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SIGNIFICANCE OF TINTINNID GRAZING DURING BLOOMS OF PHAEOCYSTIS POUCHETII (HAPTOPHYCEAE) IN DUTCH COASTAL WATERS*

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ABSTRACT

Extremely dense populations of tintinnids (24 000 to 118 000·dm^{-3}) were found during the spring bloom of the alga *Phaeocystis pouchetii* in the Dutch Wadden Sea and coastal North Sea. Microscopic observations showed that these Protozoa grazed on the single-cell stage of the colony-forming *Phaeocystis*. At the end of the bloom, the biovolume of the tintinnid population equalled or even exceeded that of the *Phaeocystis* population, indicating that microfaunal grazing prevented further growth of the *Phaeocystis* spring bloom.

1. INTRODUCTION

The haptophycean alga *Phaeocystis pouchetii* (Hariot) Lagerheim forms massive blooms in the North Sea along the coasts of Belgium (LANCELOT, 1983), the Netherlands (GIESKES & KRAAY, 1977; VELDHUIS *et al.*, 1986) and Germany (WEISSE *et al.*, 1986). A series of studies have been initiated in these countries to answer numerous questions regarding the biology of *Phaeocystis*. The sudden disappearance of this organism after dense blooms is rather puzzling. Do the colonies sediment as a consequence of senescence at the end of the bloom? Is the life-cycle of *Phaeocystis* with its alternation of single cells and colonies interrupted? Or is the bloom grazed upon by herbivores? The grazing question has been tackled by observations and tests with calanoid copepods. WEISSE (1983) demonstrated that *Phaeocystis* is a suitable food source for *Acartia* spp and *Temora longicornis*, which explains the growth of copepod populations during *Phaeocystis* blooms (BAKKER, personal communication). In contrast, FRANSZ (unpublished, 1985) found that copepod populations diminished or only maintained their numbers during *Phaeocystis* blooms in the coastal zone of the North Sea. JOIRIS *et al.*, (1982) and FRANSZ & GIESKES (1983) have drawn attention to the incomplete conversion of the phytoplankton production by herbivores in coastal waters of the North Sea. In fact it is difficult to imagine that blooms of *Phaeocystis*, which last only a few weeks, can be effectively exploited by herbivorous copepods that have relatively long reproduction cycles. It would seem more likely that the exploitation of the bloom, if it occurs at all, involves rapidly growing unicellular herbivores. We have carried out a series of microscopic observations at various stations along the Dutch coast to test whether microfaunal grazing is of any importance in the disappearance of *Phaeocystis* blooms.

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2. MATERIAL AND METHODS

2.1. SAMPLING

Observations were carried out during 4 sampling programmes in the Dutch coastal waters. In 1980, the Stations B and Q in the Ems-estuary (Fig. 1) were visited on several occasions during

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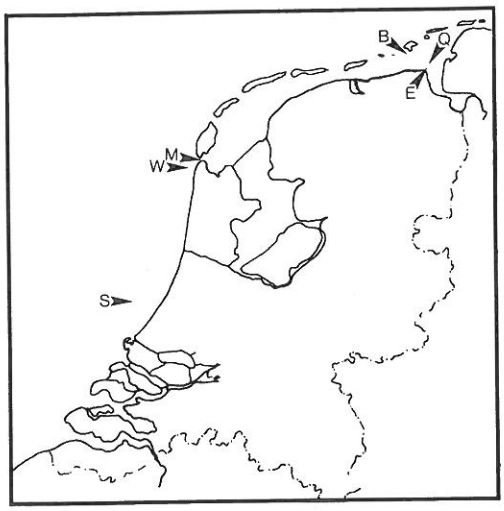

Fig. 1. Map of the Dutch coast, showing the position of the 6 sampling stations Q, E, B, W, M and S.

spring, and in 1983 the Stations B and E were sampled throughout the year. In 1984, samples were collected at one near-shore station in the North Sea (S) near to the plume of the river Rhine. Finally, in spring 1985 samples were taken at Stations W and M.

In all sampling programmes the water was collected from the surface of the vertically mixed water column, in 1980 and 1985 by means of a pump and in 1983 and 1984 by means of a bucket. Information on water temperature, concentrations of suspended matter *etc.* is presented in other publications (COLIJN & LUDDEN, 1986; ADMIRAAL *et al.*, 1985; VELDHUIS *et al.*, 1986).

2.2. MICROSCOPIC OBSERVATIONS

Microscopic observations were carried out on preserved material with an inverted microscope to identify and count the phytoplankton and also to assess numbers of microfaunal organisms. Sodium-acetate buffered lugol was used as a preservative. *Phaeocystis* colonies disintegrated under the fixation and concentration procedures, and therefore they were counted by the total number of single cells. In 1983, the organisms were observed in freshly collected unpreserved water samples and the number of colonies of *Phaeocystis* less than 50 μm in diameter and those larger than 50 μm were counted; after removal of the colonies and fixation the remaining single cells were counted. Fresh samples were used for microscopic observations on tintinnid grazing.

Tintinnids were identified according to HOFKER (1922), MARSHALL (1969) and BAKKER & PHAFF (1976). The loricate tintinnids were counted, but in those cases where naked tintinnids could be identified they were included as well.

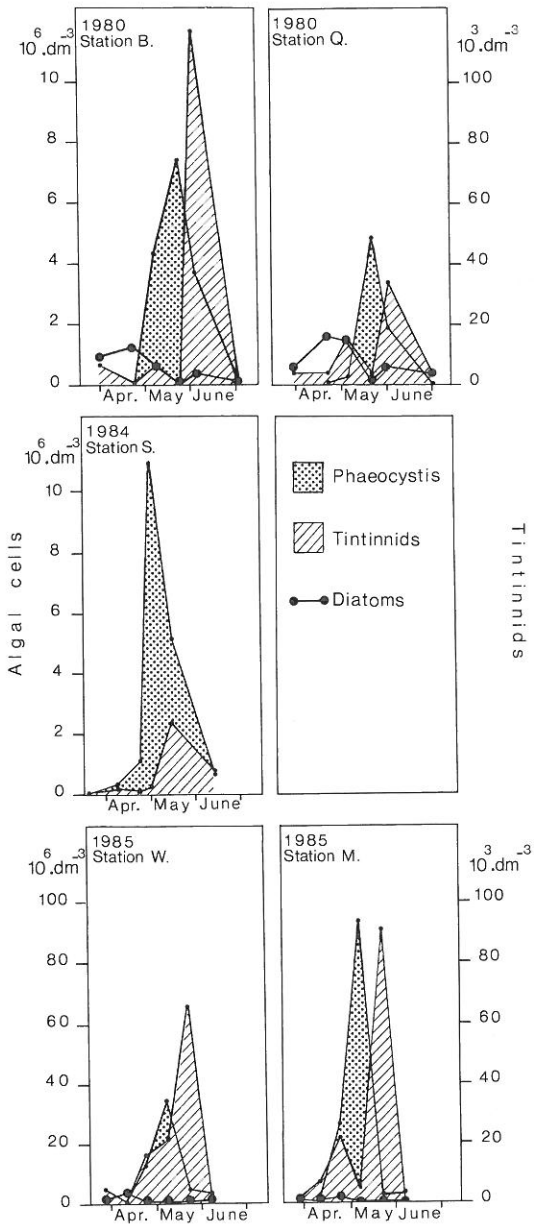

Fig. 2. Development of the populations of *Phaeocystis pouchetii* and of tintinnids and diatoms at 5 stations. Note the different scales for the tintinnids and the algae.

3. RESULTS

3.1. CO-OCCURRENCE OF TINTINNIDS AND PHAEOCYSTIS

Fig. 2 shows that in 3 series of independent observations a peak in the number of *Phaeocystis* cells was followed by a peak in the number of tintinnids. The blooms of *Phaeocystis* usually lasted less than one month and high numbers of tintinnids were seen only during a very short period (*cf.* JOHANSEN, 1976). The timing of algal blooms and microfauna development could not be analyzed in detail, since the populations were sampled only once per 2 weeks. Highest numbers of *Phaeocystis* cells were between 5 and 90×10^6 cells·dm^{-3} and highest numbers of tintinnids were between 24 000 and 118 000 cells·dm^{-3}, values that are higher than the highest values usually found in well-developed tintinnid populations (HEINBOKEL & BEERS, 1979; HARGRAVES, 1981; VERITY & STOECKER, 1982). Only JOHANSEN (1976) observed concentrations of tintinnids as high as 44 to 72 000 cells·dm^{-3} in a Canadian estuary. PARANJAPE (1980) found, during peak abundance of *Helicostomella subulata*, several thousands of cells·dm^{-3}, but occasionally as many as 100 000 cells·dm^{-3} were counted.

In 1983, maximum numbers of tintinnids were found in the Ems estuary (Fig. 3) during the spring bloom of *Phaeocystis* in April rather than during the later phytoplankton (chlorophyll-a) maximum in May-June, which consisted mainly of large-celled diatoms (ADMIRAAL *et al.*, 1985). The microscopic observations at Station B started too late to measure the onset of the blooms so that both *Phaeocystis* and tintinnids were present in considerable numbers during the first sampling. However, the early decline in the tintinnid numbers was clear at a time when *Phaeocystis* was still rising. At Station E, the tintinnids bloomed massively at the time of low chlorophyll concentration, when modest numbers of *Phaeocystis* cells were present. Both tintinnids and *Phaeocystis* had disappeared by the time of the following sampling. So, the observations in 1983 (Fig. 3) differed from those in other years (Fig. 2) by the early development of dense tintinnid populations and by the relatively low concentrations of *Phaeocystis* cells.

At Station B, tintinnids bloomed before the bacteria (another potential food source) reached their first biomass peak (Fig. 3). The results for Station E (Fig. 3) and D (Fig. 2) and data in VELDHUIS *et al.* (1986) show co-dominance of tintinnids and bacteria. This co-occurrence needs further consideration since normally bacteria bloom at the time when *Phaeocystis* colonies disintegrate (LAANBROEK *et al.*, 1985) and large numbers of single-celled *Phaeocystis* are also liberated (see Discussion section).

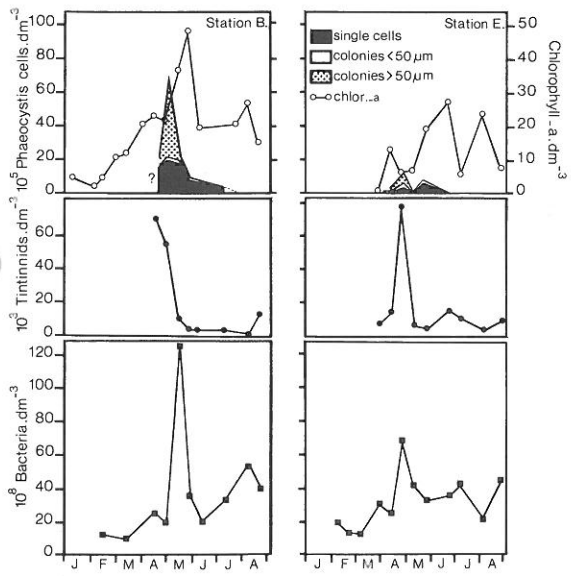

Fig. 3. Seasonal fluctuation in chlorophyll a concentration, and the numbers of tintinnids, bacteria and *Phaeocystis* cells in the Ems estuary in 1983. The numbers of *Phaeocystis* cells were arbitrarily calculated, assuming that one colony <50 µm contained 20 cells and that one colony >50 µm harboured 1000 cells.

3.2. IDENTIFICATION OF TINTINNIDS AND GRAZING

The dense tintinnid community observed in the Ems estuary in 1983 was dominated by *Helicostomella subulata*; in 1985, *Tintinnopsis beroidea* was abundant in the western Wadden Sea. The high variability in the shape of this latter species (BAKKER & PHAFF, 1976) precludes the identification of other similar species, but these samples (and those of Station S) contained other species, too.

In 1983, tintinnids grazing on *Phaeocystis* were observed under the microscope. The tintinnids (mainly *H. subulata*) browsed on the surface of bladder-like colonies scavenging single cells, but were unable to disrupt the colonies. Free-

TABLE 1

Dimensions of tintinnids in μm, with standard deviations between parentheses, as observed during spring 1985 in the western Wadden Sea. n = number of measurements.

	Station W			*Station M*		
Date	*Lorica-length*	*Oral diameter*	*n*	*Lorica-length*	*Oral diameter*	*n*
March 27	–	–		80 (19)	54 (12)	7
April 9	69 (69)	24 (13)	8	40 (18)	25 (18)	7
April 23	28 (14)	21 (10)	13	39 (12)	20 (3)	12
May 7	23 (11)	17 (5)	16	28 (11)	13 (7)	13
May 23	33 (6)	25 (7)	10	31 (4)	28 (7)	8
June 6	45 (15)	26 (8)	5	122 (58)	25 (6)	5

swimming *Phaeocystis* cells were also captured by tintinnids. Digestion of *Phaeocystis* cells taken up by the tintinnids lasted about 30 min.

The average cell size in the tintinnid populations changed during the spring bloom. The smallest cells were observed in early May during the peak in the *Phaeocystis* populations (Table 1, *cf.* Fig. 2), whereas their average size had increased at the end of May and in early June by the time of and after the tintinnid bloom.

4. DISCUSSION

Microflagellate algae are a suitable food source for tintinnids (HEINBOKEL, 1978a; HARGRAVES, 1981; VERITY, 1985). The size of food particles ingested by tintinnids is less than 43% of the diameter of the oral field (HEINBOKEL, 1978b), which is usually between 10 and 50 μm. However, CAPRIULO (1982) observed that natural populations of tintinnids occasionally ingested particles approximating the size of their oral fields. On the other hand, single-celled bacteria are considered too small to be grazed effectively by tintinnids. SPITTLER (1973, cited in HEINBOKEL, 1978b) presented evidence that tintinnids would not ingest particles less than 2 μm in diameter except under temperature stress. HOLLIBAUGH *et al.* (1980) found that ^{3}H-thymidin labelled bacteria were taken up by the tintinnid *Helicostomella subulata*, but the apparent clearance rates were one or two orders of magnitude lower than JOHANSEN (1976) measured when the same tintinnid species grazed on microflagellates.

The cells of *Phaeocystis* have an average

TABLE 2

Biovolume of phytoplankton and protozoa during the spring bloom 1985 in the western Wadden Sea. Data in 10^6 $\mu m^3 \cdot dm^{-3}$. n.d. = not detected.

	Phytoplankton				*Protozoa*	
	Diatoms	*Dinoflagellates*	*Phaeocystis*	*Other algae*	*Tintinnids*	*Other protozoa*
Station W						
March 27	412	<5	195	37	n.d.	n.d.
April 9	1825	75	180	187	<35	n.d.
April 23	1884	184	3600	94	52	n.d.
May 7	159	549	4465	38	36	22
May 23	223	32	428	158	353	14
June 6	4520	454	39	282	<32	45
Station M						
March 27	539	9	84	183	133	n.d.
April 9	758	<5	38	140	48	n.d.
April 23	3530	135	1742	302	92	23
May 7	428	341	14175	42	7	79
May 23	238	431	241	99	581	43
June 6	552	566	145	642	86	124

diameter of 3 to 8 μm and hence are within the size range suitable for tintinnid grazing. The tintinnid populations found in our samples adjusted their lorica length and oral diameter in the course of the *Phaeocystis* blooms; minimal oral diameters of 13 to 17μm were observed during the peak of *Phaeocystis* blooms (Table 1). This reduction of the tintinnids oral fields, resulting either from rapid growth of small-celled individuals (BAKKER, personal communication) or from succession by small-celled species, may perhaps be interpreted as an optimalization of feeding on the relatively small *Phaeocystis* cells. However, during the degradation phase of *Phaeocystis* colonies at the end of the blooms, the tintinnid populations again had larger oral fields (Table 1).

The phytoplankton at the sampling stations consisted mainly of diatoms and *Phaeocystis*. The diatom species (mainly *Biddulphia sinensis* and *Thalassiosira excentrica*) succeeding *Phaeocystis* (ADMIRAAL *et al.*, 1985) were too large for tintinnid grazing, but the small-celled diatoms of the earlier phase of the spring bloom did not support blooms of tintinnids either.

It seems likely that the large numbers of single-celled *Phaeocystis* provide an extremely rich temporary food source for microfaunal herbivores enabling them to form large populations. Analogously, JOHANSEN (1976) found that tintinnid populations dominated by *H. subulata,* closely followed the summer blooms of micro-flagellates, such as *Rhodomonas, Isochrysis* and *Pyramimonas*. CAPRIULO & CARPENTER (1983) found a positive relation between the numbers of tintinnids and nanophytoplankton density. The experiments by JOHANSEN (1976) and our microscopic observations showed intensive feeding of *H. subulata* on microflagellates. These observations accord with the rapid development of *H. subulata* in an early phase of relative sparse *Phaeocystis* blooms (Fig. 3). In contrast, we observed that populations of *T. beroidea* developed massively during a later phase of more dense *Phaeocystis* blooms; a different efficiency of feeding on microflagellates could be responsible for the different occurrence of *H. subulata* and *T. beroida*.

Phaeocystis has often been supposed to produce toxic substances that may deter herbivores, but the tintinnids in the present study were neither inhibited nor poisoned by *Phaeocystis* such as reported by VERITY & STOECKER (1982) during blooms of *Olisthodiscus luteus*. BURKILL (1982) calculated that very sparse populations of tintinnids were able to consume most of the seasonal primary production of nanophytoplankton. Potential grazing rates of tintinnids could be criticized as a means of calculating *in situ* grazing rates. Nevertheless, in the present study the biovolume of the tintinnids equalled or even exceeded the biovolume of the micro-flagellates, *in casu Phaeocystis* cells. In this case it is evident that tintinnid grazing occurring at a rate much lower than the maximum of 10 to 20% of the body weight per h (HEINBOKEL, 1978a; VERITY, 1985) can have a dramatic impact on the micro-flagellates.

KORNMANN (1955) and KAYSER (1970) demonstrated that the life-cycle of *Phaeocystis* begins with unicellular stages leading to colonies which then release numerous unicells after maturation. The various stages seem to co-exist in natural blooms as is indicated in Fig. 3.

One of the features of *Phaeocystis* colonies is that their size offers some degree of mechanical resistence against microfauna and copepod grazing. However, the unicells, when released by the disintegration of large colonies, are vulnerable to tintinnid grazing, and this is probably severe in view of the massive numbers of tintinnids as compared to the total biovolume of *Phaeocystis* cells (colonial and unicellular combined, Table 2). One can imagine the *Phaeocystis* bloom being undermined by a severe reduction of the number of unicells capable of forming the next generation of colonies. In fact, VELDHUIS *et al.* (1986) observed that low numbers of so-called micro-zoospores (diameter 3 μm) remained in the last stages of *Phaeocystis* blooms; we are now tempted to ascribe this to losses due to tintinnid grazing.

Dense tintinnid populations may be associated with dense blooms of *Phaeocystis* near-shore only. Examination of samples taken at offshore stations in the North Sea by VELDHUIS *et al.* (1986) did not show conspicuous tintinnid populations. Consistently, the *Phaeocystis* blooms did not decline as abruptly as at the near-shore stations, possibly due to reduced grazing. BAKKER (personal communication) found per dm^3 70 to 160 000 ciliates (10 to 30 μm in diameter) during *Phaeocystis* blooms; these Protozoa possibly graze on *Phaeocystis* cells as tintinnids do. We found that tintinnid populations during *Phaeocystis* blooms were dominated by different species. Hence, it seems likely that a variety of microfauna species may graze on

Phaeocystis, thereby interacting in a species-specific way with this alga. We recommend that future studies on grazing in *Phaeocystis* blooms should consider the contribution by the microfauna as well as that of copepods.

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