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ORIGINAL ARTICLE

# 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

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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 microalgal biomass and controls the formation of blooms (Ohman and Hirche, 2001; Armbrust, 2009).

Based on inverted light microscopy (Supplementary Video S1) the microalgae feeding on the nauplius larva resembled members of the relatively 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 phagocytise microalgal 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 ichtyotoxicity (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 Fernandéz-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.

**Table 1** *Karlodinium* strains used in the present study. Species, strain identification tag, mixotrophic tendency, cell densities used in copepod immobilisation and mortality experiment, whether or not the cultures caused mortality within 24 h and the strains showed swarming and accumulation behaviour towards a killed copepod (the copepods were killed using a needle and offered to *Karlodinium* cultures in multidish wells (24-well plate)). The strain ID refers to the Scandinavian Culture Collection for Algae and Protozoa (www.sccap.dk). Y and N refer to yes and no, respectively.

| *Species/ strain ID* | *Mixotrophic tendency (% cells containing food vacuoles)* | | *Concentration in* Acartia tonsa *experiment* ($10^4$ *cells* $ml^{-1}$) | Acartia tonsa *24-h mortality* | *Swarming behaviour* |
|---|---|---|---|---|---|
| | R. salina | H. triquetra | | | |
| *K. armiger* K-0668 | 91 | 57 | 1.0 | Y | Y |
| *K. veneficum* K-1385 | 0 | 0 | 2.5 | N | N |
| *K. veneficum* K-1635 | 9 | 3 | 3.6 | N | N |
| *K. veneficum* K-1640 | 7 | 3 | 5.0 | N | N |
| *K. veneficum* K-1386 | 10 | 5 | 2.7 | N | N |

*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-plate 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 trochophore 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 nauplius 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, $\mu = (\ln x_{t_2} - \ln x_{t_1})/(t_2 - t_1)$, where $x_{t_2}$ and $x_{t_1}$ 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. veneficum 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. veneficum* 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 *Cochlodiinium 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. veneficum* (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. veneficum* strains tested here attacked *Acartia tonsa* agrees well with previous experimental studies on other toxic strains of this species, reporting no short-term (<48h) 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

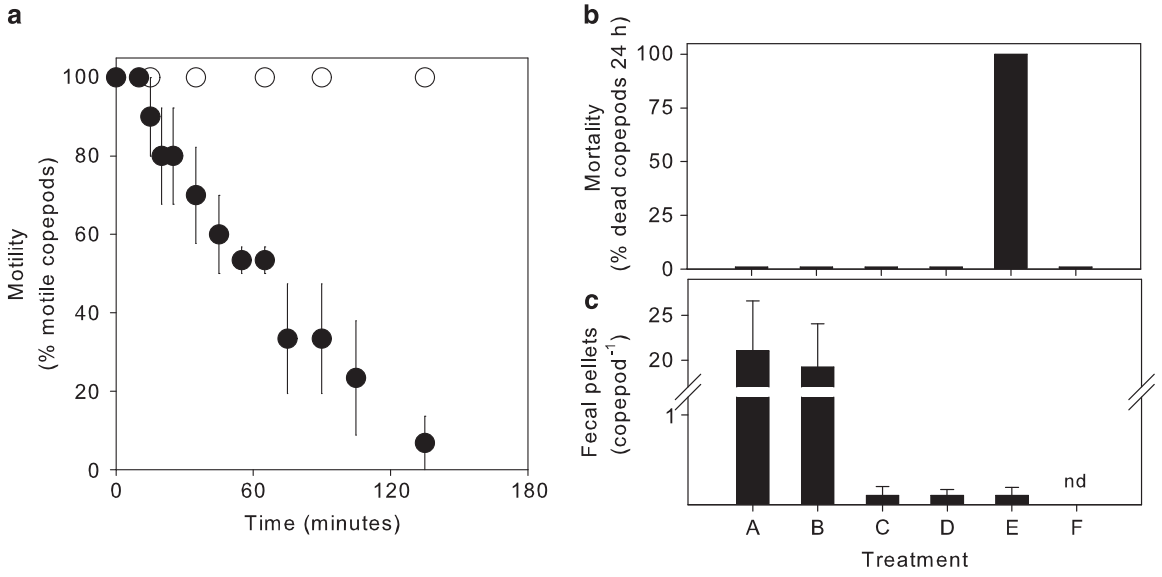


**Figure 1** Effects on copepod motility and mortality of *K. veneficum* and *K. armiger* strains and control treatments. (**a**) Short-term immobilisation in treatments with four *K. veneficum* strains (white circles) and with one *K. armiger* strain (black circles). White circle also includes data from all copepod control treatments, that is, with and without microalgal food and cell-free *K. armiger* culture filtrate (10 000 cells ml$^{-1}$); (**b**) Mortality and; (**c**) grazing of adult female copepods incubated in treatments: (A) *Rhodomonas salina* (ca. 20 000 cells ml$^{-1}$), (B) and unidentified gymnodinoid dinoflagellate (ca. 20 000 cells ml$^{-1}$), (C) without food (unfed control), (D) in the cell-free *K. armiger* culture filtrate of the (E) live *K. armiger* cell treatment (10 000 cells ml$^{-1}$) and (F) all four *K. veneficum* strains (See Table 1 for cell densities). Data in (**a**, **b**) represent means of five replicate units containing two copepods each and error bars indicate standard error s.e. ($n = 5$). (nd) indicate that the data for faecal pellet production were not determined in the treatments with *K. veneficum* strains.

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 urosome 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; Kiørboe and Visser, 1999). Depending on the swimming speed and size of an encountering organism, copepods can respond to predators by performing powerful escape jumps (Kiørboe and Visser, 1999). The microscopic size of *K. armiger* may disguise it as a prey and enable the predator to

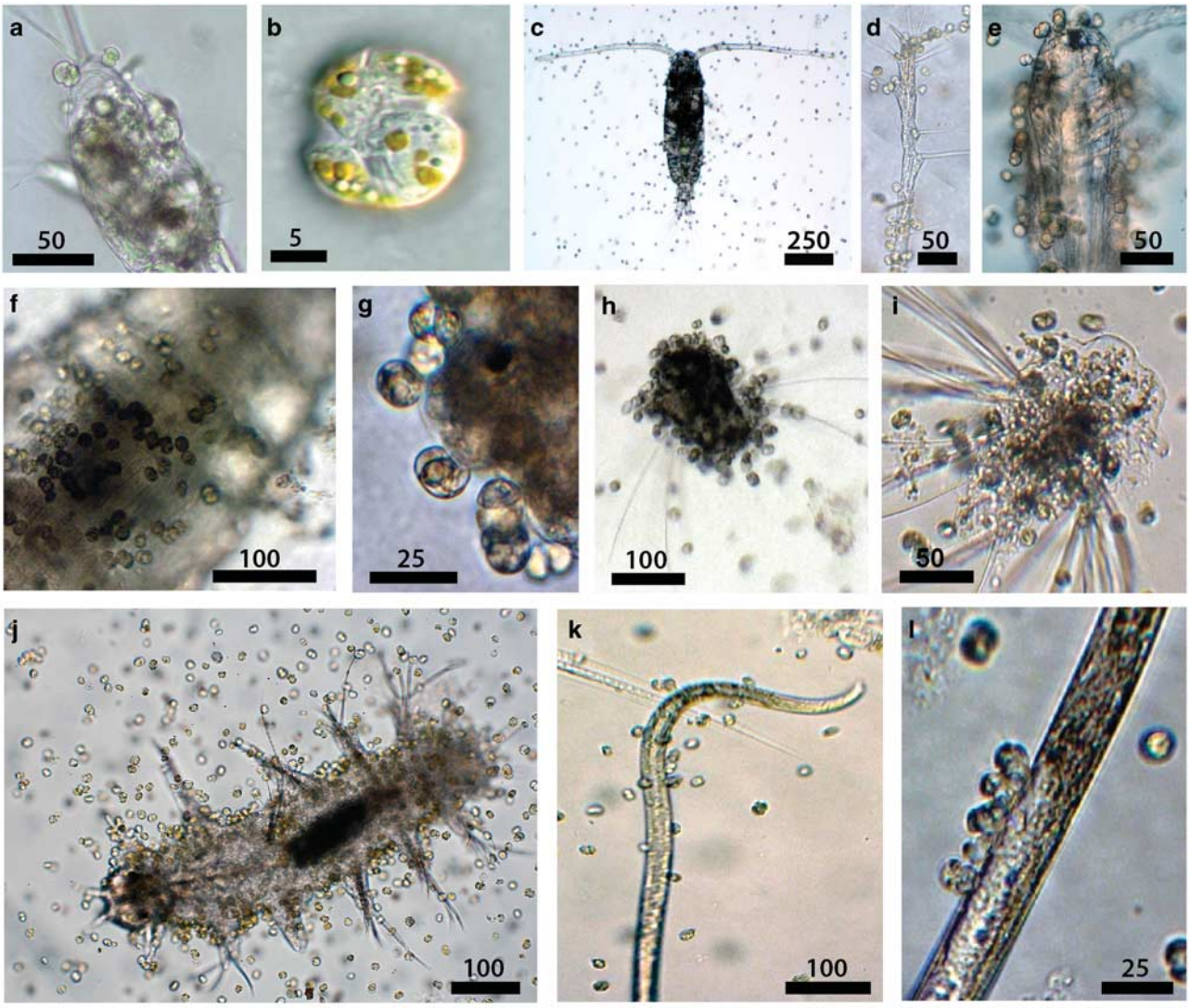


**Figure 2** Microalgae immobilising and eating metazooplankton. (**a**) Frame grabbed micrographs of an unidentified microalgal dinoflagellate tube feeding on an immobilised copepod nauplius larvae in a natural seawater sample from Denmark; (**b**) Micrograph of *K. armiger* in ventral view (type material). Note many small yellow–green chloroplasts (1–2 μm long); (**c**) Frame grabbed micrograph of the common marine copepod *Acartia tonsa* mixed with a culture of *K. armiger*; (**d**) Frame grabbed micrographs of initial microalgal attacks on the sensory antennae of the copepod (compound image); (**e**) *K. armiger* cells swarming around and attaching to an immobilised copepod; (**f**) Accumulations of feeding *K. armiger* cells on the surface of *A. tonsa*; (**g**) Tube feeding on an unidentified planktonic polychaete trochophore larva of *K. armiger*. Note the large food vacuole as dark globules in the central part of *K. armiger*; (**h**) *K. armiger* immobilising and; (**i**) food remnants of the trochophore larva after a day of incubation. A substantial part was removed by tube feeding; (**j**) *K. armiger* swarming around and forming feeding aggregates on an immobilised later stage polychaete-larva; (**k**) *K. armiger* attacking and attaching; (**l**) to an unidentified marine nematode. Units on scale bars μm. Video sequences can be viewed as Supplementary Information on *The ISME Journal* website (http://www.nature.com/ismej).

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

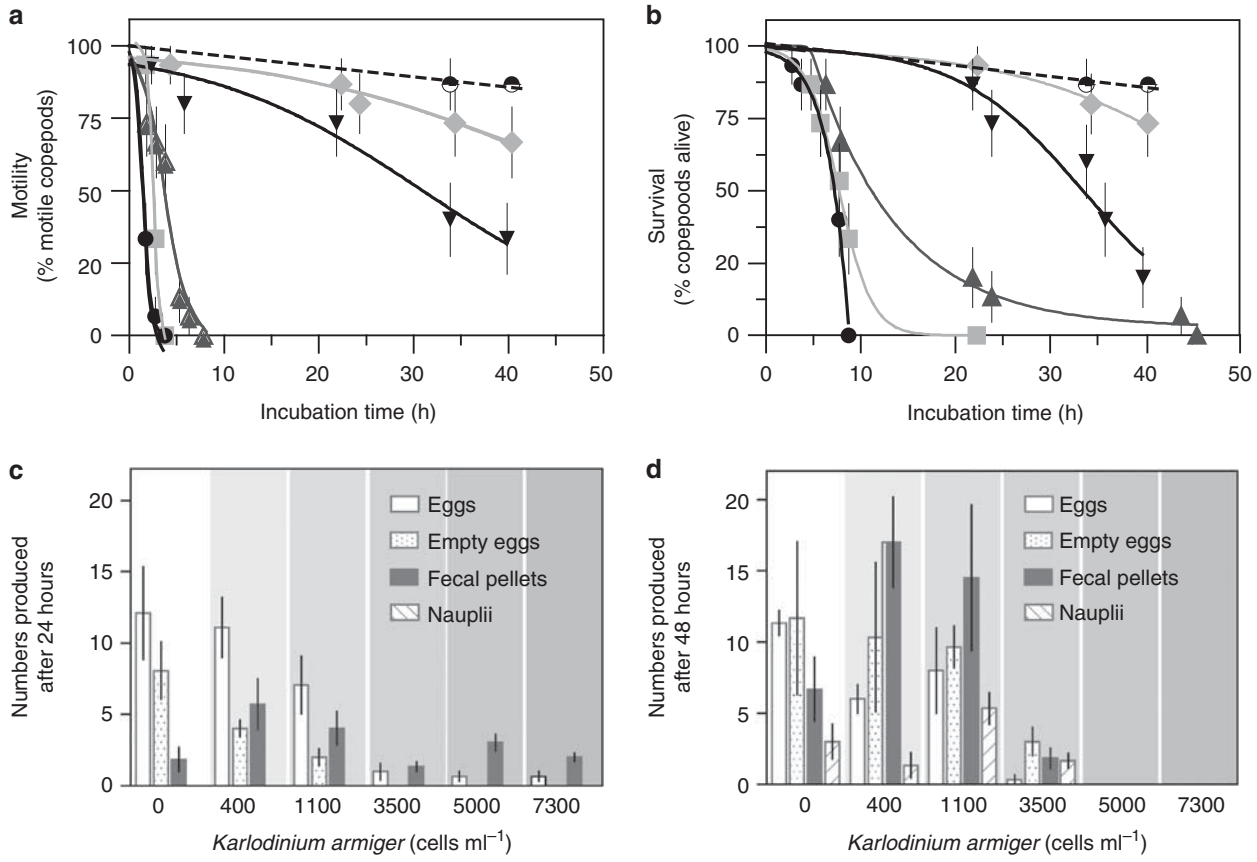


**Figure 3** Immobilisation and survival of *Acartia tonsa,* and trophic roles of microalgae and copepods in a range of *K. armiger* cell densities. (**a**) copepod immobilisation and; (**b**) survival as a function of time when incubated with different *K. armiger* cell densities of 0 (black and white divided circles), 400 (grey tilted squares), 1100 (black triangles), 3500 (dark grey triangles), 5000 (grey squares) and 7300 cells ml$^{-1}$ (black circles); (**c**, **d**) number of copepod faecal pellets, eggs, empty egg-shells and nauplii larvae produced after 24 h (**c**) and 48 h (**d**) of incubation with different densities of *K. armiger.* Visual gradient in (**c**, **d**), indicate microalgal density. Suppressed 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. ($n = 3$).

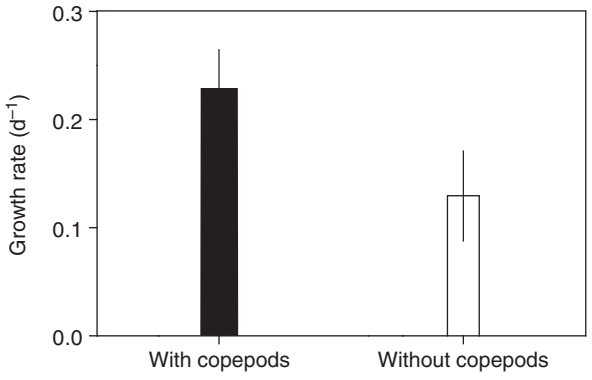


**Figure 4** Growth in *K. armiger* culture with and without copepods. Growth rate ($d^{-1}$) measured during incubation with copepods (black bar) as potential food was 85% higher than purely phototrophic growth rates without copepods (white bar) (*t*-test, $P>0.05$). Growth rate was calculated as the change in log-transformed cell density divided by the incubation time. Bars represent means of three replicates and error bars are s.e. ($n=3$).

were immobilised within 2 h of exposure (Figure 3a), and after ca. 8 h, 50% of the copepods were dead (Figure 3b). At densities below 1100 cells $ml^{-1}$, the copepods grazed and reproduced on a diet of *K. armiger*, but above 3500 cells $ml^{-1}$, copepod grazing and egg-production were suppressed (Figures 3c and d). The number of nauplius larvae, eggs and faecal pellets produced in the lower *K. armiger* densities, were lower after 40 h than after 24 h (Figures 3c and d). This suggests that *K. armiger* also ingested copepod eggs and faecal pellets (Supplementary Video S5). Ingestion of copepod faecal pellets by *K. armiger* and other phagotrophic dinoflagellates has recently been reported (Poulsen *et al*., 2011).

Our data show that the trophic roles of *K. armiger* as prey and copepods as consumers are switched, when densities of the mixotrophic microalgae reach levels above ca. 1000 cells $ml^{-1}$. *K. armiger* is known to occur at higher cell densities in eutrophic estuarine waters (Fernandez-Tejedor *et al*., 2004; Garcés *et al*., 2006). Field studies have reported maximum densities in the range 10 000–100 000 cells $ml^{-1}$ (Delgado and Alcaraz, 1999; Deeds *et al*., 2002) for *K. armiger* and *K. veneficum*. This indicates that microalgal feeding on copepods and other metazoans can occur in coastal areas.

The carnivorous strategy of *K. armiger* on copepods is likely to be an important mechanism when high cell densities have developed, coupling population growth to reduced grazing and promoting the persistence of blooms. It may be less important during the initiation of blooms at lower cell densities, but *K. armiger* may also benefit from food uptake of other metazooplankton organisms at lower cell densities without being able of immobilisation. This is because, although *K. armiger* harbours permanent chloroplast and is unable to survive without light, its growth rate is very low when growing entirely phototrophic ($<0.1$ $day^{-1}$). Fast 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 suggests 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 producers, 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 grani* (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 swarming 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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