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GRAZING RATE AND PARTICLE SIZE SELECTION BY THE CHOANOFLAGELLATE *DIAPHANOECA GRANDIS* FROM THE SEA-ICE OF LAGOON SAROMA KO, HOKKAIDO

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Abstract: *Diaphanoeca grandis*, a loricate choanoflagellate, is an abundant microheterotroph in many localities, including Antarctica. Little is known of its feeding selectivity or ingestion rates. In order to investigate the role of choanoflagellates in ice-covered marine environments, the grazing rates and particle size selection by *D. grandis* from the bottom-ice community of lagoon Saroma Ko were measured using fluorescent latex microspheres (FM). Maximum uptake rate was around 2 FM·cell^{-1}·h^{-1} and maximum clearance rate was 0.7 nl·cell^{-1}·h^{-1}. *D. grandis* was not able to ingest microspheres larger than 1 μm. These data are discussed in relation to their abundance, grazing rates by other protozoa and food availability in under-ice environments.

1. Introduction

Choanoflagellate protozoa are an ubiquitous and conspicuous constituent of marine environments, particularly in polar regions where their numbers range from 10^3–10^7 cells·l^{-1} (SILVER *et al.*, 1980; BOOTH *et al.*, 1982; BUCK and GARRISON, 1983, 1988; FRYXELL *et al.*, 1984; MARCHANT, 1985; GARRISON and BUCK, 1989). The abundance of choanoflagellates in a variety of marine environments suggests that these microheterotrophs play an important role in pelagic food webs. In the sub-Arctic Pacific BOOTH *et al.* (1982) found them to be the fifth most abundant group of nanoplankton and in the Weddell Sea, Antarctica they were the third most abundant group (BUCK and GARRISON, 1983). DAVIS and SIEBURTH (1982) reported that choanoflagellates accounted for 36% of the heterotrophs in marine environments and BUCK and GARRISON (1988) found that they constituted 8–20% of the total heterotrophic flagellate biomass at the ice edge in the Weddell Sea.

Choanoflagellates feed principally on bacteria but they have also been reported to ingest pico- and nanoautotrophs as well as detritus and high molecular weight dissolved organic material (LAVAL, 1971; LEADBEATER and MORTON, 1974; MARCHANT, 1985; SHERR, 1988). They bear a single flagellum surrounded by a collar of tentacles with which they trap food particles. Data on their feeding rates and selection of the size of food particles are scanty and apparently lacking altogether from ice-covered marine environments where they are most abundant.

The uptake of fluorescent paint particles and fluorescently labelled latex microspheres, bacteria and high molecular weight dextrans have been employed in investiga-

tions of the grazing rates of microheterotrophs (McMANUS and FUHRMAN, 1986; CYNAR and SIEBURTH, 1986; SHERR *et al.*, 1987; SHERR, 1988). However, reports of grazing rates of choanoflagellates have concentrated on aloricate genera (FENCHEL, 1982; CYNAR and SIEBURTH, 1986; SALONEN and JOKINEN, 1988).

The sea-ice of lagoon Saroma Ko, northern Hokkaido, Japan, has a rich community of autotrophs and microheterotrophs (TAKAHASHI, 1981). He found *Diaphanoeca grandis* to be present under the ice together with the other loricate choanoflagellates *Acanthocorbis unguiculata* and *Stephanoeca* sp. *D. grandis* from lagoon Saroma Ko appeared identical to this species from various Antarctic localities including lakes (MARCHANT and PERRIN, 1986), pack-ice (GARRISON and BUCK, 1989) and coastal sites where it occurs throughout the year (MARCHANT, 1985; MARCHANT and PERRIN, 1990) as well as other parts of the world including arctic Greenland (THOMSEN, 1982). At oceanic ice-edge sites in Antarctic waters the distribution of choanoflagellates was strongly negatively correlated with depth (GARRISON and BUCK, 1989). This correlation was not evident at shallow coastal sites where the choanoflagellate population exhibited seasonal species succession and marked seasonality in their abundance (MARCHANT and PERRIN, 1990).

This paper reports an investigation of the size of particles ingested and the rate of their uptake by *D. grandis* from lagoon Saroma Ko to gain insights into the feeding behavior and ecological role of this organism in ice-covered environments.

2. Materials and Methods

Samples were taken from the colored bottom layer of sea-ice from about 1 km offshore in lagoon Saroma Ko on 7 March 1988. This lagoon has connections with the Sea of Okhotsk and was covered with 20 cm of ice at the time of sampling. The concentration of *Diaphanoeca grandis* in the bottom-ice community was around 10^5 cells $\cdot$ l^{-1}. The sea-ice samples were melted in a large volume of filtered lake water at 0°C and transported to Tokyo by air where they were maintained at -1°C. *D. grandis* isolated from this culture were incubated at 0°C in suspensions of fluorescent carboxylated latex microspheres (FM) (Polysciences) at concentrations in the order of 10^4–10^7 FM $\cdot$ ml^{-1}. To prevent the FM from clumping when diluted in filtered lake water, they were treated with 10 mg $\cdot$ ml^{-1} bovine serum albumin (Sigma) before incubations (PACE and BAILIFF, 1987). Separate time-course incubations were made using FM 0.25 μm, 0.50 μm, 1.0 μm, 1.97 μm in diameter. At various times aliquots were taken and the number of FM incorporated by between 20 and 81 (mean 47) unfixed cells was counted using an Olympus fluorescence microscope. Cells were counted directly, without concentration on filters, thus overcoming the problem of distinguishing whether FM have been ingested or attached to the cell surface (CYNAR and SIEBURTH, 1986). It was found unnecessary to fix the cells as flagellar motion stopped, the protoplast remained intact and none of the FM were egested when cells were brought to room temperature. By avoiding use of a fixative, the fixative-induced egestion reported by SIERACKI *et al.* (1987) was obviated. Cell volumes were calculated from measurements made using an ocular micrometer on unfixed cells.

3. Results

Diaphanoeca grandis accumulated FM on the feeding tentacles and ingested these

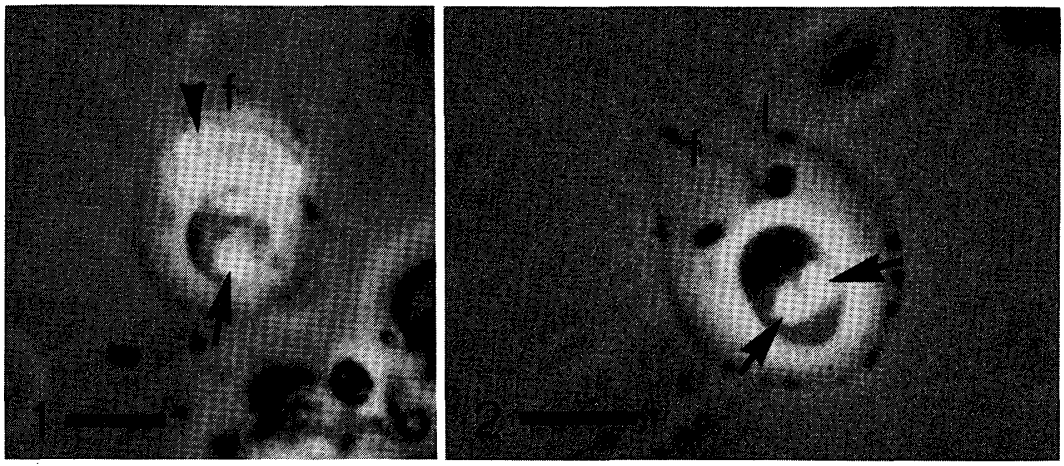

Fig. 1. Light micrograph using combined phase contrast and fluorescence showing FM both in food vacuoles (arrow) and attached to the feeding tentacles (arrowhead) that surround the flagellum (f) at the anterior end of the cell. Scale marker=5 μm.

Fig. 2. FM in two food vacuoles (arrows) which lie posteriorly in the cell. Note the flagellum (f) and the lorica (l) that surrounds the cell. Scale marker=5 μm.

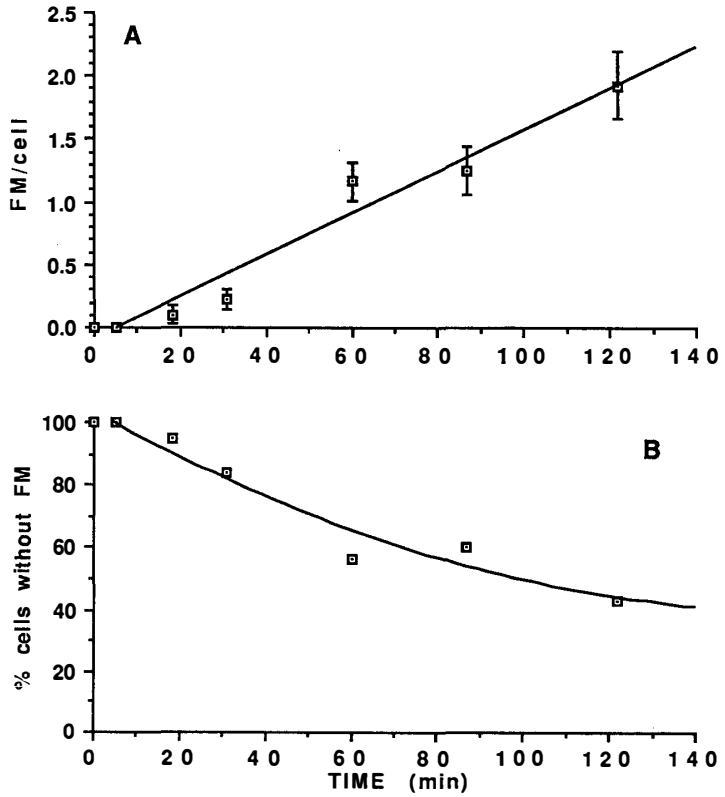

Fig. 3. Time course of ingestion of FM 0.5 μm in diameter at a concentration of 1.25×10^6 FM·ml^{-1} at 0°C. (A) Linear regression ($r^2=0.96$) of uptake of FM ingested per cell with standard deviation of each uptake measurement. (B) Percent of cells not having ingested FM.

particles into two food vacuoles which lie at the posterior end of the cell (Figs. 1 and 2). Figure 3 indicates the time course of the uptake of 0.5 μm diameter FM at a concentration of 1.25×10^6 FM$\cdot$ml^{-1} at 0°C as well as the percentage of cells not having taken up FM during the incubation. Similar plots were constructed from time course incubations of different concentrations of the different sized FM. In all cases the uptake of FM was linear for at least the first 60 min of incubation and fitted a linear regression ($r^2 > 0.9$). Uptake rates of the different sized FM plotted against concentration of the FM showed considerable variability and indicated that maximum uptake rate was about 2 FM$\cdot$cell^{-1}h^{-1} (Fig. 4). The clearance rate (the volume of water filtered per unit time) is calculated as the uptake rate divided by FM concentration. The slope near the origin of the uptake against FM concentration curve (Fig. 4) indicates a clearance rate of around 0.7 n$l\cdot$cell$^{-1}\cdot$h^{-1}. Specific clearance is the number body volumes cleared per hour. The mean body volume of *D. grandis* was calculated as 150 μm^3, thus this

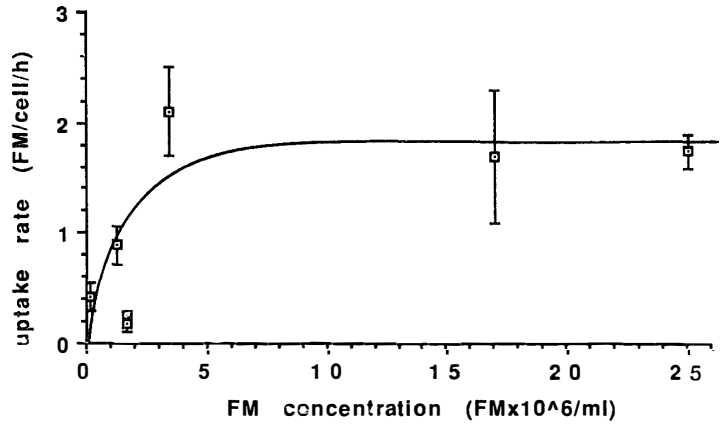

Fig. 4. The uptake rate of FM as a function of FM concentration with 95% confidence limits for each uptake rate measurement.

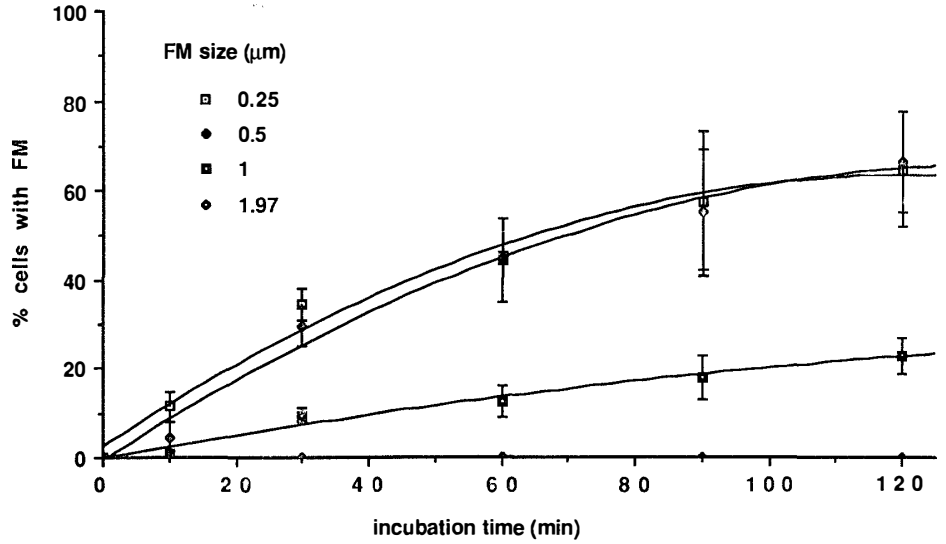

Fig. 5. Time course of the percentage of D. grandis *populations ingesting FM of different sizes. Error bars indicate the range.*

choanoflagellate species clears about 5×10^3 times its cell volume per hour.

D. grandis ingested FM of different sizes at different rates. Figure 5 shows: (a) There was no difference in the uptake of 0.25 μm and 0.5 μm diameter FM. After two hours both sized particles were taken up by about 65% of the cells. (b) After two hours only about 20% of the cells had taken up 1 μm diameter FM and (c) FM 1.97 μm in diameter were not ingested.

4. Discussion

In this study unfixed cells of *D. grandis* exhibited a clearance rate of around 0.7 $nl \cdot cell^{-1} \cdot h^{-1}$ at 0°C. FENCHEL (1982) calculated the clearance rate of *Monosiga* to be 2.0 $nl \cdot cell^{-1} \cdot h^{-1}$ at 20°C when fed on natural bacterial populations and SALONEN and JOKINEN (1988) estimated the clearance rate of this aloricate choanoflagellate from a small humic lake to be 2.5 $nl \cdot cell^{-1} \cdot h^{-1}$ at 15–23°C from *in situ* incubations with FM. The clearance rate of another aloricate choanoflagellate, *Codosiga* was estimated to be 0.4 $nl \cdot cell^{-1} \cdot h^{-1}$ at 20°C using FM (CYNAR and SIEBURTH, 1986). However, as these organisms were fixed with glutaraldehyde before the number of ingested FM was counted, the reported clearance rates may be an underestimate (SIERACKI *et al.*, 1987). Thus, choanoflagellates in under-ice environments apparently feed at rates within the range of those found in warmer waters.

In comparison, DAVIS and SIEBURTH (1984) found clearance rates of flagellates feeding on bacteria were 40–650 $nl \cdot cell^{-1} \cdot h^{-1}$ while clearance rates of 400–6000 $nl \cdot cell^{-1} \cdot h^{-1}$ for heterotrophic dinoflagellates, 800–2000 $nl \cdot cell^{-1} \cdot h^{-1}$ for ciliates and 400–1000 $nl \cdot cell^{-1} \cdot h^{-1}$ for tintinnids were reported by LESSARD *et al.* (1987). Thus clearance rates of the aloricate choanoflagellates and *D. grandis* are considerably lower than other microheterotrophs. However, as FENCHEL (1987) indicates, filter feeding organisms, including choanoflagellates, sieve food particles from the feeding current that they generate rather than directly impacting them.

That *Monosiga* has been reported to ingest particles 0.21 μm in diameter (SALONEN and JOKINEN, 1988) and *D. grandis* ingests microspheres at least as small as 0.25 μm in diameter indicates these organisms are able to utilize the smallest of the prokaryotic organisms as food. FENCHEL (1982) concludes that the low clearance rates (low water velocity) through the feeding basket of choanoflagellates may be related to the pressure drop that is a function of the porosity of the filter but the performance of choanoflagellates in trapping food particles will, to a large extent, be a function of the size of food particles encountered.

Choanoflagellates differ considerably in the spacing between the costal strips of the lorica. In some, the protoplast is virtually completely exposed (*e.g.* in *Crinolina aperta* the protoplast is suspended in an open-ended barrel-shaped lorica and in *Bicosta* the protoplast lies in the fork of a Y-shaped lorica). The protoplasts of others, such as *Stephanoeca*, *Acanthoeca*, *Acanthocorbis* and to a lesser extent *D. grandis*, lie within a lorica constructed from closely arranged costal strips. The spacing between the costal strips of some species of *Stephanoeca* is less than 0.2 μm and around 2 μm at the posterior end of the lorica of *D. grandis* (MARCHANT, 1985). If the flagellar beat generates a water current, bearing food particles, flowing from the posterior to anterior end

of the organism to reach the feeding tentacles (FENCHEL, 1987), the food particles apparently have to pass through the fenestrae of the lorica. If this is so, the lorica would function as a sieve, only permitting particles smaller than a certain size to reach the feeding basket. Differences in lorica structure indicate that different species of choanoflagellates may be selective in the size of food particles ingested.

In consideration of the ecological role of choanoflagellates it must be noted that as well as ingesting organisms they are able to take up high molecular weight "dissolved organic carbon". Recently, SHERR (1988) demonstrated that heterotrophic flagellates, including the aloricate choanoflagellate, *Codosiga*, ingested polysaccharides with a molecular weight greater than 500000. *D. grandis* from Antarctica ingests polysaccharides with a molecular weight as low as 4000 at a concentration of $2\,mg \cdot l^{-1}$ (H. J. MARCHANT, unpublished). This concentration is within the range reported for dissolved organic carbon in Antarctic waters (BÖLTER and DAWSON, 1982). High molecular weight material is often a considerable fraction of total dissolved organic carbon in natural waters (WHEELER, 1976; OGURA, 1977). Therefore, choanoflagellates may short circuit the microbial loop of the food web by utilizing some of the detrital carbon that would otherwise be broken down and assimilated by bacteria before they are grazed by protozoa, including choanoflagellates. In Antarctic waters choanoflagellates are a conspicuous component in the diet of *Euphausia superba* (MARCHANT and NASH, 1986; TANOUE and HARA, 1986). Thus, at least in cold water environments, choanoflagellates may directly link both high molecular weight dissolved organic material and the picoplankton with metazoan grazers.

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