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NOTE

Grazing of *Calanus helgolandicus* on *Dinophysis norvegica* during bloom conditions in the North Sea: evidence from investigations of faecal pellets

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ABSTRACT: Toxic dinoflagellate blooms are a common phenomenon in the North Sea, but the fate of the toxins in the food web is largely unknown. Herbivorous copepods may play a key role in the transport of toxins through the food web, but it is still uncertain to what extent toxic algae are grazed. The present experiment was carried out during an autumn bloom of *Dinophysis norvegica* in the North Sea, to study whether *Calanus helgolandicus* feed on *Dinophysis* spp. under natural conditions. The experiment showed that *C. helgolandicus* fed very efficiently on *D. norvegica* at high algal concentrations (>9000 cells l^{-1}), as 98% of the faecal pellets (FP) produced contained *Dinophysis* spp. cells (up to 32 cells FP^{-1}). Therefore, *C. helgolandicus* feeds on *D. norvegica* under natural conditions, and its FP may be an important vehicle transferring toxins within the pelagic and to the benthic community.

KEY WORDS: Zooplankton · *Dinophysis* spp. · *Calanus helgolandicus* · Grazing · Faecal pellet

In marine ecosystems the transfer of toxins to higher trophic levels such as fishes, birds and whales has been suggested to occur via tintinnids (Maneiro et al. 2000), clams (Prepas et al. 1997) and copepods (Teegarden & Cembella 1996, Tester et al. 2000). Herbivorous zooplankton may produce toxin-containing faecal pellets (FP) after ingesting toxic algae; these can be consumed by other zooplankton in the water column or they can sink out of the water column (Maneiro et al. 2000). Since one of the largest loss factors of phytoplankton is due to zooplankton grazing, it is important to understand how harmful algae species affect zooplankton grazing. Avoidance of toxic dinoflagellates may enhance bloom development. Toxic phytoplankton cells are thought to be less attractive to zooplankton grazers, and toxic cells should be selectively avoided by zooplankton when feeding on mixtures of different prey species (Fiedler 1982, Teegarden et al. 2001).

Due to their high sinking rates (Smayda 1969, Komar et al. 1981) copepod FP often comprise a significant fraction of the vertical carbon export out of the euphotic zone (Wassmann et al. 1999, Wexels Riser et al. 2002). The membrane-enclosed FP that all copepods produce can contain recognisable remains of their food. Therefore, the FP production approach was chosen to study whether or not *Calanus helgolandicus* feeds on toxic dinoflagellates of the genus *Dinophysis*. The ecology and ecophysiology of *Dinophysis* spp. remains poorly understood, mostly because it has not been possible to culture these species. Field experiments are therefore important for increasing the knowledge of the interactions between *Dinophysis* spp. and their potential predators.

Materials and methods. The present work was carried out during a Lagrangian experiment aboard RV 'Heincke' in the North Sea in August 2001 (cruise HE-152). A patch with enhanced dinoflagellate concentrations was marked with a surface float equipped with a GPS radio transmitter. Copepods for the experiment were collected from 30 m to the surface at 56° 18.57′ N, 6° 38.91′ E by vertical net tows with a WP2 zooplankton net (180 µm mesh size). Adult *Calanus helgolandicus* females were placed in 5 experimental bottles (1180 ml volume, containing 5 individuals each), and 1 control bottle without copepods was run simultaneously. The control bottle was used to correct for initial FP numbers in the incubation water. Incubation water was collected from the chlorophyll maximum (17 m depth) using Niskin bottles, and pre-screened through a

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200 µm mesh to remove larger zooplankton. The experimental bottles were carefully closed to prevent any air bubbles and placed onto a plankton wheel in an on-deck flow-through incubator to maintain uniform cell distribution at *in situ* temperature (17 ± 0.5°C). The experiment was carried out in dim light for 24 h. At the end of the incubation experiment, the copepods were removed and the contents from the bottles were carefully sieved through a 20 µm Nitex mesh and preserved with buffered formaldehyde (2% final concentration). All FP were enumerated using an inverted microscope with phase contrast and ocular micrometer (Zeiss IM 35). The length and width (or diameter) of the pellets was measured and the FP volume (FPV) calculated using appropriate stereometrical configurations according to Edler (1979). A volumetric carbon conversion factor of 69.4 µg C mm^{-3} (Riebesell et al. 1995) was applied to estimate FP carbon (FPC). Photographs of FP were taken with a Nikon 500 digital camera at 100 to 150× magnification. In addition, samples were prepared for scanning electron microscopy (SEM). These samples were filtered on 0.2 µm polycarbonate membrane filters, rinsed with deionised water to remove salt and formaldehyde and then air-dried. Filters were placed on SEM stubs and sputtered with gold/palladium for 3 min (25 mA, 5 nm). SEM photographs were taken at 400× magnification.

Results and discussion. The total cell concentration of dinoflagellates (*Dinophysis* spp. and *Ceratium* spp.) ranged between 1000 and 18 000 cells l^{-1} during the Lagrangian experiment and *D. norvegica* was the predominant dinoflagellate species during the drift experiment. *Dinophysis* spp. produced Dinophysis Toxin 1 (DTX1), but no okadaic acid (OA) (Catherine Legrand pers. comm.). *Dinophysis* spp. was the principal dinoflagellate genus in the incubation water and *D. norvegica* was by far the most numerous species, with 9580 cells l^{-1}, or 82% of the number of cells (Figs. 1 & 2a). *C. furca* was the most numerous species of the genus *Ceratium*, comprising 10% of the cells, or 1140 cells l^{-1}.

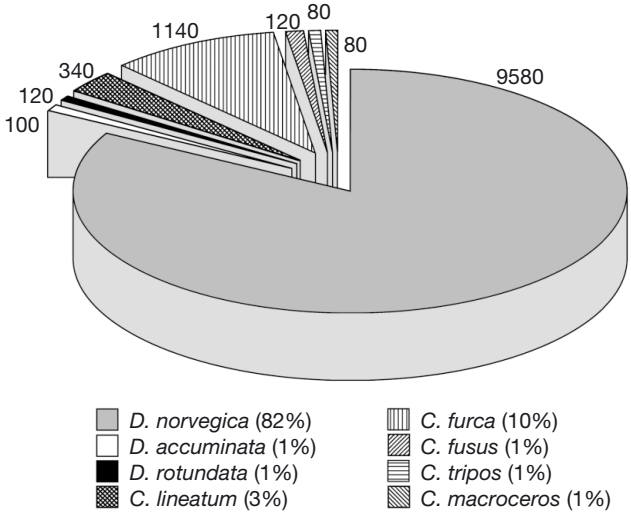

Fig. 1. Dinoflagellate species composition in the incubation water (cells l^{-1})

Table 1. *Calanus helgolandicus.* Faecal pellet (FP) production rate, volume and carbon production (means ± 95% confidence intervals)

Production rate (FP $ind.^{-1}$ d^{-1}):	23.6 ± 1.6
FP volume produced (mm^3 $ind.^{-1}$ d^{-1}):	0.026 ± 0.010
FP carbon produced (µg C $ind.^{-1}$ d^{-1}):	1.82 ± 0.75

Table 2. Length, diameter and volume (means ± SE) of *Calanus helgolandicus* faecal pellets (FP) produced by adult females. Only intact FP are included (n = 466); these were 86.8% of all FP (n = 537)

FP length (µm):	374 ± 5.0
FP diameter (µm):	65.9 ± 0.4
FP volume (×10^4 $µm^3$):	137 ± 3.29

The FP production rate of *Calanus helgolandicus* was ~24 FP $female^{-1}$ d^{-1} (Table 1), which was relatively low compared to other studies (e.g. Corner et al. 1972). Table 2 shows the average FP length, diameter and volume. The average volume of the FP was 137 × 10^4 $µm^3$, similar to that reported by Harris (1994). No 'ghost pellets' were observed; 87% of the FP were intact and only 13% showed signs of fragmentation (Table 2). No *Ceratium* spp. cells or remains of *Ceratium* spp. were detected within the FP. According to Teegarden et al. (2001), *Ceratium* spp. are unsuitable food for copepods and avoided by grazers; however, from investigations of FP content only, it cannot be concluded that *C. helgolandicus* did not feed on *Ceratium* spp. Nevertheless, *Ceratium* spp. probably did not make up a large fraction of the diet, and the cells must have been destroyed before or during egestion.

Photographs of the FP suggest that *Calanus helgolandicus* ingested but did not digest (no empty cells observed) *Dinophysis norvegica* cells (Fig. 2b–f). A total of 98% of the FP produced during the experiment contained *D. norvegica*. The number of *D. norvegica* cells within one FP ranged from 0 to 32, with an average of 19 ± 2.5 SE. This represents an ingestion rate of 400 to 500 *Dinophysis* spp. cells $ind.^{-1}$ d^{-1}. Earlier experiments indicate that *Temora longicornis* is able to ingest *Dinophysis* spp. cells, but the ingestion rate was too low to affect a bloom or the fate of diarrhetic shellfish poisoning (DSP) toxins in the food web (Maneiro et al. 2000, 2002).

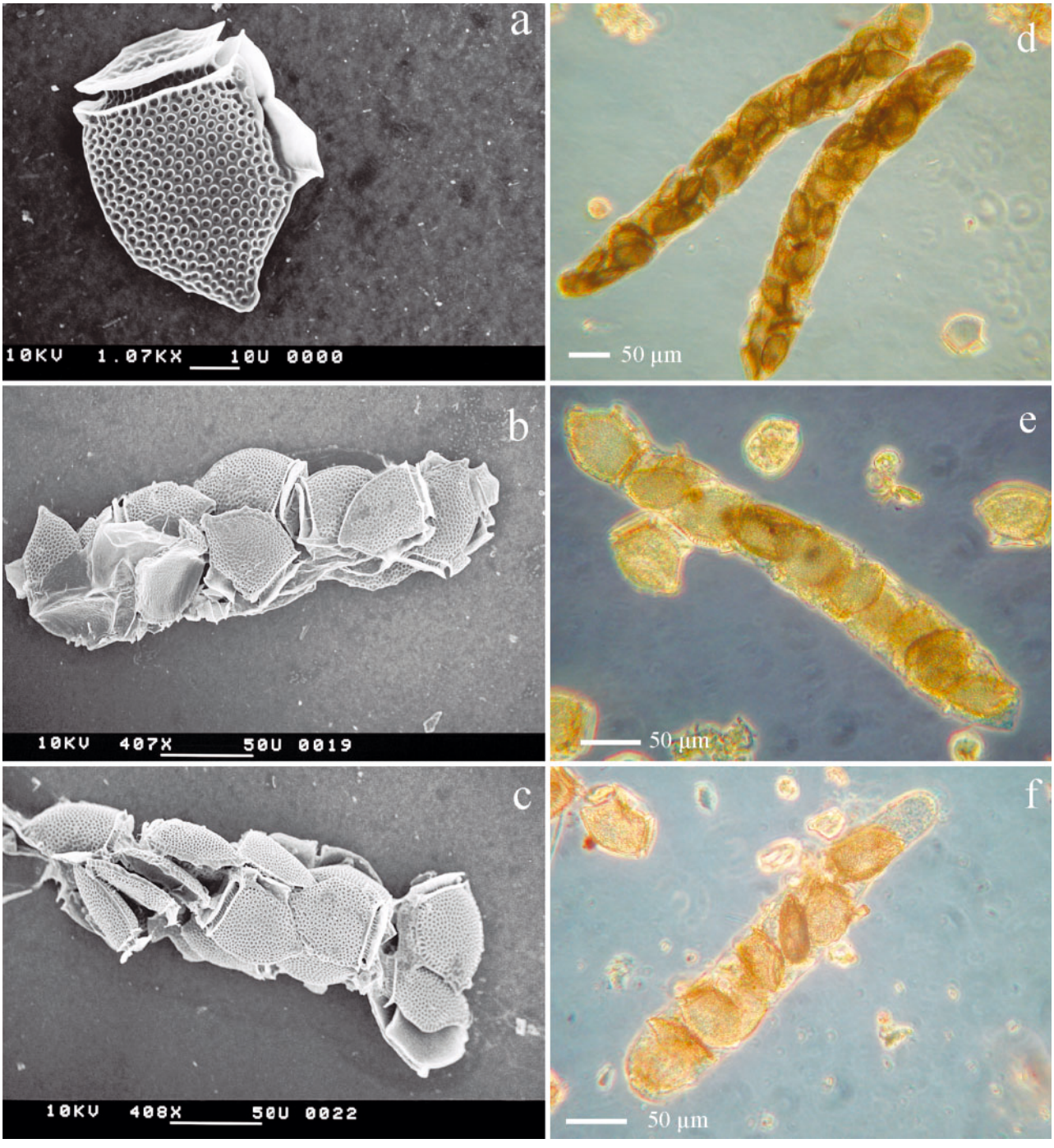

Fig. 2. Scanning electron micrographs (SEM) of (a) *Dinophysis norvegica*, (b,c) *Calanus helgolandicus* faecal pellet containing *D. norvegica*; (d–f) light micrographs of *C. helgolandicus* faecal pellets containing *D. norvegica*

Our experiment shows that *Calanus helgolandicus* feeds efficiently on *Dinophysis norvegica* under natural conditions. This may play an important role in the transport of DSP toxins through the food web and in the fate of *Dinophysis* spp. blooms, particularly in periods when *Dinophysis* experience sub-optimal growth conditions. Due to the very high concentration of *Dinophysis* and relatively low abundance of *C. helgolandicus* (~2800 adults m^{-2} at 0 to 30 m depth) their grazing could not have had a great impact on the fate of the bloom or the transport of DSP toxins through the food web at the time of the experiment. The experiment also shows that studies of phytoplankton remains in FP may provide important information on toxic blooms.

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