Copepod predation on phagotrophic ciliates in Oregon coastal waters

Lynne Fessenden, Timothy J. Cowles

College of Oceanic and Atmospheric Sciences, Oceanography Admin. Bldg 104, Oregon State University,
Corvallis, Oregon 97331-5503, USA

ABSTRACT: Clearance rates on natural assemblages of aloricate, phagotrophic ciliates (20 to 100 µm) were measured for calanoid copepods in Oregon, USA, coastal waters. Experiments were conducted prior to and throughout the 1991 upwelling season in an effort to determine the trophic significance of ciliates as copepod prey in a system often dominated by large phytoplankton. Copepods cleared ciliates at higher rates than they cleared phytoplankton, except during upwelling-induced diatom blooms. Clearance rates on ciliates (ml copepod^{-1} h^{-1}) were measured for *Calanus pacificus* (12.6 to 32.4), *Pseudocalanus* sp. (4.8 to 7.4) and *Centropages abdominalis* (1.2 to 7.1). The highest clearance rates on ciliates were measured when the phytoplankton standing stock was <5 µg chl a l^{-1} No detectable clearance of ciliates was measured for *Acartia longiremis* or *C. pacificus* during upwelling bloom events (45 µg chl a l^{-1}). Ciliate biomass ranged from 3 to 32 µg C l^{-1} and was significantly less than phytoplankton biomass (100 to 2200 µg C l^{-1}) on all dates. During non-upwelling months and between diatom blooms, ciliates contributed 16 to 100 % of the estimated carbon ingested by copepods. Calculated ingestion of ciliate carbon (µg C copepod^{-1} d^{-1}) averaged 6.5 (±2.4) for *C. pacificus*, 4.6 (±1.1) for *Pseudocalanus* sp. and 0.7 (±0.4) for *C. abdominalis*. Ingestion of ciliates alone provided enough carbon to meet the basic respiration requirements of *C. pacificus* and *Pseudocalanus* sp. in January and February respectively. Estimates of predation, based on average copepod densities, indicate 25 to 45 % of the phagotrophic ciliate population is cleared from surface waters per day.

KEY WORDS: Microzooplankton · Copepod diet · Upwelling

INTRODUCTION

Phagotrophic protists are now recognized as a trophic link between <5 µm cells (bacteria, picophytoplankton and nanoflagellates) and metazoans such as copepods (Sherr & Sherr 1988, Stoecker & Capuzzo 1990, Gifford 1991). Bactivory by phagotrophic protists has been well documented (reviewed by Sherr & Sherr 1989), and the quantitative importance of protists as consumers of phytoplankton may equal or exceed that of metazoan grazers (Sherr & Sherr 1992). S. Neuer & T. J. Cowles (unpubl.) recently measured protist herbivory in the upwelling region off the Oregon, USA, coast and found that the protist assemblage of heterotrophic and mixotrophic ciliates, and heterotrophic dinoflagellates and nanoflagellates grazed 26 to 50 % of the potential primary production. The ultimate fate of the primary production grazed by protists depends on whether the protists themselves are eaten by higher consumers. Due to the small size of their fecal material (Stoecker 1984, Gowing & Silver 1985), protists contribute to the recycling and increased residence time of organic matter within surface waters (Welschmeyer & Lorenzen 1985). It is not clear if the protist-copepod trophic link operates under the diatom bloom conditions typical of coastal upwelling regions. Determining the magnitude of this link in productive coastal waters is relevant not only to trophic studies but also to the quantification of carbon flux.

Phagotrophic protists, including heterotrophic nanoflagellates, heterotrophic dinoflagellates, and heterotrophic and mixotrophic ciliates, often dominate marine nano- (2 to 20 µm) and microplankton (20 to 200 µm) assemblages (Sherr et al. 1986, Stoecker & Capuzzo 1990, Gifford 1991). The C:N ratio of the heterotrophic forms (3–5:1) is lower than that of phytoplankton (6–15:1),

suggesting that protists are a protein-rich prey source for zooplankton predators (reviewed by Stoecker & Capuzzo 1990). Kleppel et al. (1991) have found the variability in egg production of copepods in the field (coastal and open ocean) to be better correlated with changes in microzooplankton biomass than with diatom biomass. They argue that protists may be responsible for providing a major portion of copepod nutrition, especially that associated with reproductive energy. In the lab, the calanoid copepod *Acartia tonsa* increases its egg production by as much as 25 % when tintinnid ciliates are included in its diet (Stoecker & Egloff 1987).

Aloricate, choreotrichous ciliates are commonly the dominant phagotrophic protists in the size range most efficiently grazed by adult copepods (Sherr et al. 1986, Berggreen et al. 1988). Several laboratory studies have demonstrated predation by calanoid copepods on both loricate and aloricate ciliates (Robertson 1983, Ayukai 1987, Jonsson & Tiselius 1990). When offered a ciliate/phytoplankton mixture, copepod clearance rates are found to be significantly higher on ciliates (Stoecker & Sanders 1985, Stoecker & Egloff 1987, Wiadnyana & Rassoulzadegan 1989). Recent studies of copepods feeding on natural microplankton assemblages, from estuarine to oceanic waters, suggest ciliates supply from 1 to 80 % of total carbon ingested (Tiselius 1989, Dolan 1991, Gifford & Dagg 1991, Gifford 1993a). Generally, a greater percentage of ingested carbon is supplied by ciliates when phytoplankton stocks are low or dominated by small cells. Kleppel et al. (1988) found phagotrophic protist carbon (detected by the presence of specific carotenoid pigments) dominated gut contents of copepods off southern California, USA, when phytoplankton biomass and productivity were relatively low.

This paper presents results from a series of copepod grazing experiments conducted with natural microplankton assemblages from Oregon coastal waters between January and August of 1991. We found that calanoid copepods grazed phagotrophic, aloricate ciliates on most sampling dates, those exceptions occurring in late July and August during upwelling-induced diatom blooms. The highest copepod clearance rates on ciliates were measured when the phytoplankton standing stock was < 5 µg chl a l^{-1}, which was the case during the winter months and between upwelling blooms. The results suggest that the degree of trophic linking between protists and copepods in these waters may vary as a function of timing and intensity of upwelling events.

METHODS

Copepods and microplankton assemblages were collected 5 miles off Yaquina Head, Oregon, in 70 m of water. The station is within the upwelling zone on the Newport Hydrographic line (44° 40' N), and was chosen for its historical record of hydrographic and zooplankton population data. Sampling cruises were conducted monthly during January, February, April and June to August of 1991 (twice in July). Copepods were collected in gentle oblique tows from 40 m with a 0.75 m ring net of 335 µm mesh and a 4 l cod end. Water for the grazing experiments was collected from 8 m in Niskin bottles with interior silicon springs. This depth was commonly within the chlorophyll and primary productivity maxima, and was the depth from which water was obtained for protistan herbivory experiments conducted on the same dates (Neuer & Cowles unpubl.). The microplankton assemblage was collected by gently filtering water through a submerged 200 µm mesh to remove macrozooplankton. The filtrate was stored in a water bath under a neutral density screen during transport to the shore-based laboratory. A 200 ml aliquot was preserved with 10 % (v/v) acid Lugols solution for microzooplankton enumeration. Samples for *in situ* chlorophyll analysis were size-fractioned in the lab, collected on GF/F filters, and extracted in 90 % acetone for 24 h at 0 °C. Temperature and salinity were recorded using a portable CTD.

Copepod clearance rates were determined by measuring the change in chlorophyll concentration and ciliate density during bottle incubations (Gifford 1993b). Copepods were kept in a cooler and sorted under a dissecting scope immediately upon returning to the lab. The most abundant calanoid copepod, > 335 µm, was chosen for the grazing experiment on each sampling date. Adult females or copepodite stage CVs were placed in 1 l beakers containing the microplankton assemblage, kept in the dark at 10 °C, and allowed to acclimate for 24 h prior to experimental incubations. As the counting variation typically encountered when enumerating protists is 20 %, it is necessary to separate the copepod feeding signal from the variation (Gifford 1993b). Therefore, body size and known phytoplankton clearance rates of the copepod species selected for each experiment were taken into consideration when determining how many copepods to add to the experimental bottles (10 to 25 copepods bottle^{-1}).

The microplankton assemblage was kept in the dark at 10 °C for 24 h while copepods acclimated, and at the start of an experiment was mixed gently and siphoned into 2 l polycarbonate bottles using silicon tubing. Three control (microplankton assemblage only) and 3 or 4 experimental (microplankton assemblage plus copepods) bottles were filled along with 1 'initial assemblage' bottle. Aliquots for chlorophyll analysis were taken immediately from the 'initial assemblage' bottle, size-fractioned, collected on GF/Fs, and extracted in 90 % acetone. A 200 ml sample (preserved in

10 % acid Lugols solution) was also taken from the initial assemblage for enumeration of ciliates. As treatment bottles were not sampled until the end of an experiment, they were free of air space or bubbles which are a known cause of protist mortality (Gifford 1993b). The bottles were secured to a plankton wheel rotating at 0.3 rpm. Experimental incubations were conducted at 10 °C (range of *in situ* temperature at 8 m: 8 to 11 °C). The winter and spring experiments were incubated for 8 h, at night, in the dark. The summer incubations ran for 12 h (evening and night) and were exposed to the local light regime, resulting in 7 h of darkness and 5 h of light (100 µE).

At the end of the experiments, chlorophyll samples were taken in triplicate from each bottle, filtered onto GF/F filters, and extracted in 90 % acetone. Chlorophyll and phaeopigment were analyzed using a Turner Designs Model-10 fluorometer (Strickland & Parsons 1972). Microzooplankton samples were collected from all bottles and preserved in Lugols solution (as above). Preserved microzooplankton were concentrated by settling 50 to 100 ml sample^{-1} for 24 h. The entire settling chamber was enumerated for all ciliates >10 µm using an inverted microscope at 200× magnification. A minimum of 100 aloricate, phagotrophic ciliates was counted for each replicate. Assuming the ciliate distribution in the sample is Poisson, confidence intervals can be obtained for single counts (Lund et al. 1958, Venrick 1978). A count of 100 cells will give a 95 % confidence interval of the estimate within ±20 % of the mean (Lund et al. 1958).

Ciliates were measured and categorized by geometrical shape in order to convert to biovolume, and identified to genus when possible. Five to ten size categories were used for each of 2 shapes: spheres and cones. Aloricate ciliate carbon was estimated using the ratio of 0.19 pg C µm^{-3} (Putt & Stoecker 1989). As this relationship was determined for ciliates preserved in 2 % Lugols solution, an additional shrinkage factor of 1.5 was applied to the ciliate biovolumes in these experiments (D. Stoecker pers. comm.). It should be mentioned that the ciliate carbon estimates presented here are conservative, as oral membranelles were not always included in the biovolume estimates. A carbon:chlorophyll ratio of 50:1 was used for estimating phytoplankton biomass. Dickson (1993) measured particulate nitrogen at the same station, on the same dates, and estimated carbon:chlorophyll ratios were 45 to 50 during both the winter and summer of 1991. Landry & Lorenzen (1989) also used a ratio of 50:1 when calculating zooplankton grazing estimates in Washington, USA, coastal waters.

Copepod clearance rates on phytoplankton (based on chlorophyll) and ciliates were calculated from Frost (1972). Clearance rates were calculated only when the difference in concentration between control and experimental bottles proved significant (*t*-test, p < 0.05). The observed changes in ciliate density exceeded 20 %. This difference is representative of the entire ciliate count (ciliates ranging in size from 10 to 100 µm). Copepod clearance rates on specific size categories of ciliates and chlorophyll were not analyzed in these experiments. The coefficient of variation between replicate chlorophyll samples was 5 %. Ingestion rates were calculated by multiplying clearance rate by the initial standing stock (Marin et al. 1986). Ingestion rates calculated in this way will be slightly higher than those calculated using the logarithmic mean prey concentration during a given experiment (Frost 1972).

The Bakun upwelling index (Bakun 1973; made available by the NOAA/NMFS Pacific Fisheries Environmental Group, Monterey, CA, USA) was used as a measure of the strength of the wind-induced coastal upwelling on all sampling dates (Fig. 1). The index, presented as weekly averages at 45° N, 125° W, has units of m^3 s^{-1} (100 m of coastline)$^{-1}$. A positive index indicates water is driven offshore, while a negative index indicates downwelling. Indices >50 reflect strong upwelling along this coastline (Small & Menzies 1981).

RESULTS

The microplankton assemblage used in the experiments was collected on the same day as the copepods, due to constraints on boat time, and thus sat for 24 h (in the dark at 10 °C), while the copepods were acclimating. The phytoplankton biomass increased on average by 50 % in the absence of large grazers. The increase occurred in both the 3 to 20 µm and >20 µm chlorophyll size ranges. The ciliate density increased on average by 43 %, but showed little change in size distribution. On the April and late July dates, ciliate mortality occurred in the microplankton assemblage and ciliate density was ca 45 % lower in the experimental assemblage than in the field.

Chlorophyll concentrations ranged from 2 to 45 µg l^{-1} in the experimental microplankton assemblages (2 to 33 µg l^{-1} *in situ*) between January and August of 1991 (Table 1). The late July and August sampling dates coincided with strong upwelling events (Fig. 1). On these dates, chlorophyll concentration in the experimental assemblage was ca 45 µg l^{-1} and more than 80 % of the phytoplankton biomass was composed of diatoms >20 µm (Fig. 2). On all other dates, with the exception of June (11.2 µg chl *a* l^{-1}), the chlorophyll concentration was <5 µg l^{-1}. During January, February and early July, the majority of the phytoplankton biomass was in the <20 µm fraction (Fig. 2).
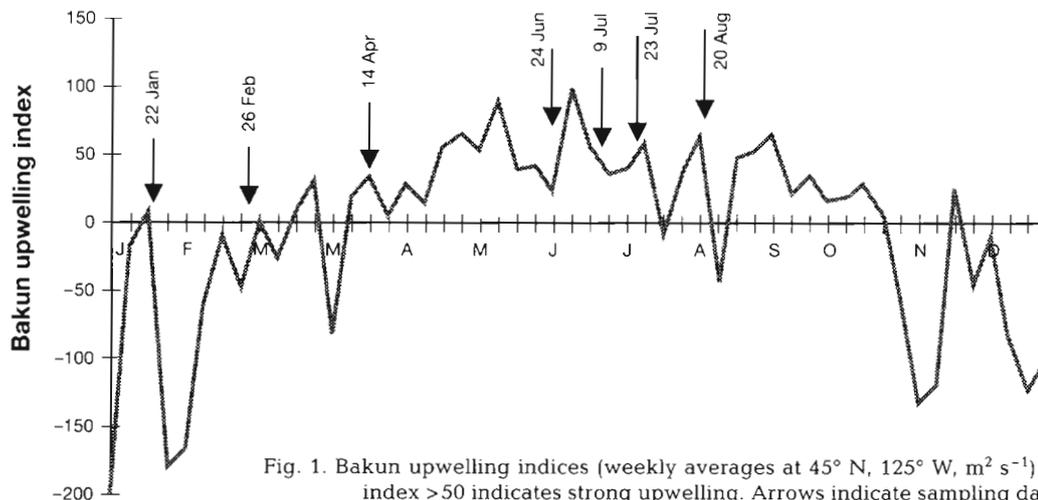
Fig. 1. Bakun upwelling indices (weekly averages at 45° N, 125° W, m² s⁻¹) for 1991. An index > 50 indicates strong upwelling. Arrows indicate sampling dates

Phagotrophic ciliate carbon was ≪10% of the phytoplankton carbon on all but the February sampling date, when it was 32%. Ciliate densities ranged from 1200 to 7700 cells l^{-1} (1100 to 6500 *in situ*), with highest abundances in February and during the August upwelling bloom (Table 1). Aloricate choreotrichous genera were most prominent. Tintinnids were present but not abundant (<100 l^{-1}). The prominent mixotrophs were *Laboea* spp. and *Tontonia* spp., and their contribution to total numbers was greatest in January and February (ca 50%). No large predatory ciliates, such as *Dindinium* spp., were observed on any of the sampling dates.

Growth of ciliates in the controls was observed in January, June and late July, and ranged from 8 to 35% (mean 25%). Mortality was observed in the February, April, July and August experiments, ranging from 6 to 46% (mean 20%). There was no apparent response of copepod clearance rates to either mortality or growth of the ciliates (Table 2).

Copepods cleared ciliates during the 5 grazing experiments conducted between January and early July, when chlorophyll concentrations did not exceed

Table 1. Initial conditions for copepod grazing experiments conducted in 1991

Date	Chl a (µg l^{-1})	Aloricate ciliates >10 µm (ind. l^{-1})	Phytoplankton carbon (µg l^{-1})	Ciliate carbon (µg l^{-1})
22 Jan	3.4	4810	170	11
26 Feb	2.0	7370	100	32
14 Apr	4.6	1200	230	3
24 Jun	11.2	1700	560	3
9 Jul	4.7	2200	235	6
23 Jul	44.3	3500	2215	8
20 Aug	44.6	7700	2230	15

12 µg l^{-1} (Table 2). In late July and August, when upwelling events induced diatom bloom conditions (44 µg chl $a\,l^{-1}$), no significant clearance of ciliates was measured.

The highest clearance rates on ciliates were measured for *Calanus pacificus* in January, 12.6 to 32.4 ml copepod⁻¹ h⁻¹ (Table 2). However, no clearance of phytoplankton by *C. pacificus* was detected. In February, *Pseudocalanus* sp. cleared ciliates at rates of 4.7 to 7.4 ml copepod⁻¹ h⁻¹, and chlorophyll at rates of 0.8 to 3.5 ml copepod⁻¹ h⁻¹. *Centropages abdominalis* was the most abundant mesozooplankton grazer available for experiments in April, June and early July. This copepod cleared aloricate ciliates at rates of 6.3 to 7.1 ml copepod⁻¹ h⁻¹ in April and early July, and at somewhat lower rates, 1.2 to 3.4 ml copepod⁻¹ h⁻¹, in June. *Centropages abdominalis* cleared phytoplankton at measurable rates only on the July date (0.4 to 1.2 ml copepod⁻¹ h⁻¹). Only 2 replicates are reported for 9 July due to copepod mortality in one of the bottles.

No detectable clearance of ciliates was measured for *Acartia longiremis* and *Calanus pacificus* during the diatom blooms in late July and August respectively. Clearance rates based on chlorophyll removal were 0.0 to 0.7 ml copepod⁻¹ h⁻¹ for *A. longiremis* and 0.3 to 0.9 ml copepod⁻¹ h⁻¹ for *C. pacificus* (Table 2).

Ingestion of ciliate carbon, in µg C copepod⁻¹ d⁻¹, averaged 6.5 (±2.4) for *Calanus pacificus*, 4.6 (±1.1) for *Pseudocalanus* sp. and 0.7 (±0.4) for *Centropages abdominalis* (Table 3). The highest weight-specific ingestion rate of ciliates was found for *Pseudocalanus* spp., which ingested 58% of its body carbon. This was an order of magnitude higher than the weight-specific ingestion measured for *Calanus pacificus* (6% of body carbon) and *Centropages abdominalis* (1 to 7% body carbon).
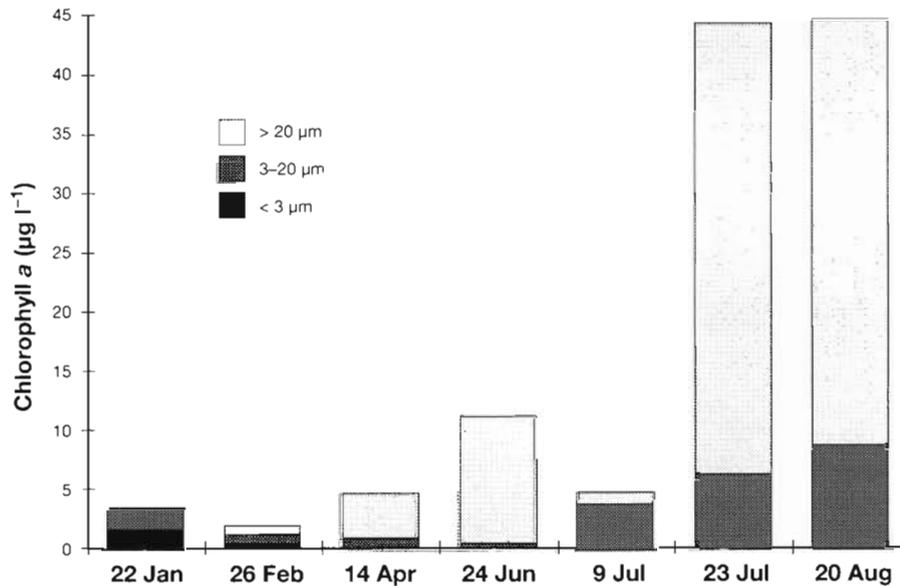
Fig. 2. Initial phytoplankton biomass for grazing experiments conducted in 1991 (in terms of size-fractioned chlorophyll *a*)

Table 2. Measured and calculated results of copepod grazing experiments conducted in 1991: phytoplankton (in terms of chl *a*) and ciliate growth rate (*k* ± SD), decline rate (*g*) in the presence of copepods, and copepod clearance rates. All experimental replicates presented. ns: no significant difference between control and experimental prey densities, p > 0.05

Date	Copepod species	k (h^{-1})		g (h^{-1})		Clearance rates (ml copepod^{-1} h^{-1})	
		Chl	Ciliates	Chl	Ciliates	Chl	Ciliates
22 Jan	*Calanus pacificus* (female)	−0.0169 (0.0000)	0.0442 (0.0035)	ns ns ns ns	0.1318 0.1392 0.0992 0.0541	ns ns ns ns	32.4 30.7 23.1 12.6
26 Feb	*Pseudocalanus* sp. (female)	−0.0239 (0.0055)	−0.0248 (0.0260)	0.0228 0.0151 0.0052 0.0114	0.0312 0.0306 0.0474 0.0461	3.5 2.3 0.8 1.8	4.8 4.7 7.4 7.2
14 Apr	*Centropages abdominalis* (female)	−0.0010 (0.0116)	−0.0578 (0.0230)	ns ns ns	0.0459 0.0443 0.0443	ns ns ns	7.1 6.9 6.9
24 Jun	*Centropages abdominalis* (female)	0.0096 (0.0015)	0.0065 (0.0147)	ns ns ns	0.0360 0.0250 0.0128	ns ns ns	3.4 2.3 1.2
9 Jul	*Centropages abdominalis* (female)	0.0146 (0.0020)	−0.0052 (0.0043)	0.0033 0.0099	0.0542 0.0559	0.4 1.2	6.3 6.5
23 Jul	*Acartia longiremis* (female)	0.0238 (0.0006)	0.0270 (0.0253)	0.0063 0.0035 −0.0002	ns ns ns	0.7 0.4 0.0	ns ns ns
20 Aug	*Calanus pacificus* (CV)	0.0025 (0.0033)	−0.0076 (0.0217)	0.0065 0.0065 0.0022	ns ns ns	0.9 0.9 0.3	ns ns ns

Table 3. Copepod ingestion rates (± SD) on phytoplankton (chl a) and ciliate prey in Oregon coastal waters, total carbon ingested per day, and carbon required for respiration[a]. ns: no significant clearance rate measured, p = 0.005

Date	Copepod species	Ingestion rates (μg C copepod^{-1} d^{-1})		Total carbon ingested (μg C copepod^{-1} d^{-1})	Respiration requirement[a] (μg C copepod^{-1} d^{-1})
		Chl a	Ciliates		
22 Jan	*Calanus pacificus*	ns	6.5 (2.4)	6.5 (2.4)	6.5
26 Feb	*Pseudocalanus* sp.	5.0 (2.7)	4.6 (1.1)	9.6 (1.9)	0.6
14 Apr	*Centropages abdominalis*	ns	0.5 (0.01)	0.5 (0.01)	1.1
24 Jun	*Centropages abdominalis*	ns	0.2 (0.06)	0.2 (0.06)	1.1
9 Jul	*Centropages abdominalis*	4.6 (3.2)	0.9 (0.02)	5.5 (3.0)	1.1
23 Jul	*Acartia longiremis*	23.1 (16.2)	ns	23.1 (16.2)	0.3
20 Aug	*Calanus pacificus*	37.3 (18.5)	ns	37.3 (18.5)	6.5

[a] $R = 0.101\ W_c^{0.884}$ (Dagg et al. 1982)

DISCUSSION

Omnivory is especially apparent among copepods living in highly dynamic environments which exhibit numerous phytoplankton bloom cycles (Landry 1981). Our results reinforce previous observations of a protist-copepod trophic link being tied into such cycles (Gifford & Dagg 1991). We found that calanoid copepods in Oregon coastal waters cleared aloricate, phagotrophic ciliates from natural microplankton assemblages at considerably higher rates than they cleared phytoplankton, except during upwelling-induced diatom blooms. During non-upwelling months and between bloom events, ciliates contributed 16 to 100 % of the estimated carbon ingested by copepods, even though ciliate biomass was significantly less than phytoplankton biomass.

Ciliates comprised 100 % of the estimated carbon ingested by *Calanus pacificus* in January when the entire phytoplankton assemblage was < 20 μm in size and about half of it was < 3 μm (see Fig. 2). In February, *Pseudocalanus* sp. averaged 52 % phytoplankton and 48 % ciliate carbon in its daily ration (Table 3). The chlorophyll biomass was still low, but diatoms > 20 μm accounted for 42 % of it. *Pseudocalanus* sp. cleared ciliates at rates 1.3 to 9.3 times higher than rates measured for clearance of chlorophyll. On 9 July, 16 % of the carbon ingested by *Centropages abdominalis* was attributable to ciliates and 84 % to phytoplankton. On that date, 20 % of the chlorophyll biomass was > 20 μm. *C. abdominalis* cleared ciliates at rates 5 to 15 times higher than they cleared chlorophyll. Similar results were found for *Acartia tonsa* in Terrebonne Bay, on the inshore Gulf of Mexico (Gifford & Dagg 1991). *A. tonsa* cleared protists at rates an order of magnitude higher than those measured for chlorophyll. Aloricate ciliates dominated the protist population in the Gulf, and they contributed 50 % of the carbon ingested by *A. tonsa* when the chlorophyll

standing stock averaged < 5 μm in size (August). Ciliates comprised only 2 % of the carbon ingested by *A. tonsa* in January, when the chlorophyll standing stock was dominated by large diatoms > 20 μm. During both seasons in Terrebonne Bay, the chlorophyll biomass was high (± 30 μg l^{-1}).

High chlorophyll biomass in our experiments was only associated with diatom bloom conditions. In general, ciliates made the largest contribution to copepod diet when large diatoms (> 20 μm) composed < 50 % of the chlorophyll biomass. These results are consistent with the observed grazing behavior of 3 species of *Calanus* in open and ice-edge areas of the Greenland Sea (Barthel 1988). In the diatom bloom conditions of the shelf polyna, copepods ingested diatoms, although ciliate biomass was substantial. In pack-ice regions where the phytoplankton was dominated by small flagellates, copepods primarily ate ciliates. In open waters, where diatom and ciliate biomass were roughly equal, copepods ingested both diatoms and ciliates.

We observed an exception to this trend in the April and June experiments, when *Centropages abdominalis* did not clear chlorophyll at detectable rates even though large diatoms were present. Ingestion of ciliates alone did not meet the estimated respiration requirements (Dagg et al. 1982) of *C. abdominalis* on these dates (Table 3). Copepods in all other experiments satisfied their respiration requirements with the ingestion of ciliates or phytoplankton or the combination of both. *Centropages* is known to tend toward carnivory (Paffenhoffer & Knowles 1980), and it is likely that they were feeding on protists or nauplii that weren't accounted for in these experiments.

Clearance rates on ciliates for *Centropages abdominalis* ranged from 28 to 170 ml copepod^{-1} d^{-1}. These rates are in the same range as those measured for *C. hamatus* (32 to 125 ml copepod^{-1} d^{-1}) feeding on ciliates in a natural microplankton assemblage in coastal

Sweden (Tiselius 1989). In June, clearance rates on ciliates for *C. abdominalis* were much lower (25 to 50 ml copepod^{-1} d^{-1}) than those measured in April and early July (150 to 170 ml copepod^{-1} d^{-1}). The lower clearance rates in June may be due to a shift in the prey size distribution (50% of the ciliates were < 20 µm in size). With the exception of June, cell size of the aloricate ciliate populations during all other experiments averaged 30 to 60 µm.

Copepod clearance rates on dinoflagellates were not determined. Neuer (1992), using epifluorescence microscopy, found heterotrophic gymnodinoid dinoflagellates were more numerous than ciliates in these waters on the same sampling dates. However, ciliate biomass equaled or exceeded the dinoflagellate biomass on all but the August date. Assuming copepods cleared heterotrophic dinoflagellates at the same rate as they cleared ciliates, the additional protist carbon ingested would come close to balancing the respiration requirements of *Centropages abdominalis*.

Copepod omnivory is to be likely unnecessary during diatom bloom conditions. In contrast to the high clearance rates of *Calanus pacificus* on ciliates in January, no clearance on ciliates was detected for either *C. pacificus* or *Acartia longiremis* during the upwelling-induced diatom blooms in August and late July respectively. Clearance rates on chlorophyll were low for both species (see Table 2), but the chlorophyll biomass was so high (44 µg l^{-1}) that phytoplankton carbon ingested averaged 23 µg copepod^{-1} d^{-1} for *A. longiremis* (578% of body carbon) and 37 µg copepod^{-1} d^{-1} for *C. pacificus* (33% of body carbon; Table 3).

It is important to restate that the experimental food concentrations in our study did not exactly replicate field conditions. Because of the 24 h acclimation period, the experimental microplankton assemblages often had higher concentrations of both ciliates and phytoplankton than the *in situ* assemblages on the actual sampling dates. The composition of the assemblage was not greatly changed, however, and the increased prey concentrations still fell in the range of what copepods experience in these waters. The largest increase between experimental and *in situ* phytoplankton biomass occurred during the June experiment (138%), but as no clearance rate on phytoplankton for *Centropages abdominalis* was detected, results were not affected. A complete doubling of the ciliate population occurred between the August sampling and experimental incubation. In this case, no clearance on ciliates was detected for *Calanus pacificus* so the extreme increase again did not sway experimental results. In the 3 experiments done with *Centropages abdominalis*, similar clearance rates on ciliates were measured on 2 dates (April and early July) when the

ciliate density differed by a factor of 2 (Table 1). Although the prey densities in the incubations differed somewhat from the field, we feel the results can still be safely applied to field conditions.

The presence of predatory ciliates (2 predator-prey systems in the same bottle) and mixotrophic ciliates (counted twice, once as chlorophyll, once as protist) in the natural assemblages could bias the outcome of this type of grazing experiment. We did not observe large predatory ciliate species on any of our sampling dates. Phytoplankton biomass was much greater than ciliate biomass, with the exception of the February date, so that the mixotroph contribution to the total chlorophyll was probably small. In February, however, mixotrophs represented 50% of the phagotrophic ciliate count (according to epifluorescence counts by Neuer 1992) and as chlorophyll biomass was low, the clearance rates on chlorophyll for *Pseudocalanus* sp. may be biased by the presence of mixotrophs. The autotrophic ciliate *Mesodinium rubrum* was also abundant in February (4000 l^{-1}; Neuer 1992), and some individuals may have been mistakenly included in the phagotrophic protists counts.

Ciliate densities observed in this study are in the range of those previously reported for coastal waters (Sherr et al. 1986, Burkill et al. 1987, Gifford 1988, Montagnes et al. 1988, Tiselius 1989), although somewhat lower than the high end of the range (2700 to 27800) reported by Landry & Hassett (1982) in Washington coastal waters. An estimate of copepod predation on ciliate biomass was obtained by applying average clearance rates to historical average abundance estimates of *Pseudocalanus* spp., *Calanus marshallae* (assume similar rate to *Calanus pacificus*), and *Centropages abdominalis* at the same station (Peterson & Miller 1976). Ignoring fine-scale vertical differences, and considering only these 3 species, the estimated grazing impact is 25% of the ciliate population removed per day. This is likely an underestimate, since *Acartia clausii* and *A. longiremis* both play a numerically important role in the copepod assemblage at this location during the summer months (Peterson & Miller 1976). Including them in the estimate would no doubt increase the impact. Also, copepod nauplii are known to consume phagotrophic protists. Dolan (1991) measured clearance rates of 0.4 ml nauplius^{-1} h^{-1} for *A. tonsa* nauplii on phagotrophic protists in Chesapeake Bay. Applying this clearance rate to abundance estimates of the feeding stages of copepod nauplii (Peterson & Miller 1976, Landry & Lorenzen 1989) adds an additional impact of 10 to 20% clearance per day on the ciliate population.

Neuer (1992) found that although ciliates and heterotrophic dinoflagellates ingested both phototrophic and heterotrophic prey in Oregon coastal waters in

1991, gymnodinoid dinoflagellates ingested a greater percentage of phototrophic food sources during upwelling bloom periods, whereas the ciliate impact was on heterotrophic food sources. We are presently working up the results of a second field season in which the contribution of heterotrophic dinoflagellates to copepod diet in these waters is examined.

An altered view of pelagic carbon flux is suggested by the concept of the microbial food web serving as the principal food resource for metazooplankton (Sherr & Sherr 1988). In productive coastal waters, the consumption of primary production by heterotrophic protists versus rapid sinking of a diatom bloom implies carbon is available in the water column to metazoan consumers, or for remineralization, for a longer period of time. If protists are consuming as much as 50 % of the primary production in neritic waters, the existence of a protist-metazoan link would greatly influence the fate of that carbon. Copepods contribute disproportionately to carbon flux due to the rapid sinking of their fecal pellets. Grazing and sediment trap measurements made in Washington shelf waters show micro-crustaceans contributing up to 21 % of the grazing pressure on phytoplankton, but 65 % of the vertical flux of organic carbon (Landry & Lorenzen 1989).

There is no evidence for mass sinking of diatom blooms in the Washington and Oregon coastal upwelling system. Landry & Lorenzen (1989) maintain that the vast majority of Washington shelf production is utilized locally and that up to 50 % is grazed by protists. Neuer & Cowles (unpubl.) found the protist grazing impact to be of similar magnitude on the Oregon shelf. Our results illustrate the potential for a substantial portion of protist secondary production to be consumed by copepods between diatom bloom events, and therefore to be linked to the carbon flux to deeper water or shelf sediments.

LITERATURE CITED

Ayukai, T (1987). Predation by *Acartia clausi* (Copepoda, Calanoida) on two species of tintinnids. Mar. microb. Food Webs 2: 45–52

Bakun, A. (1973). Coastal upwelling indices, west coasts of North America, 1946–71. U.S. Dept Commerce, NOAA Tech. Rep. NMFS-SSRF 471

Barthel, K.-G. (1988). Feeding of three *Calanus* species on different phytoplankton assemblages in the Greenland Sea. Meeresforsch. 32: 92–106

Berggreen, U., Hansen, B., Kiørboe, T. (1988). Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar. Biol. 99: 341–352

Burkill, P. H., Mantoura, R. F. C., Llewellyn, C. A., Owens, N. J. P. (1987). Microzooplankton grazing and selectivity of phytoplankton in coastal waters. Mar. Biol. 93: 581–590

Dagg, M. J., Vidal, J., Whitledge, T. E, Iverson, R. L., Goering, J. J. (1982). The feeding, respiration, and excretion of zooplankton in the Bering Sea during a spring bloom. Deep Sea Res. 29: 45–63

Dickson, M. L. (1994). Nitrogen dynamics in a coastal upwelling regime. Ph.D. thesis, Oregon State University, Corvallis

Dolan, J. R. (1991). Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods. Mar. Biol. 111: 303–309

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 17: 805–815

Gifford, D. J. (1988). Impact of grazing by microzooplankton in the northwest arm of Halifax Harbour, Nova Scotia. Mar. Ecol. Prog. Ser. 47: 249–258

Gifford, D. J. (1991). The protozoa-metazoan trophic link in pelagic ecosystems. J. Protozool. 38: 81–86

Gifford, D. J. (1993a). Protozoa in the diets of *Neocalanus* spp. in the Oceanic Subarctic Pacific Ocean. Progr. Oceanogr 32: 223–237

Gifford, D. J. (1993b). Consumption of protozoa by copepods feeding on natural microplankton assemblages. In: Kemp, P. F., Sherr, B. F., Sherr, E. B., Cole, J. J. (eds.) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p. 723–729

Gifford, D. J., Dagg, M. J. (1991). The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchrus* Murukawa. Mar. microb. Food Webs. 5: 161–177

Gowing, M. M., Silver, M. W. (1985). Minipellets: a new and abundant size class of marine fecal pellets. J. mar. Res. 43: 395–418

Jonsson, P. R., Tiselius, P. (1990). Feeding behavior, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. Mar. Ecol. Prog. Ser. 60: 35–44

Kleppel, G. S., Frazel, D., Pieper, R. E., Holliday, D. V. (1988). Natural diets of zooplankton off southern California. Mar. Ecol. Prog. Ser. 49: 231–241

Kleppel, G. S., Holliday, D. V., Pieper, R. E. (1991). Trophic interactions between copepods and microplankton: a question about the role of diatoms. Limnol. Oceanogr. 36: 172–178

Landry, M. R. (1981). Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. Mar. Biol. 65: 77–82

Landry, M. R., Hassett, R. P. (1982). Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67: 283–288

Landry, M. R., Lorenzen, C. J. (1989). Abundance, distribution, and grazing impact of zooplankton on the Washington shelf. In: Landry, M. R., Hickey, B. M. (eds.) Coastal oceanography of Washington and Oregon. Elsevier, Amsterdam, p. 175–210

Lund, J. W. G., Kipling, C., Le Cren, E. D. (1958). The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143–170

Marin, V., Huntley, M. E., Frost, B. (1986). Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. Mar. Biol. 93: 49–58

Montagnes, D. J. S., Lynn, D. H., Roff, J. C., Taylor, W. D. (1988). The annual cycle of heterotrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assessment of their trophic role. Mar. Biol. 99: 21–30

Neuer, S. (1992). Role of protist grazing in the Oregon Upwelling System. Ph.D. thesis. Oregon State University, Corvallis

Paffenhöfer, G. A., Knowles, S. C. (1980). Omniverousness in marine copepods. J. Plankton Res. 2: 355–365

Peterson, W. T., Miller, C. B. (1976). Zooplankton along the continental shelf off Newport, Oregon, 1969–1972: distribution, abundance, seasonal cycle and year-to-year variations. Publication no. ORESU-T-76-002, Oregon State University Sea Grant College Program, Corvallis

Putt, M., Stoecker, D. K. (1989). An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34: 1097–1107

Robertson, J. R. (1983). Predation by estuarine zooplankton on tintinnid ciliates. Estuar. coast. Shelf Sci. 16: 27–36

Sherr, B., Sherr, E. (1989). Trophic impacts phagotrophic protozoa in pelagic food webs. In: Hatton, T., et al. (eds.) Recent advances in microbial ecology. Japan Scientific Societies Press, Tokyo, p. 388–394

Sherr, E., Sherr, B. (1988). Role of microbes in pelagic food webs: a revised concept. Limnol. Oceanogr. 33: 1225–1227

Sherr, E., Sherr, B. (1992). Trophic roles of pelagic protists: phagotrophic flagellates as herbivores. Arch. Hydrobiol. Beih. Ergebn. Limnol. 37: 165–172

Sherr, E., Sherr, B. F., Paffenhöfer, G. A. (1986). Phagotrophic protozoa as food for metazoans: a 'missing' trophic link in marine pelagic food webs? Mar. microb. Food Webs 1 61–80

Small, L. F., Menzies, D. W. (1981). Patterns of primary productivity and biomass in a coastal upwelling region. Deep Sea Res. 28: 123–149

Stoecker, D. K. (1984). Particle production by planktonic ciliates. Limnol. Oceanogr. 29: 930–940

Stoecker, D. K., Capuzzo, J. M. (1990). Predation on protozoa: its importance to zooplankton. J. Plankton Res. 12: 891–908

Stoecker, D. K., Egloff, D. E. (1987). Predation by Acartia tonsa Dana on planktonic ciliates and rotifers. J. exp. mar. Biol. Ecol. 110: 53–68

Stoecker, D. K., Sanders, N. K. (1985). Differential grazing by Acartia tonsa on a dinoflagellate and a tintinnid. J. Plankton Res. 7: 85–100

Strickland, J. H. D., Parsons, T. R. (1972). A practical handbook of seawater analysis, 2nd edn. Bull. Fish. Res. Bd Can. 167: 1–310

Tiselius, P. (1989). Contribution of aloricate ciliates to the diet of Acartia clausi and Centropages hamatus in coastal waters. Mar. Ecol. Prog. Ser. 56: 49–56

Venrick, E. L. (1978). Statistical considerations. In: Sournia, A. (ed.) Phytoplankton manual. UNESCO, Paris, p. 69–74

Welschmeyer, N. A., Lorenzen, C. A. (1985). Pigment budgets: zooplankton grazing and growth in a temperate fjord and the Central Pacific Gyre. Limnol. Oceanogr. 30: 1–21

Wiadnyana, N. N., Rassoulzadegan, F. (1989). Selective feeding of Acartia clausi and Centropages typicus on microzooplankton. Mar. Ecol. Prog. Ser. 53: 37–45