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The microbial loop in a eutrophic bay and its contribution to bivalve aquaculture

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Abstract Information on planktonic food webs around bivalve farms is important, because bivalves utilize natural suspended matter as food. Not only phytoplankton but also other heterotrophic protists are occasionally essential to bivalves. Oysters cannot use bacterioplankton, but they can ingest protists that feed on bacterioplankton, thus using microbial energy indirectly through this microbial loop. To evaluate the importance of the microbial loop in planktonic food webs, occurrences of bacteria and heterotrophic protists were studied in the eutrophic Hiroshima Bay, where oyster aquaculture is economically important. Temporal changes in microbial loop components suggested that energy flow within the microbial loop was enhanced at the end of a phytoplankton bloom. The distribution of microbes and other protists implies that transfer efficiencies within planktonic food webs including the microbial loop differed among regions of the bay. Thus, the microbial loop may play an important role in planktonic food webs in Hiroshima Bay. In some oyster ponds in France, the importance of microbial energy flow to oyster production was actually evaluated. Possibly, energy flow within the microbial loop is also important to oyster production in Japan.

Key words: microbial loop, bacteria, flagellate, ciliate, oyster

In various oceanic regions, significant energy flows from bacteria to higher trophic levels and return flows from such organisms to dissolved organic matter pool, that is an original sense of the microbial loop (Azam *et al.*, 1983), have been clarified (eg. Sieburth, 1984; Pierce and Turner, 1992). In eutrophic bays, where the bacterial biomass and production are very high, possibly the bacterial production strongly influences planktonic food webs, which may enhance the productivity of higher trophic levels (Sherr *et al.*, 1986; Sherr and Sherr, 1988; Fukami *et al.*, 1999).

Hiroshima Bay is one of the eutrophic areas in the Seto Inland Sea of Japan. The northern, inner region of the bay is strongly influenced by riverine inputs and has a very low seawater exchange rate with the outer region. Also, like chlorophyll-*a* concentrations, the biomass and production of bacteria are high in the Seto Inland Sea (Yamaguchi *et al.*, 1995; Imai and Yamaguchi, 1996; Tada *et al.*, 1998), implying that microbial food webs play important roles in the ecosystem of the bay. There are many rafts for aquaculture of oysters throughout the whole bay and adjacent areas, and oyster production in these areas constitutes 50-60 % of the total Japanese production. To utilize the oyster productivity effectively and sustainably, it is necessary to clarify the food web components and the energy flow between them, including the microbial loop system.

In the present study, firstly, the temporal change of the microbial loop components in the coarse of a phytoplankton bloom was elucidated. Secondly, the characteristics of the

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distributions of the main microbial loop components [bacteria, heterotrophic nanoflagellates (HNF) and ciliates] as well as the environmental conditions in Hiroshima Bay in summer were evaluated. Thirdly, the function of the microbial loop to oyster production was reviewed and then the importance of the energy flow from ciliates to oyster was discussed.

Temporal changes in the microbial loop components in the coarse of a phytoplankton bloom

The investigation was conducted in a harbor (ca. 5-m water depth) in the northern part of Hiroshima Bay, the Seto Inland Sea of Japan (Fig. 1), to clarify the temporal changes in the microbial loop components in the coarse of the harmful alga *Heterosigma akashiwo*. Seawater sampling was carried out at surface and 1 m above the bottom every one to four days in the mornings from 6 June to 17 July in 1995. *Heterosigma akashiwo* cells were counted in fresh seawater from each depth. A seawater subsample was fixed with Lugol's iodine solution (final concentration : 2 %), and then concentrated by settling. Microzooplankton (zooplankton with body widths less than 200 μm) of each subsample were counted in 1-2 mL of concentrated samples with a phase contrast microscope using a Sedgwick-Rafter chamber. Another seawater in each sample was fixed by glutaraldehyde (final concentration: 1 %), and fixed bacteria and HNF were stained with DAPI (Porter and Feig, 1980) and DAPI and FITC (Sherr and Sherr, 1983a; 1983b), respectively, and then counted with epifluorescence microscopy.

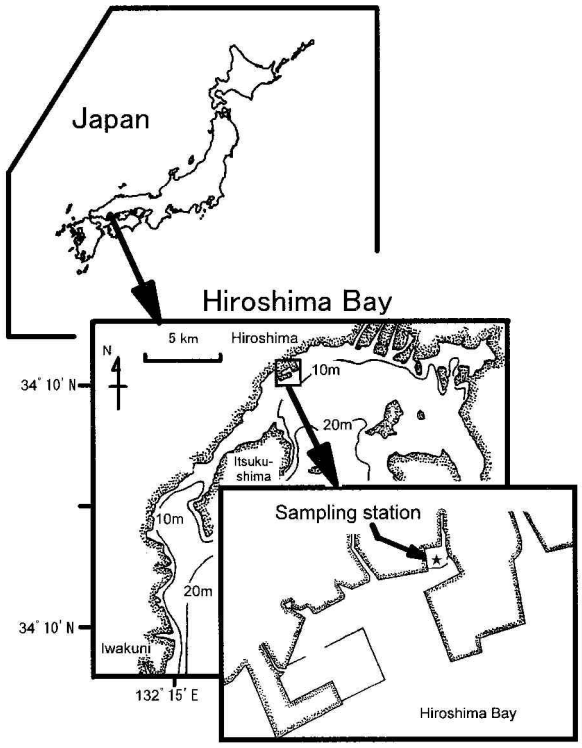

Fig. 1. Sampling station for investigating the change in the microbial loop components in a harbor in Hiroshima Bay, the Seto Inland Sea of Japan

Decay of tintinnid-ciliate population

A *H. akashiwo* bloom with a cell density of over 10^4 cells mL^{-1} in the surface water occurred from 23 June to 1 July 1995 (Fig. 2). A typical negative effect of the *H. akashiwo* bloom on the microzooplankton community was the decay of tintinnid ciliate population. During the bloom period, the abundance of tintinnid ciliates in the surface layer markedly decreased by one order of magnitude, compared to the abundance before the bloom (Fig. 2). The species diversity (Shannon-Wiener's H') of tintinnids in the surface layer also decreased during the bloom. Similar phenomena during *H. akashiwo* blooms have been reported in previous studies (Verity and Stoecker, 1982; Kamiyama, 1995).

Response of the microbial loop components

The microbial loop components fluctuated in response to the formation and decay of the bloom. Two abundance peaks of bacteria in surface water were recorded just after the beginning and just after the end of the bloom (Fig. 3). Also, the fluctuations in HNF abundance were found to be tightly coupled with those of bacterial abundance; there were two abundance peaks detected after 1 - 3 days of the bacterial abundance peaks. Although conspicuous change of aloricate ciliates was not observed

during the process of bloom formation, the abundance increased drastically at the end of the bloom, and temporarily became two orders higher in magnitude than before the bloom.

These fluctuations of the microbial loop components reveals a drastic change in the food web system during the course of the *H. akashiwo* bloom. In particular, it is clear that the decay of the bloom enhanced the flow of energy within the microbial loop from the bacteria through the HNF to the aloricate ciliates. This process is interpreted as follows. The decay of the *H. akashiwo* bloom probably caused increase in dissolved organic matter (DOM) because of cell lysis of *H. akashiwo*. Increased DOM possibly stimulated the production of heterotrophic bacteria (Cole *et al.*, 1988). Consequently, bacterivorous organisms such as HNF and small aloricate ciliates increased in response to the food supply. Partly, production of aloricate ciliates may be also promoted by the increase of HNF. It is interesting that it took only a few days for the process from decay of the bloom to the increase of aloricate ciliates. The microbial loop system probably responds the change of the plankton community quickly.

Characteristics of the distributions of bacteria, HNF and ciliates in Hiroshima Bay in summer

Investigation was performed in 4 - 6 layers in the water column at 6 sites in Hiroshima Bay (Fig. 4) from a research vessel in summer, (13-15 June and 20-22 August 1996, and 1 - 3 July 1997). Environmental parameters were measured and analyzed (temperature, salinity, chlo-

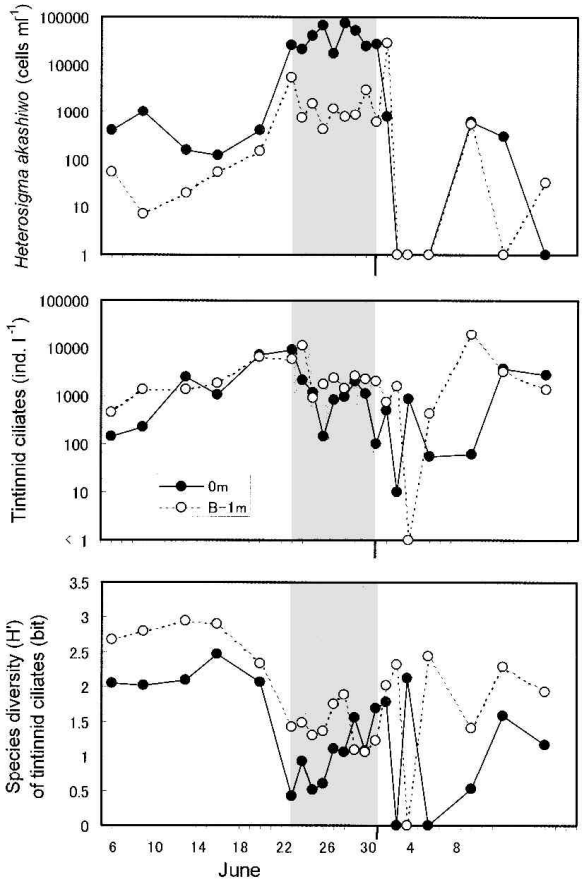

Fig. 2. Temporal changes in abundance of *Heterosigma akashiwo*, and abundance and species diversity of tintinnid ciliates during the course of the bloom. Shaded areas indicate the bloom period (23 June to 1 July 1995) when the density of *H. akashiwo* exceeded 10^4 cells mL^{-1}

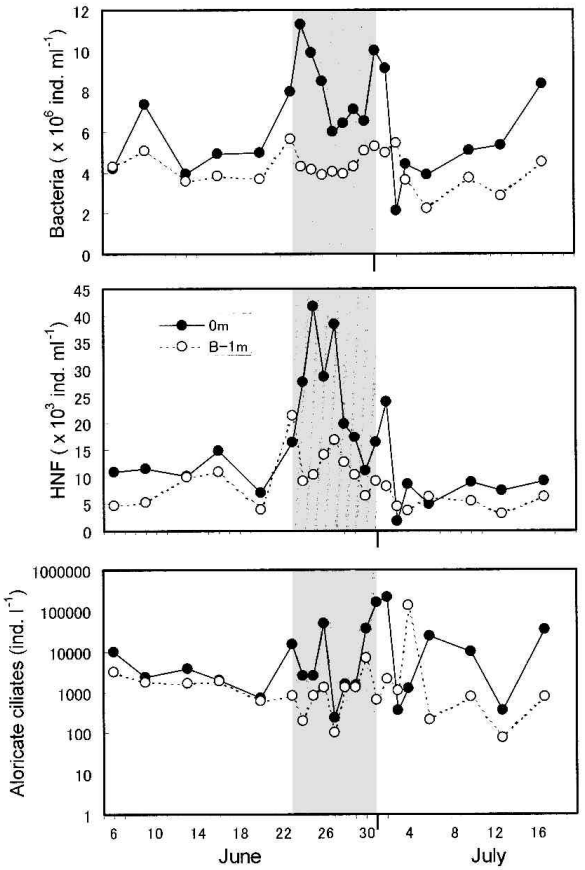

Fig. 3. Temporal changes in the abundance of microbial loop components of bacteria, heterotrophic nanoflagellate (HNF) and aloricate ciliates during the course of a *Heterosigma* akashiwo bloom. Shaded areas indicate the bloom period (23 June to 1 July 1995) when the density of *H. akashiwo* exceeded 10^4 cells mL^{-1}

rophyll-*a* and nutrients). Based on unfractionated seawater (total faction) and the seawater filtered through a 20-μm mesh screen (<20-μm fraction), the chlorophyll-*a* concentration in each fraction was measured with a Turner Designs fluorometer according to Suzuki and Ishimaru (1990). From another filtered seawater sample, the concentrations of dissolved organic nitrogen (DIN) that consists of NO_2-N, NO_3-N, NH_4-N, and PO_4-P and SiO_2-Si were determined using a TrAAcs 800 autoanalyzer (Bran-Luebbe Co.)

The main microbial loop components (bacteria, HNF and ciliates) were also investigated at 3 of the 6 sites: the inner (Stn. 1), central (Stn. 3) and outer (Stn. 5) regions of the bay (Fig. 4). Abundances of them were generally measured with the same method as the above. Simultaneously the cell dimensions of each taxon for HNF and aloricate ciliates and the lorica dimension of each tintinnid-ciliate species were measured, and the cell volume of them was calculated by approximating the shape of each taxon to a standard geometric configuration. Bacterial carbon biomass was estimated based on the average cell volume (0.098 μm^3 $cell^{-1}$) from annual Hiroshima Bay data (Imai and Yamaguchi, 1996) and an allometric conversion equation (29.6 f gC $cell^{-1}$; Loferer-Krößbacher *et al.*, 1998). The carbon biomass of HNF and aloricate ciliates were estimated based on the cell volume data and the carbon-volume conversion factors for HNF (0.22 pgC $cell^{-1}$; Børsheim and Bratbak, 1987) and for aloricate ciliates (0.19 pgC μm^{-3}; Putt and Stoecker, 1989), and that of tintinnid ciliates were calculated based on the volume of the lorica according to Verity and Langdon (1984).

Environmental conditions

The strong influence of riverine inputs on the seawater environment in the inner region of the bay was recognized by the low salinity in the surface on all occasions. Similarly, in July 1997, the concentrations of nutrients (DIN, PO_4-P and SiO_2-Si) were comparatively high in the surface layer at the innermost site (Stn. 1; distribution of only DIN concentration shown in Fig. 5). However, high concentrations of DIN in the surface layer were limited to the innermost site where chlorophyll-*a* concentrations were high (Stn. 1, 2), suggesting that riverine-nutrient inputs may rapidly be taken up by phytoplankton in the vicinity of the river mouth areas in Hiroshima Bay. Generally, higher concentrations of chlorophyll-*a* were recorded in the surface and near-surface layers in the inner regions of the bay (Stn. 1 and 2; Fig. 6). The contribution of the <20-μm fraction to total chlorophyll-*a* concentration mostly exceeded 60 % in June 1996 and in July 1997. In August 1996, also more than 60 % of the contribution of the <20-μm fraction was recorded in the almost all layers at Stn. 1 and 2 although less than 40 % of the contribution was partly

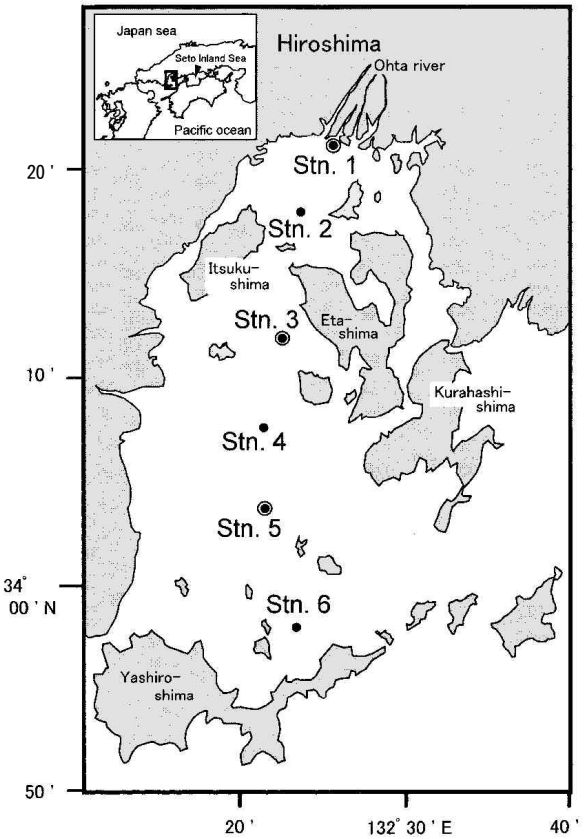

Fig. 4. Study sites in Hiroshima Bay for investigating the distribution of the microbial loop components. Environmental conditions were surveyed at all sites, while abundance and biomass of the microbial loop components (bacteria, heterotrophic nanoflagellates and ciliates) were investigated at Stn. 1, 3 and 5 only (◉)

observed in the central and outer regions of the bay. This result indicates that the phytoplankton assemblage is generally dominated by nano- and picoplankton in the inner region of the bay in summer.

Abundance of the main microbial loop components

In the inner region of the bay, abundances of the main microbial loop components were higher than in the other regions. Abundances of bacteria and HNF ranged from 1.10×10^6 cells ml^{-1} to 5.75×10^6 cells ml^{-1} and from 0.52×10^3 cells L^{-1} to 11.06×10^3 cells L^{-1}, respectively. The highest abundances of both organisms on each occasion were recorded in the surface, 2-m or 5-m layers at Stn. 1 (Fig. 7). The abundance of total ciliates ranged from 0.6×10^2 ind. L^{-1} to 20.5×10^3 ind. L^{-1} during the study, and distributed abundantly in the surface and 2-m layers at Stn. 1 in all months. Ciliate assemblages were generally dominated by aloricate forms except for samples collected in August 1996 at Stn. 1 and 5 (Fig. 8). In July 1997, the maximum abundance of ciliates, especially the tintinnid forms at Stn. 1, was considerably lower than in June and August 1996 (Fig. 8).

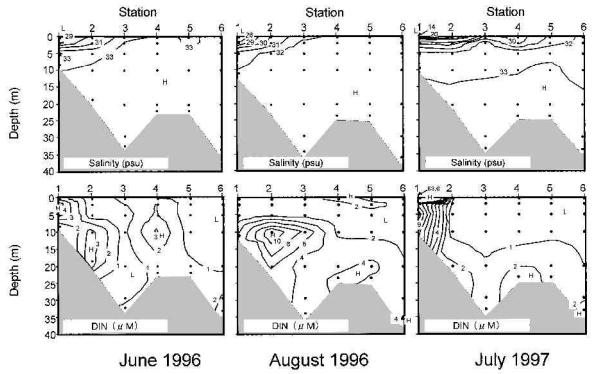

Fig. 5. Horizontal and vertical distributions of salinity and dissolved inorganic nitrogen (DIN) concentration.

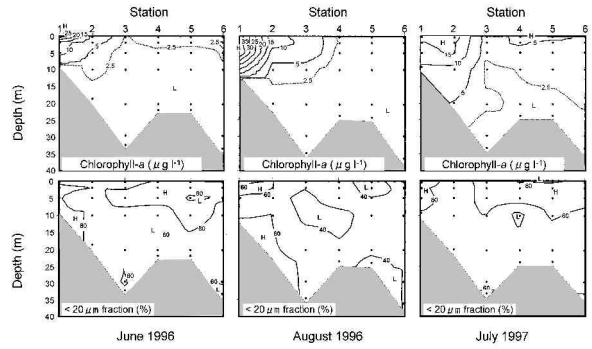

Fig. 6. Horizontal and vertical distributions of the chlorophyll-*a* concentration and the percentage of the chlorophyll-*a* concentration accounted for by the <20-μm fraction

Transfer efficiency from primary producers to their grazers

Among Stn. 1, 3 and 5 in the present study, Stn. 1 was characterized by high chlorophyll-*a* concentrations and high abundance of the microbial loop components. High biomass of primary producers may sustain the high production of the grazer plankton. Further, to evaluate the transfer efficiency of energy from the primary producers to their grazers, the relationships of the carbon biomass between each ciliate assemblage (tintinnid or aloricate ciliate) and the prey organisms (bacteria, HNF, and <20-μm chlorophyll-*a*) were analyzed, assuming that the carbon conversion factor of the <20-μm chlorophyll-*a* was 30 (Riley, 1965; Parsons, 1984). For this analysis, stable prey-predator conditions are necessary but at Stn. 1 in July 1997, environmental conditions indicat-

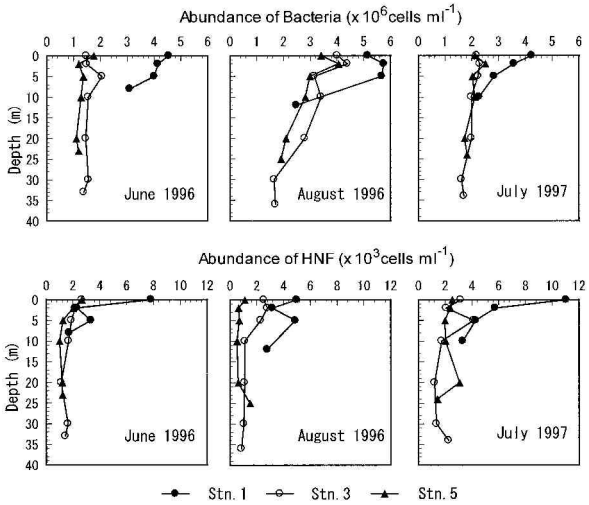

Fig. 7. Vertical distributions of the abundances of bacteria and heterotrophic nanoflagellates (HNF) at Stn. 1, 3 and 5 in Hiroshima Bay

ing extremely low salinity from riverine inputs imply that the stable prey-predator conditions was not applicable for the ciliate assemblage. Hence, data from Stn. 1 in July was not used for the analysis.

Regarding the relationships between aloricates and the total prey organisms, the significant positive linear correlations ($r^2=0.45$ - 0.73, $p<0.05$) were observed at all sites (Fig. 9). The slope of the linear regression at Stn. 3 was significantly higher than those at Stn. 1 and 5 ($p<0.01$). Further, the X-axis intercept at Stn. 1 in Fig. 9 was significantly higher than those at Stn. 3 and 5 ($p<0.01$), implying that the biomass of primary producers and microbes not utilized by aloricate ciliates was higher at Stn. 1 than at the other sites. These interpretations demonstrate that the energy transfer efficiency from primary production and microbes to aloricate ciliates may be low in the inner region of the bay (Stn. 1) compared to the central region of the bay (Stn. 3).

To consider the energy transfer efficiency, it is necessary to practically measure the primary production and subsequent grazing impact, because information on biomass only represents a scene of dynamic relationships between prey and predator. In spite of such limitations, biomass relationships between prey and predators in this study were clear and characteristics differed among areas in the bay, suggesting that the biomass relationship may be one of the factors to evaluate the efficiency of energy flow in planktonic food webs.

Contribution of the microbial loop to production of bivalve aquaculture

The size of prey that bivalves can feed upon depends on the bivalve species. Some mussels can retain the particles of bacterial size (Kreeger and Newell, 1996) but oysters cannot efficiently utilize the picoplankters (Langdon and Newell, 1990). However, the oysters have the ability to efficiently retain the particles larger than 6μm (Palmer and Williams, 1980), which included the size of bacterivorous protists. Hence, it is a possibility that oysters can utilize the picoplanktonic energy source by ingesting bacterivorous protists. Le Gall *et al.* (1997) proved this scenario. The cyanobacteria *Synechococcus* sp. retaining specific yellow-gold autofluorescence was supplied for the ciliate *Uronema* sp. as food source. This protist that

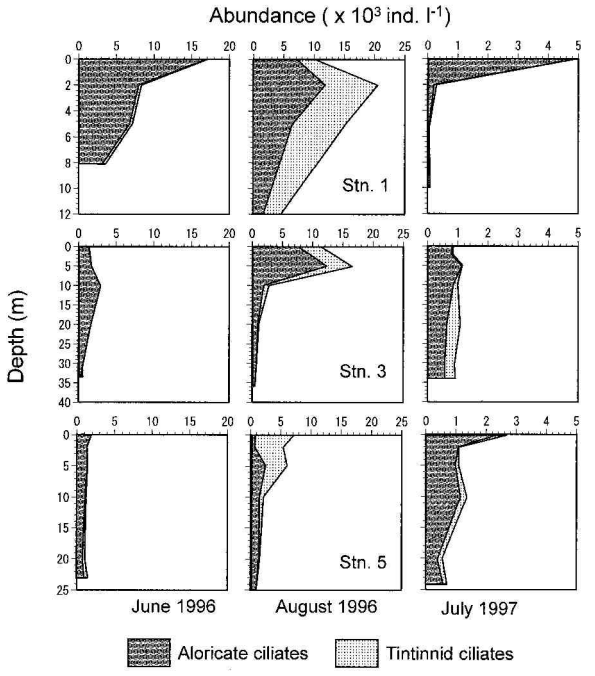

Fig. 8. Vertical distributions of the abundance of ciliates at Stn. 1, 3 and 5 in Hiroshima Bay

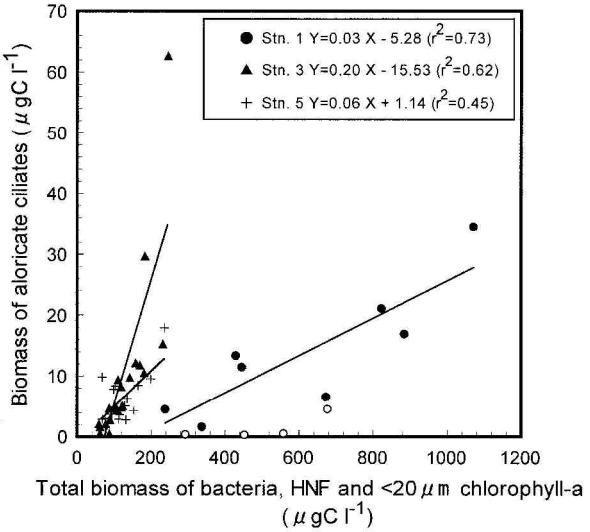

Fig. 9. Relationships between the carbon biomass of aloricate ciliates and all prey organisms that consist of bacteria, heterotrophic nanoflagellates (HNF) and <20-μm fraction of chlorophyll-*a*. ○ (data at Stn. 1 in July 1996) was omitted from the calculation of the regression line

fed on *Synechococcus* was labeled by many spots of the yellow-gold autofluorescence observed by epifluorescence microscopy under blue-light excitation. Then, the labeled *Uronema* was supplied to oysters, large amount of autofluorescent particles clumped on residual membranes of labeled ciliates was observed in the stomach of oysters, although few parcels could be found in the stomach of oysters which were provided with only *Synechoccus* suspension. The results suggest that the ciliates play a role as a trophic intermediation between picoplankton and oysters. This result is evidence that oysters can receive from not only phytoplankton energy source but also from the microbial loop energy flow.

Actually, the effects of oyster feeding on protists and picoplankton were compared under laboratory experiments (Dupuy *et al.*, 2000b). While the abundance of picophytoplankton was constant under both conditions with a filtering oyster and without oyster, in the case of ciliates as prey, the abundance decreased under the conditions with a filtering oyster for the first 15 minutes (Fig. 10). Also, the feeding activity of oysters was compared between food sources. The clearance rate of oysters on ciliates was in the same level as that on diatoms as shown in Fig. 11 (Dupuy *et al.*, 2000b). Furthermore, based on the feeding activities of the oyster *Crassostrea gigas* on the ciliates, the carbon specific feeding impacts of oysters on ciliate assemblages in some oyster ponds in France were estimated and compared with the impact on phytoplankton assemblages (Table 1). The results indicated that the ratio of removed carbon contents from ciliate assemblage to that from phytoplankton assemblage ranged from 0.30 to 4.5, implying that importance of ciliates for oyster probably depends on the environmental conditions and the food-web systems in the oyster farming area. Ciliate may not be always important food source for oysters, but there is a possibility that they play an important role as a food source if the microbial loop activity was high and the phytoplankton biomass is insufficient for oyster food.

The role of the heterotrophic protists as food source for oysters has been pointed out in the some ponds where the oyster was cultured on the bottom (Dupuy *et al.*, 1999; 2000a; 2000b). In Japan, generally cultured oysters are suspended into the seawater from the specific rafts or line to get higher production of oyster in a unit area. Hence, probably the oyster production more strongly depends on the planktonic food webs in the oyster farming areas. In some areas in Japan, unknown death in summer, decreasing production in a unit area and damage due to red tides have caused many problems for

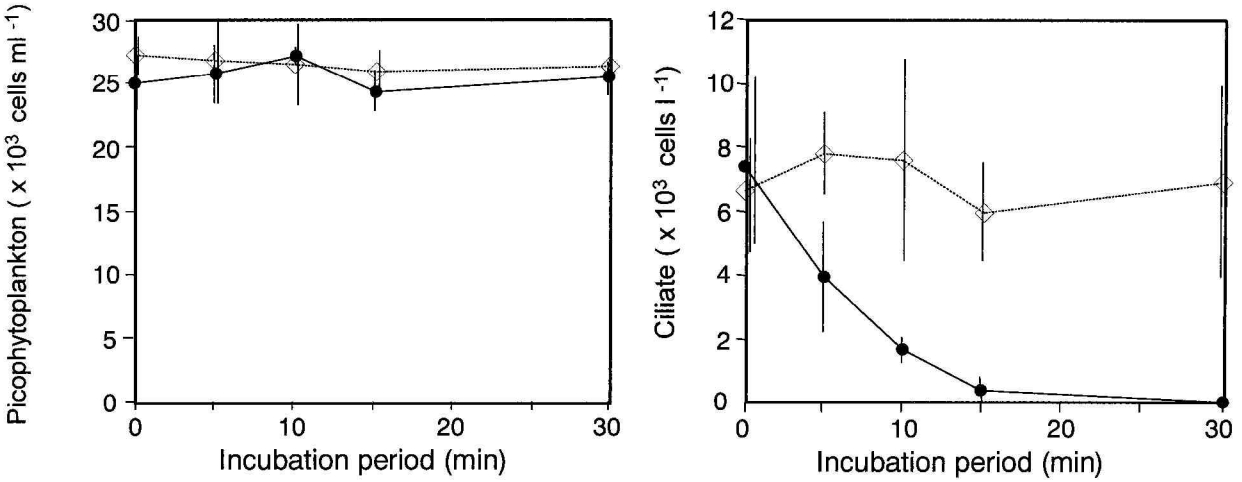

Fig. 10. Change in abundances (mean±SD, n=3) of picophytoplankton and ciliates under the conditions with a filtering oyster (●) and without oyster (◇)(Modified from Dupuy *et al.*, 2000b)

Table 1. Feeding impact by *Crassostrea gigas* on ciliates and phytoplankton

Area	Ciliates			Phytoplankton			
	Prey	Biomass (μgC L^{-1})	Removed(**A**) (μgC $h^{-1}g^{-1}$)	Prey	Biomass (μgC L^{-1})	Removed(B) (μgC $h^{-1}g^{-1}$)	A/B
French Atlantic coastal ponds*1	FA	63.5	126	CD	—	27.5	4.5
French Atlantic coastal ponds*2	FA	7-32	48-218	FA	161-648	641-2652	0.07-0.21
Mediterranean Thau Lagoon*3	FA	3	38.6	FD	161.5	1307	0.03

*1: Dupuy et al. 1999, *2: Dupuy et al. 2000a, *3: Dupuy et al. 2000b.
FA: field assemblage, FD: field diatoms, CD: cultured diatom (*Phaedactylum tricornatum* 1×10^6 cells L^{-1})

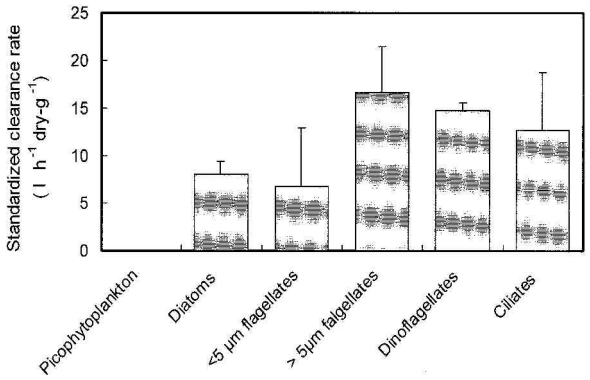

Fig. 11. Standardized clearance rates (mean ± SD, n=3) of the oyster *Crassostrea gigas* on the various taxonomic groups. Data taken from Dupuy *et al.* (2000b)

oyster aquaculture. One of the reasons is too high density of oyster in the farming area, which probably exceeded the carrying capacity for oyster cultivation suitable for the particular environmental conditions. To use the bivalve farming area efficiently and lastingly, it is essential to estimate the carrying capacity for oyster aquaculture. For that, it is necessary to quantitatively clarify the food-web system (biomass of each planktonic group, its production and the trophic interaction) and to monitor environmental characteristics in oyster farming area as well.

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