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Grazing Pressure by Ciliates on the Nanoflagellate Community in a Subtropical Pelagic Continental Shelf Ecosystem: Small Ciliates (of < 45 μm) are Major Consumers of the Nanoflagellate Community

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Jun-Yu Chen, An-Yi Tsai, Gwo-Ching Gong, and Kuo-Ping Chiang (2012) Grazing pressure by ciliates on the nanoflagellate community in a subtropical pelagic continental shelf ecosystem: small ciliates (of < 45 μm) are major consumers of the nanoflagellate community. *Zoological Studies* **51**(8): 1308-1318. Trophic relations between nanoflagellates and ciliates in the southern East China Sea (ECS) were studied along a cross-shelf transect in Aug. 2010. Short-term experiments with 4-μm fluorescently labeled beads (FLBs) were used to estimate ingestion rates of the ciliate community at 6 stations. There were 3 zones along the transect: inner-shelf, mid-shelf, and upwelling zones. At all sites, ciliates of < 45 μm in equivalent spherical diameter (ESD), including *Strombidium* spp., *Strobilidium* spp., *Laboea* spp., *Tontonia* spp., and tintinnids, were the most abundant group (79%-95%) of the ciliate community. *Strombidium* spp. ingestion rates ranged 59-374 flagellates/ciliate/d, *Strobilidium* spp. ranged 26-268 flagellates/ciliate/d, *Laboea* spp. and *Tontonia* spp. ranged 18-289 flagellates/ciliate/d, and tintinnids ranged 58-249 flagellates/ciliate/d. Ciliate of < 45 μm in ESD were the most important nanoplankton grazers, consuming nearly 100% of nanoflagellate production in inner-shelf and 43% in offshore oceanic waters. In summer, ciliates are one of the main trophic level consumers of nanoflagellates, especially in inner-shelf waters. http://zoolstud.sinica.edu.tw/Journals/51.8/1308.pdf

Key words: Nanoflagellates, Fluorescently labeled beads, Ciliates, Southern East China Sea, Ingestion rate.

The classical view of a short food chain (grazing food chain) where phytoplankton are consumed by large zooplankton which are in turn preyed on by larger zooplankton and larval and small fishes, has been modified to incorporate what is termed the 'microbial loop' or 'microbial food web' (Pomeroy 1974, Azam et al. 1983). Marine planktonic ciliates dominate the microzooplankton in most marine ecosystems (Beers et al. 1980, James and Hall 1995), serve as a trophic link between the microbial food web and grazing food chain (Sherr et al. 1986, Stoecker and Capuzzo 1990, Gifford 1991, Pierce and Turner 1992), and thus play major roles in carbon and energy fluxes in marine ecosystems, making them ecologically a very important community.

Theoretical studies of oceanic food-web dynamics suggest that ciliates are capable of consuming a significant proportion of primary production (Frost 1991), and field studies showed that ciliates indeed consume 10%-86% of daily primary production (Pierce and Turner 1992, Ota and Taniguchi 2003). Moreover, high metabolic rates of ciliates suggest that they contribute to nutrient remineralization at a quantitative level sufficient to support primary production (Ota and Taniguchi 2003). Although several studies contributed greatly to our understanding of the grazing behavior of ciliates on phytoplankton (of < 20 μm) using a dilution technique (Gaul and

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Antia 2001, Paterson et al. 2007, Quinlan 2009), other studies investigated the possible importance of ciliate grazing on picoplankton (bacteria, *Prochlorococcus*, and *Synechococcus*) and nanoplankton (*Nannochloropsis* sp., *Rhodomonas* sp., and *Isochrysis galbana*) (Christaki et al. 1999, Kisand and Zingel 2000, Suzuki and Miyabe 2007, Saccà et al. 2009, Chen et al. 2010).

Oligotrich ciliates and tintinnids frequently dominate ciliate communities and are grazers of nanoplankton and picoplankton in marine ecosystems (Bernard and Rassoulzadegan 1990). Most species of the ciliate community are better able to effectively ingest nano-sized rather than pico-sized food particles (Kivi and Setälä 1995). In previous studies, it was also demonstrated that ciliates were the most important predators of nanoflagellates, as they consume 32%-80% of nanoflagellate production (Verity 1985, Nakano et al. 2001). Still, the importance of grazing pressure of ciliates on the nanoflagellate community has not been clarified in many ecosystems.

Oligotrich ciliates mainly consume food particles of < 10 μm in diameter (Jonsson 1986). In the East China Sea (ECS), 62%-97% of ciliates are reported to be < 50 μm in equivalent spherical diameter (ESD), most often being 20-40 μm in ESD (Ota and Taniguchi 2003). Based on an optimum predator: prey size ratio of about 8: 1 established by Jonsson (1986), it appears that prey for these ciliates should be within the 2.5-5-μm size range. A study by Tsai et al. (2010) showed that > 90% of the total nanoflagellate abundance was made up of 2-5-μm-sized nanoflagellates. Therefore, these ciliates may play an important role linking the nanoflagellate carbon source to higher trophic levels in the ECS.

The southern ECS, which extends from the coast of China to the offshore region northeast of Taiwan, is a highly dynamic region because of interactions of different water types (Gong et al. 1996). Hydrographic and nutrient conditions in the southern ECS regulate the phytoplankton biomass and primary production (Liu et al. 1995). Compared to physicochemical knowledge of the region, few ecological studies on microbial population distributions in the southern ECS have been carried out. This study was undertaken to analyze the contribution of the ciliate community to the oceanic carbon flux in eutrophic upwelling and oligotrophic oceanic waters in a southern section of the ECS. We assessed the importance of nanoplankton as a food source for ciliates in each water mass and quantified the strength of the nanoflagellate-ciliate link from the point of view of carbon fluxes in this marine ecosystem.

MATERIALS AND METHODS

Sampling

We collected samples from 12 stations at 5 m in depth in the southern ECS along an inshore-offshore transect on board the R/V *Ocean Researcher II* in Aug. 2010 (Fig. 1). The hydrography along this transect is fairly well studied (Gong et al. 1996, Chiang et al. 1997), and the area is known to be influenced by different water masses, including China coastal water, Taiwan Warm Current water, and upwelling water (Gong et al. 1996). Six stations for inshore-to-offshore grazing experiments were located along a eutrophic-oligotrophic gradient or coastal-to-oceanic environment. Stations I1 and I2 were located in the inner-shelf zone, which is affected by bottom-flow water of the South China Sea and China coastal water. Stations M1 and M2 were located in the mid-shelf zone, which is made up of Taiwan Warm Current water, and stations U1 and U2 were near the upwelling system (Fig. 1). Seawater was collected using a SeaBird CTD-General Oceanic Rosette assembly (NE 20th Street, Bellevue, Washington, USA) with 20-L Go-Flo bottles at different water depths. Temperature and salinity were measured in depth profiles with a SeaBird CTD. Nitrate was measured according to Gong et al. (1995). Water samples were filtered (25 mm GF/F) for the chlorophyll (Chl) *a* analysis which was measured after extraction with an *in vitro* fluorometer (Turner Design 10-AU-005,

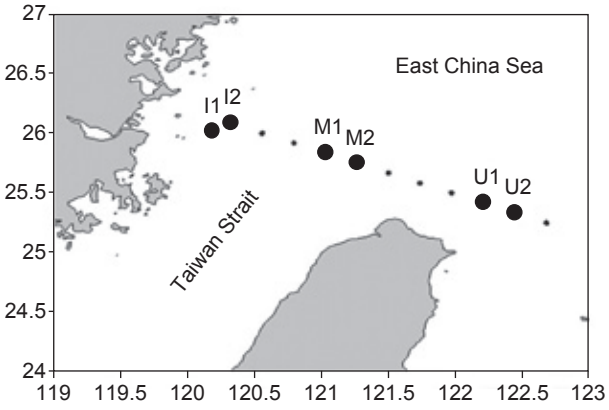

Fig. 1. Chart of the sampling stations. The 6 stations used for grazing experiments are indicated.

W. Maude Avenue Sunnyvale, California, USA) (Parsons et al. 1984).

Abundance and biomass measurements

For nanoflagellate microscopic counts, samples for estimating cell densities were immediately fixed by adding glutaraldehyde to a final concentration of 1% (v/v) (Christaki et al. 1999, Sanders et al. 2000). Samples (20 ml each) for pigmented and non-pigmented nanoflagellates were filtered onto a 0.8-μm black Nuclepore filter (Whatman, USA) under low pressure (< 100 mmHg). A 0.45-μm-pore-size Millipore filter (Whatman, USA) was used as a pad to obtain a uniform distribution of cells. Cells left on the filter membranes were stained with 4'6-diamidino-2-phenylindole (DAPI) at a final concentration of 1 μg/ml (Porter and Feig 1980) and examined by epifluorescence microscopy at 1000× (Nikon Optiphot-2, Japan). Non-pigmented nanoflagellates were identified by their blue fluorescence under ultraviolet illumination, while pigmented nanoflagellates were identified by their orange and red autofluorescence under blue excitation light. To obtain reliable estimates of abundances, at least 100 nanoflagellates were counted per sample.

For ciliates, 500-ml water samples from the surface were fixed with neutralized formaldehyde (at a final concentration of 2%) (Gifford 1985, Sherr et al. 1986, Brownlee and Jacob 1987, Stoecker et al. 1989) and kept at 4°C until analysis. To obtain a reliable ciliate abundance count, a 500-ml water sample was concentrated into a 100-ml sample with a 20-μm-mesh net, then the concentrated sample (100 ml) was settled in an Utermöhl chamber (Utermöhl 1958). The entire area of the Utermöhl chamber was examined at 200× or 400× using an inverted microscope (TMD 300, Nikon, Japan). Based on the cell shape, lorica, and collar appearance, ciliates were categorized into *Strombidium* spp., *Strobilidium* spp., *Laboea* spp., *Tontonia* spp., *Strombidinopsis* spp., *Mesodinium* spp., and tintinnids. Furthermore, ciliates were divided into 4 size groups, of 15-30, 30-45, 45-60, and > 60 μm in ESD. To separate coastal and oceanic stations, we used a Bray Curtis similarity analysis (Van den Brink and Ter Braak 1998) based on cell sizes of the ciliates communities.

To estimate the carbon biomass, cell sizes were measured with an ocular micrometer and converted into cell volumes according to Taniguchi (1984) and then transformed to ESD values. Biomass was calculated from the average biovolume of particular groups of organisms using conversion factors from biovolume to carbon biomass: 220 fgC/μm^3 for nanoflagellates (Borsheim and Bratbak 1987) and 140 fgC/μm^3 for ciliates (Putt and Stoecker 1989).

For *Synechoccocus* and heterotrophic bacteria, 2 ml sampled from 5 m in depth was fixed with 40 μl paraformaldehyde (at a final concentration of 0.2%), quickly frozen in liquid nitrogen, and stored in a freezer at -75°C for later analysis (Campbell and Vaulot 1993). Abundances of heterotrophic bacteria and *Synechococcus* spp. were calculated with a Becton-Dickinson LSR 6 Flow Cytometer (Franklin Lakes, New Jersey, USA) (Marie et al. 1997). Samples were run on a low setting for 2 min. Specimens of *Synechococcus* spp. were distinguished by their positions in plots of orange (FL2) and red fluorescence (FL3). SYBR Green I (Molecular Probes, Willow Creek Road, Eugene, USA) was used as a nucleic acid stain (Marie et al. 1997) to identify heterotrophic bacteria in a plot of FL3 (red) versus FL1 (green fluorescence). Internal calibration beads (1 μm in size, with yellowish-green fluorescence) were added as a standard to samples.

Grazing experiments

Grazing rates of ciliates feeding on nanoplankton were determined using 4-μm fluorescently labeled beads (FLBs) following the method of Sherr et al. (1991). FLBs of 4 μm were distinguished from natural nanoplankton by the bright-green color under blue light excitation. For these experiments, 500-ml water samples were poured into each triplicate polycarbonate bottle. FLBs (4 μm) were added to each bottle at about 20% of the *in situ* nanoflagellate abundance (including heterotrophic and pigmented nanoflagellates) and incubated in a water bath at the *in situ* temperature under natural light intensities for 30 min. Preliminary experiments indicated that ingestion of 4-μm FLBs by ciliates became saturated after 30 min. After incubation, samples were preserved with neutralized formaldehyde (at a final concentration of 2%), and numbers of FLBs ingested by ciliates were examined at 200× or 400× under an inverted microscope.

Ingestion rates of ciliates (flagellates/ciliate/d) were calculated by multiplying the ingestion rate of FLBs by the ratio of nanoflagellates to added FLBs. Community consumption rates (flagellates/

L/d) were estimated by multiplying the average ingestion rates of nanoflagellates by the total ciliate abundance.

We estimated growth rates of nanoflagellates as given in Tsai et al. (2005) by a fractionation method (Wright and Coffin 1984).

RESULTS

Environmental conditions

During this study, the marine environment along the transect was categorized into 3 zones based on physical and chemical properties of the seawater. Offshore stations (stations U1 and U2) had upwelling water (Fig. 2A, B), and the mid-shelf zone (stations M1 and M2) consisted of Taiwan Strait water with high temperatures and low salinities, and a nearly homogeneous water column (Fig. 2A, B). The coastal zone (stations I1 and I2), which was influenced by bottom flow water of the South China Sea Current, had high salinities (> 34 psu) and low temperatures (< 25°C) (Jan et al. 2002) (Fig. 2A, B). As a result of these hydrographical conditions, the highest value of Chl *a* in the water column of 3.15 mg/m^3 was observed at station I1 at 10 m in depth (Fig. 2C).

Water temperatures at 5 m in depth during the study period fluctuated between 24.77 (I1) and 28.23°C (U2) (Table 1). Salinity and Chl *a* concentrations respectively ranged from 33.38 (M2) to 34.30 ppt (U1) and from 0.12 (U2) to 1.95 mg/m^3 (M1) (Table 1). Nitrate concentrations were generally below detection limits (Table 1).

Spatial variations of nanoflagellates and ciliates

The total abundance of nanoflagellates ranged 0.47-1.37 × 10^6 cells/L (Table 1). Higher values (> 10^6 cells/L) were found in coastal (I1) and Taiwan Strait waters (M1). These values coincided with peaks of bacterial and *Synechococcus* spp. abundances (Table 1). The nanoflagellate population was dominated by the smaller-sized fraction (2-5 μm), which accounted for > 98% of the total abundance of nanoflagellates measured at each site (Table 1).

Surface abundances of total ciliates were high in inner-shelf waters at stations I1 and I2 (823-1296 cells/L) and decreased toward the offshore upwelling region (61-206 cells/L) (Table 1). The ciliate community consisted of oligotrichs of the genera *Strombidium* spp., *Laboea* spp., and *Tontonia* spp. (Table 1, Fig. 3A). Tintinnids were the most abundant genera found at station I1 (439 cells/L). However, in Taiwan Strait water at station M1, the ciliate community consisted mostly of *Strombidinopsis* spp. and *Mesodinium* spp., which made up > 50% of the total abundance of ciliates (Table 1, Fig. 3A). Ciliate cells ranged in size from 15 to 85 μm in ESD. Ciliates of < 45 μm in ESD were relatively abundant, making up 79%-96% of total cell abundances (Fig. 3B).

Grazing experiment

Ingestion rate of ciliates ranged between 47 (M2) and 333 flagellates/ciliate/d (I2) in the

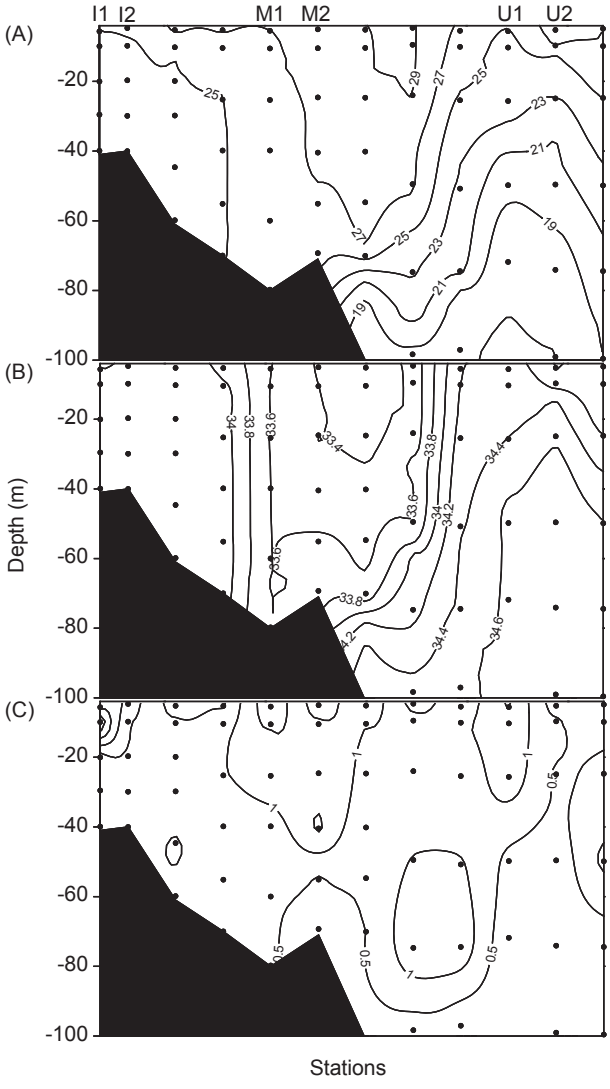

Fig. 2. Spatial variations in temperature (A), salinity (B), and chlorophyll (Chl) *a* concentrations (C) in the southern East China Sea.

inner-shelf zone (Fig. 4A). Furthermore, daily consumption of nanoflagellates by ciliates varied from 0.03 × 10^5 (U2) to 4.2 × 10^5 flagellates/L/d (I2) (Fig. 4B). *Strombidium* spp. ingested 59-374, *Strobilidium* spp. 26-268, *Laboea* spp. and *Tontonia* spp. 18-289, and tintinnids 58-249 flagellates/ciliate/d (Fig. 5A). *Strombidium* spp. had the largest variation in daily consumption of nanoflagellates (at 0.18-2.2 × 10^5 flagellates/L/d), with the highest value recorded at station I2. Consumption rates of other taxa were low (< 10^5 flagellates/L/d) at all stations during the study period (Fig. 5B). Not including station I1, *Strombidium* spp. were responsible for 37%-72%

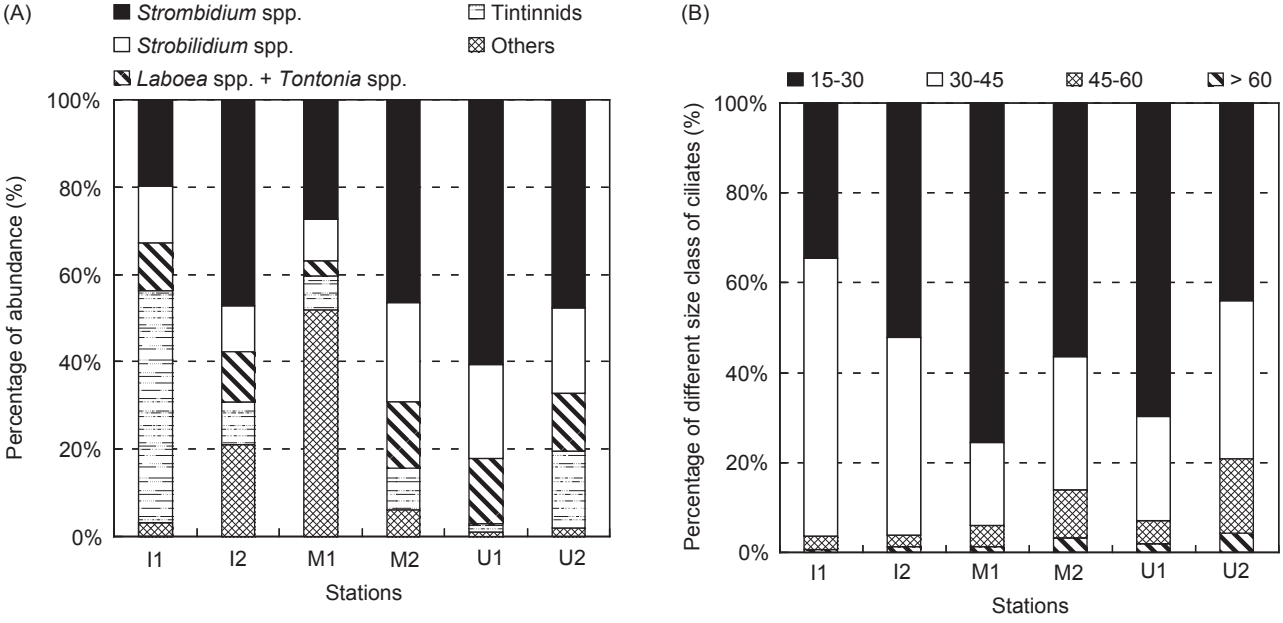

Fig. 3. Percentage contributions of ciliate taxa (A) and 4 size classes (15-30, 30-45, 45-60, and > 60 μm). (B) to the total ciliate abundance.

Table 1. Physical and chemical factors, and microbial standing stocks at each sampling station used for the grazing experiments

Station	I1	I2	M1	M2	U1	U2
Temperature (°C)	24.77	25.55	27.14	27.92	24.85	28.23
Salinity (psu)	34.02	34.04	33.61	33.38	34.30	33.99
Chlorophyll *a* (mg/m^3)	1.72	1.40	1.95	0.60	1.60	0.12
NO_3 (μm)	0.0	0.2	0.0	0.0	0.1	0.0
Bacteria (10^8 cells/L)	5.44	2.74	4.57	3.97	2.32	1.65
Synechococcus spp. (10^7 cells/L)	5.92	4.39	43.64	9.38	2.57	0.89
Total nanoflagellates (10^6 cells/L)	1.37	0.93	1.07	0.47	0.59	0.79
Pigmented nanoflagellates (10^6 cells/L)	0.56	0.35	0.43	0.13	0.15	0.28
Heterotrophic nanoflagellates (10^6 cells/L)	0.81	0.58	0.64	0.35	0.43	0.51
2-5-μm nanoflagellates (10^6 cells/L)	1.35	0.93	1.07	0.47	0.58	0.79
> 5-μm nanoflagellates (10^6 cells/L)	0.012	0.006	0.004	0.005	0.008	0.006
Total ciliates (cells/L)	823	1296	726	403	206	61
Strombidium spp. (cells/L)	162	613	199	187	125	34
Strobilidium spp. (cells/L)	108	134	69	92	45	14
Laboea spp. + *Tontonia* spp. (cells/L)	89	150	24	61	31	9
Tintinnids (cells/L)	439	126	57	39	4	13
Others[1] (cells/L)	25	273	377	25	2	1

[1]*Strombidinopsis* spp. and *Mesodinium* spp.

of the ciliate grazing impact on nanoflagellates in the southern ESC (Fig. 5C). Abundances of tintinnids were determined at each sampling site throughout the study period. They were dominant at station I1 where they accounted for about 54% of the grazing impact on nanoflagellates in the inter-shelf area (Fig. 5C).

Ciliates at 15-30 μm in ESD were considerably less-efficient feeders on nanoflagellates and had lower ingestion rates (23-45 flagellates/ciliate/d) than larger ciliates in the Taiwan Strait and upwelling area (M1, M2, and U1) (one-way ANOVA, $p < 0.05$) (Fig. 5D). Ciliates of < 45 μm in ESD were responsible for 63%-94% of ciliate grazing on nanoflagellates, making them major consumers of nanoflagellates in the ciliate community (Fig. 5E). Ciliates of 30-45 μm in ESD had a greater impact than those of 15-30 μm in ESD in the inner-shelf area (stations I1 and I2) (Fig. 5E). Furthermore, we found that small ciliates (< 45 μm in ESD) were the most important nanoplankton grazers (Fig. 5F), and abundances of nanoflagellates were positively correlated with ingestion rates of ciliates of 15-30 μm in ESD (Fig. 6).

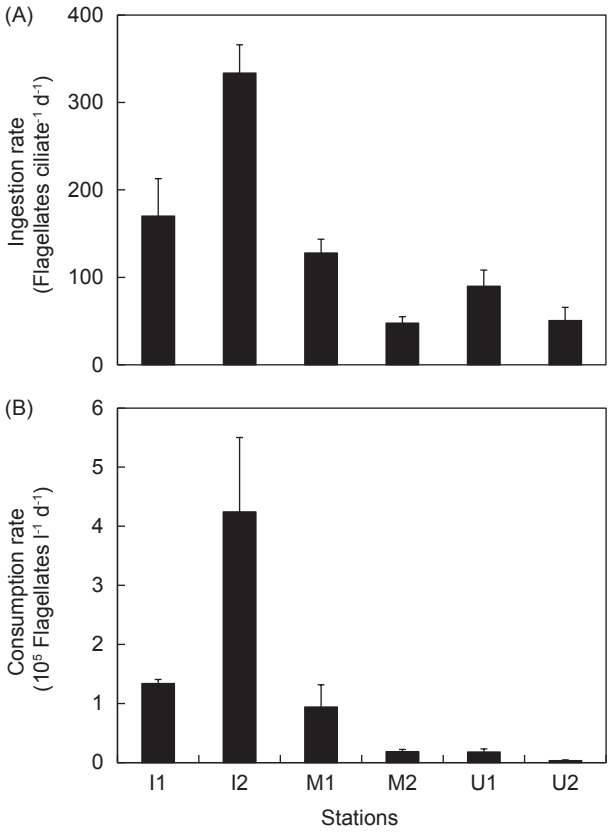

Fig. 4. Spatial variations in ingestion (A) and consumption rates (B) of ciliates.

Our cluster analysis of the similarity of ciliate communities among 6 experimental stations noted 2 distinct groups of ciliates: one dominated by ciliates 30-45 μm in ESD in inner-shelf waters (I1 and I2) and the other dominated by ciliates 15-30 μm in ESD in offshore waters (M1, M2, U1, and U2) (Fig. 7).

DISCUSSION

Previous studies (Capriulo and Carpenter 1980, Rassoulzadegan and Etiennl 1981, Kivi and Setälä 1995) of particulate food size ranges and feeding rates in oligotrichous ciliates suggested that ciliates of ≤ 200 μm can control the production of nanoplankton in the sea (Verity 1985), while small oligotrichous ciliates (of ≤ 30 μm) probably control the production of picoplankton (Sherr and Sherr 1987, Sherr et al. 1991, Kisand and Zingel 2000, Suzuki and Miyabe 2007). Our study found a predominance of ciliates of 15-45 μm in ESD in the ciliate community in the southern ECS (Fig. 3B), where they exerted high grazing pressures on nanoflagellates (of > 100 flagellates/ciliate/d) (Fig. 5A).

Based on an optimum predator: prey size ratio of about 8: 1 established by Jonsson (1986), oligotrich ciliates of 15-45 μm in ESD could graze on prey 2-5.6 μm in size. Riegman and Kraay (2001) reported that phytoplankton of 2-5 μm in size in the Faroe-Shetland Channel were mostly of the Prasinophyceae and Chrysophyceae, which were mainly grazed by ciliates. However, according to Jonsson (1986), studies on optimal prey size spectra have not been conducted on oligotrich ciliates. Smetacek (1984) and Kivi and Setälä (1995) showed that *Strobilidium* sp. can consume food items almost as large as themselves, and Heinbokel (1978) reported that tintinnids can ingest prey at a predator/prey size ratio of 2.5: 1. As a whole, the planktonic ciliate community seems to be adapted to grazing on a large size range of food organisms.

The method used in this study to quantify feeding rates was simple and enabled the *in vivo* food vacuole content to be examined by induced FLBs (Sherr et al. 1987) using epifluorescence microscopy. Fluorescent-labeled prey were previously used to determine feeding rates of different-sized particles by ciliates (Borsheim 1984, Jonsson 1986). However, to the present, there are few reports on *in situ* feeding rates of

ciliates on nanoflagellates. Jürgens et al. (1996) showed that ciliates, particularly small oligotrich ciliates which are known to have high grazing rates on heterotrophic nanoflagellates (HNFs), are abundant in surface waters and ingest HNFs at a rate of 240 HNFs/ciliate/d/. In the present study, *Strombidium* spp. effectively fed on nanoflagellates at rates ranging 59-374 flagellates/ciliate/d, *Strobilidium* spp. at 26-268 flagellates/ciliate/d, and tintinnids at 58-249 flagellates/ciliate/d. These rates are similar to those reported by Jürgens et al. (1996).

We used particles of 4 μm in size to estimate ingestion rates of ciliates in the present study. This size is well within the cell size range (2-5 μm) of predominate nanoflagellates (Table 1). We found clear evidence that the ciliate community, especially ciliate species such as *Strombidium*

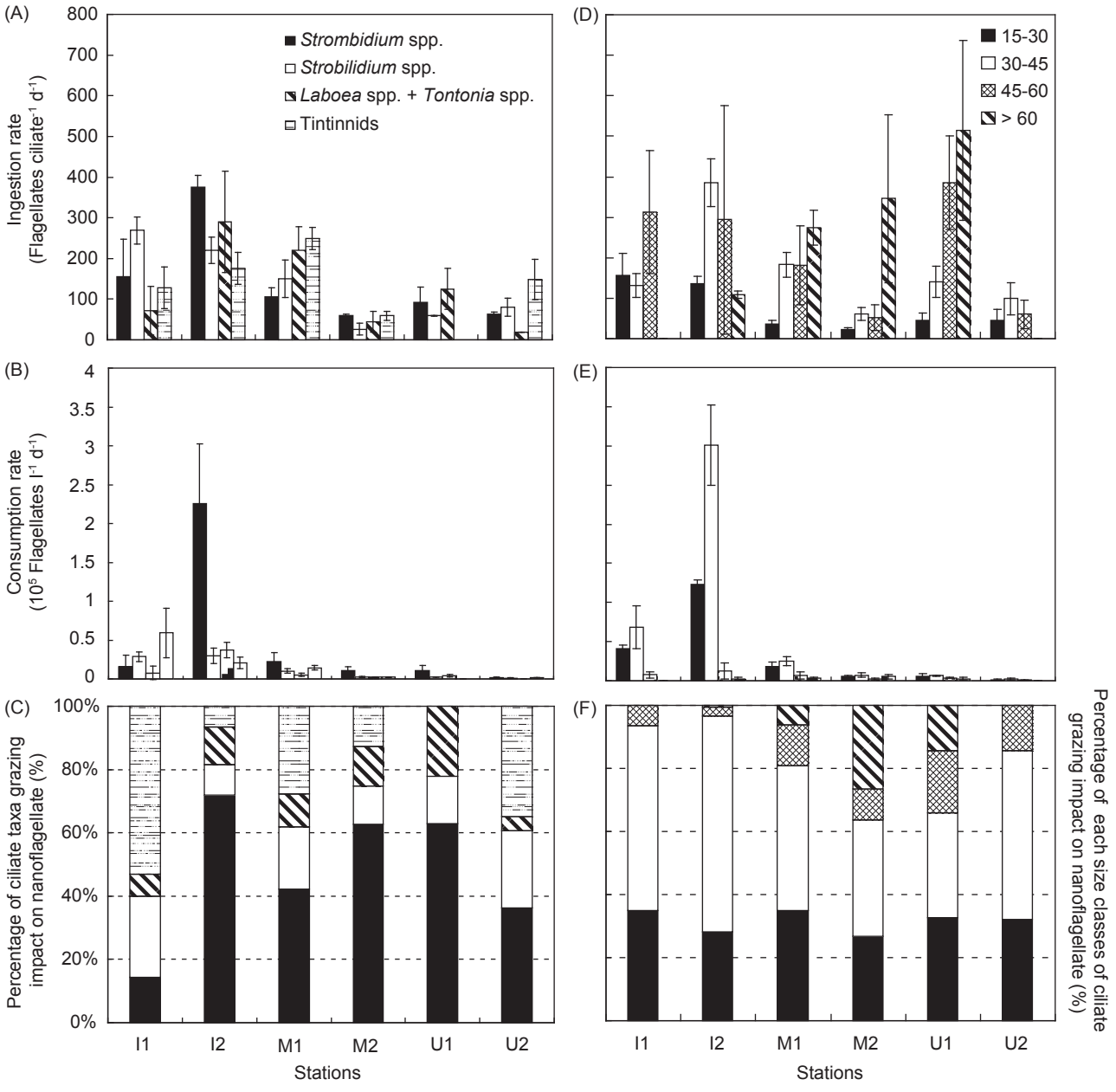

Fig. 5. Ingestion rates (A, D), consumption rates (B, E), and percent contributions of different ciliate taxa and size classes (C, F) to grazing on nanoflagellates. (% is the consumption rate of each species and size class / total consumption rate, with the total consumption rate acquired from the consumption rate of each station).

spp., *Strobilidium* spp., *Tontonia* spp., *Laboea* spp., and tintinnids, are able to effectively graze on nanoflagellates. For example, ingestion rates of *Strombidium* spp. ranged 59-374 flagellates/ciliate/d, with a maximum consumption rate of 2.2 × 10^5 flagellates/L/d in inner-shelf waters (station I2) (Fig. 5A, B). The presence of *Strombidium* spp. in our experimental samples collected from station I2 suggested potential consumption of about 25% of the standing stock of nanoflagellates each day. Ciliates of 30-45 μm in ESD were estimated to have potential consumption of about 33% of the standing stock of nanoflagellates per day in inner-shelf waters (station I2) (Fig. 5E). Apart from the coastal ecosystem, *Strombidium* spp. were major grazers of nanoflagellates, accounting for 37%-72% of the total ciliate grazing impact on nanoflagellates in the southern ESC.

Moreover, as clearance rates are a function of ingestion rates and prey concentrations, they may be a more-conservative indicator of the feeding behavior of an organism than ingestion rates. Ciliate clearance rates obtained in this study ranged 1.0-16.80 μl/ciliate/h, consistent with observations from other reports, for example, 1.2-8.3 μl/ciliate/h in Sherr et al. (1991), 0.8-1.5 μl/ciliate/h in Vargas and Martínez (2009), 2.6 μl/ciliate/h in Jonsson (1986), and 2.3 μl/ciliate/h in Bernard and Rassoulzadegan (1990). The use of FLBs to assess short-term clearance rates of ciliates can provide valuable information regarding which components of the *in situ* ciliate community are capable of ingesting nano-sized prey particles and relative magnitudes of clearance rates of various ciliate species.

The relationship between ingestion rates and nanoflagellate abundances by cell size (Fig. 6) indicates subsaturation of grazing on nanoflagellates by these small ciliates (of 15-30 μm in ESD). However, previous studies suggested that small ciliates with cell sizes of < 30 μm in ESD are probably consumers of bacteria (Rassoulzadegan et al. 1988, Sherr et al. 1988, Ichinotsuka et al. 2006). Although some of the dominant ciliate species in this study were small (*Strombidium* spp. and *Strobilidium* spp., at < 30 μm in ESD), we did not measure grazing of ciliates on bacteria. A previous study showed that most of the isolates of *Strombidium* and *Strobilidium* examined so far did not effectively ingest particles of < 2 μm (Jonsson 1986). Another study (Lynn and Montagnes 1991) reported that ciliates mainly ingest prey larger than bacteria and similar in size to small nanoflagellates (2.2-5 μm). Therefore, while it is likely that ciliates consist of diverse species with various feeding modes which allow grazing on a variety of food items, the major prey in the nanoflagellate community of most species are < 5 μm.

Based simply on a consideration of cell sizes in the ciliate community, we would expect to find 2 different marine ecosystems of carbon flux in surface waters of the southern ECS (Fig. 7), and we previously used size fractionation to estimate nanoflagellate growth rates at the same sampling sites (Chiang, unpubl. data). They ranged 0.85/d (I1) to 1.54/d (M2), and averaged 1/d. There was no significant difference in growth rates between nanoflagellates of inner-shelf and middle-shelf waters (*t*-test, $p > 0.05$). In inner-shelf waters, we found that ciliates of 30-45 μm in ESD were responsible for grazing 62% of nanoflagellate production, and ciliates of 15-

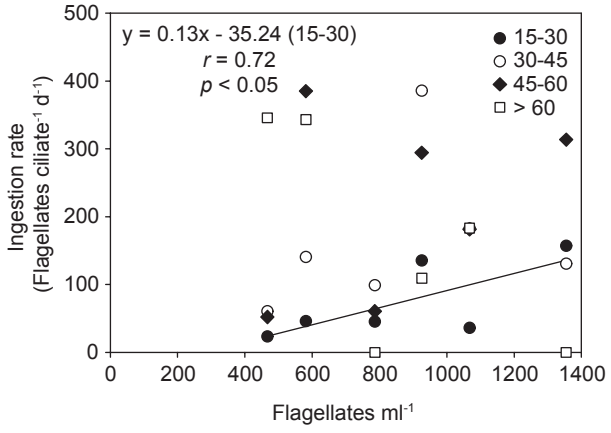

Fig. 6. Relationship between ingestion rates and nanoflagellate abundances by cell size.

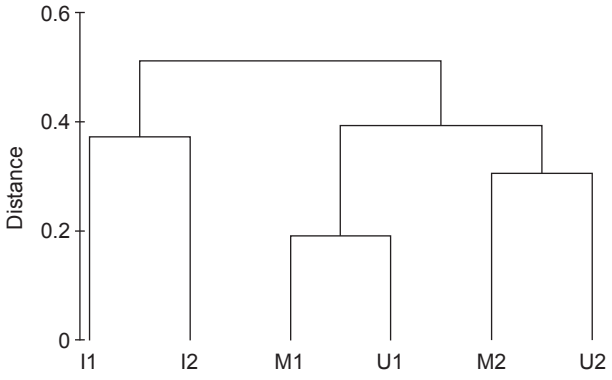

Fig. 7. Cluster analysis of ciliate cell sizes (15-30, 30-45, 45-60, and > 60 μm) calculated by the Bray-Curtis similarity analysis.

30 μm in ESD for grazing 23% (Fig. 8). Almost 100% of nanoflagellate production was consumed by ciliates. These findings are similar to those of Nakano et al. (2001), who found ciliates to be the most important predators of nanoflagellates, consuming about 80% of nanoflagellate production.

The present study found that at offshore stations (M and U), ciliates of 15-30 μm in ESD consumed 13% of nanoflagellate production, those of 30-45 μm in ESD consumed 12%, and those of 45-60 μm in ESD consumed 11% (Fig. 8), showing that the ciliate community grazed about 43% of nanoflagellate production, and thus did not significantly contribute to nanoflagellate mortality. There may be other alternative sources of nanoflagellate mortality, including grazing by dinoflagellates (Bjørnsen and Kuparinen 1991, Strom 1991). Wu et al. (2010) reported a high abundance of dinoflagellates (> 3000 cells/L) in offshore waters of the southern ECS. Strom (1991) reported that small heterotrophic dinoflagellates mainly feed on nanoflagellates, so they potentially compete with ciliates for prey.

In conclusion, this study supported the hypothesis that ciliates are major consumers of nanoflagellates and transfer 43%-100% of nanoflagellate production into higher trophic levels via the microbial food web in the southern ECS, especially in inner-shelf waters. Ciliates of < 45 μm in ESD are the most important nanoplankton grazers. Ingestion rates of nanoflagellates in diets of various taxa of ciliates considerably vary. Their *in situ* feeding behaviors and ecology remain poorly documented in aquatic systems.

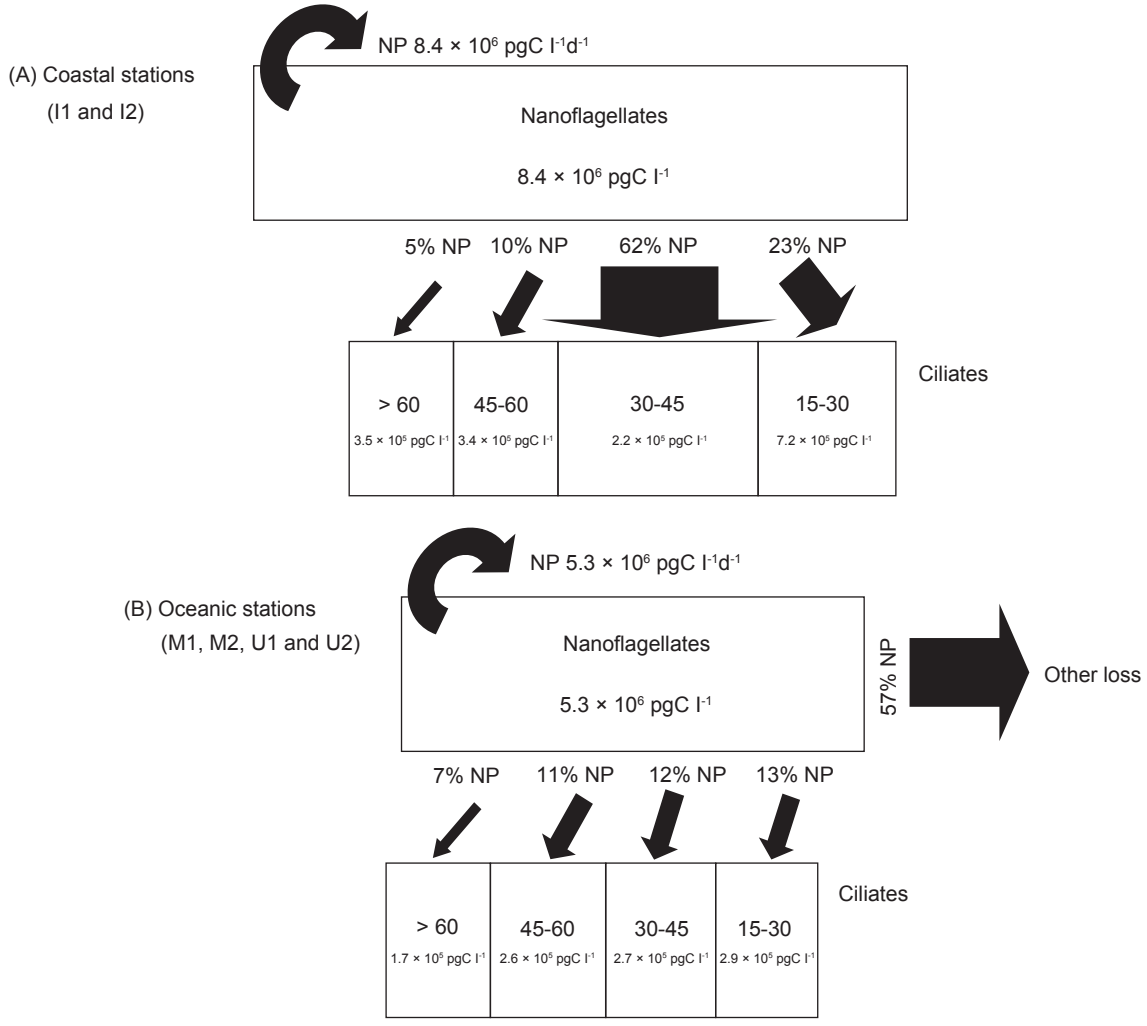

Fig. 8. Schematic carbon flow diagrams depicting spatial variations in energy transfer between ciliates and nanoflagellates in the southern East China Sea in summer. Numbers next to looped arrows represent nanoflagellate production (NP). Straight arrows pointing to ciliates indicate grazing rates.

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