

Inhibition of copepod grazing by diatom exudates: a factor in the development of mucus aggregates?

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ABSTRACT: Dissolved polymers, especially polysaccharides, are thought to play an important role in formation of larger organic aggregates in the sea. Phytoplankton may exude considerable quantities of polymers, although the particular conditions conducive to release of these substances are not known. Such processes are implicated in the formation of large-scale gelatinous mucus aggregations in the Adriatic Sea. We investigated extracellular carbohydrate release in the diatoms *Coscinodiscus granii*, *Stephanopyxis palmeriana*, *Skeletonema costatum*, *Nitzschia delicatissima*, *Thalassiosira weissflogii*, and 2 species of *Chaetoceros*, under different culture conditions, and examined whether these exudates interfered with copepod grazing. The copepod species studied are typical of Adriatic coastal environments. Our results indicate that during the exponential growth phase the contribution of the high-molecular-weight carbohydrate (HMWCHO) fraction to extracellular release was minor, except in phosphorus limited medium. A shift towards an increased significance of HMWCHO was observed in the stationary phase for all species tested. Grazing experiments with *Temora longicornis* and *Pseudocalanus elongatus* demonstrated that copepod feeding was inhibited by HMWCHO diatom exudate and also stationary phase culture filtrate. Feeding rates were also adversely affected by the presence of pure HMW dextran used as a model substance. Copepod feeding was more strongly inhibited by diatom HMWCHO exudates than an equivalent concentration of pure dextran, suggesting an additional response to the chemical stimuli of algal exudates.

INTRODUCTION

Evidence has accumulated that both healthy and stressed phytoplankton may release considerable amounts of organic compounds, among which carbohydrates are quantitatively important (Fogg 1983, Myklesstad et al. 1989). High-molecular-weight carbohydrates represent the main constituent of slime secretion of phytoplankton and bacteria (Decho 1990) and have been shown to be involved in the attachment of cells to surfaces and cell-cell adhesion, producing multicellular aggregates (Yoshida 1991). Interparticle bridging by polysaccharide polymers is thought to be important in formation of larger organic aggregates in the sea (Allredge & McGillivray 1991).

Some phytoplankton species, particularly dinoflagellates, have been shown to produce compounds which deter copepod feeding (Huntley 1982, Huntley et al. 1986, Uye & Takamatsu 1990) although the chemical

nature of the specific compounds has not been identified. Exudates produced during phytoplankton blooms may interfere with copepod grazing. Chemically or mechanically mediated inhibition of grazing upon mucilage producing plankton may thus have potential significance for particle aggregation processes. Increased particle attachment due to algal exudates (Allredge & Silver 1988) together with specific combinations of physical conditions in the pelagic environment (Riebesell 1991) may also contribute significantly to macroaggregate formation.

Several recent studies have dealt with macroaggregate formation in different coastal and open waters; however, gelatinous (mucus) aggregates in some Mediterranean regions (Adriatic Sea, Thyrranian Sea, Aegean Sea) have attracted special attention due to their widespread and massive appearance (Stachowitsch et al. 1990). Historical records of such occurrences date back to the 18th and 19th centuries (Fonda

Umani et al. 1989, Molin et al. 1992) so eutrophication can hardly be considered as a direct cause of the mucus phenomenon. Several authors have tried to explain the phenomenon either by a microbial approach to 'solve the mystery of floating mucilage' (Herndl et al. 1992), by analysing the biochemical (Malej & Faganeli 1988, Marchetti et al. 1989, Posedel & Faganeli 1991) and the phytoplankton composition (Revelante & Gilmartin 1991, Cabrini et al. 1992) of mucus, or by analysing environmental factors before and during the outbreaks (Fanuko & Turk 1990). None of these explanations has proved convincing.

The purpose of our study was to estimate the extracellular carbohydrate release in selected diatom cultures under different culture conditions. The species studied were from the genera *Nitzschia*, *Skeletonema* and *Chaetoceros*, which have been shown to be associated with the formation of aggregates in the Adriatic (Marchetti et al. 1989, Stachowitsch et al. 1990, Revelante & Gilmartin 1991), and were in most cases isolated from the field. In addition *Coscinodiscus granii* and *Stephanopyxis palmeriana* were studied as representatives of larger diatoms, as the former in particular has been shown to produce mucilage *in situ* and in culture (Boalch & Harbour 1977). *Isochrysis galbana* and *Tetraselmis suecica* were considered as representatives of the microflagellates, which have also been associated with mucilage in the field (Stachowitsch et al. 1990). We then carried out a series of feeding experiments to examine whether these exudates interfere with grazing of copepods. The copepod species used, *Temora longicornis* and *Pseudocalanus elongatus*, are frequent species in the coastal zone of the Adriatic, and also in open waters, while *Calanus helgolandicus* is less abundant and typical of the winter spring period (Fonda Urmani et al. 1993). To test the thickening (mechanical) effect of polysaccharides we also examined the influence of pure high-molecular-weight carbohydrates on copepod feeding. The importance of these processes in large-scale mucus accumulations is discussed.

MATERIALS AND METHODS

Phytoplankton cultures. Stock cultures of the diatoms *Coscinodiscus granii* and *Stephanopyxis palmeriana* from the Plymouth Marine Laboratory Culture Collection were maintained in f/2 medium (Guillard & Ryther 1962) in batch culture using a 2 wk transfer cycle. For experiments, diatoms were grown in 3 l Pyrex flasks containing 2.5 l of prefiltered sterile (0.22 µm) medium of 3 different strengths: f/2; fN₁₀/P₅₀ (N/P atomic ratio 122, referred to as P-lim); and f/200. Cultures were not aerated, but were gently agitated manually once per day. The culture temperature was

14 °C with a light:dark cycle of 12:12 h. The cultures in f/2 medium were exposed to 2 different light (cool-white fluorescent tubes) conditions: high light (105 µE m⁻² s⁻¹; f/2 HL) and low light (46 µE m⁻² s⁻¹; f/2 LL) while the others were kept at low light conditions.

Two species of *Chaetoceros*, and *Skeletonema costatum* and *Nitzschia delicatissima*, were isolated from samples collected in March 1991 from the Gulf of Trieste (northern Adriatic). Cultures were kept in f/2 medium at low and high light; otherwise the conditions were the same as for *Coscinodiscus granii* and *Stephanopyxis palmeriana* cultures. All experiments were carried out in duplicate.

Samples for cell counts and for extracellular carbohydrate analyses were collected approximately every 48 h for *Coscinodiscus granii* and *Stephanopyxis palmeriana* and daily for *Chaetoceros* species. *C. granii* and *S. palmeriana* cells were enumerated, after fixation in Lugol's solution by inverted microscope using the Utermöhl method, while *Chaetoceros* concentrations were determined with a Coulter Multisizer. Cell count data were used to define the growth curve for each species, and these were used to separate the sampling into the exponential and stationary phases. Samples from *Skeletonema costatum*, *Nitzschia delicatissima* and *Thalassiosira weissflogii* cultures were withdrawn only twice: during the exponential growth and stationary phase.

Chemical analyses. For extracellular carbohydrate analysis, aliquots (4 × 50 ml) were first centrifuged (4500 × g; 45 min) to remove cells, and then the supernatant containing water soluble components was analysed for total carbohydrate (TCHO). The high-molecular-weight fraction (HMWCHO) was further determined after dialysis (12 000 MW cutoff; Visking dialysis tubing) against distilled water (> 36 h).

High- and low-molecular-weight fractions of dissolved CHO exudate were also separated by gel filtration on PD-10 Sephadex columns (Pharmacia Corp.). Elution curves of standards (blue dextran with molecular weight of 2 000 000, FITC dextran with MW 35 600 and 4400, glucose, saccharose, and CoCl₂, Sigma Chemical Comp.) are shown in Fig. 1. Samples for gel filtration from *Skeletonema costatum* cultures were taken 4 times: on Days 2, 5, 8, and 28. Two non-diatom cultures (*Isochrysis galbana* and *Tetraselmis suecica*) in a stationary growth phase maintained under the same conditions as diatoms (f/2 medium, low light) were also analyzed. Total, and high-molecular-weight, dissolved carbohydrates were determined using the phenol-sulphuric acid reaction with glucose as a standard (Strickland & Parsons 1968). Larger volumes (9 l) of stationary diatom cultures were centrifuged (4500 × g, 45 min), supernatant dialysed against distilled water to isolate HMW components, the solution concentrated

at 80 to 90°C under reduced pressure and used as an addition in the grazing experiments.

Carbohydrate release rates were calculated from a combination of cell counts and CHO analyses for time points during exponential and stationary growth phases. Two replicate determinations were made for each species for 4 time intervals during exponential phase, and 2 intervals during stationary phase. The possible influence of bacteria was investigated in initial experiments in which cultures were treated with $30 \mu\text{g ml}^{-1}$ of streptomycin. No significant differences were observed compared to untreated controls.

Grazing experiments. The copepods *Temora longicornis*, *Pseudocalanus elongatus* and *Calanus helgolandicus* were sorted from zooplankton samples collected with a $250 \mu\text{m}$ net off Plymouth. Copepods were starved overnight at 14°C in filtered seawater prior to experimentation. Grazing experiments were done at 13 to 14°C under dim light using triplicate 250 ml glass bottles containing 20 (*T. longicornis* or *P. elongatus*) or 5 (*C. helgolandicus*) copepods for each treatment. The bottles were placed together with controls, containing only food suspensions, on a plankton wheel (1 rpm). The duration of the experiments ranged from 4 h (experiments with dextran) to 6 h (experiments with cell-free stationary phase medium). Feeding rates were calculated from the difference between initial (C_0) and final (C_t) particle concentrations determined with a Coulter Multisizer using the equations of Marin et al. (1986). Analyses of initial and final particle concentrations in control bottles showed no significant difference, therefore no correction for algal growth was necessary.

In a preliminary experiment, a mixture of adult *Temora longicornis* and *Pseudocalanus elongatus* (10 of each species) were used as grazers and *Thalassiosira weissflogii* offered as food (Plymouth Marine Laboratory culture collection, average cell volume $1255 \mu\text{m}^3$). Feeding bottles contained either food suspension of algal cells (average food concentration $2845 \times 10^3 \mu\text{m}^3 \text{ml}^{-1}$, ca $2000 \text{ cells ml}^{-1}$) in filtered seawater or food suspension with the addition of HMW-CHO concentrate obtained from *Coscinodiscus granii* cultures (final HMWCHO concentration of approximately 4 mg l^{-1}). Fecal pellets produced were pipetted into a counting tray and counted to provide an additional indication of grazing activity. Further grazing experiments were carried out to check the possible effects on copepod feeding of pure high-molecular-weight carbohydrate using dextran MW 40 000 (Sigma Chemical Co.) at concentrations of 4 and 20 mg l^{-1} , and also the influence of HMWCHO (concentrate from *C. granii* culture; final concentrations in HMWCHO-treated bottles = $3.6 \pm 0.5 \text{ mg l}^{-1}$; control bottles $< 1 \text{ mg l}^{-1}$ of total CHO). Copepods used in this experiment were 20 adult *T. longicornis* or 5 *C. helgolandicus* per

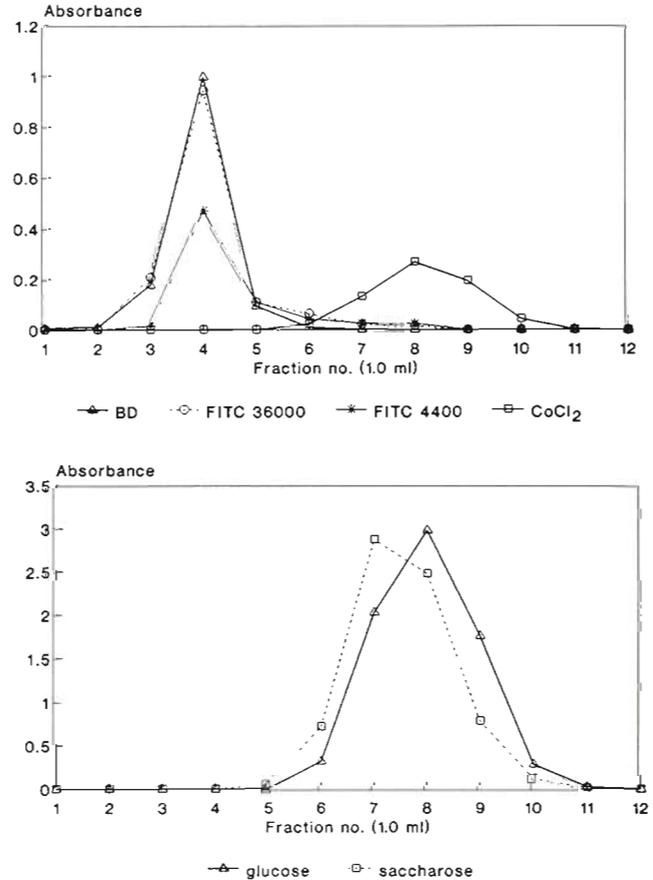


Fig. 1. Elution curves of standards: Sephadex gel-fractionation of high-molecular-weight dextran (BD = blue dextran with MW 2 000 000, FITC = fluorescein isothiocyanate dextran with MW 36 000 and 4400) and CoCl_2 (upper panel), and saccharose and glucose (lower panel). x-axis shows spectrophotometer reading for carbohydrates following standard methods for CHO determination

250 ml bottle. The experiment was carried out the same way as the previous one, offering *T. weissflogii* as a food (average food concentration $5800 \times 10^3 \mu\text{m}^3 \text{ml}^{-1}$, ca $4100 \text{ cells l}^{-1}$). Fecal pellets were counted and the length and width measured for 20, selected randomly from each feeding bottle.

Finally, we tested the effect of algal exudate on *Temora longicornis* feeding using cell-free culture medium from a stationary *Skeletonema costatum* culture. The algal food (*S. costatum* isolated from the Gulf of Trieste samples; average food concentration $4180 \times 10^3 \mu\text{m}^3 \text{l}^{-1}$, ca $14900 \text{ cells l}^{-1}$) was suspended either in filtered seawater or in a filtrate containing algal exudates of a stationary culture of the same species. An additional experiment (average food concentration $7140 \times 10^3 \mu\text{m}^3 \text{l}^{-1}$, ca $24500 \text{ cells l}^{-1}$) was carried out with a 50% dilution of exponentially growing *S. costatum* culture medium and 50% dilution of stationary *S. costatum* culture medium.

Table 1. Rates of TCHO release ($\mu\text{g TCHO} \times 10^{-7} \mu\text{m}^{-3} \text{d}^{-1}$) for different diatom species harvested in exponential growth phase (see 'Materials and methods' for details of culture media), and normalized to cell volume

Species	Volume (μm^3)	f/2 LL	f/2 HL	P-lim	f/200
<i>Coscinodiscus granii</i> ^a	2.2×10^6	1.2	0.5	0.6	0.8
<i>Stephanopyxis palmeriana</i> ^a	1.7×10^5	1.3	0.3	0.5	0.8
<i>Skeletonema costatum</i> ^{a, b}	0.28×10^3	16.7	8.6	–	0.9
<i>Nitzschia delicatissima</i> ^{a, b}	0.1×10^3	12.1	4.7	1.0	2.0
<i>Chaetoceros</i> sp. 1 ^{a, b}	50	5.9	2.5	2.4	–
<i>Chaetoceros</i> sp. 2 ^{a, b}	2.3×10^3	0.4	–	0.2	–
<i>Thalassiosira weissflogii</i> ^b	1.3×10^3	0.6	0.4	–	–

Cell volumes determined by: ^ainverted microscope measurement; ^bCoulter Counter measurement

RESULTS

The rates of TCHO release for different diatom species harvested during exponential growth and normalized to cell volumes are given in the Table 1 and the proportion of the HMWCHO in exponential and stationary phase in Table 2. TCHO release rates during exponential growth varied from <1 to $16.7 \times 10^{-7} \mu\text{g TCHO} \mu\text{m}^{-3} \text{d}^{-1}$. *Skeletonema costatum*, *Nitzschia delicatissima* and one species of *Chaetoceros* produced significantly more ($p < 0.01$) soluble TCHO per unit cell volume than the other diatom species, the amounts being higher in high nutrient medium. TCHO concentrations over time in f/200 and P-lim culture media were generally proportional to cell numbers (Fig. 2), indicating that extracellular TCHO release took place in all growth phases. In f/2 media (LL and HL) the

TCHO concentration initially increased with increasing cell numbers, but ultimately reached a plateau.

During the initial growth period the contribution of the high-molecular-weight fraction was minor except in the P-limited medium. A higher proportion of HMWCHO fraction was also observed in f/2 medium under high light conditions for *Coscinodiscus granii* and *Stephanopyxis palmeriana*, while a shift towards an increased significance of HMWCHO was observed in the stationary phase for all species tested (Table 2). Gel permeation chromatography of soluble extracellular carbohydrates also showed an increasing importance of the HMWCHO fraction with ageing of the culture of *Skeletonema costatum* (Fig. 3a) and in the stationary cultures of *Isochrysis galbana* and *Tetraselmis suecica* (Fig. 3b). Small quantities of low-molecular-weight products were observed during the stationary phase in all experiments.

Table 2. Proportions (as % of TCHO release) of HMWCHO released during exponential and stationary phase (growth conditions as for Table 1). Values are means \pm SD, which are based on 8 measurements during the exponential phase, and 4 during the stationary phase; single values, for which no replicates were obtained, are also shown

Species Medium	f/2 LL	f/2 HL	P-lim	f/200
<i>Coscinodiscus granii</i>				
Exponential	38 \pm 12	44 \pm 17	57 \pm 4	31 \pm 11
Stationary	58 \pm 9	68 \pm 12	72 \pm 14	76 \pm 3
<i>Stephanopyxis palmeriana</i>				
Exponential	31 \pm 14	33 \pm 16	68 \pm 5	39 \pm 12
Stationary	72 \pm 9	75 \pm 19	79 \pm 11	97
<i>Chaetoceros</i> sp. 1				
Exponential	44 \pm 5	38 \pm 6	68 \pm 9	–
Stationary	50 \pm 6	41 \pm 1	–	–
<i>Chaetoceros</i> sp. 2				
Exponential	29 \pm 9	–	66 \pm 9	–
Stationary	50	–	86	–
<i>Nitzschia delicatissima</i>				
Exponential	24 \pm 13	–	–	–
Stationary	60 \pm 13	62	87 \pm 7	–
<i>Skeletonema costatum</i>				
Exponential	20 \pm 11	–	–	–
Stationary	72 \pm 18	68	74 \pm 9	–

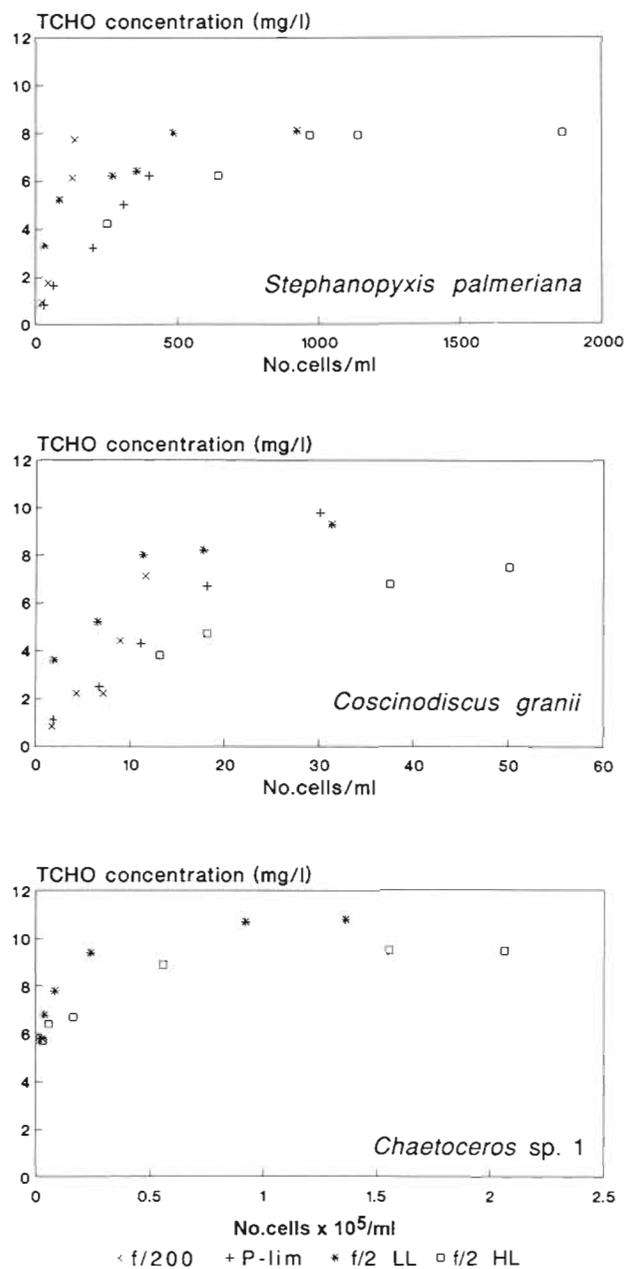


Fig. 2. *Stephanopyxis palmeriana*, *Coscinodiscus granii*, *Chaetoceros sp. 1*. Relations between total carbohydrate (TCHO) concentration (mg l^{-1}) and cell concentration in different culture media. f/2 LL = f/2 medium under low light; f/2 HL = f/2 medium under high light; f/200 = f/200 medium; P lim = phosphorus limiting medium. See text for details of culture conditions

The results of the preliminary feeding experiment with mixed *Temora longicornis* and *Pseudocalanus elongatus* and addition of concentrated diatom HMWCHO are shown in Fig. 4. Feeding rate and the number of fecal pellets produced were significantly lower in

experimental bottles with addition of the diatom HMWCHO.

We then examined the effect of pure HMW dextran and diatom HMWCHO on feeding and egestion rate of *Temora longicornis* and *Calanus helgolandicus*. No influence of dextran addition on *C. helgolandicus* feeding was detected. In contrast, we found that *T. longicornis* ingestion rates were significantly lower ($p < 0.01$, t -test between means) when exposed to dextran (Fig. 5), and the number of fecal pellets produced in the presence of dextrans and diatom HMWCHO was also lower (Fig. 5). *T. longicornis* produced pellets with mean length of $336 \pm 52 \mu\text{m}$ and mean width of $54 \pm 10 \mu\text{m}$. The size of fecal pellets varied under different experimental treatments and hence egestion rates in terms of the volume of fecal pellets produced per copepod per hour are given in Table 3.

Feeding rates of *Temora longicornis* were then determined under 3 different treatments: food (*Skeletonema costatum*) was suspended in filtrate of the stationary *S. costatum* culture (Fig. 6a) and in diluted

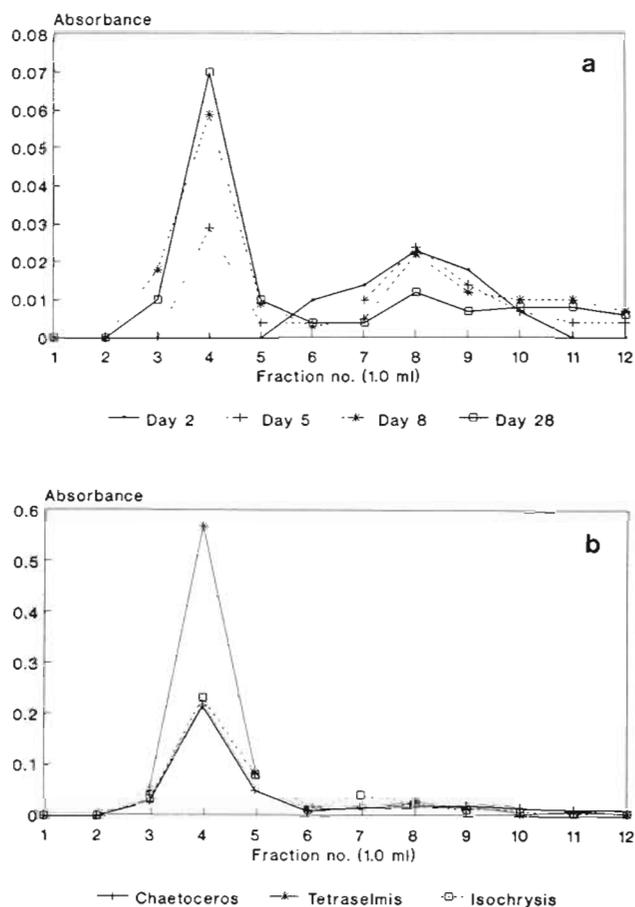


Fig. 3. (a) Sephadex gel-fractionation of *Skeletonema costatum* culture over time. (b) Sephadex gel-fractionation of stationary phase cultures of *Chaetoceros sp. 1*, *Tetraselmis suecica* and *Isochrysis galbana*

(50%) filtrate of exponentially growing and diluted (50%) filtrate of stationary *S. costatum* culture (Fig. 6b). While we found no effect of the addition of exponentially growing algal culture filtrate, *T. longicornis* ingested food at significantly ($p < 0.01$, *t*-test between means) lower rates in the presence of the stationary culture filtrate, both concentrated (Fig. 6a) and diluted (Fig. 6b).

DISCUSSION AND CONCLUSIONS

Extracellular release of organic matter by phytoplankton has been the subject of numerous studies during the last 20 yr, however, the significance and ecological role of this process are still matters of uncertainty (Wood & van Valen 1990). Although Sharp (1977) concluded that there was no good evidence that

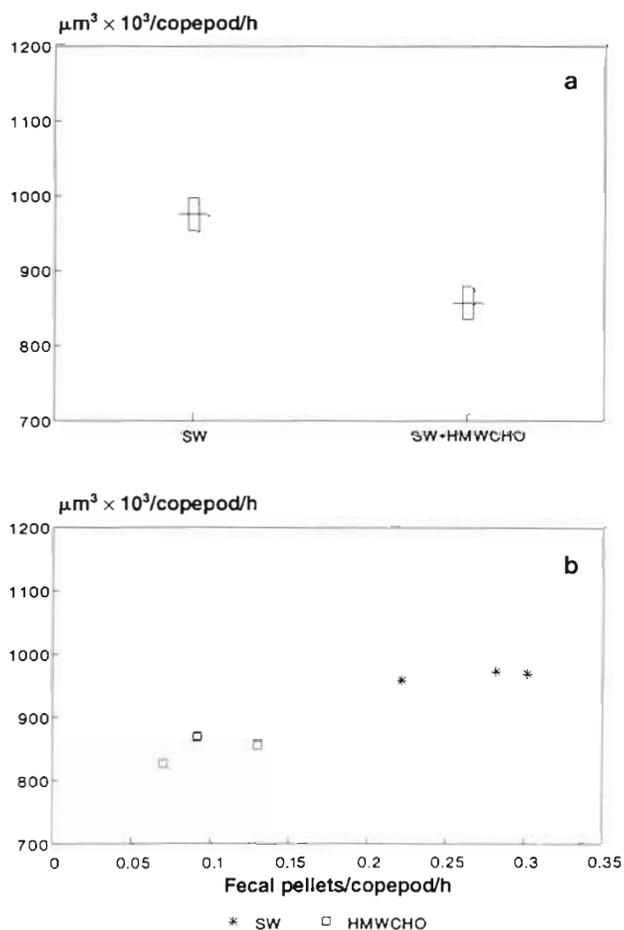


Fig. 4. Mixed copepods (*Temora longicornis*, *Pseudocalanus elongatus*) feeding on *Thalassiosira weissflogii*. The effect of HMWCHO concentrate from stationary phase *Coscinodiscus granii* culture on (a) feeding (mean \pm SD) and (b) fecal pellet production

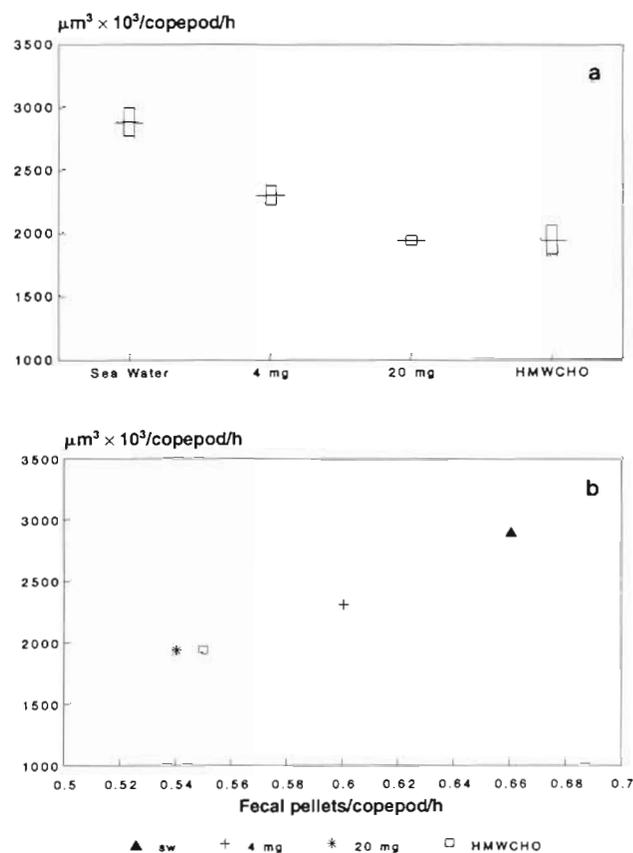


Fig. 5. *Temora longicornis* feeding on *Thalassiosira weissflogii*. Comparative effects of pure HMW dextran and HMW-CHO diatom concentrate on (a) feeding (mean \pm SD) and (b) egestion rate

healthy phytoplankton excrete organic matter, it is now generally accepted that extracellular release of organic compounds occurs during both exponential and stationary phases of growth (Myklesstad et al. 1989). Extracellular release of organic matter has also been shown to occur in natural phytoplankton populations (Ittekkot et al. 1981, Lancelot 1983).

A wide variety of biochemical products have been found in phytoplankton exudates (Lancelot 1984), although there is still considerable uncertainty as to

Table 3. *Temora longicornis*. Egestion rates in relation to different additions of pure dextran and HMWCHO (mean, with standard deviation in parentheses)

Treatment	Egestion rate ($\times 10^6 \mu\text{m}^3 \text{copepod}^{-1} \text{h}^{-1}$)
Seawater control	0.50 (0.10)
4 mg dextran	0.46 (0.09)
20 mg dextran	0.33 (0.07)
HMWCHO	0.45 (0.10)

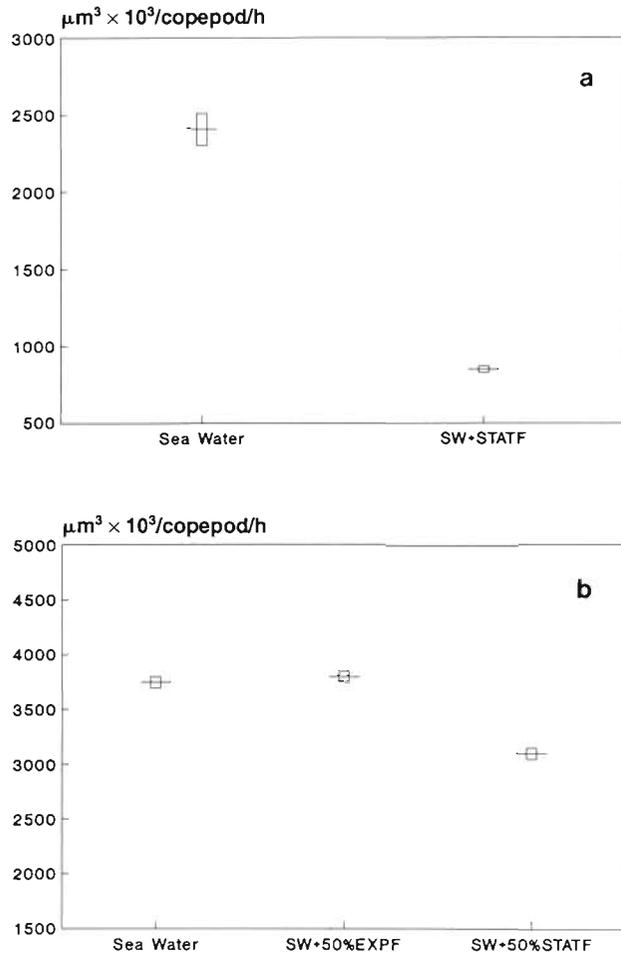


Fig. 6. *Temora longicornis* feeding on *Skeletonema costatum*. (a) Effect of cell-free stationary culture medium from *S. costatum* (STATF) on feeding rate (mean \pm SD). (b) Effect of diluted (50%) cell-free culture medium from exponentially growing (EXPF) and stationary (STATF) culture of *S. costatum* on feeding rate (mean \pm SD)

the precise nature of material released. Some studies have indicated that exudates from actively growing phytoplankton are predominantly low-molecular-weight (<700) compounds (Sondergaard & Schierup 1982). Møller Jensen (1983) reported that extracellular products with low MW (<900) constituted 69 to 100 % before, and 39 to 56 % during, the spring diatom bloom. In contrast, studying freshwater phytoplankton, Nalewajko & Schindler (1976) found low-molecular-weight (<1500) material representing less than 10 % of total extracellular products in all cases, even after incubation times of only 1 h. Similarly, Lancelot (1984) showed that the amount of small excreted molecules (MW <500) was always lower than extracellular polymers and suggested that polysaccharides were the main excreted products of natural phytoplankton. Moreover, Ittekkot et al. (1981) measured large amounts

of carbohydrates released into seawater during a phytoplankton bloom and more than 75 % consisted of combined carbohydrates. They concluded that CHO monomers were not the major excretion products of phytoplankton but were formed mainly by *in situ* hydrolysis of dissolved combined CHO. On the other hand, it has been suggested that rapid bacterial uptake of low-molecular-weight products and subsequent release of high-molecular-weight compounds by bacteria may be responsible for the predominance of the latter in natural waters (Chrost & Faust 1983, Gomes et al. 1991).

Our results indicate an increased significance of HMWCHO release in the stationary phase of culture growth, and also during exponential growth in P-limited medium. We did not follow the presence of bacteria during our experiments and it remains unclear to what extent the shift towards HMWCHO in stationary cultures is due to bacterial uptake of smaller molecules.

Smetacek (1985) proposed that mucus production, flocculation and mass sinking represent an important part of diatom survival strategy from a surface growing stage to a benthic or deep-water resting stage. Declining growth in conjunction with mucus secretion presumably leads to increasing sinking rate and aggregate formation. Indeed, Kiørboe et al. (1990) have demonstrated that stickiness of diatom cells may increase by more than 2 orders of magnitude as cell growth ceases, while Alldredge & McGillivray (1991), who studied attachment probabilities of marine snow, found the highest probabilities of aggregate formation for diatom flocs. We suggest that production of mucus, which is predominantly carbohydrate, may act as a grazer deterrent enabling diatoms to maintain their biomass and to conserve essential elements (N, P, Si) which are in short supply. Increased HMWCHO production during stationary growth phase, and also in P-limited environments, enhances the stickiness of diatom cells and consequently increases the attachment probability leading to macroaggregate formation.

The grazing potential of copepods has long been viewed as a major factor controlling seasonal diatom blooms in coastal waters (Cushing 1989, Bathmann et al. 1990). There are indications that the age of phytoplankton cultures may negatively affect copepod grazing (Mullin 1963). Cowles et al. (1988) demonstrated that copepods selectively ingested faster growing algal cells when a mixture of fast and slow growing diatom cells was offered as food. They concluded that physiological differences between algal cells were critical for selection and suggested that copepods may use extracellular gradients in exudates to discriminate between the 2 algal types. The grazing rates of the copepod spe-

cies used in the present experiments, under control conditions, are typical of those reported for these species, using similar experimental techniques, feeding on natural particulate material *in situ*. For example, for *Pseudocalanus elongatus*, Harris (1982) measured rates ranging from 21.9 to $30.4 \times 10^6 \mu\text{m}^3$ copepod⁻¹ d⁻¹, and for *Temora longicornis*, O'Connors et al. (1980) reported maximum ingestion rates in the range from 20.6 to $71.8 \times 10^6 \mu\text{m}^3$ copepod⁻¹ d⁻¹.

Our study demonstrates that copepod feeding, under the experimental conditions used, employing species characteristic of the Adriatic, is inhibited by HMW-CHO diatom exudate as well as stationary (aged) culture filtrate. The feeding rate of *Temora longicornis* was also adversely affected by the presence of pure HMW dextran used as a model substance. This implies that inhibition may be at least partly mechanically mediated. It has been demonstrated that during phytoplankton blooms the thickening behaviour of algal polysaccharides may change rheological properties (Jenkinson 1986), and the study of bacterial extracellular polysaccharides indicated high apparent viscosity increasing markedly with bacterial concentration (Anton et al. 1988). Jenkinson (1989) suggested that increased viscosity may even kill fish in some plankton blooms due to the high energy requirement for pumping the more viscous liquid. Copepods use comparatively more energy for swimming than some other zooplankton (Davenport & Trueman 1985). Increased viscosity due to polysaccharides may reduce turbulence (Jenkinson 1990) and consequently encounter rate, leading to lower ingestion rates. In more viscous and less turbulent environments, copepods would require higher energy output when feeding (Granata & Dickey 1991).

Our experiments indicate that copepod feeding is more strongly inhibited by diatom HMWCHO exudates from senescent cells than by an equivalent concentration of the pure HMW dextran (Fig. 5) pointing to an additional response to chemical stimuli of algal exudates. There are several studies demonstrating rejection or avoidance of some dinoflagellate prey by copepods (Huntley 1982, Huntley et al. 1986, Gill & Harris 1987, Uye & Takamatsu 1990). These authors also suggested that reduced grazing pressure was an important factor in development of monospecific dinoflagellate blooms. Our results suggest that diatom cells may also produce distasteful compounds. Inhibition of copepod feeding on diatoms may have important repercussions for spring phytoplankton dynamics in temperate waters. The potential of herbivorous copepods to control the development of diatom blooms and production of large quantities of mucus is thus dependent on the presence of sufficient copepod stock during the time of the exponentially growing diatom

population, when the cells produce deterrent mucus only in limited quantities. Reduction of copepod feeding rates due to algal exudates in transitional and stationary phase will allow diatom stocks to persist and continue producing mucus. Eventually, this process may enhance macroaggregate formation and mass sedimentation.

There is as yet no direct evidence from the field that these processes are crucial for the massive mucus accumulations in the Adriatic Sea during recent years. However, monitoring of the zooplankton in the northern Adriatic (Anon. 1991) has indicated substantially lower late winter-spring copepod stocks during years when mucus accumulated. Future work should establish whether combining HMWCHO production rates for Adriatic phytoplankton species with observed algal biomass in the area (e.g. Anon. 1991) can account for comparable production figures to the field-estimated mucus biomass (e.g. Stachowitsch et al. 1990).

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