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Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods

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Abstract. Growth rates of microphagous ciliates (forms which feed primarily on picoplankton-sized prey) were estimated, along with rates of their consumption by copepods, in shipboard experiments conducted in the mesohaline portion of Chesapeake Bay, USA, under contrasting water column conditions in April, June, and August 1987. Estimates were based on temporal changes in cell densities in size-fractionated water samples incubated under in situ conditions. In April, at low temperatures (7 to 10 °C) and with oxygen present throughout the water column, similar generation times of ca. 1 to 1.5 d were estimated for surface and deep water (24 m) ciliate populations. In June, water was anoxic below 12 m and a distinct anoxic microphage community grew at about twice the rate of the surface community with generation times of ca. 7 and 14 h, respectively. In August, bottom water was again anoxic, but the same *Strobilidium* sp. dominated both surface and deep waters with low or no growth apparent in anoxic waters and a generation time of ca. 8 h in surface waters. Copepod (primarily *Acartia tonsa* Dana nauplii) clearance rates for microphagous ciliates in surface waters were 0.11, 0.56, and 0.53 ml h^{-1} copepod^{-1} for April, June and August, respectively. Calculation of removal rates, based on average densities, indicated that from 34 to 200% of surface waters were cleared d^{-1} of microphagous ciliates by copepods.

Introduction

Marine protists have long been recognized as a possible trophic link between bacteria and metazoan zooplankton (e.g. Lackey 1936), but recently many workers have concluded that, while of great importance in nutrient regeneration, microbial communities are probably "sinks", i.e., the amount of carbon which reaches trophic levels above bacteriovores is quantitatively insignificant due to respiratory losses (Ducklow 1983, Pomeroy and Wiebe 1988). While some experimental data have been presented (Ducklow et al. 1986), this viewpoint is based on the idea that bacterial carbon must pass though at least two trophic levels before reaching a mesozooplankton-nekton food web. The assumption (formally stated in Azam et al. 1983, Sieburth 1984) is that bacteria are consumed by microflagellates which must in turn be consumed by larger protists such as ciliates before bacterial carbon is in a particle large enough to be efficiently grazed by common zooplankters such as copepods. This argument suffers from some misconceptions (Sherr et al. 1986b, Sherr and Sherr 1988). First, bacteriovorous forms should be more accurately termed microphagous since they probably exploit a wide variety of picoplankton-sized organisms: heterotrophic, autotrophic, prokaryotic and eukaryotic whose combined production may be considerably larger than that of bacterioplankton alone (Sherr et al. 1986b, Sherr and Sherr 1988). Secondly, recent work (Sherr et al. 1986a, Sherr and Sherr 1987, B. Sherr et al. 1989, E. Sherr et al. 1989) has revealed the ubiquity of microphagous ciliate microzooplankters in a variety of coastal waters.

In Chesapeake Bay, microphagous ciliates, forms which consume picoplankton-size prey, generally dominate the ciliate microzooplankton in terms of cell numbers and form ca. 25% of the biomass; they reach peak abundances in early spring and mid-summer (Dolan 1991). Although largely composed of small (10 to 20 μm) oligotrichs and scuticociliates, moderate sized (40 to 60 μm long) scuticociliates and peritrichs are also seasonally abundant (Dolan 1991). Consumption of these microphagous ciliates by metazoans could pass carbon into higher trophic levels via a "simple, two-step food chain" (Sherr and Sherr 1987) and constitute a direct role for metazoans, such as copepods, in structuring microbial communities.

There have been many laboratory investigations of copepod grazing on nanoplankton-consuming or herbivorous ciliates, generally large oligotrichs and tintinnids (Robertson 1983, Turner and Anderson 1983, Stoecker and Sanders 1985, Ayukai 1987, Stoecker and Egloff 1987, Wiadnyana and Rassoulzadegan 1989, Jonsson and Tiselius 1990) and two field studies of copepod

grazing on ciliate communities (Gifford and Dagg 1988, Tiselius 1989). There are, however, few data on metazoan zooplankton grazing of microphagous ciliates.

Studies of natural populations of microphagous ciliates have concentrated on obtaining estimates of picoplankton consumption rates (Sherr et al. 1987, B. Sherr et al. 1989, E. Sherr et al. 1989) and estimates of abundances (Sherr et al. 1986a, Dolan 1991). There has been only one study on copepod predation on microphagous ciliates, a laboratory investigation of adults presented with one ciliate species and no alternative food source (Berk et al. 1977).

In the present study I examined the growth and loss rates of estuarine microphagous ciliates under in situ conditions. Three sets of field experiments, conducted under contrasting water column conditions, investigated growth in different parts of the water column and grazing losses in surface waters associated with the copepod *Acartia tonsa*.

Materials and methods

General approach

Experiments were carried out onboard the R. V. "Ridgely Warfield" while anchored at 39°58'N; 76°20'W in the mesohaline portion of the Chesapeake Bay on 19 April, 23 June and 21 August 1987. A detailed description of the study site can be found elsewhere (Dolan and Coats 1990). Microphagous ciliate growth rates and grazing losses were measured by monitoring changes in ciliate numbers in water passed through Nitex screening to remove predators, in untreated water with in situ concentrations of copepods, and in water to which copepods were added. This approach estimates a predator's impact on a prey population which may not equal numbers of prey actually ingested (Dolan and Coats 1991a). Growth rates and grazing losses of nanoplankton-consuming ciliates (large oligotrichs and tintinnids) were not estimated, as the size fractionation method greatly reduced their concentrations in control and predator-free containers.

Depth profile sampling

Preceding experimental manipulations depth profiles of physical parameters and biological samples were obtained. Sampling protocol and processing have been described previously (Dolan and Coats 1990). Briefly, a CTDFO$_2$-Niskin bottle cast provided depth profiles of physical parameters and nine or ten Niskin bottles were tripped to given three or four samples each from surface, transition and bottom waters. Water was drawn from each Niskin bottle to provide samples for dissolved oxygen and chlorophyll *a* concentrations; whole water aliquots were preserved with glutaraldeyde for microflagellate counts and with Bouin's (Coats and Hienbokel 1982) for ciliate enumerations. The fixative, like most formalin-based solutions, likely results in cell shrinkage. Two-liter aliquots from each bottle were concentrated to 20 ml over 20-μm Nitex screens and preserved with Bouin's for metazoan zooplankton counts. Standard methods were employed in processing samples for oxygen (Carpenter 1965) and chlorophyll (Strickland and Parsons 1972). Microflagellate counts were made following the procedure of Caron (1983). Ciliates were enumerated following the Utermöhl (1958) technique and categorized as either microphagous, macrophagous or predacious following previously described (Dolan 1991) species categorizations based on microsphere uptake experiments, food vacuole content and literature reports. Metazoans were enumerated in a settled sample, 5 to 10 ml of the concentrate representing 0.5 to 1 liter of whole water, examined with an inverted microscope at ca. 50× magnification.

Experimental protocol

After depth profiles were completed, water samples were taken from surface waters using a 5-liter plastic bucket. Bottom waters (April) or anoxic waters from the oxic/anoxic transition layer (June and August) were sampled with a Niskin bottle. The following manipulations were performed to yield three distinct subsamples for each water sample: (1) 750 ml were gently screened through either a 64-μm Nitex (April) or a 10-μm Nitex (June and August, respectively, (2) 750 ml were untreated, (3) 750 ml of water were treated with the addition of organisms retained by a 64-μm Nitex screen through which 2 liters of sample water had been gently passed. The treatments provided samples that contained: (1) only small ciliates with no copepods or large predacious ciliates present, (2) the in situ ciliate and copepod community, (3) water with an elevated concentration of copepods. Ca. 150 ml were removed from each 750 ml sample and preserved with Bouin's for determinations of ciliate abundances at time zero. The remaining water was placed in a dialysis sac (90 mm diam., 15 000 MW cutoff: Spectrapor Corp.) which had previously been rinsed and autoclaved. The dialysis sacs were placed in a circulating water bath flushed with water pumped from the depth of the sample origin. The action of the circulating water kept the samples in constant motion with no obvious settling of material. After 12 h, a 150 ml sample from each sac was preserved for ciliate and copepod enumerations.

The following special precautions and procedures were taken to insure that anoxic water samples (June and August) were maintained under in situ conditions. Teflon tubing was run between the sampling port of the Niskin bottle and the interior of a glove box flushed with N_2 gas, where all manipulations were performed. Aliquots taken at the beginning and end of manipulation indicated that no oxygenation had taken place during sample manipulation. The water bath used for incubating anoxic water samples was a sealed box with a small overflow port that admitted very little air. Water circulating through the box was obtained from the same depth as the sample using a submersible pump. Oxygen concentrations were determined from the overflow of the bath at ca. 3 h intervals and showed no oxygenation of bath waters.

Sample processing took ca. 30 min and was performed simultaneously on surface and subsurface waters. Experiments were run in duplicate in April and June and in triplicate in August. Replicate experiments represented sequential repetitions of the entire procedure with new water samples, containers and screens. In all the experiments, incubations commenced between 6:00 and 8:00 hrs Eastern Standard Time.

Sample processing and data analysis

Subsamples (25 to 50 ml) of time zero and 12 h samples were sedimented and the entire surface of the settling chamber examined at 200×. Duplicate counts generally yielded a CV of ca. 10%. Copepod and other metazoan abundances were determined for each dialysis sac by screening 100 ml from both the time zero and time 12 h samples through a 20-μm Nitex, rinsing the screen into a settling chamber and scanning the chamber surface at 50× with an inverted microscope. All copepod stages were assumed to be *Acartia tonsa* Dana, the spring-summer dominant copepod species in mesohaline Chesapeake Bay waters (Brownlee and Jacobs 1987).

Ciliate growth and copepod grazing parameters were calculated following the system of equations devised by Frost (1972). Calculations were performed separately for each replicate with a microphagous ciliate growth constant (K, h^{-1}) based on the net increase in cell density with time in the container without grazers (screened sample) and a grazing coefficient (g, h^{-1}) calculated for each container with grazers (in situ and increased copepod concentrations) based on changes in microphagous ciliate density corrected for growth.

Results

Experimental conditions (Table 1) varied between the three dates reflecting the different oxygen and chlorophyll *a* concentrations and organism distributions (Fig. 1) which were encountered. The results of the experiments (summarized in Tables 2 and 3) are presented in chronological order, each preceded by a description of the water column conditions.

Table 1. Summary of experimental conditions

Date	Water depth (m)	Temperature (C°)	Salinity (ppt)	Oxygen (ml l^{-1})	Chlorophyll *a* (µg l^{-1})
19 April	0	10.2	7.3	7.1	6.8
	27	7.4	17.5	3.8	37.3
23 June	0	24.3	10.7	5.1	7.3
	13	19.7	15.2	0.0	6.2
21 August	0	26.8	12.0	4.1	6.7
	10	26.6	16.5	0.0	5.4

April

On 19 April 1987, the water column was strongly stratified (Fig. 1) with a salinity gradient of ca. 10 ppt and temperatures ranging from ca. 7 to 10 °C. Dissolved oxygen ranged from 7.0 ml l^{-1} near the surface to 3.8 ml l^{-1} at 27 m depth. Chlorophyll *a* increased with depth from 6.8 to 37 µg l^{-1}, the highest concentration observed; casual observations indicated that the dominant phytoplankter was a *Prorocentrum* sp. Copepod nauplii were abundant (58 to 92 l^{-1}) in the surface layer but abruptly decreased to low concentrations (14 to 20 l^{-1}) in transition and bottom waters. The densities of post-naupliar copepods were low (0 to 18 l^{-1}) with peak concentrations in deep water, corresponding with the chlorophyll maximum. Heterotrophic microflagellate concentrations were relatively invariant (2.3 to 4.2 × 10^3 cells ml^{-1}). Numbers of microphagous and herbivorous ciliates were about equal and their distributions were roughly parallel, with distinct mid-water maxima (ca. 10 cells ml^{-1}) coinciding with the abrupt decrease in naupliar copepod density. The microphagous ciliate assemblage was numerically dominated by a *Strombidium* sp. (ca. 30 µm diam.) at all

Table 2. Summary data from microphagous ciliate growth experiments. Number of replicate water samples (*n*), initial cell concentrations ml^{-1} (t_0), generation time of the entire microphagous ciliate community in hours, and the identity, size (based on Bouin's fixed specimens) and generation time of the dominant ciliate species. SD: standard deviation, L: length, W: width

Date	Depth (m)	*n*	t_0 (SD)	Community generation time (SD)	Dominant species	L × W (µm)	Generation time (SD)
19 April	0	2	3.1 (0.65)	21.4 (8.75)	*Strombidium* sp.	30 × 20	22.1 (1.48)
	27	2	5.5 (2.71)	34.8 (5.72)	*Strombidium* sp.	30 × 20	17.9 (11.40)
23 June	0	2	0.6 (0.05)	14.3 (10.53)	*Strobilidium* sp.	10 × 10	10.7 (9.07)
	13	2	3.3 (0.59)	6.5 (1.28)	*Pleuronema* sp.	55 × 25	8.4 (5.03)
21 August	0	3	10.4 (0.69)	7.8 (1.27)	*Strobilidium* sp.	10 × 10	11.4 (8.10)
	10	3	1.7 (1.23)	no growth	*Strobilidium* sp.	10 × 10	no growth

Table 3. Measured and calculated results of copepod grazing experiments. Rep: replicate number. Time zero (t_0) and time 12 h (t_{12}) are in cells ml^{-1}, ciliate growth rate (*K*) and decline rate (*g*) in h^{-1}. Grazer concentrations (*n*) are numbers of nauplii/copepods l^{-1}; filtration (*F*) is in ml copepod^{-1} h^{-1}; [*C*] is the average prey concentration to which the grazers were exposed; calculated ingestion rates (Ing) based on *F* and [*C*] are in cells copepod h^{-1}

Date	Rep	Control			Grazer						
		t_0	t_{12}	*K*	t_0	t_{12}	*g*	*n*	*F*	[*C*]	Ing
19 April	1	3.6	4.9	0.0256	2.5	2.1	0.0402	105/13	0.341	2.3	0.80
	1				1.7	1.9	0.0164	529/49	0.028	1.6	0.10
	2	2.7	4.6	0.0440	1.7	2.1	0.0264	116/28	0.183	1.9	0.38
	2				5.5	2.2	0.1204	686/71	0.159	3.1	0.50
23 June	1	0.6	0.9	0.0338	1.2	0.6	0.09155	270/3	0.335	0.9	0.29
	1				0.9	0.1	0.24381	489/0	0.443	0.3	0.16
	2	0.5	1.8	0.1067	0.8	0.1	0.28003	290/0	0.965	0.3	0.33
	2				0.4	0.2	0.13521	338/0	0.487	0.3	0.12
21 August	1	10.5	26.9	0.0801	15.1	24.5	0.04048	57/0	0.710	19.4	13.08
	1				22.2	27.2	0.06132	54/32	0.751	24.6	3.25
	2	10.4	38.3	0.1086	13.0	26.2	0.05064	88/0	0.571	18.9	10.9
	2				30.3	21.8	0.13265	210/23	0.584	25.9	15.1
	3	10.5	29.4	0.0882	15.5	30.5	0.03181	133/0	0.239	22.1	4.8
	3				20.7	18.6	0.09479	240/52	0.332	19.6	6.4

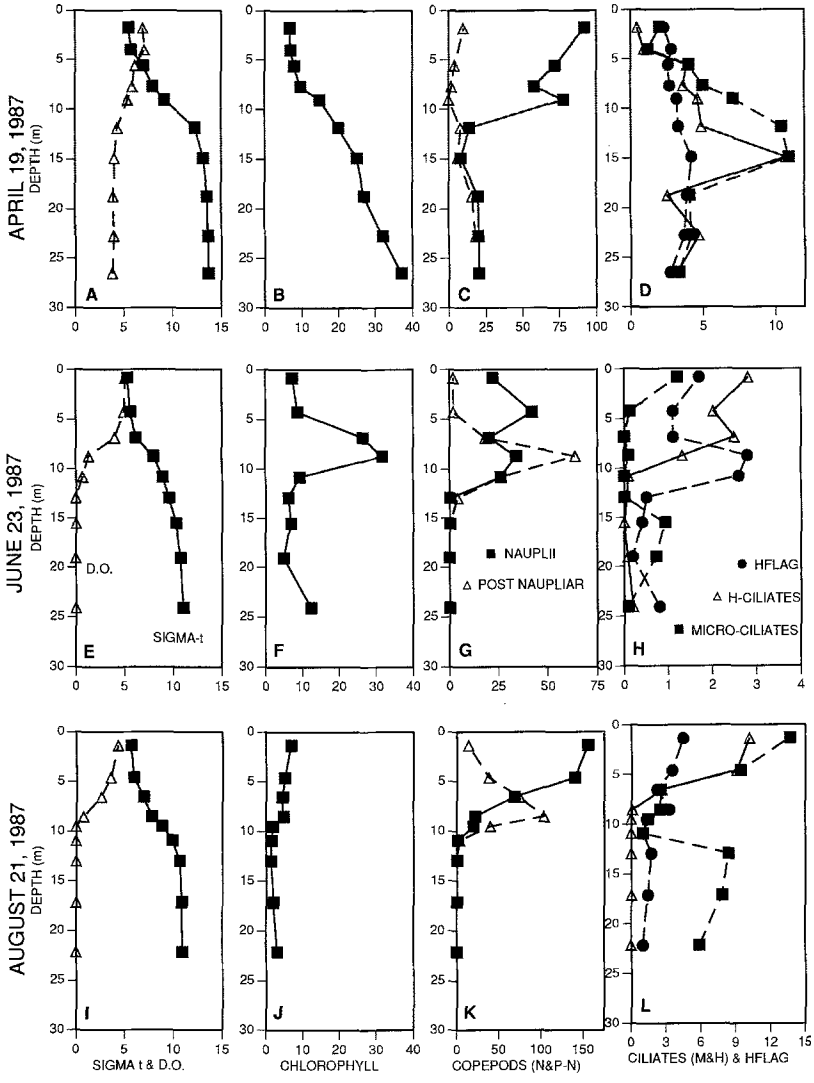

Fig. 1. Vertical profiles of physical and biological parameters at experimental Chesapeake Bay station on 19 April, 23 June and 21 August 1987. (A), (E) and (I): dissolved oxygen (D.O.) (ml l^{-1}) and sigma-t. (B), (F) and (J): chlorophyll *a*, µg l^{-1}. (C), (G) and (K): naupliar and post-naupliar copepods l^{-1}. (D), (H) and (L): heterotrophic microflagellates (HFLAG) $\times 10^3$ ml^{-1}, herbivorous ciliates (H-CILIATES) ml^{-1}, and microphagous ciliates (MICRO-CILIATES) ml^{-1}

depths. Herbivorous ciliates consisted largely of *Tintinnopsis baltica* and *T. levigata*.

Microphagous ciliate growth, measured in 64-µm screened water, gave estimates of community generation times of 21.4 h (avg. of 27.1 and 15.8) and 34.8 h (avg. of 30.7 and 38.8 h), for surface and deep waters, respectively. Declines in microphagous ciliate densities in containers with natural and increased copepod concentrations gave average filtration rate estimates of 0.34 and 0.03 ml copepod^{-1} h^{-1}, respectively.

June

On 23 June 1987, waters were anoxic below 12 m in the presence of gradients (0 to 27 m) of salinity (10.7 to 16.0 ppt) and temperature (24.3 to 18.1 °C). A distinct chlorophyll maximum layer (26 to 32 µg l^{-1}) was present at the base of the pycnocline at ca. 9 m. This depth also represented the peak in post-naupliar copepods (64 l^{-1}) and heterotrophic microflagellates (2.8×10^3 cells ml^{-1}) as well as a minimum in microphagous ciliate density (0.1 cells ml^{-1}). Microphagous ciliates were highest near the surface (1.2 cells ml^{-1}) with a secondary maximum (0.9 cells ml^{-1}) at ca. 12 m, below the peak copepod density. The surface layer microphagous ciliate community was dominated by a small *Strobilidium* sp. (ca. 10 µm diam.); the anoxic community was composed of a *Pleuronema* sp. and a *Cyclidium* sp. In surface waters naupliar copepod density ranged from 22 to 42 l^{-1}; herbivorous ciliates (largely a *Strombidium* sp. ca. 45 µm in length and *Tintinnopsis acuminata*), dominated surface waters; densities ranged from 1.3 to 2.8 cells ml^{-1} with both nauplii and herbivorous ciliates virtually absent from transition and bottom waters.

Due to the presence of the predatory ciliate *Didinium* sp. in surface and anoxic waters in June, 10-µm screening was used to estimate the growth of microphagous ciliates. The average ciliate generation time in surface waters was 13.5 h (avg. of 6.5 and 20.5 h), approximately twice the average generation time for anoxic waters of 6.5 h (avg. of 5.6 and 7.4 h). Unlike the April experiments, the June grazing treatments included very few postnaupliar copepods (Table 3). The in situ copepod concentration treatments yielded an average filtration rate estimate of 0.65 ml copepod^{-1} h^{-1} vs 0.47 ml copepod^{-1} h^{-1} in the increased copepod density treatment.

August

On 21 August 1987, waters below 9 m were anoxic. There was no chlorophyll maximum layer present and concentrations ranged from 4.8 to 6.7 μg l^{-1} in the surface layer and from 1.3 to 3.1 μg l^{-1} in transition and bottom waters. The vertical distributions of copepods and ciliates were similar to that found in June. Post-naupliar copepods showed a distinct maximum (104 l^{-1}) at the base of the pycnocline, corresponding with a subsurface minimum of microphagous ciliate abundance. Microphagous ciliate densities were highest (13.7 cells ml^{-1}) at the surface, declined to a depth of ca. 11 m and showed a secondary maximum (8.3 cells ml^{-1}) at ca. 13 m, below the peak copepod density. Unlike June conditions there was no distinct anoxic water microphagous ciliate community, and both surface and deep waters were dominated by the same small *Strobilidium* sp. (ca. 10 μm diam.) seen in June. Herbivorous ciliates (*Eutintinnus pectinus*, *Tintinnopsis acuminata*), and the mixotrophic *Laboea strobila* declined with depth from 10.2 cells ml^{-1} to undetectable levels below the oxic/anoxic layer, similar to naupliar copepods which declined from a surface concentration of 156 l^{-1}.

As in the June experiments, 10-μm screening was used to estimate the growth of microphagous ciliates in August. The predatory ciliate *Didinium* sp. was found in surface samples and *Euplotes woodruffi* was present in anoxic waters. The average generation time recorded from surface waters was 7.6 h (mean of 6.4, 7.8 and 8.7 h); no growth was recorded in anoxic water samples. From the dialysis sacs with in situ densities of copepods an average filtration rate of 0.51 ml copepod^{-1} h^{-1} was calculated compared to 0.56 ml copepod^{-1} h^{-1} from the containers with increased grazer densities.

Discussion

Community generation times of microphagous ciliates found in surface and deep, well-oxygenated, waters ranged from an average of ca. 35 h at 7.4 °C to 8 h at 26.8 °C (Fig. 2). These growth rates, and the fact that growth rates increased with temperature (i.e., a Q_{10} of ca. 2) are very similar to the data presented by Verity (1986, Fig. 7b) for community growth rates of tintinnids in Narragansett Bay. The high abundances of microphagous ciliates and rapid growth potential support the idea that, like tintinnids in Narragansett Bay (Verity 1986), microphagous ciliates in the Chesapeake Bay are productive and rapidly enter the planktonic food web. The picture is not quite so clear with regard to the microphagous ciliates in anoxic waters. The experiments in anoxic waters gave different results with two qualitatively distinct communities of microphagous ciliates.

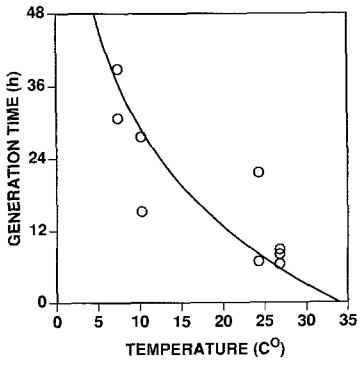

Fig. 2. Generation time and temperature of microphagous ciliates from oxygenated waters. The data set did not allow statistical distinction among alternate models

In the June experiment, the anoxic community was a distinct assemblage (*Pleuronema* sp. and *Cyclidium* sp.) from that found in oxygenated surface waters; it was doubling ca. every 6.5 h. In August, the same small *Strobilidium* species dominated both surface and deep water communities of microphages; rapid growth was estimated from surface samples and no growth from anoxic water samples. Anoxic waters may at times be inhabited by an adapted anoxic or microaerophilic community and at other times consist of cells which are not growing and have presumably sunk or been mixed from shallower depths. Judging from vertical distributions, anoxic water represents a zone of low predation pressure from copepods, regardless of the source of the ciliate community. It should be noted, however, that the copepod distributions were based on samples gathered during morning hours and may not be representative of all hours. Predacious ciliate grazing of microphagous ciliates in anoxic water has been documented (Dolan and Coats 1991b) and could represent a density control mechanism.

Copepod grazing may have a significant impact on surface layer populations of microphagous ciliates. An approximation can be made by considering what portion of the surface waters are cleared of microphagous ciliates by copepods, using estimates of average clearance rates and copepod densities (naupliar and post-naupliar combined). This approach, while ignoring important fine-scale vertical differences within the surface layer, indicates that significant grazing losses probably occur. For April, with an average clearance rate of 0.18 ml copepod^{-1} h^{-1} (Table 3) and a copepod density of ca. 80 l^{-1} (Fig. 1C) this yields an estimate of 35% d^{-1} of the surface water cleared of microphagous ciliates. Similar calculations for June and August yield estimates of 48% and 200%, respectively, of surface waters cleared d^{-1}. These estimates reflect the relatively high abundances of copepods in mesohaline Chesapeake Bay waters.

It is interesting to note that the high copepod impact estimate is associated with the date when chlorophyll concentrations were lowest (Fig. 1). This tends to support the contention made in a number of studies that ciliates could be an important component of the diet of metazoan zooplankters such as copepods when phytoplankton stocks are low (reviewed in Stoecker and Capuzzo 1990, Gifford 1991). However, it should be pointed out that my experiments did not measure ingestion, they estimated ciliate mortality associated with copepods. Further experiments with field populations using techniques designed to estimate ingestion and assimilation are needed (e.g., radiolabeled prey).

These approximations of grazing impact are large despite the fact that clearance rates were low compared to previous work with adult copepods. For example, laboratory studies on *Acartia tonsa*, have provided estimates ranging from 1.2 to 12 ml copepod^{-1} h^{-1} (reviewed in Stoecker and Capuzzo 1990, Gifford 1991). Overall, higher filtration rates appear to be associated with larger prey items (Tiselius 1989). For adults feeding on natural assemblages, clearance rates on small oligotrichs (10 to 20 μm diam.), likely microphagous forms, were estimated as ca. 1 to 2 ml copepod^{-1} h^{-1} over temperatures ranging from 11.5 to 29.5 °C (Gifford and Dagg 1988) compared to my average estimates of 0.18 to 0.56 ml copepod^{-1} h^{-1} over a temperature range of 10.2 to 26.8 °C. Estimates from the present study are probably lower because they were based on the abundances of naupliar and post-naupliar copepods combined, and clearance rates are up to two orders of magnitude lower for naupliar stages relative to adults (Berggreen et al. 1988).

The grazing rates reported here could also be underestimates due to experimental artifacts. No attempt was made to control the possibly confounding effect of heterotrophic or mixotrophic dinoflagellate grazing, although they were not obviously present in any of the experiments. Container effects may also have occurred. Incubations were carried out in relatively small volumes (650 ml) and some containers with increased copepod concentrations showed lower grazing rates relative to their counterparts with in situ abundances (three out of seven). In addition the experiments were perfomed during daylight hours and there is evidence that for adult copepods grazing rates are highest during the night (Durbin et al. 1990); there are no comparable data for pre-adult stages.

Conceding that grazing rates reported here may be underestimates, it is apparent that copepods probably exert a significant control on microphagous ciliate density in surface waters of the mesohaline Chesapeake Bay during summer months. Given these estimates of microphagous ciliate loss rates, one may postulate that copepod grazing activity could influence the relative proportions of picoplankton-consuming microflagellates and ciliates, which could in turn affect the composition of the picoplankton community. There is some evidence for this in the vertical distributions of copepods, microphagous ciliates and heterotrophic microflagellates. On all three sampling dates copepod maxima corresponded with microphagous ciliate minima, and not with heterotrophic microflagellate minima (Fig. 1). For example, the chlorophyll *a* maximum layer encountered on 23 June coincided with maxima in copepod and heterotrophic microflagellate densities and minimum microphagous ciliate concentrations (Fig. 1F to H).

This study shows that a microbial loop link with metazoan-nekton food webs via microphagous ciliates is not only plausible but likely in environments with high microphagous ciliate concentrations. In addition to providing a route for carbon flow to higher trophic levels, the "simple two-step pathway" (Sherr and Sherr 1987), copepods eating microphagous ciliates, may be of importance in structuring microbial communities.

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