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Abundance and biomass of copepod nauplii and ciliates and herbivorous activity of microzooplankton in the East China Sea

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Abstract: The abundance and biomass of copepod nauplii and ciliates and herbivorous activity of microzooplankton were studied in July 1998 in the East China Sea as a part of the China JGOFS–LOICZ study. The total abundance and biomass of ciliates and copepod nauplii ranged between 10 and 1970 indiv. l^{-1} and between 0.08 and 16 μgC l^{-1}, respectively. The community was dominated by ciliates. When integrated over the surface 50-m layer, the total biomass fell in the range of 5–283 mgC m^{-2}. Microzooplankton herbivory, determined using the dilution technique, corresponded to 20 to 48% of the phytoplankton standing stock and 54 to 89% of the primary production per day.

Key words: ciliate, microzooplankton grazing, dilution method, East China Sea

Introduction

Only half of the CO_2 emitted from the burning of fossil fuels is accumulated in the atmosphere (Takahashi 1989). It is supposed that a large part of the CO_2 is transported down into the deep ocean by the "biological pump". The efficiency of the biological pump is influenced by the size-structure of trophic pathways. The fecal pellets of macrozooplankton are large and sink rapidly to the deep ocean before being broken down by bacterial degradation and other processes. Microzooplankton are heterotrophic protists and metazoans that pass through a 200-μm mesh. Because the fecal debris of microzooplankton are small, they sink slowly and are largely remineralized within the euphotic zone. Therefore, microzooplankton grazing on phytoplankton remineralize the particulate carbon back to CO_2 and result in less vertical flux of particulate matter into the deep sea. The JGOFS (Joint Global Ocean Flux Study) programs have taken microzooplankton and their grazing activity as one of their areas of study (Landry et al. 1995; Burkill et al. 1995; Verity et al. 1993, 1996; Garrison et al. 1998; Rivkin et al. 1999).

The East China Sea is a typical mid-latitude marginal sea in the western North Pacific. It covers 7.5×10^5 km^2. Seventy percent of the East China Sea is on the continental shelf and the remaining 30% is basin bordered by the Ryukyu Island Arc. The Yangtze River annually discharges about 9×10^{11} m^3 of fresh water into the East China Sea (Guan & Mao 1982). The river water contains very high concentrations of nitrate and silicate but low phosphate (Gong & Liu 1995). The East China Sea is one of the most productive areas of the world (Wang & Fan 1995). This biological production must have a strong influence on the magnitude of carbon fluxes. The nutrient distributions (Watanabe et al. 1995; Gong & Liu 1995), phytoplankton communities (Kurita et al. 1995) and primary production (Furuya et al. 1995) have been studied in the East China Sea. In the first phase of the Chinese JGOFS study from 1990 to 1994 in the East China Sea, macrozooplankton was found to graze less than 10% of the daily phytoplankton primary production (Wang & Fan 1995). However, data about microzooplankton are sparse in this area. Taniguchi & Ota (1995) studied the biomass and production of microzooplanktonic ciliates in the East China Sea in winter 1992 and summer 1993. As part of the Chinese JGOFS-LOICZ study, we studied the abundance and biomass of copepod nauplii and ciliates, which are important components of microzooplankton. The grazing pressure of microzooplankton on phytoplankton was also studied using the dilution method.

Materials and Methods

Measurements were made on board the R.V. *Science No. 1* during 4–22 July 1998. The study stations are shown in

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Fig. 1A. The sampling was done following JGOFS Core Measurement Protocols (Longhurst et al. 1990). Water samples were collected with 6-liter Niskin bottles on a Rossette CTD from the surface, 10-, 30- and 50-m depths. A 1-liter water sample from each depth was poured into a 1-liter plastic bottle and fixed in 1% Lugol's iodine solution. The bottles were stored cool for less than 2 months in the dark until analyzed in the laboratory. Water temperature and salinity were recorded by a CTD system at each station.

In the laboratory, the fixed water samples were settled for at least 24 h in the plastic bottles. The overlying water was siphoned out to leave a 100-ml aliquot. Then 25 ml (50 or 100 ml if necessary) of each sample was settled in sedimentation chambers and counted using a light microscope (Zeiss) at 150× magnification. In each sample, ciliates (including aloricate ciliates and tintinnids) and copepod nauplii with a maximum preserved dimension of >20 μm were counted.

The dimensions of the ciliates and nauplii were measured using a microscope (Olympus) at high magnification. The cell volume of each species was estimated using appropriate geometric shapes (cones, balls, cylinders, cuniforms and their combinations). The total plasma volume of tintinnids is assumed to occupy 0.3 of the lorica volume (Verity & Langdon 1984; Gilron & Lynn 1989). The carbon : volume ratios used to calculate biomass were 0.05 pgC μm^{-3} for copepod nauplii (Mullin 1969) and 0.19 pgC μm^{-3} for ciliates (Putt & Stoecker 1989).

The dilution incubation experiments were carried out at two anchored (Stns 410, 111) and four grid stations (Stns 102, 105, 205, 909). The experimental protocols of Landry & Hassett (1982) and Burkill et al. (1990) were followed. Experimental equipment including 25-liter polycarbonate carboys, 1.5-liter polycarbonate bottles, glass filter bottles etc. was soaked with 10% HCl and rinsed with filtered seawater before use. At each of the above stations, 24 liters of seawater from 0.5-m depth were collected using Niskin bottles and transferred to a polycarbonate carboy. Part of this water was filtered through GF/F filters. The filered seawater (FSW) was assumed to be free of predators and prey. The FSW was added to make concentrations of 100, 75, 50 and 25% ambient seawater in the respective polycarbonate bottles. It was easy to eliminate the macrozooplankton that were rare in the water samples. From each concentration, 500-ml water samples were taken for the determination of initial chlorophyll-*a* (Chl-*a*) concentration. The remaining water was poured into two 1.5-liter polycarbonate bottles. Caution was taken to avoid the introduction of air bubbles in the bottles. The bottles were incubated in two plastic boxes at 0.5-m depth suspended from the ship or in a temperature-controlled container onboard the ship. In the latter case, surface water was pumped through the container to maintain the temperature. Further samples for Chl-*a* concentration were taken after 24 h of incubation.

Chlorophyll-*a* concentration was determined as follows. Water samples were filtered through GF/F glassfiber filters.

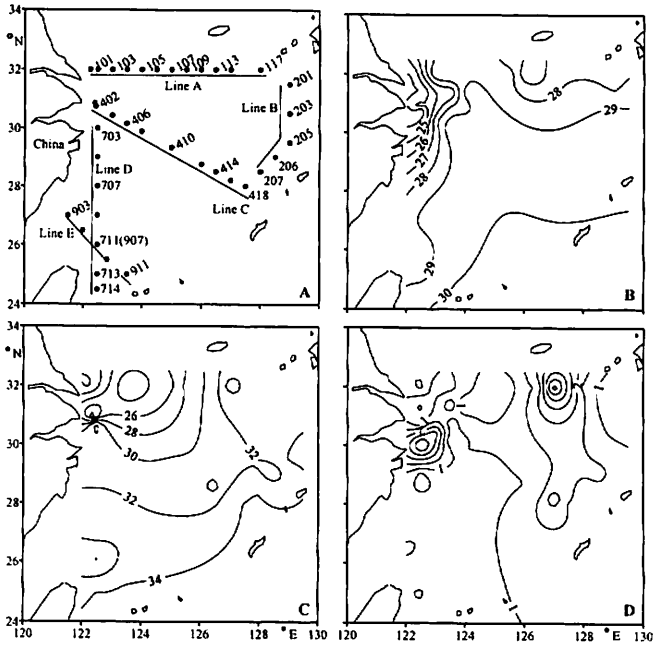

Fig. 1. The study stations (**A**) and the surface temperature (**B**, °C), salinity (**C**) and chlorophyll-*a* concentrations (**D**, $\mu g\,l^{-1}$).

The filters were extracted with 90% acetone at −20°C in darkness for 24 h. The concentration of Chl *a* was determined using a Turner Designs (Model II) fluorometer that was calibrated with pure Chl *a* (Sigma).

According to Landry & Hassett (1982), microzooplankton grazing rate (g, d^{-1}) and the potential phytoplankton growth rate (k, d^{-1}) can be expressed as:

$$1/t \ln (P_t/P_0)=k-c\times g$$

where P_t is the Chl-*a* concentration at time t; P_0 is the initial Chl-*a* concentration; c is the dilution factor. Values of k and g were determined from linear regression of the apparent phytoplankton growth rate against the dilution factor. Microzooplankton grazing pressure on phytoplankton standing stock (Pi) and primary production (Pp) were calculated according to Verity et al. (1993):

$$\mathrm{Pi}=(1-e^{-gt})\times 100\%$$

$$\mathrm{Pp}=(e^{kt}-e^{(k-g)t})/(e^{kt}-1)\times 100\%$$

Results

Water temperature, salinity and chlorophyll-*a* concentration

The surface water temperature was between 24 and 30°C (Fig. 1B). Temperatures near the mouth of the Yangtze River were lower than in the open sea. The vertical distributions of temperature at some selected stations are presented in Fig. 2. Along Line A, there was a thermocline at 0–20 m at stations west of Stn 111. No obvious thermocline was present in the other sections. Surface salinity was in the

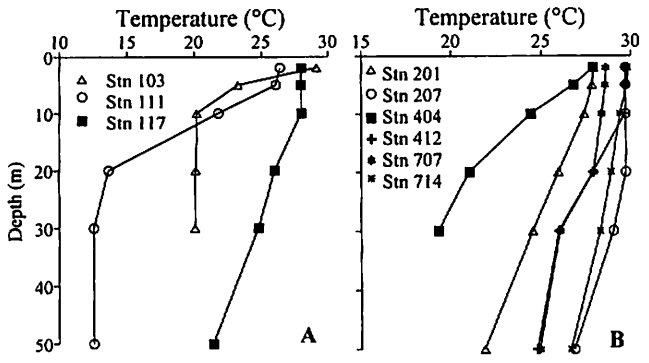

Fig. 2. Vertical profile of temperature at representative stations on Line A (A) and Lines B–D (B).

range of 19–34 (Fig. 1C). The lowest salinity values were at the Yangtze River mouth. No halocline was found at any stations. The surface Chl-*a* concentration ranged between 0.32 (Stn 705) and 4.38 (Stn 115) $\mu g l^{-1}$. The distribution of Chl *a* was obviously patchy (Fig. 1D). For example, Chl-*a* concentration reached its peak of 4.38 $\mu g l^{-1}$ at Stn 115 but fell to 0.44 and 0.53 $\mu g l^{-1}$ at the adjacent stations (Stn 116 and Stn 113, respectively). Chlorophyll-*a* concentration was 3.70 and 0.32 $\mu g l^{-1}$ respectively at Stn 703 and neighboring Stn 705.

Abundance and biomass of copepod nauplii and ciliates

Copepod nauplii were fewer than 20 indiv. l^{-1} at most of the stations. However, at Stns 402 and 703 (Yangtze River mouth), their abundances were 240 and 70 indiv. l^{-1}, respectively. Most of the aloricate ciliates belong to the Family Strombidiidae, Order Oligotrichida. The concentration of aloricate ciliates was high in the surface waters of Stns 103, 105 and 106 with concentrations of 980, 840 and 1970 indiv. l^{-1}, respectively. The other peak was of 730 indiv. l^{-1} at 10-m depth at Stn 705. The concentration of aloricate ciliates at other stations ranged from 0 to 270 indiv. l^{-1}. Tintinnid ciliates were rare at all the stations. The highest concentration was 730 indiv. l^{-1} at Stn 401 where the dominant tintinnid species belonged to the genus *Tintinnopsis*. At the other stations, tintinnid abundances ranged between 0 and 150 indiv. l^{-1}. Total abundances of copepod nauplii and ciliates ranged between 10 and 1970 indiv. l^{-1} (Fig. 3). Except between Stns 103 and 107, total abundances were less than 300 indiv. l^{-1} along Line A. Total abundances were less than 120 indiv. l^{-1} along Line B and decreased from north to south. Along Line C, total abundances were less than 200 indiv. l^{-1} except between Stns 401 and 403. Total abundances decreased from north to south along Line D with the largest concentration of 730 indiv. l^{-1} at 10-m depth at Stn 705. Total abundances were consistently low (50 indiv. l^{-1}) along Line E.

The biomass of copepod nauplii ranged between 0 and 5.7 $\mu gC l^{-1}$. The ciliate biomass ranged between 0.02 and 12.4 $\mu gC l^{-1}$. Total combined biomass of copepod nauplii

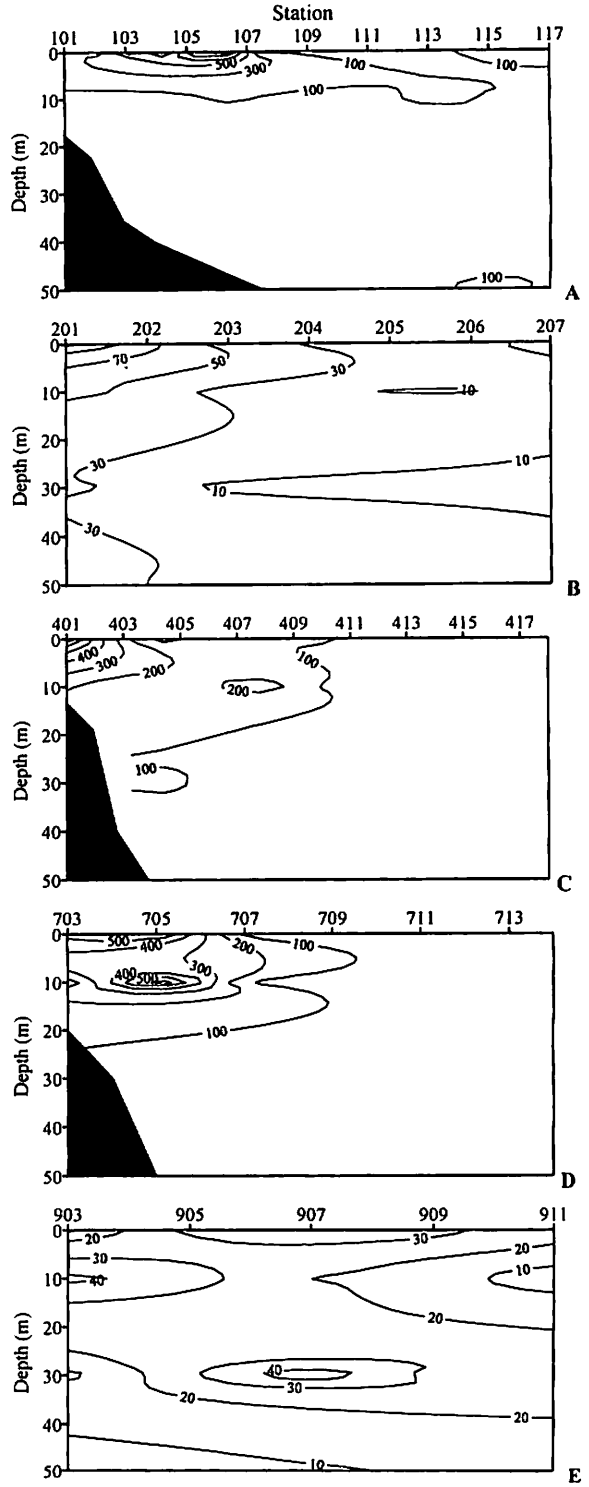

Fig. 3. Total abundance (indiv. l^{-1}) of ciliates and nauplii along Lines A–E (A–E).

and ciliates ranged from 0.08 (30 m at Stn 911) to 16 $\mu gC l^{-1}$ (10 m at Stn 705) as shown in Fig. 4. When integrated, the water column (from 50 m to surface) standing stocks of nauplii and ciliates ranged between 5 and 283 mgC m^{-2} (Fig. 5). Standing stocks higher than 200 mgC m^{-2} were recorded at Stns 401, 404 and 705. At more than 50% of

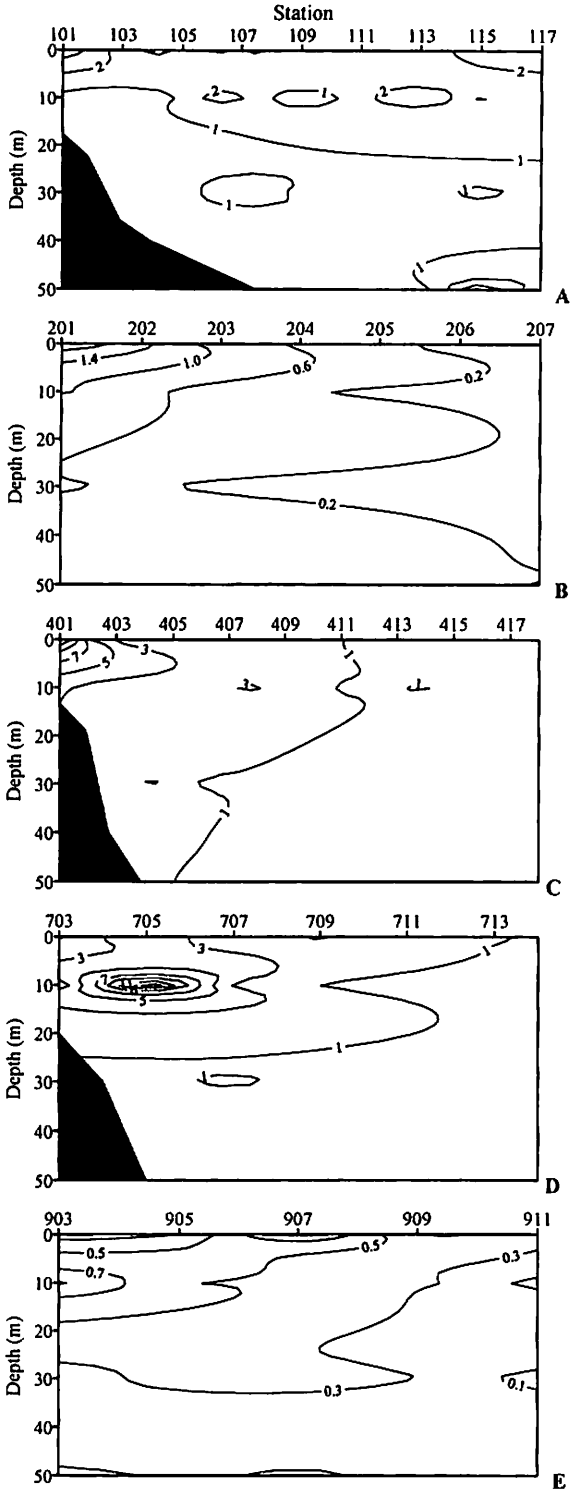

Fig. 4. Total biomass (μgC l^{-1}) of ciliates and nauplii along Lines A–E (A–E).

the stations, the standing stocks were <50 mgC m^{-2}. The water column standing stocks were higher in coastal areas than in offshore oceanic areas.

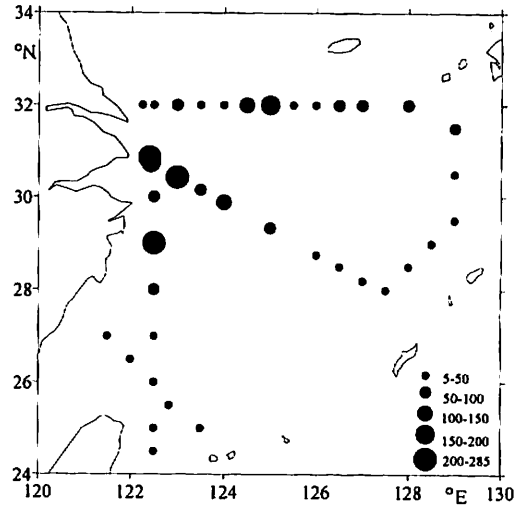

Fig. 5. The surface 50 m integrated biomass (mgC m^{-2}) of ciliates and nauplii. Refer to Fig. 1A for station locations.

Microzooplankton herbivorous activity

Among the seven dilution incubation experiments, three showed microzooplankton grazing, the other four showed no grazing (Fig. 6). According to the three positive experiments, the grazing rates by microzooplankton were 0.22–0.66 d^{-1} (Table 1). The grazing pressure by microzooplankton ranged from 20% to 48% of the phytoplankton standing stock and 54% to 89% of the primary production per day.

Discussion

The microzoopankton biomass ranged between 0.08 and 16 μgC l^{-1} in the surface waters. These values are quantitatively similar to the values between 1 and 30 μgC l^{-1} reported from other oceanic waters (reviewed by Garrison et al. 1998; Howell-Kubler et al. 1996; Porter et al. 1985; Lynn & Montagnes 1991). It has been reported that the biomass of microzooplankton is tightly correlated to that of phytoplankton in estuarine and coastal waters (Burkill 1982; Verity 1987) and in oceanic waters (Burkill et al. 1993). Suzuki & Taniguchi (1998) and Uye et al. (1998) found a positive correlation between total ciliate abundance and Chl-*a* concentration (or <20 μm Chl-*a* concentration). We did not find this relationship in the East China Sea (Fig. 7). Dolan & Coats (1990) were also unable to find a significant correlation in Chesapeake Bay. In general, since ciliates are likely to consume a limited range of the phytoplankton available in natural ecosystems, Chl-*a* concentration is probably a poor indicator of potential ciliate food availability.

Taniguchi & Ota (1995) estimated the water column biomass of microplanktonic ciliates in the East China Sea. In their study, the water column biomass was calculated from the surface to the bottom (200 m when depth >200 m). Their results were 8–90 mgC m^{-2} in winter (February to

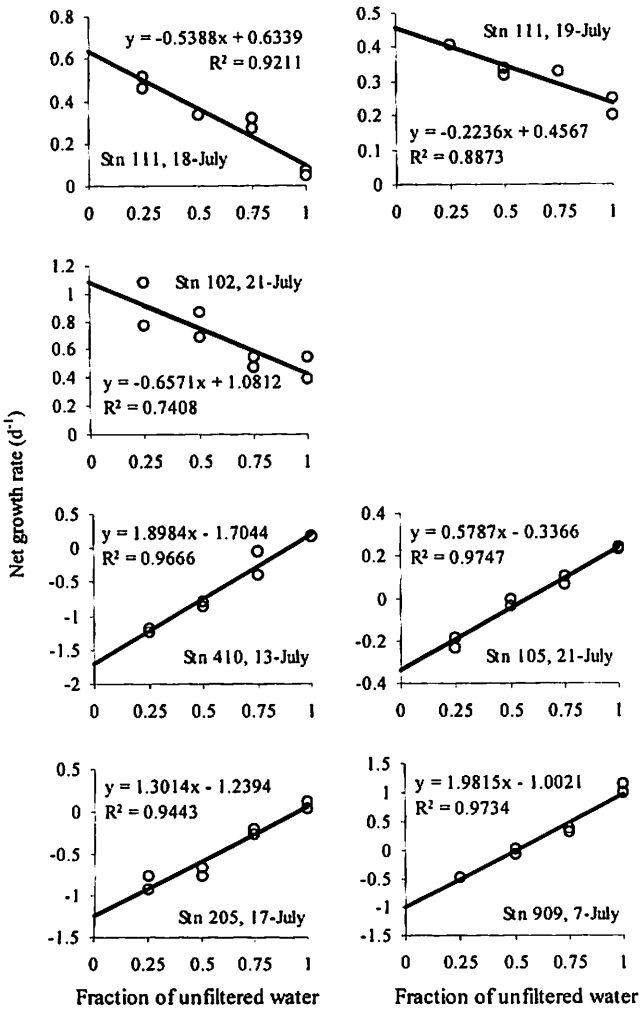

Fig. 6. Apparent growth rate as a function of the dilution factor in the dilution experiments.

Table 1. Results of dilution experiments. *g*: microzooplankton grazing rate; *k*: potential phytoplankton growth rate; Pi and Pp: microzooplankton grazing pressure on phytoplankton standing stock and primary production, respectively.

Station	Date	g (d^{-1})	k (d^{-1})	Pi (%)	Pp (%)	r^2
111	18 July	0.54	0.63	42	89	0.92
111	19 July	0.22	0.46	20	54	0.89
102	21 July	0.66	1.08	48	73	0.74

March, 1992) and 12–50 mgC m^{-2} in summer (September–October, 1993). Our estimation from 4–22 July (5–283 mgC m^{-2}) was comparatively higher.

In order to interpret the results of the dilution experiments, several limitations should be pointed out. First of all, only three of the seven dilution experiments showed microzooplankton grazing. The three experiments were carried out at the coastal stations. Therefore, the results are not representative of the whole of the East China Sea but rather only the coastal waters. Secondly, one of the factors that affects dilution experiments is the nutrient concentration (Landry & Hassett 1982). The nutrient concentrations of dilution incubations in this study are listed in Table 2. According to Paasche & Erga (1988), nutrient limitation is unlikely when nitrate plus ammonium concentration $>1.00\,\mu M$ and phosphate concentration $>0.10\,\mu M$. If this is indeed the case, there was no nutrient limitation in our experiments.

Although four of the seven dilution experiments showed no grazing, the results were of interest. After incubation for 1 d, Chl-*a* concentrations decreased (not increased) sharply in the more diluted treatments, and the regression showed very good linear relationship. This kind of regression has also been observed in the studies of Andersen et al. (1991) and Kamiyama (1994). In the experiments of Andersen et al. (1991), the negative net growth rates were attributed to the low nutrient concentrations. We can not explain why this phenomenon happened in our study despite the comparatively high nutrient concentrations.

Many authors have used dilution methods to study microzooplankton grazing pressure on phytoplankton (Rivkin

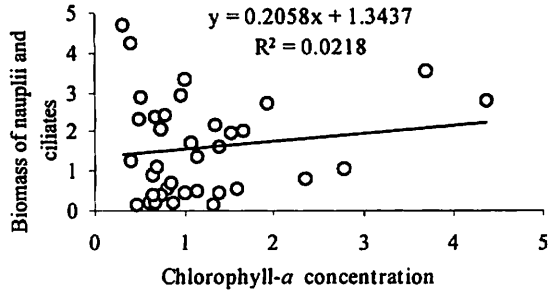

Fig. 7. Inter-relationship between total biomass ($\mu gC\,l^{-1}$) of nauplii and ciliates and chlorophyll-*a* concentration ($\mu g\,l^{-1}$) in the East China Sea surface waters. Station 401 is omitted because there is no chlorophyll-*a* data for this station.

Table 2. Initial chlorophyll *a* ($\mu g\,l^{-1}$) and nutrient concentrations (μM) of the dilution experiments.

Station	111 (18 July)	111 (19 July)	102	105	205	410	909
Chlorophyll *a*	1.45	1.66	1.65	1.39	1.39	0.94	0.64
Nitrate	0.12	0.24	9.95	4.83	0.33	0.24	0.25
Ammonium	2.15	1.29	4.8	1.13	1.61	2.38	3.94
Phosphate	0.23	0.21	0.47	0.22	0.19	0.32	0.29

et al. 1999; Lehrter et al. 1999; Landry et al. 1998; Froneman & Perissinotto 1996; Burkill et al. 1995). According to these authors, microzooplankton usually graze on the phytoplankton at a rate of 0–79% of the standing stock per day. The range for percentage of daily primary production is 0–271%. The results of this study lie in the middle of these ranges.

No data on macrozooplankton grazing on phytoplankton were collected on this cruise. On the China JGOFS cruises in April and October 1994, Wang & Fan (1995) calculated with the gut pigment method that copepods grazed <10% of the daily primary production in the East China Sea. It is clear that the major herbivorous populations are the microzooplankton. As discussed by Burkill et al. (1993), the significance of high microzooplankton grazing pressure is both complex and important. Firstly, since few microzooplankton taxa produce fecal material with significant sinking velocities, phytogenic carbon will merely be recycled in the water column by respiratory processes. Secondly, since microzooplankton can be grazed by organisms that do produce rapidly sinking fecal pellets (e.g. pelagic tunicates), a proportion of the phytoplankton will be exported into deep waters via a two or more step food chain. In view of the significant impact of microzooplankton herbivory on phytoplankton, quantitative measurements are needed on the fate of microzooplankton in the East China Sea.

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