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Changes in microbial loop components: effects of a harmful algal bloom formation and its decay

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ABSTRACT: Temporal changes in microbial loop components during the formation and decay of a bloom caused by the harmful microalga *Heterosigma akashiwo* (Raphidophyceae) were investigated in 1995 at a site in northern Hiroshima Bay, the Seto Inland Sea of Japan. A surface bloom of *H. akashiwo*, the density of which exceeded 10^4 cells ml^{-1}, was recorded in early summer at surface temperatures ranging from 21.6 to 23.2°C. Although the abundance and species diversity of tintinnid ciliates decreased in the surface layer when the density of *H. akashiwo* exceeded 10^4 cells ml^{-1}, aloricate ciliates increased evidently at the end of the bloom. In addition, the mean ciliate biomass in the surface and near bottom layers reached the same level as the bacterial biomass. Two peaks in bacterial abundance and biomass were recorded during the formation and at the end of the bloom, and fluctuations in the abundance of heterotrophic nanoflagellates (HNF) clearly corresponded with fluctuations in bacterial abundance, with a lag period of 1 to 3 d. The increase of each microbial loop component during the course of the *H. akashiwo* bloom suggests that the dissolved organic matter produced from *H. akashiwo* cells temporarily enhanced the energy flow from bacteria through HNF to bacterivorous aloricate ciliates.

KEY WORDS: *Heterosigma akashiwo* · Bloom · Red tide · Microbial loop · Bacteria · Flagellate · Ciliate · Abundance

INTRODUCTION

Since the concept of the microbial loop was proposed by Azam et al. (1983), the roles of its components, such as bacteria, heterotrophic nanoflagellates (HNF) and ciliates, have been investigated in coastal waters. Consequently, previous studies have demonstrated that the biomass of bacteria is equal to or exceeds that of phytoplankton (Simon et al. 1992), and its production is equivalent to 30% of the primary production based on phytoplankton (Cole et al. 1988). Simultaneously, ecological studies on the other microbial loop components have revealed that they can play important roles as bacteria grazers (Fenchel 1982, Sieburth 1984, Sherr & Sherr 1987) and that they are also consumed by various net-zooplankton (Stoecker & Capuzzo 1990, Pierce & Turner 1992). These results suggest that some parts within the microbial loop effectively link the grazing food chain and may enhance fisheries production (Sherr et al. 1986, Sherr & Sherr 1988). However, there is a possibility that bacterial production is not linked to higher trophic levels because of many steps of transfer loss (Ducklow et al. 1986). In eutrophic marine ecosystems, it is likely that bacterial production strongly influences planktonic food webs, which enhance the productivity of higher trophic levels, even if there are many steps of transfer loss. This is because bacterial biomass and production are very high in such areas. From this point of view, the function of microbial loops is important in coastal fisheries production.

The blooms of *Heterosigma akashiwo* (previously referred to as *Heterosigma carterae* or *Olithodiscus luteus*) have been observed in coastal waters throughout the world. Heavy blooms of *H. akashiwo* have been associated with cultured fish mortality (Chang et al. 1990, Honjo 1993). Furthermore, *H. akashiwo* blooms probably weaken trophic links within grazing food

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webs in coastal waters. Many micro- and macrozooplankton reject *H. akashiwo* cells as a food source. The collapse of tintinnid ciliate populations due to blooms of *H. akashiwo* is well documented (Verity & Stoecker 1982, Kamiyama 1995). The calanoid copepod *Acartia omorii* has been found to almost completely reject *H. akashiwo* due to the intracellular chemical compounds in this alga (Uye & Takamatsu 1990). Inhibition of the feeding activity and reproduction of the rotifer *Synchaeta cecila* caused by *H. akashiwo* has also been reported when more than 1000 cells ml^{-1} of this alga are present (Egloff 1986).

The northern part of Hiroshima Bay is one of the most highly enclosed and eutrophic areas in the Seto Inland Sea. In this area, blooms of *Heterosigma akashiwo* often occur in early summer, as well as other harmful algal blooms (e.g. Matsuyama et al. 1997). Nevertheless, the productivity of planktivorous fish such as anchovy and sardine in Hiroshima Bay is very high (Hashimoto et al. 1997). It is therefore essential to clarify the effects of these blooms on the structure of planktonic food webs in order to elucidate the fisheries production system in this bay. To our knowledge this is the first study of temporal changes in microbial loop components in the whole process of a *H. akashiwo* bloom.

MATERIAL AND METHODS

The investigation was conducted in a harbor (ca 5 m water depth, Stn V in Nagasaki et al. 1994) in the northern part of Hiroshima Bay, the Seto Inland Sea. Sampling was carried out every 1 to 4 d between 09:00 and 11:00 h during the course of a *Heterosigma akashiwo* bloom from June 6 to July 17, 1995. This site is located in a closed area where water currents from outer areas are negligible. Vertical profiles of temperature and salinity were measured with a temperature-salinity bridge (YEO-KAL model 602). Seawater was collected at the surface with a plastic bucket and at 1 m above the bottom (B-1 m) with a Niskin bottle sampler. Water samples were transported to the laboratory within 2 h. *H. akashiwo* and other dominant species of phytoplankton were counted in triplicate in 0.05 to 1 ml of fresh seawater from each depth.

Part of the seawater from each depth was filtered through a glass-fiber filter (Whatman GF/F). The chlorophyll a (chl *a*) on the filter was extracted with N, N-dimethylformamide at –20°C under dark conditions (Suzuki & Ishimaru 1990), and then measured with a Turner Designs fluorometer. In the filtrate obtained by filtration of another seawater sample with Millipore Milex HV-filter (pore size: 0.45 µm), concentrations of nutrient salts (NO_2-N, NO_3-N, NH_4-N, PO_4-P, SiO_2-Si) were measured using a TrAAcs 800 autoanalyzer (Bran-Luebbe Co.), based on the method of Strickland & Parsons (1968).

A 200 ml seawater sample was fixed with Lugol's iodine solution (final concentration: 2%), and then concentrated by settling to a volume of 1 to 6 ml. Microzooplankton (zooplankton with body widths less than 200 µm) of each subsample were counted in 1 to 2 ml of concentrated samples with a phase contrast microscope at a magnification of ×150, using a Sedgwick-Rafter chamber. The large mixotrophic dinoflagellate *Gymnodinium sanguineum* was identified and counted in fixed samples. However, it was not possible to identify the other mixotrophic and heterotrophic dinoflagellates. In the ciliate community, most of the tintinnids were distinguished into species by lorica morphology based on the same references used by Kamiyama & Tsujino (1996). The species diversity (H') of tintinnids for each sample was calculated using the Shannon-Wiener function (Shannon & Weaver 1963):

$$H' = -\sum_{i=1}^{s} (n_i/N)\log_2(n_i/N)$$

where n_i is the abundance (ind. l^{-1}) of i species, N is the total abundance (ind. l^{-1}), and s is the number of species.

Since heterotrophic and mixotrophic aloricate ciliates could not be readily identified into genus or species, they were grouped into separate morphotypes which were based on size and shape. In the present study, the autotrophic ciliate *Mesodinium rubrum* (= *Myrionecta rubura*) was not included in the aloricate ciliates. The length of an appropriate lorica or cell dimension was measured using a calibrated ocular micrometer for up to a maximum of 10 individuals for each taxon. The mean lorica volume for tintinnids and the mean cell volume for aloricate ciliates were calculated by approximating the shape of each ciliate taxon to a standard geometric configuration.

Seawater in each sample was fixed by glutaraldehyde (final concentration: 1%), and then bacteria and HNF abundance were quantified with an epifluorescent microscope according to the method of Iwamoto et al. (1994), who basically followed the procedure of Porter & Feig (1980) and Sherr & Sherr (1983a,b). Each day, bacteria in 0.3 ml of the fixed subsample were stained with 4', 6-diamidino-2-phenylindole (DAPI; final concentration: 0.5 µg ml^{-1}), and then filtered onto a Sudan black B-stained Nucleopore polycarbonate filter (pore size: 0.2 µm) with less than 300 mm Hg vacuum pressure. The HNF in 5 to 20 ml of the fixed subsample was stained with DAPI (final concentration: 0.1 µg ml^{-1}) and fluorescein isothiocyanate (FITC; final concentration: 1 µg ml^{-1}) solutions and then filtered onto a Sudan black B-stained Nucleopore filter (pore size: 1.0 µm) with less than 100 mm Hg vacuum pressure. With regard to bacteria, at least 200 cells in more

than 10 fields, and for HNF at least 50 cells in more than 25 fields, were counted with an epifluorescent microscope at 1250× magnification. The length and width of each HNF cell were measured using a calibrated ocular micrometer, and then the cell volume was calculated as an ellipse sphere shape.

The carbon contents of tintinnid ciliates (Ct, pg) were estimated by fitting the lorica volume (LV, μm^3) of each species in the equation: Ct = 444.5 + 0.053 LV (Verity & Langdon 1984), and the Ct of aloricate ciliate cells preserved in 2% Lugol's iodine solution were calculated from cell volume data using a carbon conversion factor of 0.19 pg C μm^{-3} (Putt & Stoecker 1989). The Ct of bacteria were converted from the mean cell volume (0.098 μm^3 $cell^{-1}$) from annual Hiroshima Bay data (Imai & Yamaguchi 1996), using a carbon conversion factor of 0.14 pg C μm^{-3} (Nagata & Watanabe 1990). The carbon values of HNF were estimated from measured cell volumes, using a carbon conversion factor of 0.07 pg C μm^{-3} (Sorokin 1979).

RESULTS

Phytoplankton and environmental conditions

A *Heterosigma akashiwo* bloom with a cell density of over 10^4 cells ml^{-1} occurred from June 23 to July 1 (hereafter we define this period as the bloom period), with surface temperatures ranging from 21.6 to 23.2°C (Fig. 1A,D). In the other phytoplankton, *Prorocentrum* spp. also increased in density to more than 10^4 cells ml^{-1} during the bloom period of *H. akashiwo* (Fig. 1E). The abundance of diatoms was low before and during the bloom period, probably indicating that diatoms did not influence the population dynamics of *H. akashiwo*. During the bloom period, the chl *a* concentration in the surface layer reached 454 μg l^{-1} (Fig. 1C). After the end of the bloom period, the salinity markedly decreased due to rainfall (Fig. 1B). After July 10, when the surface salinity recovered more than 12 psu, the chl *a* concentration increased again. This was caused by increasing diatoms in the phytoplankton community.

All nutrient concentrations changed similarly in the surface and B-1 m layers. The DIN (= NO_2-N + NO_3-N + NH_4-N) concentrations in both layers were almost exhausted by phytoplankton during the bloom period (Fig. 1F). Phosphorous concentrations were also low during the bloom period but spiked increases in the surface layer were detected occasionally and a faint increase in the B-1 m layer was observed around the end of the bloom (Fig. 1G). No evident fluctuation of SiO_2-Si concentration was observed before or during the bloom period (Fig. 1H). Clear increases of SiO_2-Si after the end of bloom were caused by the large influx of inland fresh water. In the bloom period, the DIN:P ratio decreased (Fig. 1I) and the Si:DIN ratio increased, suggesting that the DIN concentration strongly limited the growth of phytoplankton rather than the PO_4-P or SiO_2-Si concentrations. The Si:P ratio gradually decreased in the latter half and after the bloom, particularly in the B-1 m layer (Fig. 1J). This is likely due to the increase of the PO_4-P concentration during this period although the SiO_2-Si concentration did not change obviously.

Microzooplankton community

Before the bloom of *Heterosigma akashiwo*, the abundance of tintinnids in both layers ranged from 1.5×10^2 to 7.0×10^3 ind. l^{-1} (Fig. 2A). At the initial stage of the bloom (June 24), the abundance reached a maximum of 1.1×10^4 ind. l^{-1} in B-1 m layer, and this group temporarily numerically dominated in the ciliate community. However, the abundance of tintinnids in the surface layer markedly decreased by 1 order of magnitude during the bloom period compared to before the bloom. For about 1 wk after the bloom, the abundance of tintinnids remained at a low level and then recovered to the same level as before the bloom. The H' of tintinnids in the surface layer also decreased during the bloom (Fig. 2B). The decrease of diversity was observed at the same time as the bloom formation, which was earlier than the point of time when the decrease in abundance of tintinnids started.

Before the bloom, the abundance of aloricate ciliates, which numerically dominated the ciliate community except on June 20, ranged from 7.2×10^2 to 10.0×10^3 ind. l^{-1} (Fig. 2C). Although a conspicuous change was not observed during the process of bloom formation, the abundance increased drastically at the end of the bloom, and temporarily became 2 orders of magnitude higher than before the bloom. After the end of the bloom, aloricate ciliate abundance in the surface layer decreased for 2 d and then recovered to the same level as before the bloom, a similar pattern as for other organisms.

Abundance of the mixotrophic dinoflagellate *Gymnodinium sanguineum* increased in the early stage of the bloom to a maximum of 1.3×10^3 ind. ml^{-1} on June 25 (Fig. 2F). However, this dinoflagellate decreased rapidly before the end of the surface bloom (July 30).

Densities of copepod nauplii and other metazoans (small copepods, copepodites, appendicularian, rotifers, larvae of bivalves, etc) were in the order of magnitude of 10^2 ind. l^{-1} before the bloom formation, although the other metazoans often exceeded 10^3 ind. l^{-1} during and after the bloom (Fig. 2D,E). In general, the variation in the abundance of the other metazoans was similar to that in temperature.

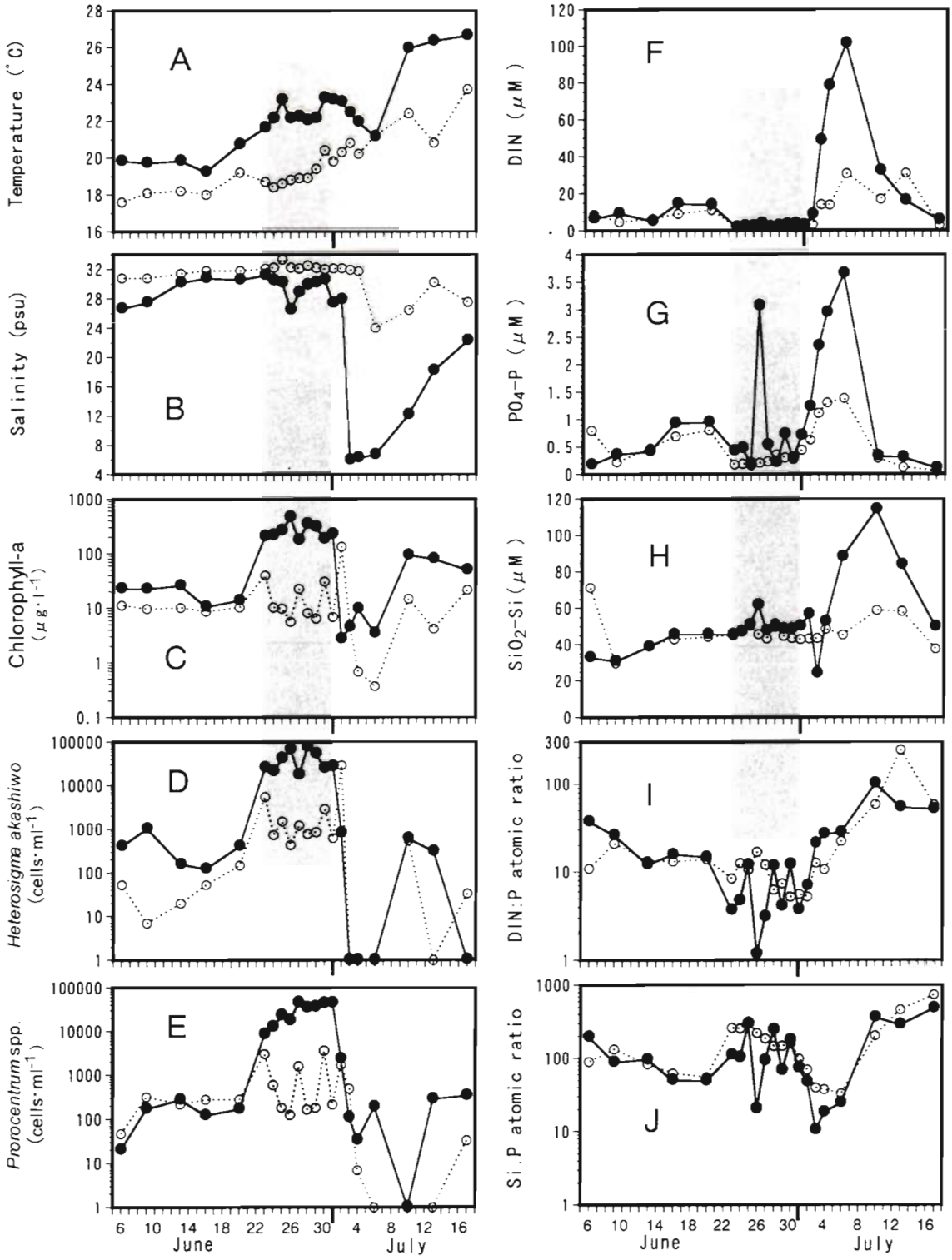

Fig. 1 Temporal changes in the hydrographic conditions and phytoplankton abundance at the surface (●) and 1 m above the bottom (○) during the course of a *Heterosigma akashiwo* bloom. Shaded areas indicate the bloom period (June 23 to July 1, 1995) when the density of *H. akashiwo* exceeded 10^4 cells ml^{-1}

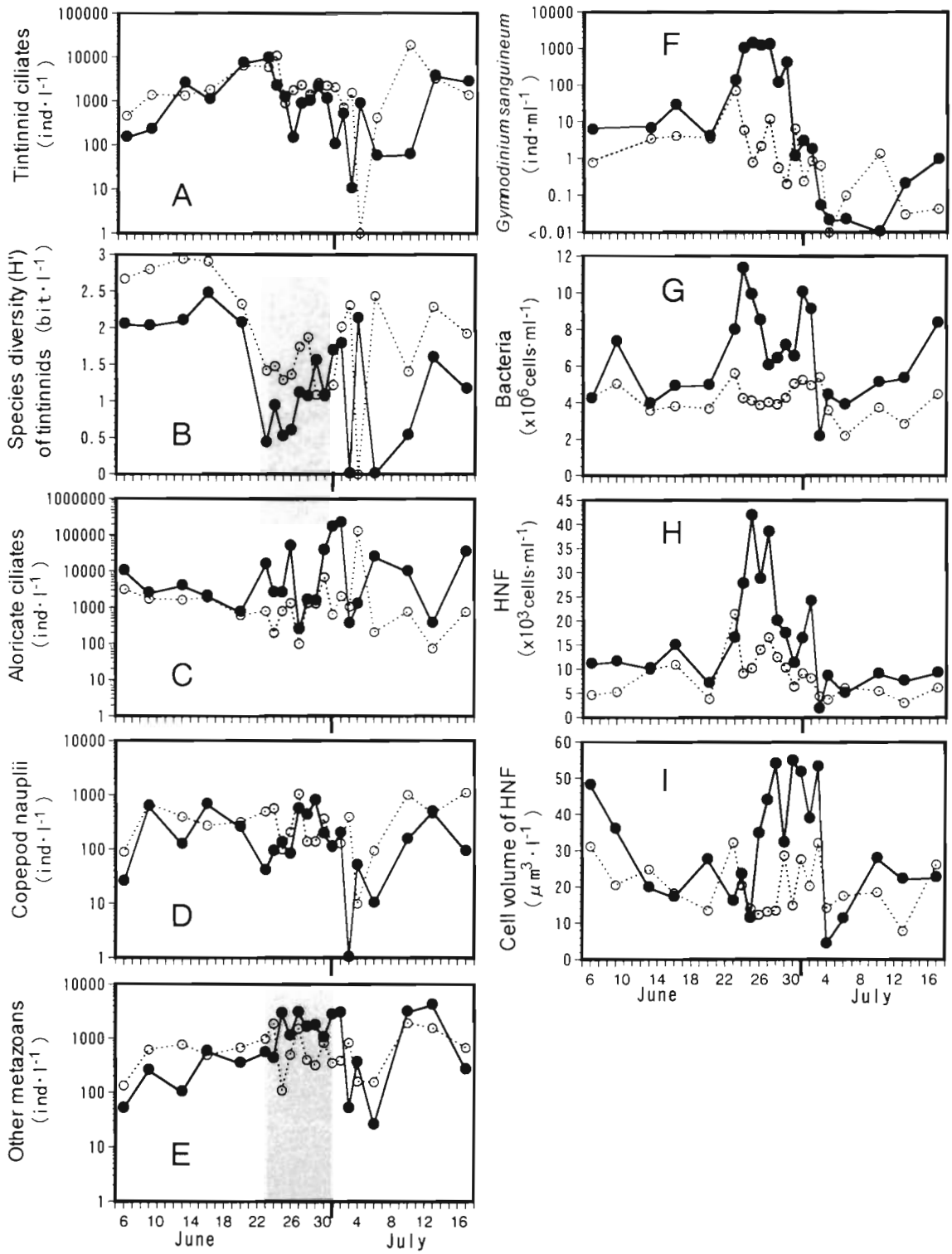

Fig. 2. Temporal changes in the abundance of microbial loop components (bacteria, heterotrophic nanoflagellates [HNF] and ciliates), species diversity of tintinnids, metazoan abundance and cell volume of HNF at the surface (●) and 1 m above the bottom (○) during the course of a *Heterosigma akashiwo* bloom. Shaded areas indicate the bloom period (June 23 to July 1, 1995) when the density of *H. akashiwo* exceeded 10^4 cells ml^{-1}

Bacteria and HNF

Bacterial abundance ranged from 3.5 to 7.3 × 10^6 cells ml^{-1} in both layers before the bloom. In the surface layer, the bacterial population increased during the course of the bloom (Fig. 2G). Two abundance peaks were recorded just after the beginning and just after the end of the bloom. A maximum abundance of 11.3 × 10^6 cells ml^{-1} was detected in the surface layer on June 24.

HNF abundance ranged from 3.9 to 14.9 × 10^3 cells ml^{-1} in both layers before the bloom (Fig. 2H). The bloom formation led to an increase of HNF abundance in a similar pattern as for bacteria. Fluctuations in HNF abundance were found to be tightly coupled with those of bacterial abundance; there were 2 abundance peaks detected after 1 to 3 d of the bacterial abundance peaks. During the bloom, the maximum abundance in the surface layer exceeded 41.7 × 10^3 cells ml^{-1} on June 25. The cell volume of HNF during the later stage and at the end of the bloom increased to a level 2 or 3 times higher than that in the early stage of the bloom (Fig. 2I).

Changes in the biomass of microbial loop components

The biomass of bacteria and HNF in both layers ranged from 29 to 149 µg C l^{-1} (mean value in both layers: 40 to 103 µg C l^{-1}) and from 3 to 117 µg C l^{-1} (mean value in both layers: 40 to 66 µg C l^{-1}), respectively (Fig. 3). Although the mean biomass ratio of HNF to bacteria in both layers ranged from 6 to 42% during the periods before and after the bloom, the ratio increased during the bloom and reached 100% on June 27.

The biomass of the ciliate community was dominated by tintinnids before the bloom. At the early stage of the bloom, the maximum tintinnid biomass was recorded at 35 µg C l^{-1}—this was 10 times as large as the biomass of aloricate ciliates. However, the dominance of ciliated protozoans shifted from tintinnids to aloricate ciliates when the bloom decayed. The biomass of aloricate ciliates increased substantially on July 1 to exceed the bacterial biomass (134 µg C l^{-1}).

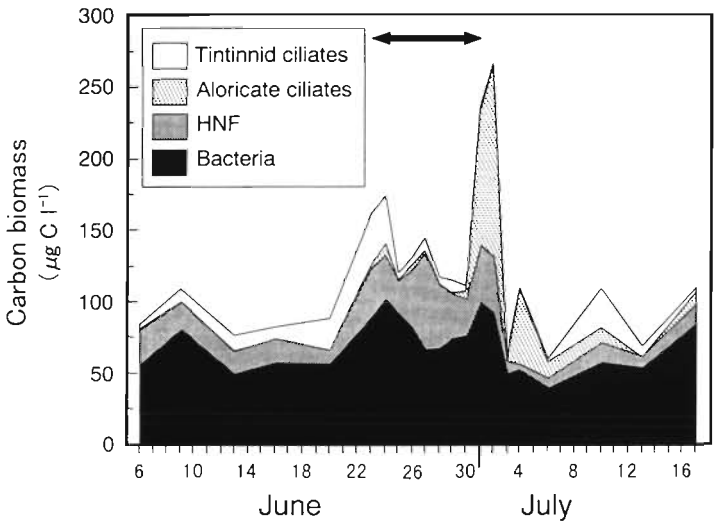

Fig. 3. Temporal changes in the carbon biomass of bacteria, HNF and ciliates during the course of a *Heterosigma akashiwo* bloom. The arrow indicates the bloom period (June 23 to July 1, 1995) when the density of *H. akashiwo* exceeded 10^4 cells ml^{-1}

DISCUSSION

The bloom of *Heterosigma akashiwo* occurred at surface temperatures between 21.6 and 23.2°C. During a different time period (May 30 to June 4) in 1994, we also observed the formation of a *H. akashiwo* bloom in the same area when surface temperatures ranged from 19 to 21°C (Kamiyama et al. unpubl. data), suggesting that the outbreak of this bloom is associated with surface temperatures around 20°C. Excystment of *H. akashiwo* occurs actively at around 20°C in marine sediments (Yamochi 1989), which might cause the appearance of vegetative cells in the water column and may be a trigger for the bloom. Before the bloom, large influxes of nutrients into the water column were not observed, indicating that nutrient conditions were not directly related to the formation of the blooms. In the present study, a large amount of rainfall could be one of the main causes of the disappearance of the *H. akashiwo* population. Before the sudden decrease of surface salinity, part of the *H. akashiwo* population moved from the surface layer to the near bottom layer on July 2, and then disappeared. This implies that there were other factors causing the collapse of the *H. akashiwo* bloom. Kim et al. (1998) demonstrated that *H. akashiwo*-killing bacteria contributed to the termination of this bloom at the same site as in this study.

The abundance of *Gymnodinium sanguineum* increased with increasing densities of *Heterosigma akashiwo*. This dinoflagellate is mixotrophic and can feed on ciliates and other phytoplankton (Bockstahler & Coats 1993, Li et al. 1996). Hence, there is a possibility that development of the *G. sanguineum* population was due to active feeding on these organisms during the formation of the bloom.

In Hiroshima Bay, 2 studies have reported that the seasonal abundance levels of bacteria and HNF were 1 to 5 × 10^6 cells ml^{-1} and 1 to

10×10^3 cells ml^{-1}, respectively (Iwamoto et al. 1994, Imai & Yamaguchi 1996). Assuming the conversion factors of 0.14 pg μm^{-3} for bacteria (Nagata & Watanabe 1990) and 0.07 pg μm^{-3} (Sorokin 1979) for HNF, the biomass of these organisms were between 12 and 65 µg C l^{-1} and between 2 and 20 µg C l^{-1}, respectively. Imai & Itoh (1984) showed that the maximum abundance and biomass of bacteria in Suo Nada, in the western part of the Seto Inland Sea, were 3.6×10^6 cells ml^{-1} and 30.8 µg C l^{-1}, respectively; those of HNF were 4.5×10^3 cells ml^{-1} and 9.0 µg C l^{-1}, respectively. The maximum values for bacteria (abundance: 11.3×10^6 cells ml^{-1}, biomass: 149 µg C l^{-1}) and HNF (abundance: 41.7×10^3 cells ml^{-1}, biomass: 117 µg C l^{-1}) in the present study exceeded those in adjacent marine areas. However, the maximum values in the present study are not excessively high when compared with the maximum abundance or biomass in Limfjorden, coastal Denmark (bacteria: 15.2×10^6 cells ml^{-1}, HNF: 15.2×10^3 cells ml^{-1}; Andersen & Sørensen 1986) or those in the Marsdiep area of the North Sea (bacteria: ca 380 µg C l^{-1}, HNF: ca 270 µg C l^{-1}; van Boekel et al. 1992). Similarly, the maximum abundance and biomass levels of tintinnid and aloricate ciliates in this study (2.15×10^5 ind. l^{-1} and 268 µg C l^{-1}) were higher than those in a eutrophic bay and in a variety of estuaries: Chesapeake Bay (16 µg C l^{-1}; Dolan & Coats 1990), Kiel Bight (56 µg C l^{-1}; Smetacek 1981), Damariscotta estuary (33 µg C l^{-1}; Revelante & Gilmartin 1987), in Southampton water (141 µg C l^{-1}; Leakey et al. 1992) and in the NW Mediterranean Sea (2.60×10^4 ind. l^{-1} and 96 µg C l^{-1}; Vaqué et al. 1997), and similar to data during a *Phaeocystis* bloom period in the Marsdiep area of the North Sea (230 µg C l^{-1}; van Boekel et al. 1992). The high abundance and biomass levels of bacteria, HNF and ciliates in this study indicate that the intense phytoplankton blooms increase to a large extent the flow of energy through the microbial loop.

The decrease of tintinnid ciliates detected during the bloom of *Heterosigma akashiwo* is a distinctive characteristic of the change in the microzooplankton community. Collapses of tintinnid ciliate populations by high densities of *H. akashiwo* have been reported in previous studies (Verity & Stoecker 1982, Kamiyama 1995). The diversity of tintinnid species also decreased during the bloom, and this diversity was found to be inversely proportional to the density of *H. akashiwo* in this study (Fig. 4). A drastic decline of species diversity in the tintinnid community was also observed during a *H. akashiwo* bloom in Hiroshima Bay in 1994 (Kamiyama 1995). After the end of the bloom in this study, the abundance of tintinnids did not recover rapidly, because the extremely low salinity in the surface layer probably prevented tintinnids

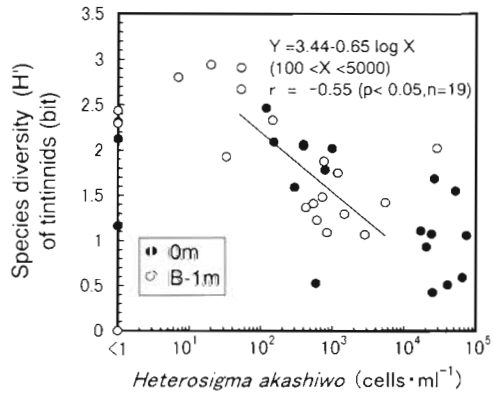

Fig. 4. Relationships between *Heterosigma akashiwo* abundance and the species diversity of tintinnid ciliates

from proliferating. Tintinnid population collapses are interpreted to be caused by the harmful effects of intracellular elements (Verity & Stoecker 1982) or mucus (Egloff 1986) of *H. akashiwo*, which probably inhibit the consumption of *H. akashiwo* (Taniguchi & Takeda 1988).

In general, an increase in bacteria coincides with an increase in chl *a* concentration indicating phytoplankton abundance (Bird & Kalff 1984). In the present study, a positive correlation was observed between abundances of *Heterosigma akashiwo* and *Prorocentrum* spp. (more than 100 cells ml^{-1}) and bacterial abundance (Fig. 5). Increases of bacterial abundance at the onset and at the end of the bloom were obvious, probably due to an increase in dissolved organic matter (DOM) produced by *H. akashiwo* and *Prorocentrum* spp. or by lysis of these algae. The increase of PO_4-P concentration and the decrease of the Si:P ratio

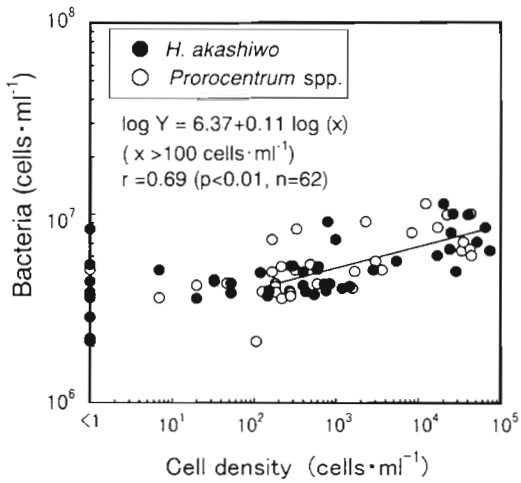

Fig. 5. Relationships between abundance of bacteria and *Heterosigma akashiwo* and *Prorocentrum* spp.

in the B-1 m layer around the end of the bloom (Fig. 1G,J) imply that bacteria actively produced PO_4-P by mineralizing organic phosphorus originated from *H. akashiwo* and *Prorocentrum* spp. Cole (1982) reported that DOM, exuded by the abundant phytoplankton, promoted the development of a bacterial population. Van Boekel et al. (1992) reported that cell lysis after a *Phaeocystis* sp. bloom caused an increase in bacterial biomass. The temporary decrease of bacteria during the midterm of the bloom observed in the present study may be caused by the grazing control of HNF.

In short-interval investigations, peaks of bacterial abundance are usually followed by increases in HNF abundance with lag periods, which is the predator-prey oscillation. In the present study, 2 peaks of bacterial abundance observed just after the bloom formation and the collapse were followed by the peaks of HNF abundance with a 1 to 3 d lag period. This lag period is somewhat shorter than data (1 to 8 d) in the other short-term investigations (Fenchel 1982, Andersen & Sørensen 1986, Nakamura et al. 1994, Tanaka et al. 1997), implying that the increase of HNF rapidly responded to the increasing bacteria under conditions after the bloom formation and the decay. After July 2, the synchronization between bacteria and HNF abundance was observed. Tanaka & Taniguchi (1999) demonstrated 6 factors that caused uncoupling and synchronization in the bacteria-HNF oscillation. Among them, physical disturbance due to much rainfall may strongly associate with the synchronization after June 2. Consequently, it is likely that short-lag and no-lag periods (synchronization) during and after the bloom caused significant correlation relationships between bacteria and HNF in the present study (Fig. 6).

An increase of aloricate ciliates at the end of the bloom is probably caused by the increases of bacteria and HNF, indicating that the trophic energy in the microbial loop from DOM, bacteria and HNF was efficiently linked to aloricate ciliates at the end of the bloom. Such increase on the biomass basis between June 30 and July 1 was from 6 to 95 µg C l^{-1}. The potential specific growth rate during this period can be estimated to be 2.65 d^{-1} (= 3.82 divisions d^{-1}), based on the temperature (23°C) and the mean cell volume of aloricate ciliates (6100 μm^3 $ind.^{-1}$) on July 1, using the equation to estimate maximum growth rate of ciliates proposed by Müller & Geller (1993). As a result, biomass of aloricate ciliates can potentially increase up to 87 µg C l^{-1}, which is similar to the actual biomass data (95 µg C l^{-1}) obtained on July 1. This suggests that aloricate ciliates were reproducing at near-maximal rates with little loss to macrozooplankton predation. Kamiyama (1995) also observed temporal increases in small aloricate ciliates at the end of a *H. akashiwo* bloom. Furthermore, Nielsen et al. (1990) reported that after the end of a harmful algal bloom of *Chrysochromulina polylepis*, microzooplankton accumulated at a specific layer where the alga had become concentrated.

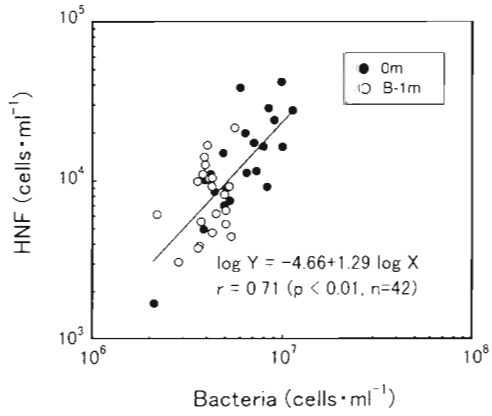

Fig. 6. Correlation between abundances of bacteria and HNF

In conclusion, a detailed investigation of microbial loop components revealed a drastic change in the food web systems during the course of the *Heterosigma akashiwo* bloom. In particular, it is clear that the bloom formation and decay enhanced the flow of energy within the microbial loop from the bacteria through the HNF to the aloricate ciliates. In spite of this, the energy flow to the tintinnids may be larger than that to the aloricate ciliates, except the bloom periods. We cannot specify the carbon budget between these organisms, since all the food sources of ciliates, such as picoplankton and autotrophic nanoflagellates, were not examined and information on heterotrophic and mixotrophic dinoflagellates except *Gymnodinium sanguineum* was lacking in the present research. Picoplankton are an important prey for HNF (Caron et al. 1991, Kuosa 1991) and ciliates (Bernard & Rassoulzadegan 1990, 1993). Heterotrophic and mixotrophic dinoflagellates are also major consumers of nano- and microplankton (Lessard & Swift 1985, Verity et al. 1993, Li et al. 1996). Further studies are needed to clarify the trophic interactions between these food web components in a highly productive marine environment where mechanisms within the food web system can be altered.

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