed in Berge et al. (2008).

CONCLUSIONS

The dinoflagellate *Karlodinium armiger* is a mixotroph which is capable of feeding on several different prey types through an inconspicuous feeding tube. We suggest that there is a similar food uptake mechanism in other mixotrophic members of *Karlodinium*, allowing ingestion of large prey. Rigid cell coverings seem to provide a barrier to grazing, making diatoms and thecate dinoflagellates less appropriate as food. Despite poor growth in light-grown laboratory cultures, light is obligatory for *K. armiger*; however, high growth rates are only obtained mixotrophically. Phagotrophy seems to serve as a means to acquire essential growth factors.

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