Marine microalgae attack and feed on metazoans

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Free-living microalgae from the dinoflagellate genus Karlodinium are known to form massive blooms in eutrophic coastal waters worldwide and are often associated with fish kills. Natural bloom populations, recently shown to consist of the two mixotrophic and toxic species Karlodinium armiger and Karlodinium veneficum have caused fast paralysis and mortality of finfish and copepods in the laboratory, and have been associated with reduced metazooplankton biomass in-situ. Here we show that a strain of K. armiger (K-0688) immobilises the common marine copepod Acartia tonsa in a density-dependent manner and collectively ingests the grazer to promote its own growth rate. In contrast, four strains of K. veneficum did not attack or affect the motility and survival of the copepods. Copepod immobilisation by the K. armiger strain was fast (within 15 min) and caused by attacks of swarming cells, likely through the transfer and action of a highly potent but uncharacterised neurotoxin. The copepods grazed and reproduced on a diet of K. armiger at densities below 1000 cells ml−1, but above 3500 cells ml−1 the mixotrophic dinoflagellates immobilised, fed on and killed the copepods. Switching the trophic role of the microalgae from prey to predator of copepods couples population growth to reduced grazing pressure, promoting the persistence of blooms at high densities. K. armiger also fed on three other metazoan organisms offered, suggesting that active predation by mixotrophic dinoflagellates may be directly involved in causing mortalities at several trophic levels in the marine food web.

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Introduction

In terms of nutritional modes the free-living dinoflagellates represent one of the most diverse groups of unicellular eukaryotes in the marine food web. Half of the species are primarily phototrophic and possess permanent chloroplasts (that is, they are microalgae), while the other half lacks pigments and are primarily heterotrophic grazers and predators. Many species of both types have mixotrophic tendencies and can combine both phototrophy and heterotrophy in different ways (Stoecker et al., 2009; Hansen, 2011). The degree of mixotrophy varies along a spectrum from purely phototrophic to purely heterotrophic species (Jones, 1994). While a few primarily heterotrophic species can acquire phototrophy through the retention of chloroplasts from ingested prey for short periods of time (days) (Burkholder and Glasgow, 1997; Skovgaard, 1998; Lewitus et al., 1999; Jakobsen et al., 2000), several primarily phototrophic species with permanent chloroplasts can supplement growth with heterotrophy by feeding on unicellular prey (Hansen, 2011). Mixotrophy in primarily phototrophic microalgae is especially common in harmful and toxic bloom species from coastal eutrophic waters (Burkholder et al., 2008; Place et al., 2012).

Typically, mixotrophic dinoflagellates catch motile prey using a so-called capture filament that functions similarly to a harpoon. Depending on the feeding mechanisms, the prey is either engulfed directly by membrane engulfment, or parts are sucked out by a microtubule supported feeding tube or peduncle (myzocytosis) (Hansen and Calado, 1999). In general, mixotrophic dinoflagellates differ from most grazers in the marine food web by having a large optimal prey size, often corresponding to their own size (Hansen and Calado, 1999). Tube feeding enables dinoflagellates to feed on very large prey (Berge et al., 2008a) and a few primarily heterotrophic dinoflagellates, generally not forming mono-specific high-density blooms, have been reported to ingest rotifers, nauplius and bivalve larvae, nematodes and even finfish by the use of a feeding tube (Calado and Moestrup, 1997; Vogelbein et al., 2002; Shumway et al., 2006; Jeong et al., 2010). This includes ingestion of fish in the primarily heterotrophic Pfiesteria piscicida, which can retain functional plastids from cryptophyte prey...
and thereby become mixotrophic (Burkholder and Glasgow 1997; Lewitus et al., 1999). In contrast, primarily phototrophic and potentially bloom forming dinoflagellates with permanent chloroplasts have not been reported to ingest metazoans (Hansen and Calado, 1999; Jeong et al., 2010). However, we observed this in a field sample from The Sound (Denmark). Unidentified dinoflagellates possessing chloroplasts were tube feeding on a copepod nauplius larva (Supplementary Video S1). Copepods are millimeter-sized abundant planktonic crustaceans and typically represent the most important metazoan grazers for the transfer of primary production to higher trophic levels in the sea (Ohman and Hirche, 2001). Generally in marine food webs, microalgae are prey and copepods grazers, and copepod grazing reduces microagal biomass and controls the formation of blooms (Ohman and Hirche, 2001; Armbrecht, 2009).

Based on inverted light microscopy (Supplementary Video S1) the microalgae feeding on the nauplius larva resembled members of the recently described genus of bloom-forming and toxic microalgae Karlodinium (Daugbjerg et al., 2000). Species of Karlodinium are less than 20 µm in length and 15 µm in width and require electron microscopy and/or DNA sequence analyses for proper species identification (Bergholtz et al., 2005; de Salas et al., 2005), which was not available for this study. Species of Karlodinium possess permanent chloroplasts of haptophyte endosymbiotic origin (Bergholtz et al., 2005) and seem to be obligate phototrophs (that is, unable to survive without light). However, the two species investigated in this study Karlodinium armiger and Karlodinium veneficum are mixotrophic and use feeding tubes to phagocytose microagal prey to obtain faster growth rates (Li et al., 1999; Berge et al., 2008a).

The widespread species K. veneficum represents one of the most problematic and well-studied toxic bloom-forming microalgae known (Garcés et al., 2006; Place et al., 2008; Mooney et al., 2009; Calbet et al., 2011) and has been recognised for its ichthyotoxicity (toxic to fish) since the 1950s (Braarud, 1957). Several aspects of its biology (Adolf et al., 2008), toxins (Deeds et al., 2002; Van Wagoner et al., 2010) and harmful effects on fish (Nielsen, 1993) have been described in detail and were recently reviewed (Place et al., 2011). The haemolytic and neurotoxic Karlotoxins (Deeds et al., 2002; Van Wagoner et al., 2010) produced by K. veneficum aid prey capture and are used directly to stun cryptophytes before ingestion (Sheng et al., 2009). Karlotoxins also have anti-grazing properties towards important microzooplankton and copepod grazers (Adolf et al., 2007; Waggett et al., 2008). Evidence suggests that fish kills because of K. veneficum are caused indirectly by cell-rupture and toxin release upon contact with the gills (Place et al., 2008; Mooney et al., 2009), as opposed to the non-toxic heterotrophic dinoflagellate Pseudopfiesteria shumwaye that may kill fish larvae by active tube-feeding (Vogelbein et al., 2002).

K. armiger is much less studied than K. veneficum (Daugbjerg et al., 2000; Bergholtz et al., 2005), and Karlotoxin production has not been reported in any strains of K. armiger. The species has been reported to produce a different substance with neurotoxic activity (Garcés et al., 2006), but the specific toxin has not been isolated and chemically characterised. While K. veneficum seems to prefer cryptophytes as food (Li et al., 1999; Adolf et al., 2008), K. armiger ingests most types of unicellular prey offered. It displays pronounced swarming behaviour and forms feeding aggregates when fed algal prey, allowing ingestion of prey several times larger than itself (Berge et al., 2008a). We studied if a strain of K. armiger and four strains of K. veneficum attacked and ingested copepods. We also describe the interactions observed via light microscopy and report the effects of an ecologically relevant range of K. armiger cell densities on the trophic flow between the mixotrophic microalgae and copepods in more detail.

Materials and methods

Microalgal strains and copepod culture

Monocultures of K. armiger (strain K-0668) and K. veneficum (strain K-1640) were obtained from the Scandinavian Culture Collection of Algae and Protozoa (SCCAP). The three other K. veneficum strains (K-1385, K-1635, K-1386) were isolated from North-East Atlantic locations (http://www.sccap.dk) and established during this work. All Karlodinium strains are available from SCCAP, except strain K-1386, which has been lost. The strain of K. armiger was the same as the one that was used to describe the species (Bergholtz et al., 2005). It was isolated from Alfacs Bay (Spain) during a harmful bloom event in 2000 and kindly submitted to SCCAP by Dr. Margarita Fernández-Tejedor. The microalgal stock cultures were grown in F/20 medium without silicate, based on sterile filtered seawater with a salinity of 30. The same medium was used for the incubation experiments with the different Karlodinium strains and copepods (see below). The copepod Acartia tonsa originated from Denmark and was grown in gently aerated, large 80-l tanks, fed the cryptophyte Rhodomonas salina (strain K-0294, SCCAP) at saturating food conditions. The salinity in the copepod stock-culture tanks was 32 and the temperature was 20°C.

As the detailed study of potential toxins involved was beyond the scope of this initial report, we did not measure toxin production in our strains. However, we tested whether the Karlodinium strains used were in a phagotrophic state under the provided experimental conditions (that is, mixotrophic and presumably toxic (Sheng et al., 2009)). We offered all five Karlodinium strains the cryptophyte Rhodomonas salina and the dinoflagellate Heterocapsa triquetra as food in predator:prey cell
density ratios of 1:5 and 2:5, respectively. For determination of mixotrophic tendency or feeding frequencies (% cells containing visible food vacuoles), the strains were mixed in triplicate microwells (24-well plate, volume 2 ml) with the microalgal prey and fixed in glutaraldehyde (1% final concentration) after 14 h of incubation under the same experimental conditions used throughout this study (see below). Feeding frequencies of the different Karlodinium strains were determined as the number of cells containing visible food vacuoles in the first 100 cells encountered, using an inverted microscope at 200–400 magnification.

Experimental conditions

The Karlodinium and microalgal prey cultures were adapted to the experimental light conditions (70 μmol photons m⁻² s⁻¹ in a light/dark cycle of 14:10 h) for at least 5 days of exponential growth before the experiments. Adaptation to experimental salinity (30) and temperature (20 °C) lasted at least 30 days, and F/20 medium was used (see above). One cohort of female Acartia tonsa was used in all experiments. Immobilisation and survival of copepods were observed via stereo and inverted microscopy. Immobilisation was recorded when the copepod was lying on the bottom of the microwell (containing from 2–15 ml total culture/medium volume; see below) with erratic movements and without the ability to swim or jump, but with internal body movements still occurring, especially gut movements. Immobilisation caused the copepods to sink to the bottom of the microwells, where they were observed lying still but alive for many hours. Death was recorded when no internal gut movement occurred. At termination of the experiments (after 24–40 h), mixtures of microalgae and copepods were fixed in acid Lugol’s solution. Microalgal cell concentrations were counted manually in acid Lugol’s fixed samples using a Sedgewick Rafter chamber (Graticules Ltd, Tonbridge, UK), and at least 300 cells were counted. To estimate copepod production during the incubation period we counted the number of eggs, empty egg-shells and nauplius larvae at the end of the incubation period (24–40 h). To estimate copepod grazing, we counted the number of faecal pellets produced during the incubation period.

Effect on copepod motility and mortality of strains of K. veneficum and K. armiger

To study potential immobilisation and mortality effects of four strains of K. veneficum and one strain of K. armiger on copepods, we incubated two adult Acartia tonsa females in five replicate 24-well microwells containing 2 ml of dense Karlodinium spp. cultures (>10 000 cells ml⁻¹) (Table 1). The densities used represent commonly occurring Karlodinium bloom densities (blooms are often <10 000 cells ml⁻¹ and sometimes 100 000 cells ml⁻¹) (Garcés et al., 2006; Place et al., 2012). As control copepod treatments, we included copepods fed 20 000 cells ml⁻¹ of an unidentified phototrophic gymnodinoid dinoflagellate (North–East Atlantic origin; strain ID unavailable) and the cryptophyte Rhodomonas salina. We also included a starved copepod control treatment without food in fresh medium. Additionally, to study potential involvement of extracellular toxins that leaked into the water, we included a treatment of cell-free culture filtrate of the live K. armiger culture (10 000 cells ml⁻¹). The cell-free culture filtrate was prepared by gently filtering the culture through 5 μm membrane filters (Poretics, Livermore, CA, USA) at a very low pressure <15 cm Hg vac and the filter did not dry during this filtration, but was always covered by the culture medium. This was done to avoid potential physical disruption of cells and release of toxins. The copepods were starved for 2 days before setting up the experiment. For the initial 130 min, we observed copepod motility in all the treatments every 5–30 min, and recorded motility and mortality after 24 h. Copepod grazing (that is, number of faecal pellets produced) was recorded in the control treatments, the culture filtrate treatment and the K. armiger live cells treatment.

Ability to immobilise and ingest other marine metazoans

Because of the absence of attacks on copepods in four out of five investigated Karlodinium strains, the rest of the incubations were only done for K. armiger (strain K-0688). To study if K. armiger immobilise and kill other metazoan organisms, seawater samples (2 l) were brought to the laboratory and a subsample was gently filtered through a 100-μm net to concentrate the organisms (the organisms were covered in water during filtration to avoid physical stress). An adult nematode, a trophophore and a later-stage polychaete larva were isolated and
transferred to multidish wells, containing K. armiger cells at densities of 1000–2000 cells ml\(^{-1}\), and observed using an inverted microscope connected to a video-camera.

**Acartia tonsa** immobilisation and survival, and trophic roles of microalgae and copepods in a range of K. armiger cell densities

The relationship between *K. armiger* cell density and copepod immobilisation, mortality, grazing and reproduction was also investigated. We exposed adult female *Acartia tonsa* to a range of cell densities in six-plate microwells. Five copepods were added in each microwell containing 15 ml of culture or F/20 medium (control) in three replicate units. *K. armiger* cell densities were 0, 400, 1100, 3500, 5000 and 7300 cells ml\(^{-1}\). The range was chose as these densities represent naturally occurring bloom densities of *K. armiger* (Delgado and Alcaraz, 1999; Fernandez-Tejedor et al., 2004; Garcés et al., 2006). Using a stereomicroscope and an inverted microscope, we recorded the number of immobile and dead copepods every 1–12 h for up to 2 days. We also measured copepod grazing (number of faecal pellet produced) and copepod reproduction (number of eggs and nauplii produced) in the different *K. armiger* cell density treatments.

At a density of 3500 *K. armiger* cells ml\(^{-1}\), we compared the population growth rate of *K. armiger* with the growth in cultures without copepods, by measuring the increase in cell density over the 2 days incubation. For comparison, *K. armiger* cells in mono-culture were grown under identical light conditions (see above) as in the copepod treatment.

**Calculations**

Mortality and immobilisation was calculated in the microwells containing two or five copepods each, as the averaged percentage of dead and immobile copepods observed in the 3–5 replicate units (see above). Copepod production and grazing rates were calculated as the change in numbers of eggs and nauplii larvae and faecal pellets, respectively, divided by the length of incubation period, or expressed as numbers produced during the incubation period. Exponential growth rates of the *K. armiger* were calculated according to \( \mu = \frac{\ln x_t - \ln x_i}{t_2 - t_1} \), where \( x_t \) and \( x_i \) is the cell concentration at end (time = \( t_2 \)) and start (time = \( t_1 \)) of the sampling interval, respectively. Cell densities were taken from three replicate units. Difference in growth rate between the cultures (3500 cells ml\(^{-1}\)) with and without copepods was tested using a \( t \)-test and evaluated at a significance level of 0.05.

**Results and discussion**

**Effects on copepod motility and survival of strains of K. veneficu**m and **K. armiger**

In the cultures with live *K. armiger* cells (10,000 cells ml\(^{-1}\)), half of the copepods were immobilised within only 1 h, and nearly all were unable to swim or perform escape jumps after 135 min (Figure 1a). We observed no effects on the motility of *Acartia tonsa* in high-density cultures of any of the four *K. veneficu*um strains, and the copepods were healthy and swam normally after the 24 h incubation in these treatments (Figures 1a and b; densities from 25 000–50 000 cells ml\(^{-1}\); Table 1). Both starved and fed control copepods (fed an unidentified gymnodinoid phototrophic dinoflagellate and the cryptophyte *Rhodomonas salina*) were all alive and healthy after 24 h, while all copepods in the treatment with *K. armiger* (10 000 cells ml\(^{-1}\)) were dead (Figure 1b) and covered with dinoflagellates tube feeding on the carcasses. The copepods grazed and produced faecal pellets in the fed control treatments (Figure 1c). No pellets were produced in the starved control or in the cell-free *K. armiger* culture filtrate, but starvation did not cause mortality or affected the motility of the copepods (Figures 1a and b).

The lack of motility and mortality effects after 24 h of the cell-free *K. armiger* culture filtrate of the same culture that caused 100% mortality (10 000 cells ml\(^{-1}\)), suggests that direct cell-contact by live *K. armiger* cells is required for copepod immobilisation and mortality to occur (Figures 1a and b). Previous studies involving harmful dinoflagellates and metazoan mortality have also reported that effects require direct contact with live cells; for example, *Heterocapsa circularisquama* caused mortality of bivalve larvae and rotifers (Nagai et al., 1996) and *Cochlodinium polykrikoides* caused rapid mortality of diverse species of bivalve larvae, only by direct cell-contact (Tang and Gobler, 2009). Similarly, natural bloom populations containing both *K. armiger* and *K. veneficu*um (Garcés et al., 2006) studied in the laboratory by Delgado and Alcaraz (1999) also required live cells to cause immobilisation and mortality of the copepod *Acartia granii*. These authors suggested a paralytic toxin as the cause and showed scanning electron microscopy of the affected copepods with attached microalgal cells. The possibility of microalgal feeding on the copepods as the cause of mortality was not discussed, probably because the causative organisms were putatively identified as *Gyrodinium corsicum* and thought to be entirely phototrophic. However, all the cells in their micrographs were facing the copepod with the ventral parts where the feeding tube emerges (Delgado and Alcaraz, 1999).

The fact that none of the *K. veneficu*um strains tested here attacked *Acartia tonsa* agrees well with previous experimental studies on other toxic strains of this species, reporting no short-term (<48 h) motility and mortality effects on different species of copepods in high-density cultures (Waggett et al., 2008; da Costa, Fernandez 2002; da Costa et al., 2005; Vaque et al., 2006). However, strain variation in cell quotas and types of Karlotoxins...
is extensive in *K. veneficum* (Bachvaroff et al., 2009). Some strains are non-toxic and these seem to be unable to catch and feed on microalgal prey and are therefore primarily phototrophic (Sheng et al., 2009; Place et al., 2011). Although, we did not investigate toxin production in our strains, three of the four *K. veneficum* strains were able to feed on both types of microalgal prey offered under the experimental conditions provided. This indicates that our three *K. veneficum* strains were probably toxic. Further studies of the presence, types and cell quotas of Karlotoxins are needed to confirm this assumption.

The lack of copepod attacks by *K. veneficum* strains may also be linked to the relatively low mixotrophic tendencies observed under the experimental conditions provided (Table 1). Toxicity of *K. veneficum* strains has been found to vary according to salinity, temperature and nutrient limitation (Adolf et al., 2009; Place et al., 2011). Also the balance between heterotrophy and phototrophy varies significantly as functions of light intensity, prey concentration and nutrient concentrations (Li et al., 1999; Adolf et al., 2006; Place et al., 2011). The mixotrophic tendency, in terms of % cells containing food vacuoles, was much higher in the *K. armiger* strain than in any of the *K. veneficum* strains when fed microalgal prey (Table 1). Therefore we cannot reject the possibility that some strains or natural populations of *K. veneficum* may be triggered to attack copepods under other environmental conditions. In fact, *K. veneficum* was recently reported to attempt feeding on *Acartia tonsa* from field observations (USA), although conclusive evidence was not provided (Place et al., 2011). Our field observation of tube feeding on a nauplius larva (Figure 2a; Supplementary Video S1), also support that feeding on copepods takes place in other species.

**Interactions between *K. armiger*, copepods and other metazoans observed in the microscope**

We directly observed copepod immobilisation by *K. armiger* (Figure 2b) using an inverted microscope. These observations confirmed the importance of direct cell-contact to cause immobilisation and mortality. Shortly after adding the copepods into the *K. armiger* culture, the cells were attracted to the copepods (Figure 2c), and frequently attached to them facing the metazoan with their ventral side (Supplementary Video S2). Initially, several cell-copepod contacts seemed to be directed towards the innervated first antennae (Figure 2d) and the uroscope or telson (Supplementary Video S2), which are involved in copepod motility and sensing. Copepods sense hydrodynamical disturbances caused by predators and prey in the surrounding fluid with hairs and setae situated at their pair of first antennae and the telson (Strickler and Bal, 1973; Krøbøe and Visser, 1999). Depending on the swimming speed and size of an encountering organism, copepods can respond to predators by performing powerful escape jumps (Krøbøe and Visser, 1999). The microscopic size of *K. armiger* may disguise it as a prey and enable the predator to...
attack the copepod unnoticed. The attacks on *Acartia tonsa* resulted in erratic cramp-like movements and inability to swim (Supplementary Video S2). This suggests that a fast-acting paralytic toxin is transferred directly into the nervous system. Potential toxin transfer could be brought about by the numerous extrusosomes covering the cell surface of *K. armiger* (Bergholtz et al., 2005) or through the feeding tube itself.

The effect of *K. armiger* attacks on the copepods was very fast, and the first immobilised individual was observed within minutes (Figure 1a, Supplementary Video S2). After some time the microalgae swarmed more aggressively, accumulated and collectively fed on the immobilised but still live copepods (Figures 2e and f) (gut movements still occurring). Finally, this collective feeding resulted in the death of the copepod (Supplementary Video S3). The ligaments between copepod carapace segments seemed to be preferential zones for initial food uptake by the microalgae (Figure 2f). This may be related to the capacity of the feeding tube to penetrate the exoskeleton. Feeding on other microalgae, *K. armiger* has difficulties ingesting heavily armoured dinoflagellate prey, such as species of *Alexandrium*, and is hardly able to ingest diatoms with tough silica cell
coverings (Berge et al., 2008a). A single tube feeding event on the copepod lasted from seconds to several minutes and a K. armiger cell was able to feed several times. After 2 days of incubation with Acartia tonsa, the microalgal population had ingested almost all of the copepod body tissue (Supplementary Video S3). Food uptake resulted in the formation of different sized food vacuoles and substantial cell swelling (a large food vacuole is shown in Figure 2g). When K. armiger feeds on microalgal prey, it can increase its cell volume to more than five times its original cell volume (Berge et al., 2008a).

We inoculated an adult nematode, a polychaete trochophore and a later stage polychaete-larva in cultures of K. armiger (1000–2000 cells ml$^{-1}$), to investigate if the carnivorous behaviour only affected the copepod Acartia tonsa. All metazoans offered were attacked and immobilised within hours (Figures 2g–l, Supplementary Video S4). The entire body content of the trochophore larvae was nearly removed by tube feeding within 24 h (Figure 2i, Supplementary Video S4). Feeding on the nematode was not directly observed, but the K. armiger cells were clearly attached to it and attempted to penetrate the cuticle with the feeding tube (Figure 2l).

The swarming behaviour of K. armiger toward metazoans was most likely by chemotaxis (Supplementary Video S3–S4), and similar swarming behaviour was reported when K. armiger fed on microalgae (Berge et al., 2008a). Chemotaxis has also been reported in primarily heterotrophic dinoflagellates (Spero, 1985; Schnepf and Drebes, 1986); for example, the tube feeding freshwater species Peridiniopsis berolinensis ingesting tissue of an injured nematode (Calado and Moestrup, 1997) and the marine Pseudopfiesteria shumwayae tube feeding on fish larvae (Vogelbein et al., 2002).

**Effect of K. armiger densities on the trophic interactions between microalgae and copepods**

Copepod immobilisation, mortality, grazing and reproduction depended on K. armiger cell densities in a dose–response manner (Figures 3a–d). At cell densities over 3500 cells ml$^{-1}$, 50% of the copepods were immobilised and survival was dramatically reduced. Grazing (faecal pellets) and production (eggs and nauplius larvae) of the copepods are occurring at cell densities $>1100$ cells ml$^{-1}$, while K. armiger is grazed by Acartia tonsa at densities $<1100$ cells ml$^{-1}$. Data points represent means of three replicate units containing five copepods each, and error bars indicate s.e. ($n=3$).
growth rates (0.5–0.7 day$^{-1}$) are only obtained when ingesting prey under mixotrophic growth. As feeding seems to stimulate the photosynthetic machinery of this mixotroph (possibly by acquiring essential growth factors), only small volumes of food may be required to significantly increase the growth rate (Berge et al., 2008a, b). In the treatment with 3500 cells ml$^{-1}$ of the density experiment, the presence of copepods resulted in 85% higher population growth rate of *K. armiger*, compared with the phototrophic growth rate in monocultures without copepods (Figure 4). This shows that ingestion of metazoan tissue may also supply the growth factors necessary for faster growth in *K. armiger*. Thus, by feeding on live individuals with no immobilisation and mortality effects on the metazoan population as a whole, *K. armiger* may obtain increased growth rates and therefore also benefit during bloom initiation at lower cell densities.

**Toxicity, prey selection and specificity** Although we did not measure toxin production in any of our tested strains, our direct observations of the interactions between *K. armiger* and different metazoans via microscopy, combined with the data on timescales of copepod immobilisation (within minutes) and the density-dependent immobilisation and mortality (dose–response relation) strongly support the involvement of a potent neurotoxin in this species. Thus, *K. armiger* seems to use toxins to stun and feed on prey in a similar manner to *K. veneficum* preying on cryptophytes (Sheng et al., 2009), but that the range of prey types extends to diverse metazoan organisms. This difference in prey specificity may indicate that the activity of the toxins produced by *K. armiger* differ from the well-known Karlotoxins produced by *K. veneficum* (Deeds et al., 2002; Mooney et al., 2009; Sheng et al., 2009). Karlotoxin specificity has been shown to be related to the sterol composition of cell membranes (Deeds et al., 2002). While most studies involving strains of *K. veneficum* have involved cryptophytes as food, recent reports suggest that the species is able to switch to other types of prey such as diatoms (Place et al., 2011). In our study, mixotrophic *K. veneficum* strains ingested both cryptophytes and thecate dinoflagellates (Table 1). These observations suggest that prey specificity may be more general in *K. veneficum* than previously recognised.

The trophic roles of the omnivorous tube feeding *K. armiger* are multiple; it may simultaneously act as a primary producer, grazer of microalgal food, a predator of unicellular (Berge et al., 2008a) and metazoan zooplankton and as a detritivore on faecal pellets (Poulsen et al., 2011). This feeding flexibility indicates that *K. armiger* competes for resources with organisms at several different trophic levels in the marine food web. Prey selection and prey preference by *K. armiger* in natural communities may affect
metazoan food web structure and function. The efficiency of ingestion by *K. armiger* feeding on phytoplankton species has been shown to be highly dependent on the food quality of the prey and ingestion rates are lower when fed armoured compared with unarmoured microalgal prey (Berge et al., 2008b). Copepods have rigid exoskeletons, which may increase prey-handling time (that is, time taken for immobilisation and piercing the prey). Thus, harmful effects of blooms of *K. armiger* on more easily handled organisms (for example, unarmoured planktonic larvae) may occur at even lower cell densities than reported here for *Acartia tonsa*. Support for this comes from an apparent higher sensitivity towards natural bloom populations containing *K. armiger* of two species of finfish compared with the copepod *Acartia granii* (Delgado and Alcaraz, 1999; Fernandez-Tejedor et al., 2004). In these studies, paralysis of juvenile finfish (2–5 cm in length) occurred within 2 min, and the fishes displayed erratic movements and loss of equilibrium and lethargy (Fernandez-Tejedor et al., 2004).

Conclusions

Four strains of *K. veneficum* (three of these were mixotrophic) tested did not attack and feed on the copepod *Acartia tonsa*. However, *K. armiger* (strain K-0688) was able to paralyse metazoan prey and feed on them when the dinoflagellate occurred at bloom densities (>1000 cells ml⁻¹). *K. armiger* thus represents the first example of mixotrophic microalgae that attack and eat live metazoans (Hansen and Calado, 1999; Jeong et al., 2010), and obtain increased growth rate from it. The strategy seems to be accomplished by means of collective swimming behaviour (chemotaxis) and the involvement of a potent but unknown neurotoxin. Following paralysis, feeding aggregates of *K. armiger* cells accumulated on the metazoan body. *K. armiger* may turn the food web upside down and release top–down control by immobilising and feeding on important copepod grazers. Most importantly, *K. armiger* ingests different types of metazooplankton organisms, suggesting that it may attack and feed on several different types of metazooplankton organisms, including larvae of commercially important bivalves and finfish. Future studies on the chemical structure and properties of the toxins produced by *K. armiger* may provide valuable new insights into the type of organisms that are potentially vulnerable. Occurrence of similar carnivorous behaviour by other harmful species and especially strains of *K. veneficum* need more attention.

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