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On the trophic coupling between protists and copepods in arctic marine ecosystems

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ABSTRACT: Grazing experiments were conducted at different seasons with the large *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*, and the small *Acartia longiremis* in Disko Bay, West Greenland and Young Sound, NE Greenland. Female copepods incubated in 200 µm screened natural water preferred large protists. Thus, particularly during the post-bloom period, the relatively large heterotrophic protists (ciliates and heterotrophic dinoflagellates) contributed substantially to the trophic coupling between protists and copepods. However, low grazing by *C. glacialis* and *C. hyperboreus* in mid-June suggests that large parts of the populations of these species had terminated feeding at this time, prior to overwintering. Clearance increased with ciliate and dinoflagellate size above 10 µm equivalent spherical diameter (ESD), equal to the size of the smallest heterotrophic protists. At a size of 30 to 40 µm ESD maximum clearance was observed. Grazing on *Phaeocystis* single cells of 5 µm by *C. finmarchicus* showed a lower size-limit for capture of this species <5 µm which contrasts with *C. glacialis* and *C. hyperboreus*, which had a lower size-limit near 10 µm. In addition to size and relative concentrations of phytoplankton and heterotrophic protists, prey and/or predator behavior is suggested to play an important role for copepod feeding.

KEY WORDS: *Calanus* spp. · *Acartia longiremis* · Omnivory · Ciliates · Heterotrophic dinoflagellates · Trophic coupling · Particle spectra · Plankton food web · Arctic

INTRODUCTION

Most arctic marine research on the fate of pelagic primary production has taken herbivory by the abundant northern copepods *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* into consideration (e.g. Conover & Huntley 1991, Conover et al. 1991). It is often assumed that the food source of these copepods consists almost entirely of phytoplankton. Accordingly, the trophic coupling between *Calanus* spp. and protists such as ciliates and heterotrophic dinoflagellates has been less thoroughly examined. An increasing number of studies have stressed, however, that ciliates are also abundant in marine pelagic food webs at high northern latitudes. This was first shown in a study from the Chucki Sea/Bering Strait by Andersen (1988) who demonstrated the importance of the 'microbial loop' at stations dominated by small-sized phytoplankton. A few subsequent studies have also suggested that heterotrophic dinoflagellates are important grazers, along with ciliates and copepods (Nielsen & Hansen 1995, Levinsen et al. 1999). This prompted a comparison of the grazing impact of different grazers. Based on biomass considerations and measurements of growth it was concluded that ciliates and heterotrophic dinoflagellates were potentially more important for the carbon flow in the Arctic than copepods during the late summer (Hansen et al. 1999). Heterotrophic dinofla-

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gellates contributed more than half of the heterotrophic protist biomass and, like copepods, were potentially important grazers of diatoms. However, it was unknown how much the heterotrophic protists contributed to the copepod diet. Thus, although heterotrophic protists were likely important for the carbon flow, knowledge about how much primary production enters higher trophic levels through heterotrophic protists in cold-water systems was generally lacking. Thus far, apparently, only a single such study from the Greenland Sea has been conducted, from which it was concluded that ciliates were indeed important food for *Calanus* spp. (Barthel 1988). There is a need for more information about the trophic coupling between copepods and heterotrophic protists, including the important heterotrophic dinoflagellates, if the fate of the primary production in arctic areas is to be fully understood.

Relatively large heterotrophic protists are abundant in the low-chlorophyll *a* surface water of stratified arctic ecosystems after the main spring/summer bloom of diatoms (Levinsen et al. 1999, 2000). During this period small-sized phytoplankton closer to the lower limit of capture by copepods dominate. In post-bloom surface waters heterotrophic protists are therefore more exposed to copepods than phytoplankton, which are presumed to be less efficiently retained. At the pycnocline, in contrast, subsurface diatom blooms composed of larger cells at high concentrations are usually characteristic post-bloom features (Nielsen & Hansen 1999). There is a need to elucidate possible differences in the coupling between heterotrophic protists and copepods under these contrasting situations.

Knowledge of copepod predation on ciliates and heterotrophic dinoflagellates based on field experiments is also pivotal if the seasonal dynamics of the heterotrophic protists are to be understood. The capability of copepods to regulate ciliates, heterotrophic dinoflagellates and other large heterotrophic protists is often interpreted as the main reason for the relative constancy of their abundance in the sea despite a high growth potential (cf. Kiørboe 1998). Compared to other trophic interactions in the planktonic food web (e.g. bacteria-heterotrophic nanoflagellates), few studies have measured ingestion of ciliates and heterotrophic dinoflagellates by copepods. Regulation has most often been suggested indirectly from predator-prey population dynamics (Smetacek 1981, Nielsen & Kiørboe 1994).

Populations of small-bodied copepods are often ignored in *Calanus*-dominated systems because plankton net with coarse mesh size is traditionally used for sampling. In contrast to *Calanus* spp. small copepods usually remain in the upper water layers, so when *Calanus* spp. descend to the bottom water to overwinter, small-bodied copepods like *Acartia longiremis* become dominant (Hansen et al. 1999). The importance of these copepods in the trophic coupling is unknown.

This paper presents results from a series of copepod grazing experiments with females of *Calanus finmarchicus*, *C. glacialis*, *C. hyperboreus* and *Acartia longiremis* conducted in Disko Bay, West Greenland and Young Sound, NE Greenland at different seasons. The goals were (1) to compare copepod clearance and ingestion on ciliates, heterotrophic dinoflagellates and phytoplankton in bloom and post-bloom situations, respectively and (2) to generate an average weight-specific clearance on heterotrophic protists, which could be applied in arctic food web models.

METHODS

Experiments were conducted in Disko Bay, West Greenland aboard the RV 'Adolf Jensen' (Greenland Institute for Natural Resources) or the RV 'Porsild' (Copenhagen University) in June 1997, April and August 1998. An additional experiment was conducted in the ice-covered Young Sound, NE Greenland in June 1999 (Fig. 1).

Copepods were collected from the upper 30 m of the water column using a 200 µm mesh size WP-2 net fitted with a large non-filtering cod-end. Within ~3 h of collection individual female *Calanus* spp. or *Acartia longiremis* were transferred to 2.33 or 2.77 l acid-washed polycarbonate bottles with 200 µm pre-filtered water. Water for incubation was collected from 2.5 m or the depth of chlorophyll maximum using a 10 l Niskin bottle. The time of incubation varied between 20 and 40 h depending on species and number of female copepods added to each grazing bottle (Table 1). Bottles were incubated at *in situ* temperature in a container with flow-through surface water and rotated intermittently by hand throughout the experiments. Initial bottles were immediately preserved with acid Lugol's to a final concentration of 2%. At the end of the incubations, controls (without animals) and grazing bottles (added copepods) were preserved and the condition of the females (dead or alive) was recorded. Subsamples for determination of chl *a* were also taken; 200 ml aliquots were filtered through GF/F filters and dark-extracted in ethanol before measurement on a Turner fluorometer (Jespersen & Christoffersen 1987).

Phytoplankton in preserved aliquots were concentrated by sedimentation and larger phytoplankton cells counted at 100 or 200×, smaller cells at 200 or 400×, in Sedgwick Rafter cells with conventional phase-contrast microscopy utilizing long-working-distance objectives. Ciliates and dinoflagellates were concen-

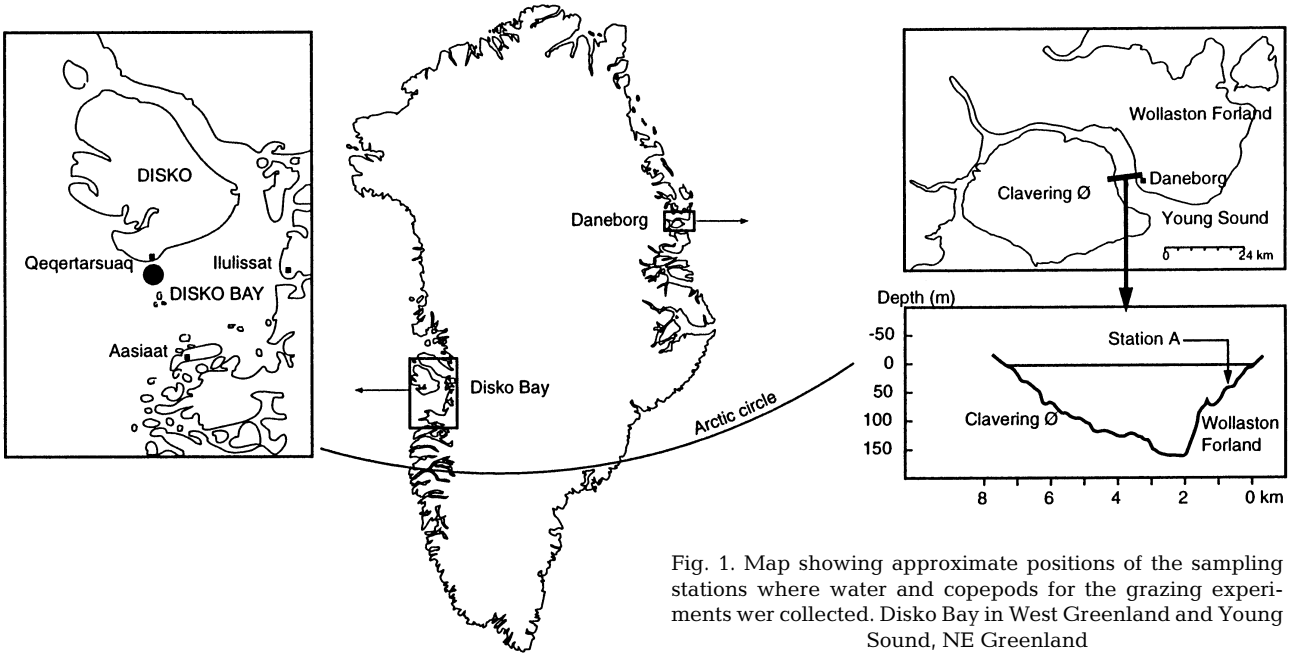

Fig. 1. Map showing approximate positions of the sampling stations where water and copepods for the grazing experiments wer collected. Disko Bay in West Greenland and Young Sound, NE Greenland

trated by sedimentation in 50 ml Utermöhl chambers and counted by inverted microscopy at 100 or 200×. Identified species and morpho-types were pooled into size-classes of the categories nanoflagellates, diatoms, dinoflagellates, the strictly autotrophic ciliate *Myrionecta rubra* and ciliates.

Protist cell volume was estimated from measurements of linear dimensions assuming simple geometric shapes of cells. Biovolume was converted to organic C content using a relation of 0.13 pg C μm^{-3} (Hansen et al. 1997). No corrections were made for fixation-induced shrinkage. Diatom plasma volume was determined according to Strathmann (1967) and converted to organic C by using the same relation as for the other protists.

Copepod clearance and ingestion rates were calculated according to Frost (1972). In experiments with several initial and/or control bottles, the results from all replicates were averaged. Clearance and ingestion rates were calculated conservatively by using initial numbers of copepods added. Copepod mortality was insignificant. Clearance was calculated only when the difference in prey concentration between control and experimental bottles proved significant (Table 2). Car-

Table 1. Grazing experiments conducted with *Calanus* spp. and *Acartia longiremis* in Disko Bay (Expts 1 to 12) and Young Sound (Expt 13) during early bloom (Expts 1a,b), bloom (Expt 13), early post-bloom (Expts 2 to 8) and late post-bloom (Expts 9 to 12) situations. n: no. of replicate grazing bottles

Expt	Date	Species	No. l^{-1}	n	Duration (h)	Temp. (°C)
1a	28–30 Apr 1998	*C. finmarchicus*	3.6	6	40	–1.7
1b	28–30 Apr 1998	*C. glacialis*	3.6	6	40	–1.7
2	12–13 Jun 1997	*C. finmarchicus*	3.6	3	25	6.9 ± 0.7
3	13–14 Jun 1997	*C. finmarchicus*	2.5	3	26	4.6 ± 1.1
4	13–14 Jun 1997	*C. hyperboreus*	0.7	3	25	4.5 ± 1.1
5	14–15 Jun 1997	*C. finmarchicus*	3.6	3	23	5.4 ± 1.4
6	15–16 Jun 1997	*C. hyperboreus*	1.1	3	24	5.1 ± 1.8
7	15–16 Jun 1997	*C. hyperboreus*	1.1	3	21	5.0 ± 1.6
8a	20–21 Jun 1997	*C. finmarchicus*	1.1–4.3	6	29	~3
8b	20–21 Jun 1997	*C. glacialis*	0.9–1.7	4	28	~3
8c	20–21 Jun 1997	*C. hyperboreus*	0.4–1.1	5	28	~3
9	21–22 Aug 1998	*A. longiremis*	48	1	24	~8
10	21–22 Aug 1998	*A. longiremis*	48	1	24	~8
11	21–22 Aug 1998	*A. longiremis*	48	1	24	~8
12	21–22 Aug 1998	*A. longiremis*	48	1	24	~8
13	12–13 Jun 1999	*C. hyperboreus*	0.4	4	27	–1.7

Table 2. Significance levels for the differences between prey densities in control and grazing bottles (*t*-test). ****p < 0.01, ***p < 0.05, **p < 0.10, *p < 0.20, ns: no significant difference. –: no data. *Calanus finmarchicus* (C. *fin*), *C. glacialis* (*C. gla*), *C. hyperboreus* (*C. hyp*)

Expt	Species	Ciliates				Dinoflagellates			Chl *a*
		Total	<20 µm	>20 µm	*Myrionecta*	Total	>20 µm	<20 µm	Total
1a	*C. fin*	****	****	–	–	ns	***	ns	****
1b	*C. gla*	****	****	–	–	ns	****	ns	****
2	*C. fin*	***	**	****	–	ns	**	ns	–
3	*C. fin*	***	***	***	*	**	**	*	–
4	*C. hyp*	ns	ns	ns	–	ns	ns	ns	–
5	*C. fin*	***	*	**	***	***	**	***	–
6	*C. hyp*	ns	ns	ns	ns	ns	ns	*	–
7	*C. hyp*	ns	ns	ns	ns	ns	ns	ns	–
8a	*C. fin*	****	***	****	****	****	****	****	****
8b	*C. gla*	ns	ns	ns	ns	ns	*	ns	ns
8c	*C. hyp*	**	**	*	ns	*	***	ns	ns
13	*C. hyp*	****	**	***	ns	–	***	–	**

bon ingestion was obtained from the product of the number of cells ingested and the weighted mean C content for that cell category in the initial bottles. The mean cell C content for each cell category was obtained by weighting the C content for each species by its relative abundance. The weight-specific clearance (ml μg^{-1} C l^{-1} d^{-1}) and ingestion (μg C μg^{-1} C l^{-1} d^{-1}) rates were estimated using the mean female carbon weight of the respective copepod species in Disko Bay (Table 3). A Q_{10} = 2.8 was used to eliminate temperature differences among the experiments (Hansen et al. 1997).

The total carbon ingestion of *Acartia longiremis* was calculated based on egg production measurements assuming a gross egg production efficiency of 33%. Egg production was converted to carbon from egg volume and a relation of 0.14 pg C μm^{-3} (Kiørboe et al. 1985).

In the following sections, we refer to bloom and post-bloom grazing experiments, respectively, based on the physical structure of the water column and the distribution of phytoplankton at the time of sampling.

RESULTS

The grazing experiments were conducted at temperatures covering the seasonal cycle observed in Disko Bay and Young Sound (–1.7 to 8°C), and contained phytoplankton and heterotrophic protist assemblages with abundances typical for spring and summer (Fig. 2). Initial chl *a* concentrations were between 0.2 and 8.6 μg l^{-1}. These were highest in April during the early spring bloom and in June at the depth of the subsurface maximum, and lowest in surface water in June (Fig. 2a,b). Cells >10 µm usually dominated the phytoplankton, although cells <10 µm contributed substantially. The ciliate and dinoflagellate biomass varied from ~1 to 20 μg C l^{-1} (Fig. 2c,d). By applying a carbon-chlorophyll *a* ratio of 50 it was estimated that ciliates and dinoflagellates on average constituted 5% of total protist carbon during the spring bloom and at the subsurface chlorophyll *a* peak, whereas they constituted more (34%) in the surface water. Naked oligotrich ciliates and athecate gymnodinoid dinoflagellates were most abundant. Heterotrophic and autotrophic dinoflagellates were not separated in this study, but it is assumed here that heterotrophic species constituted the majority of the dinoflagellates, in agreement with previous studies from the area (Levinsen et al. 1999, 2000).

Table 3. *Calanus* spp. mean ± SE of the female cephalothorax length and carbon weight (from S. D. Madsen, T. G. Nielsen & B. W. Hansen unpubl.)

	C. finmarchicus	*C. glacialis*	*C. hyperboreus*
Length (µm)	2680 ± 60	3460 ± 100	6450 ± 260
Weight (µg C)	160 ± 10	410 ± 50	940 ± 120

Clearance

Grazing of protists differed between *Calanus* spp. with the largest species performing the highest clearance (Fig. 3). A difference in their seasonal grazing patterns was also observed. Clearance by C*alanus finmarchicus* was highest during the warmer post-bloom period. Here, a rate of ~500 ml $female^{-1}$ d^{-1} on the autotrophic ciliate *Myrionecta rubra* and oligotrichous ciliates >20 µm was measured (Fig. 3a,b). Lower clearances were found on chl *a* and oligotrichs <20 µm (306 and 271 ml $female^{-1}$ d^{-1}, respectively). Dinoflagellates

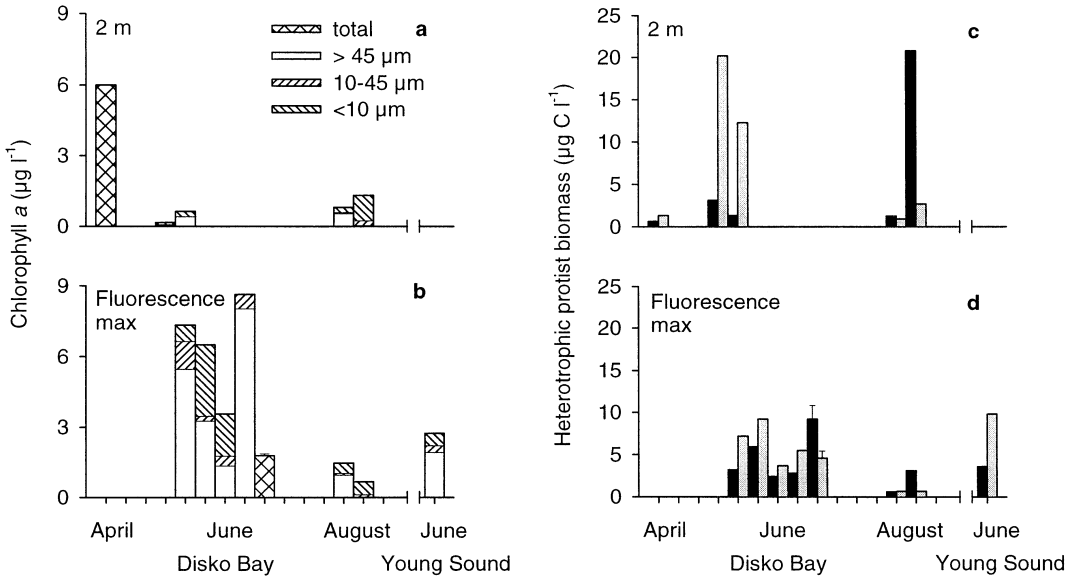

Fig. 2. Initial concentrations of total and size-fractionated phytoplankton expressed as chl *a* (a,b) and biomasses of ciliates (black) and dinoflagellates (grey) (c,d) in experimental water collected from 2 m and the depth of the fluorescence maximum (usually ~30 m)

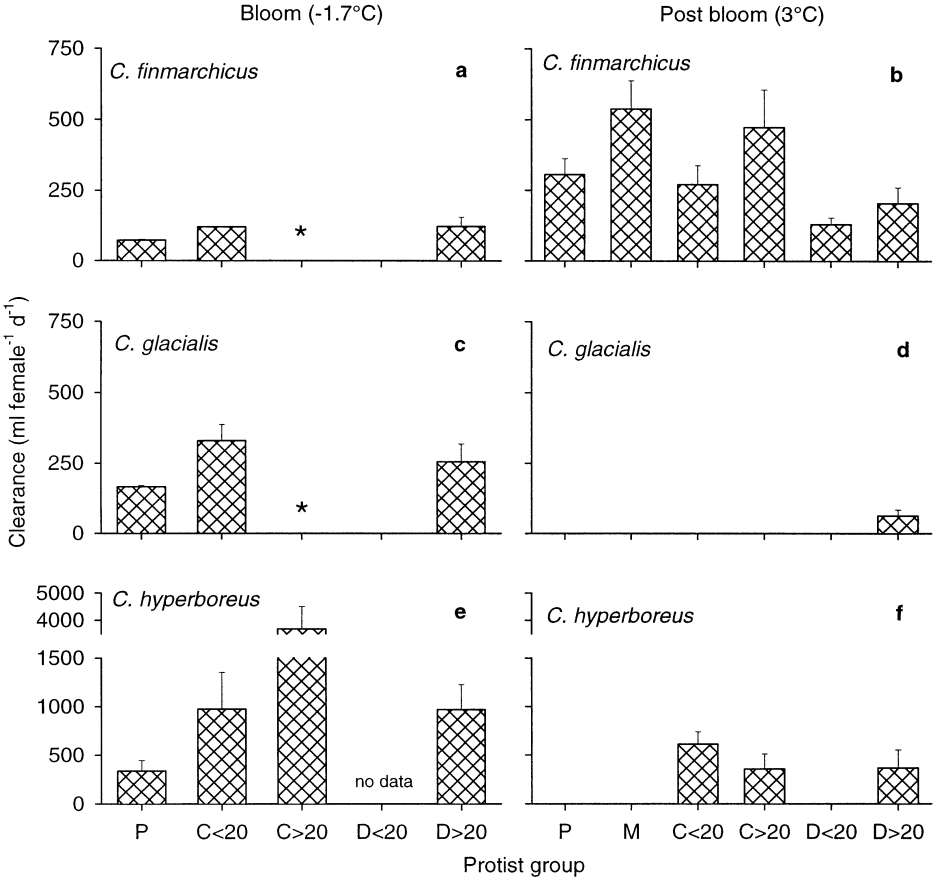

Fig. 3. Bloom and post-bloom clearance by *Calanus* spp. on chl *a* (P), *Myrionecta rubra* (M), and oligotrichous ciliates (C) and dinoflagellates (D) with ESD <20 µm and >20 µm. Experiments were initiated on 28 April (a,c), 12 June (e), and 20 June (b,d,f). Initial chl *a* concentrations on these dates were 6.0, 2.7 and 1.8 µg l^{-1}, respectively. For further information on experimental conditions, see Table 1. Error bars indicate ±SE of the mean (too small to be seen in a and c). *Concentrations of ciliates >20 µm ESD in these experiments were too low to allow calculation of clearance

were grazed least efficiently, particularly cells <20 µm. In contrast, grazing by *C. glacialis* and *C. hyperboreus* was low or undetectable during the post-bloom period compared to the bloom period (Fig. 3c–f). Grazing was consistently lower although the temperature had increased by 5°C. *Calanus glacialis* only cleared dinoflagellates >20 µm while *C. hyperboreus* also cleared ciliates at relatively low rates. Grazing of heterotrophic protists by *C. hyperboreus* was not detected in any other post-bloom incubations (Expts 4, 6, 7; Table 2). When grazing was measured, *C. glacialis* and *C. hyperboreus*, like *C. finmarchicus*, cleared ciliates at a significantly greater rate than they cleared chl *a*. The clearance of ciliates was ~300 and 1000 to 3700 ml female^{-1} d^{-1} for *C. glacialis* and *C. hyperboreus*, respectively. Corresponding values for clearance on chl *a* were 166 and 338 ml female^{-1} d^{-1} (Fig. 3c,e). All 3 species of *Calanus* generally did not graze on dinoflagellates <20 µm (Table 2).

Cell counts supported the clearance results from chl *a* measurements in that there was more grazing on phytoplankton by *Calanus finmarchicus* than by *C. glacialis* and *C. hyperboreus* during the post-bloom period. *C. finmarchicus* grazed on all major protist groups including *Myrionecta rubra* and nanoflagellates dominated by solitary *Phaeocystis* cells ~5 µm in size (Fig. 4). In contrast, there was no or only low grazing on phytoplankton by *C. glacialis* and *C. hyperboreus* (Tables 4 & 5). Low grazing on nanoflagellates during the post-bloom period was observed only once by *C. hyperboreus* (Expt 4). Generally, *C. glacialis* cleared diatoms, while *C. hyperboreus* did not and neither species cleared nanoflagellates. In fact, the nanoflagellate abundance increased significantly compared to controls during incubations with added *C. hyperboreus* (Table 5).

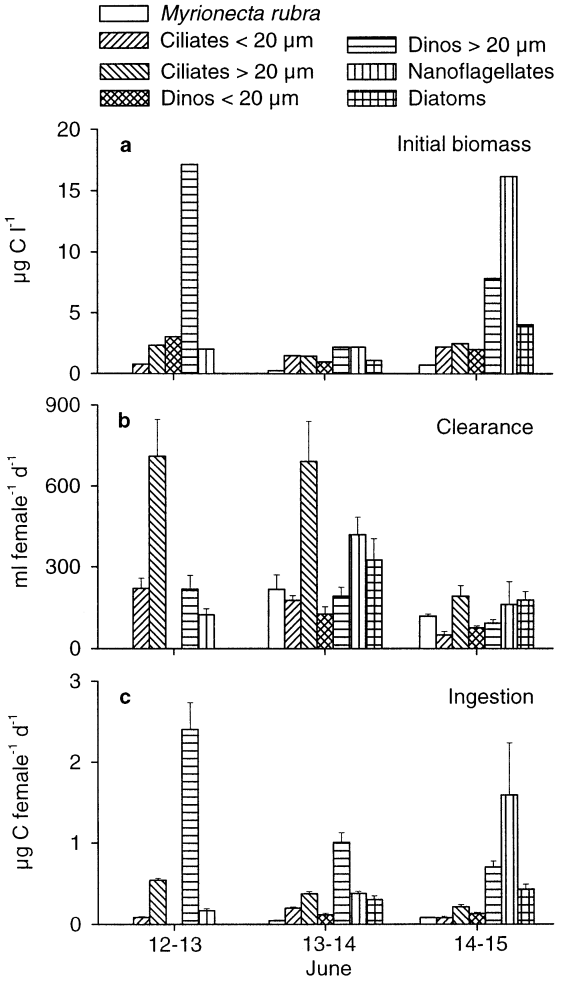

Fig. 4. *Calanus finmarchicus*. Post-bloom (a) initial biomass, (b) clearance and (c) ingestion of major protist groups in 3 grazing experiments performed with surface water (12–13 June) and water from the depth of the fluorescence maximum (13–15 June). Error bars indicate ±SE of the mean

Table 4. Post-bloom grazing of major phytoplankton groups in Disko Bay (12–16 June). Concentration in initial (Init), control (Con) and grazing bottles with added *Calanus finmarchicus* (*C. fin*) or *C. hyperboreus* (*C. hyp*). No. of replicates is given after bottle name. SE is given in parentheses. Significance levels of the difference between control and experimental prey densities as explained in Table 2

Expt	Copepod species	Phytoplankton group	Concentration (cells × 10^3 l^{-1}) Init, 1	Con, 1	Grazing, 3	p
2	*C. fin*	Nanoflagellates	31	22	14 (1)	**
		Diatoms	0	1	0 (0)	*
3	*C. fin*	Nanoflagellates	18	34	11 (2)	***
		Diatoms	7	14	6 (1)	**
4	*C. hyp*	Nanoflagellates	5	4	3 (0)	*
		Diatoms	6	5	4 (1)	*
5	*C. fin*	Nanoflagellates	250	192	119 (29)	***
		Diatoms	27	17	9 (1)	**
6	*C. hyp*	Nanoflagellates	326	262	358 (34)	ns
		Diatoms	24	9	4 (2)	ns
7	*C. hyp*	Nanoflagellates	98	100	138 (26)	ns
		Diatoms	48	148	125 (16)	ns

Table 5. Phytoplankton post-bloom grazing on 20–21 June in Disko Bay (Expt 8a–c). Concentration in initial (Init), control (Con) and grazing bottles with added *Calanus finmarchicus* (*C. fin*), *C. glacialis* (*C. gla*) or *C. hyperboreus* (*C. hyp*). SE is given in parentheses. No. of replicates is given after bottle name. Significance levels of the difference between control and experimental prey densities as explained in Table 2. neg: negative significant relationship

Species	Concentration (cells × 10^3 l^{-1})					Grazing		
	Init, 3	Con, 7	*C. fin*, 6	*C. gla*, 4	*C.hyp*, 5	*C. fin*	*C. gla*	*C. hyp*
Nitzschia spp.	43	2	1	1	2	*	ns	ns
	(7)	(0)	(0)	(1)	(1)			
Thalassiosira spp.	17	16	7	6	15	*	*	ns
	(7)	(5)	(2)	(3)	(4)			
Chaetoceros spp.	14	8	7	4	7	ns	*	ns
	(5)	(2)	(2)	(1)	(2)			
Diatoms, total	74	25	14	11	24	**	**	ns
	(14)	(5)	(3)	(2)	(6)			
Other	15	2	4	2	2	ns	ns	ns
	(5)	(1)	(3)	(1)	(1)			
Nanoflagellates	13	69	34	62	143	ns	ns	*(neg)
	(2)	(21)	(12)	(58)	(44)			
Phytoplankton, total	101	95	56	76	169	***	ns	*(neg)
	(20)	(22)	(12)	(59)	(50)			

Weight-specific, temperature-corrected clearances revealed no significant difference between clearance of ciliates and dinoflagellates. The rates varied independently with heterotrophic protist concentration averaging (± SE) 1.0 ± 0.1 ml μg^{-1} C d^{-1} at 3°C (Fig. 5).

Size of prey cells had a major effect on clearance (Fig. 6). *Calanus* spp. grazed on all sizes of ciliates and dinoflagellates but grazing was low on the smallest cells. The clearance on 10 µm ESD (equivalent spherical diameter)-sized ciliates indicate that this size is close to the lower limit for particle capture. Within a prey size range of 10 to 30 µm ESD, the clearance of ciliates and dinoflagellates by *C. finmarchicus and C. hyperboreus* increased from about 0 to 2–3 ml μg^{-1} C d^{-1}. For prey >25 to 30 µm ESD clearance was more variable (Fig. 6). Feeding rates were generally higher upon ciliates compared to upon similar-sized dinoflagellates. The range in heterotrophic protist cell size was too small in the experiments using *C. glacialis* to make a similar plot for this species, but they tended to follow the same pattern. Thus, ciliates with a mean size of 26 µm ESD were grazed with a rate of 0.9 ml μg^{-1} C d^{-1} while the corresponding rate for 17 µm ESD-sized dinoflagellates was 0.3.

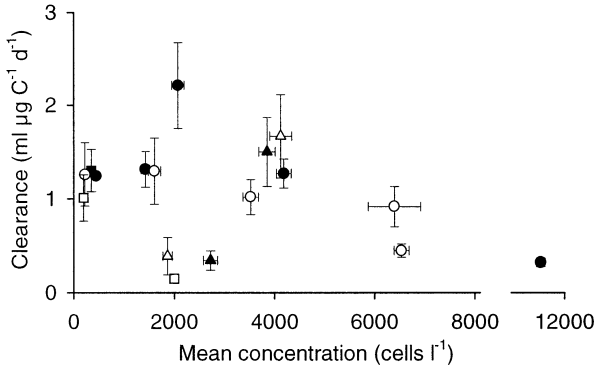

Fig. 5. Weight-specific clearance of ciliates (closed symbols) and dinoflagellates >20 µm (open symbols) by *Calanus finmarchicus* (●), *C. glacialis* (■) and *C. hyperboreus* (▲) at different concentrations of ciliates and dinoflagellates. Data were corrected to 3°C by using a Q_{10} of 2.8. Error bars indicate ±SE of the mean

The functional response of *Acartia longiremis* grazing on natural populations of heterotrophic protists demonstrated a maximum weight-specific clearance for ciliates of ~15 ml μg^{-1} C d^{-1} at 8°C (Fig. 7). Decreasing clearances were measured with increasing prey concentrations, and at concentrations higher than about 10^4 l^{-1} no grazing could be detected.

Ingestion

Because of high phytoplankton biomass as determined from chl *a*, heterotrophic protists contributed less to ingestion during the 28 to 30 April bloom experiments although they were cleared by copepods at the highest rate (Fig. 8a,b). The heterotrophic protists, which consisted almost entirely of cells <20 µm, contributed only 0.5% to the ingestion by *Calanus finmarchicus* and *C. glacialis.* A larger contribution (12%) was observed for *C. hyperboreus* during the spring

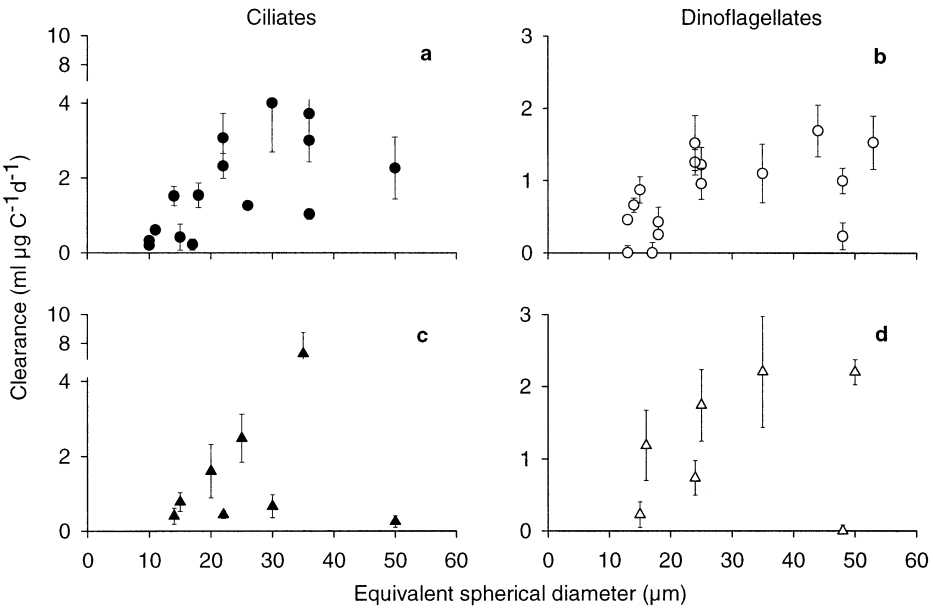

Fig. 6. Clearance by *Calanus finmarchicus* (upper panels) and *C. hyperboreus* (lower panels) on different cell sizes of (a,c) ciliates and (b,d) dinoflagellates corrected to 3°C. Cells within a size range have been pooled before calculation of the clearance. Error bars indicate ±SE of the mean

bloom in Young Sound where the concentration of chl *a* was lower (Fig. 8a,b). This latter value was similar to the post-bloom heterotrophic protist food share for *C. finmarchicus* in Disko Bay (Fig. 8c,d).

When the phytoplankton biomass was estimated from microscopic cell counts, the contribution of ciliates and dinoflagellates to total ingestion in post-bloom experiments was larger. A particularly high share was observed in the low-chl *a* surface water on 12 and 13 June (Fig. 4). Here the ciliate and dinoflagellate proportions of total food were 20 and 75%, respectively. In 2 other post-bloom grazing experiments, conducted during subsequent days with subsurface water, the heterotrophic protist shares were 72% (ciliates 25%) and 37% (ciliates 11%), respectively (Fig. 4c). During the post-bloom period, neither *C. glacialis* nor *C. hyperboreus* usually ingested phytoplankton, when measured as either chl *a* or by cell counts, nor did they usually ingest heterotrophic protists. Ingestion of heterotrophic protists when occurring was positively related to their concentration (Fig. 9). *Calanus* spp. feeding increased when presented with more heterotrophic protists. The share of ciliates and dinoflagellates to the ingestion by *Acartia longiremis* in August as estimated from egg production was <25 and <7% in August respectively (data not shown).

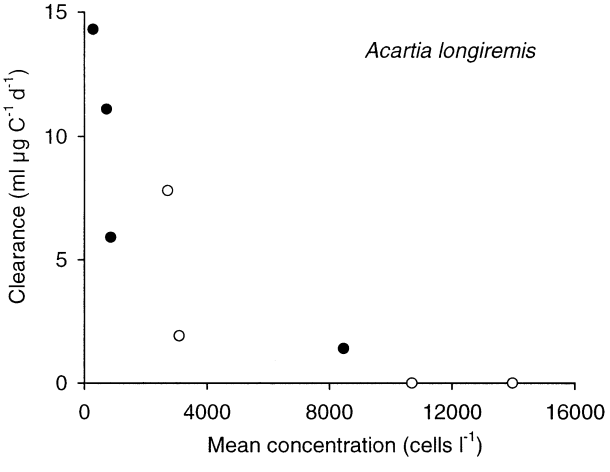

Fig. 7. Weight-specific clearance of ciliates (●) and dinoflagellates (○) by *Acartia longiremis* at different concentrations of ciliates and dinoflagellates. Weight of females was 3.7 µg C (from Hansen et al. 1999). Each point represents the mean female clearance at 8°C from a single grazing bottle

DISCUSSION

Trophic coupling between protists and copepods

In Disko Bay and Young Sound, ciliates and heterotrophic dinoflagellates are important grazers (Nielsen & Hansen 1995, Levinsen et al. 1999, Rysgaard et al. 1999). The present study reveals that these heterotrophic protists can also constitute a substantial proportion of the copepod diet. The trophic coupling was

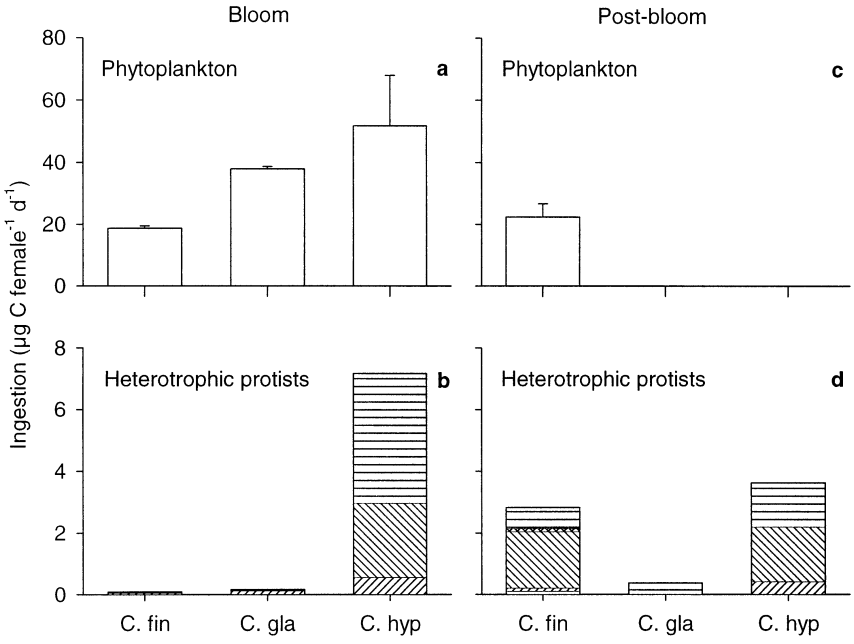

Fig. 8. *Calanus* spp. Bloom and post-bloom ingestion of (a,c) phytoplankton and (b,d) *Myrionecta rubra*, ciliates and dinoflagellates larger and smaller than 20 µm. Notice different scales on *y*-axis. Chl *a* was converted to phytoplankton carbon by using a relation of 1:50. Error bars indicate ±SE of the mean (only upper panels). Same experiments as in Fig. 3 (key to identification of protist groups as in Fig. 4)

particularly strong during the post-bloom period, when heterotrophic protists may constitute the majority of total daily ingestion by *Calanus finmarchicus* (Fig. 4). Because heterotrophic protists likely had fed upon phytoplankton, in addition to small bacteria-eating flagellates, a large part of the primary production may therefore have reached the 'herbivorous' copepods via ciliates and heterotrophic dinoflagellates. The latter 2 groups thus provided a direct trophic linkage between phytoplankton and copepods.

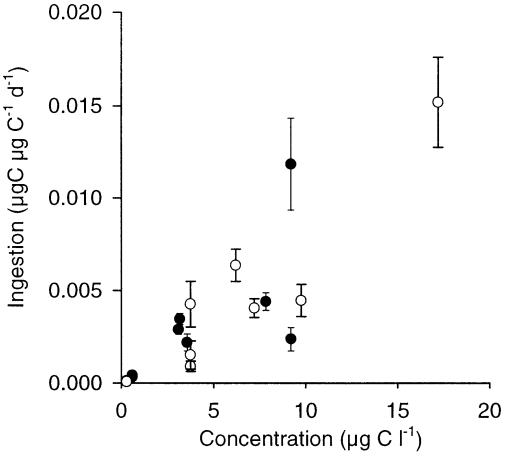

Fig. 9. Specific ingestion of ciliates (●) and dinoflagellates (○) at various heterotrophic protist concentrations in Disko Bay and Young Sound by *Calanus* spp. Data are not temperature corrected. Error bars indicate ±SE of the mean

The C-specific daily ration (µg C ingested µg^{-1} body C d^{-1} × 100) for *Calanus finmarchicus* during the post-bloom period was ~2%, close to daily rations of 1.1, 1.8 and 4.2% calculated from Ohman & Runge (1994). These authors found such food rations sufficient to support fecundity, growth and development of *C. finmarchicus* in the Gulf of St. Lawrence. Assuming a similar role for heterotrophic protists in Disko Bay may have strong implications for the demography of the copepods because of the short season for growth. It can be speculated that heterotrophic protists influence the life history of the northern/arctic long-living *Calanus* spp. by extending the period for near-surface growth and development and postponing their descent to overwintering depths.

Conversely, during the early phases of the bloom, the contribution of ciliates to the diet of *Calanus finmarchicus* in Disko Bay was negligible (~0.5%). At this time, there was abundant phytoplankton and the copepods were predominantly herbivorous (Fig. 8). In the literature, a similar seasonal pattern related to the relative distribution of size and concentration of phytoplankton and heterotrophic protists has been reported for *Acartia tonsa* in the Gulf of Mexico (Gifford & Dagg 1991).

For *Calanus glacialis* and *C. hyperboreus* the grazing pattern was different. The trophic coupling to heterotrophic protists might be important during spring,

as demonstrated for *C. hyperboreus* in Young Sound, but these species usually did not graze during the post-bloom period. In case they did, however, the food was often entirely composed of heterotrophic protists (Fig. 8). Thus, although grazing was sporadically measured and the rates were low compared to at bloom situations, they could contribute considerably to the coupling (Figs. 3 & 8). Large individual variability in gut fullness of *Calanus* spp. (up to a factor of 28) has previously been reported, even for copepods collected at the same time, locality and depth (Båmstedt 1988, Båmstedt et al. 1992). Variation in grazing activity between females was also indicated by the large standard errors of the feeding rates obtained in this study. Obviously, it is difficult to define the grazer activity of an 'average field individual' for these large copepod species. However, the observed seasonal variation, in addition to individual variability, probably reflects termination of feeding by part of the *C. hyperboreus* and *C. glacialis* populations prior to overwintering. This is supported by observations of empty (colorless) guts of post-bloom collected individuals.

The small-bodied *Acartia longiremis* also exhibited an omnivorous feeding strategy, which supports earlier suggestions from fatty acid analysis by Norrbin et al. (1990). If the observed high proportion of heterotrophic protists in the diet of *A. longiremis* is typical for this species, it could potentially sustain a trophic coupling and regulating impact on heterotrophic protists after the late stage *Calanus* spp. have descended to the bottom water in mid-summer.

The magnitude of the clearance by *Acartia longiremis* on heterotrophic protists can be evaluated by comparison with another report of their grazing on natural plankton. *A. longiremis* did not clear ciliates at detectable rates during an upwelling diatom bloom, but they cleared chl *a* at a maximum of 16.8 ml d^{-1} at 10°C (Fessenden & Cowles 1994). This value is similar to *A. longiremis* clearance on ciliates measured in the present study.

Understanding clearance in the field

From the above it follows that the 'background level' of phytoplankton influences the importance of heterotrophic protists as vectors of primary production to higher trophic levels. Other factors that potentially influence clearance are prey concentration, prey size, and behavior of prey and predator.

Concentration

The functional response of *Acartia longiremis* (Fig. 7) was close to what would be expected if food concentration was the only variable, as has been shown in laboratory studies (e.g. Kiørboe et al. 1985). This indicates a fairly stable plankton composition between experiments using *A. longiremis*, a reasonable assumption because the experimental water was collected on the same day at positions close to each other. A rough comparison of heterotrophic protist size-classes confirms that the size compositions were actually quite similar. The functional response for *Calanus* spp. (Fig. 5) lacked such relation between clearance and concentration. It probably reflects that different plankton communities from different seasons and ecosystems were being compared. Thus, parameters in addition to the concentration of heterotrophic protists played an important role.

Size

Substantial clearance by *Calanus finmarchicus* on *Phaeocystis* solitary cells indicates that the size-limit for prey capture by this copepod was <5 µm. In contrast, *Phaeocystis* was too small to be grazed by *C. hyperboreus*. Although grazer excretion can induce growth of small autotrophs leading to underestimates of their removal rates (Roman & Rublee 1980), grazing by *C. hyperboreus* in this case did not occur. Thus, *C. finmarchicus* apparently was able to feed on smaller particles than *C. hyperboreus*. A difference in the lower size-limit of the particle-retention spectra between these 2 *Calanus* species is supported by results of Hansen et al. (1994). They found that *C. finmarchicus* CIV–V efficiently collected solitary *Phaeocystis* cells of 4.5 µm in contrast to *C. hyperboreus* CV. Furthermore, Huntley (1981) found that female *C. finmarchicus* ingested particles in the 5 to 10 µm particle category, while *C. glacialis* and *C. hyperboreus* did not.

Calanus spp. generally grazed ciliates more efficiently than phytoplankton. Large ciliates were cleared at a particularly high rate. Size-dependency is well-known for copepods preying on phytoplankton (e.g. Frost 1972), but knowledge of size-dependent predation on heterotrophic protist prey is limited. Size-dependent clearance of heterotrophic protists has apparently been previously demonstrated only once for small-bodied neritic copepods preying on ciliates (Tiselius 1989). Our results, in agreement with that study, showed increasingly higher clearances with prey sizes above ~10 µm ESD, a size which corresponds to the smallest ciliates and dinoflagellates. Maximum capture efficiency for *Calanus* spp. was achieved for food with a size of approximately 30 to 40 µm ESD. By comparison, Tiselius (1989) reported increasing clearances of ciliates by *Acartia clusi* until a size of 25 µm ESD. Above this size, a fairly constant

clearance was measured. In contrast, Tiselius found that clearance by *Centropages hamatus* increased continuously with size up to the largest size fraction offered, 35 to 50 µm ESD ciliates. Frost (1972) also found a linear increase in clearance by *Calanus pacificus* with an increasing size of diatoms up to the largest tested diatom species of 67 µm ESD.

Behavior

A continuous increase in clearance with size agrees with predicted filtration efficiencies from filtration of immobile prey until a critical size is reached, above which there is nearly 100% retention efficiency (Nival & Nival 1976). However, increasing clearance with size might equally well imply that predator or prey behavior is also involved in the feeding process. On the one hand, large-sized prey emits stronger hydrodynamic signals, which should increase the capture efficiency by raptorial feeding copepods (Jonsson & Tiselius 1990). On the other hand, if the copepods were suspension-feeding, the fact that relatively fast-swimming ciliates were cleared with rates about twice as high as similar-sized slower-moving dinoflagellates (Fig. 6) may indicate that prey behavior influences the magnitude of the clearance by enhancing encounter rate. Whichever mechanism is the most important will be difficult to demonstrate with natural plankton assemblages, but should be addressed in future studies. It does not influence the conclusion of a size-dependent clearance, but it is in contrast to results of Tiselius (1989) who found that there was no difference between clearance on phytoplankton and ciliates when size was taken into account.

While small (<20 µm ESD) ciliates were grazed, clearance of small dinoflagellates was not detected for *Calanus glacialis and C. hyperboreus*. This was unexpected, based on assumptions of escape responses by ciliates similar to that demonstrated for *Myrionecta rubra* (Jonsson & Tiselius 1990), although probably less dramatic. Two lines of reasoning might explain this observation. First, the sizes of the largest ciliate and dinoflagellate dimensions in the <20 µm ESD size category were different. Dinoflagellates were (sub)spherical with diameters between ~10 and ~17.5 µm, whereas ciliates typically were almost conical or elongated ellipsoids, with lengths of up to 30 µm, twice the diameter of an average-sized dinoflagellate. Thus, the actual size perceived by the copepods might have been larger for the ciliates in this size category, and dinoflagellates may have been close to the lower size limited of the particle spectra. Second, a faster swimming speed of ciliates may increase prey-predator encounter rates.

Even *Myrionecta rubra* was cleared efficiently by *Calanus finmarchicus*, although this ciliate has a pronounced escape behavior with burst swimming velocities reaching 8.5 mm s^{-1} elicited by an approaching copepod (Jonsson & Tiselius 1990). Low clearance by *Acartia tonsa* when feeding on this ciliate was attributed to burst swimming, which equaled or exceeded the flow velocities generated by the copepod during feeding (Jonsson & Tiselius 1990). It appears reasonable to assume that the much larger *Calanus* spp. create flow velocities sufficiently strong to capture *M. rubra*. Thus, although ciliates might have adapted to compensate for an increased predator encounter rate by an increased escape behavior, our results suggest that in the context of susceptibility to predators it may be a net disadvantage for heterotrophic protists to be fast swimmers.

Ecological implications of a size-differentiated clearance

A direct implication of the size-clearance relation is that large ciliates and dinoflagellates may experience a stronger top-down regulation than small species. Therefore, it is not only because of growth scale according to body size, with a negative exponent, that large ciliates are more strongly regulated than small ones as suggested by Nielsen & Kiørboe (1994). More efficient predation on large ciliates presumably reinforces this pattern. A combination of size-selective feeding and low growth-rate of the larger species may explain why the size distribution of field ciliate and dinoflagellate communities usually are skewed towards smaller species (Fig. 10), and may reflect that top-down regulation is indeed very strong (Verity & Smetacek 1996).

An indirect consequence of size-selective feeding is that regulation of heterotrophic protists by copepods potentially varies with depth. In the low chl *a* surface waters of stratified systems, a phytoplankton cell size, in the lower range of optimum copepod filtration efficiency, usually dominates. Relatively large heterotrophic protists are therefore more exposed to capture and consumption. Conversely, the subsurface water is often dominated by diatoms and heterotrophic protists of equal size, so the size discrepancy diminishes. Such a varying degree of regulation has previously been suggested partly to explain the elevated biomass patches of ciliates and heterotrophic dinoflagellates occurring concurrently with the spring bloom and subsurface peak of chain-forming diatoms (Nielsen & Hansen 1995, Levinsen et al. 1999). A vertically-differentiated top-down regulation assumes an equal distribution of copepods in the water column.

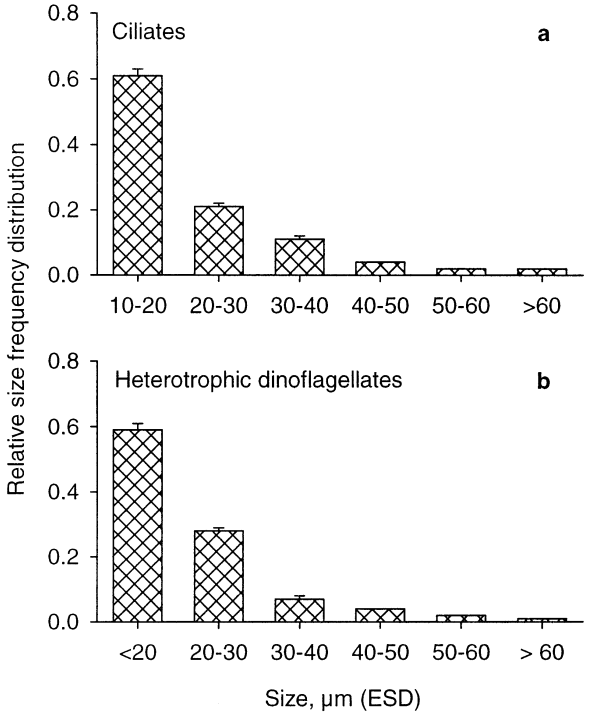

Fig. 10. Relative size distribution (mean ± SE) of (a) ciliates and (b) heterotrophic dinoflagellates. Data from samples collected from the upper 15 m of the water column during an annual study conducted in Disko Bay, West Greenland, from April 1996 to June 1997 (Levinsen et al. 2000). n = 106

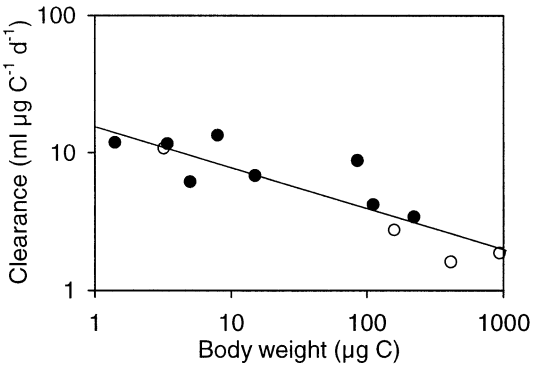

Fig. 11. Maximum weight-specific clearance at 5°C by adult female copepods on natural ciliate populations scaled to the body weight for different-sized copepods collected from the literature (●): *Acartia clausii*, *Centropages hamatus* (Tiselius 1989); *A. tonsa*, *Neocalanus plumchrus* (Gifford & Dagg 1991); *Calanus finmarchicus* (Ohman & Runge 1994); *Calanus pacificus*, *Centropages abdominalis*, *Pseudocalanus* sp. (Fessenden & Cowles 1994). *A. longiremis*, *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* from this study are shown by open dots. A Q_{10} of 2.8 was used to correct clearances to 5°C. Coefficient of determination (R^2) for the regression line = 0.79

Copepod body size and feeding activity

Over the reported range of natural heterotrophic protist concentrations and size compositions we found a mean weight-specific *Calanus* clearance of 1 ml µg^{-1} C d^{-1} at 3°C (range 0.1 to 2.2). This is very similar to a temperature corrected *C. finmarchicus* clearance on dinoflagellates and ciliates of 0.1 to 1.7 ml µg^{-1} C d^{-1} reported by Ohman & Runge (1994). Our weight-specific clearance for the smaller *C. finmarchicus* on average exceeded that of the larger *C. hyperboreus* by 35%. However, the maximum weight-specific clearance rate of the small-bodied *Acartia longiremis* was ~4 times higher than that of *Calanus* spp. Plotting maximum weight-specific clearance on ciliates as a function of adult female copepod weight (Fig. 11), including copepods of different body sizes from the literature, resulted in a log-log regression plot with a decreasing weight-specific clearance with body weight (scaling exponent –0.30). Thus, the maximum weight-specific clearance on ciliates by copepods, F_{max} (ml µg C^{-1} d^{-1}), is allometrically related to body size, W (µg C ind^{-1}), according to

$$F_{max} = a \times W^{0.30}$$

where a = 15.8. This indicates that small-bodied copepods like *A. longiremis*, and possibly also small developmental stages of *Calanus* spp. which dominate after the main bloom in the Arctic, have a great influence on the efficiency of the trophic coupling between the primary producers and the higher trophic levels.

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