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# Grazing impact of the field ciliate assemblage on a bloom of the toxic dinoflagellate *Heterocapsa circularisquama*

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**Abstract:** The ingestion rates of the toxic dinoflagellate *Heterocapsa circularisquama* by ciliate species in the field were measured using the fluorescently labeled algae (FLA) method with the vital fluorescent dye CMFDA (5-chloromethylfluorescein diacetate). Based on the results of the five in-situ feeding experiments, 16 species of tintinnid ciliates and 3 species of aloricate ciliates that can feed on *H. circularisquama* were recognized, and the mean ingestion rate of each species ranged from 0.2 to 14.5 cells indiv.$^{-1}$ h$^{-1}$. Further, abundances of ciliates and copepod nauplii were investigated when a bloom of *H. circularisquama* occurred in Hiroshima Bay, the Seto Inland Sea of Japan, and the grazing impact of the ciliate assemblage on the *H. circularisquama* concentration was estimated based on the species specific ingestion rates and their abundances. Ciliates potentially feeding on *H. circularisquama* were distributed abundantly in the northern parts of the bay where the concentration of *H. circularisquama* was in an order of magnitude of $10^2$ cells ml$^{-1}$. However, the abundance of the ciliate assemblage was low at the stations under red tide conditions (*H. circularisquama* cell concentration >10000 cells ml$^{-1}$), although copepod nauplii were abundant even under the red tide conditions. Daily grazing loss by the ciliate assemblage ranged from 3 to 53% of the *H. circularisquama* population. Results of a simulation with a short-interval prediction model for the *H. circularisquama* concentration, which takes into account these grazing impacts, indicated that information on grazer ciliates is important to improve the prediction model for the outbreak of *H. circularisquama* red tides.

**Key words:** *Heterocapsa circularisquama*, ciliate, ingestion rate, grazing impact, distribution, Hiroshima Bay

## Introduction

Since first recorded in 1988, blooms of *Heterocapsa circularisquama* Horiguchi have spread to the coastal waters of western Japan and caused serious damage to the aquaculture of pearl and common oysters, and the short-necked clam fishery (Matsuyama et al. 1995, 1996, 1997a). Recently the physiological and ecological characteristics of *H. circularisquama* have been clarified. Toxicity of this alga is specific to bivalves and does not cause any problems to fish. The main cause of the massive mortalities to bivalves is probably due to dysfunction of the heart by a toxic protein (or polypeptide) complex on the surface of *H. circularisquama* cells (Matsuyama et al. 1997b). The exact characteristics of the toxic substance are still not known in detail. This alga prefers high temperatures and salinities for growth (Yamaguchi et al. 1997).

There are only a few reports on the interactions of this alga with zooplankton. *Heterocapsa circularisquama* caused mortality of the tintinnid ciliate *Favella taraikaensis* at concentrations of more than 4000 cells ml$^{-1}$, and this was probably due to direct cell contact with the ciliate (Kamiyama & Arima 1997). Survival rates of the marine rotifer *Synchaeta* sp. and *Nocticulca scintillans* decreased during 1 to 3 d periods at dinoflagellate concentrations of ca. 100 cells ml$^{-1}$ and ca. 2000 cells ml$^{-1}$, respectively (Kamiyama 1999a). However, Kamiyama (1997) reported that two species of tintinnid ciliates *Favella azorica* and *F. taraikaensis* actively fed on *H. circularisquama* when the concentration of this alga was below 1000 cells ml$^{-1}$. The

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calanoid copepod *Acartia omorii* was also actively able to feed on *H. circularisquama* at concentrations within an order of magnitude of $10^2$ to $10^3$ algal cells $ml^{-1}$ (Kamiyama 1999a). These data from laboratory experiments imply that grazing by zooplankton may influence the population dynamics of *H. circularisquama* in the early stages (concentration$<1000$ cells $ml^{-1}$) of the blooms. In particular, ciliates are regarded as potential grazers of *H. circularisquama* because *H. circularisquama* is within the size range of nanoplankton on which ciliates can effectively feed (e.g. Kamiyama 1999b), and the potential growth rate of ciliates (generally 1–2 divisions $d^{-1}$, Pierce & Turner 1992) is equivalent to or exceeds that of *H. circularisquama* (1.3 division $d^{-1}$, Yamaguchi et al. 1997). Hence, it is important to accumulate data on the feeding activity of ciliates and information on their abundance in field seawater during *H. circularisquama* blooms.

The fluorescently labeled algae (FLA) method is effective for examining the feeding activities of protists (Rublee & Gallegos 1989). This method enables the measurement of the ingestion rates of protists, which are difficult to culture under laboratory conditions. Li et al. (1996) used a vital fluorescent dye CMFDA (5-chloromethylfluorescein diacetate) to examine the feeding of mixotrophic dinoflagellates. Further, Kamiyama (2000) modified the FLA method with CMFDA for estimating the feeding rates of field ciliate species on *H. circularisquama*.

In the present study, we measured the ingestion rates of various field ciliate species feeding on *H. circularisquama* using the FLA method with CMFDA and then estimated the grazing impacts of the field ciliate assemblage on *H. circularisquama* in the early stages of a bloom. Furthermore, the effects of grazing by the ciliate assemblage were evaluated with a prediction model based on the concentration of *H. circularisquama*.

## Materials and Methods

### Prey alga and staining

A strain of *Heterocapsa circularisquama* (HA92-1) isolated from Ago Bay, Mie Prefecture in Japan, in December 1992 was maintained in modified SWM3 medium (Chen et al. 1969; Itoh & Imai 1987). The staining method with the CMFDA dye followed Li et al. (1996). A stock solution of CMFDA (50 $\mu$M) was prepared by dissolving 1mg of CMFDA in 0.5 ml of dimethylsulfoxide and then diluting up to an appropriate volume with filtered seawater. This solution was kept in a freezer (−20°C) until the experiments were carried out. For the feeding experiments, part of the stock solution acclimated to room temperature was inoculated into 3 to 7 ml of the *H. circularisquama* cultures (cell concentration: $9.15\times10^4-2.11\times10^5$ cells $ml^{-1}$) to a final concentration of 1 $\mu$M (Li et al. 1996). This culture was then incubated for 1 h under dark conditions at the temperature for each in-situ feeding experiment. Almost all cells of *H. circularisquama* were stained by the CMFDA (Kamiyama 2000), and this dye is not harmful to either the prey alga or the grazer protists (Li et al. 1996; Kamiyama 2000).

### In-situ feeding experiment

Five feeding experiments were carried out in summer and autumn (1 and 25 August and 8 October 1997, 18 June 1998, and 1 October 1999), according to the method of Kamiyama (2000). For each experiment, a seawater sample was collected from the surface or 1-m depth layer at a coastal site in western Hiroshima Bay, the Seto Inland Sea, Japan. One liter of the seawater was poured into 1-liter polycarbonate bottles, and then the CMFDA-labeled *H. circularisquama* (3–7 ml) was added to the bottle to a final concentration of 6.4 to $7.8\times10^2$ cells $ml^{-1}$. For the experiment on 1 October, two experimental bottles were prepared. The bottles were then incubated on a rotator (1 rpm) under conditions of in situ temperature (20–25°C) and 30 $\mu$mol photon $m^{-2}$ $s^{-1}$. After 10 and 30 min of incubation, 300 ml of the water was sampled from each incubated bottle and then divided into three 100-ml bottles containing 10 ml of 20% buffered formaldehyde for fixation of the field ciliates. The sampling time intervals were set based on the results of a preliminary experiment using the CMFDA-labeled *H. circularisquama*: the number of *H. circularisquama* ingested by *Favella taraikaensis* increased linearly with increasing periods of incubation during the first 20–30 min (Fig. 2 in Kamiyama 2000).

One hundred milliliters of the fixed samples was settled in an Utermöhl chamber and the ciliates in the three concentrated samples were observed with an inverted epifluorescence microscope (Olympus IX–70). Microscopic observations were performed in two steps (Kamiyama 2000). Firstly, auto-fluorescence due to chlorophyll *a* was monitored in the food vacuoles of ciliates at a magnification of 150× using the inverted microscope with transmitted light and epifluorescence under blue (B)-light excitation (Olympus U–MWBV filter set: a 400−440 nm band pass filter, a DM500 dichroic mirror and a BA475 barrier filter). Secondly, whether or not the detected particles produced green fluorescence due to the CMFDA was examined under interference blue (IB)-light excitation (Olympus U–MNIBA filter set: a 470–490 nm band pass filter, a DM505 dichroic splitting mirror and a BA 515–550 barrier filter). Ingested *H. circularisquama* cells should be observable under both excitation regimes. Ingestion rates for each ciliate species on *H. circularisquama* were calculated from the increase in the average number of ingested cells between 10 and 30 min of incubation. Since the fluorescence is very specific for stained algae under the IB-light excitation (Kamiyama 2000), no control experiment was conducted.

### Field investigation

Investigations in Hiroshima Bay were carried out on a re-

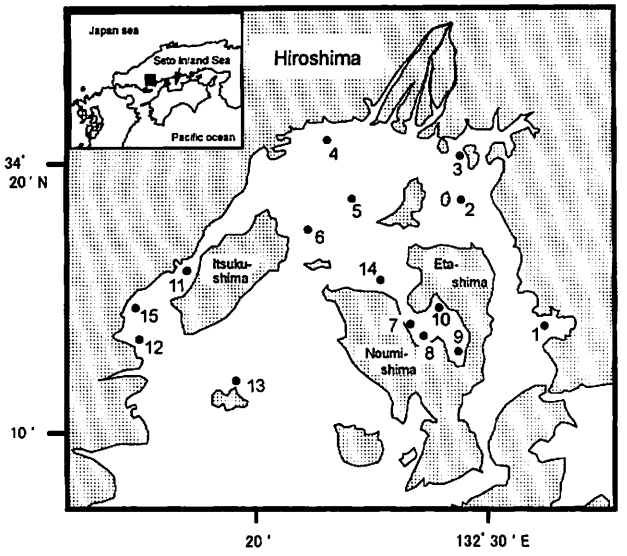


**Fig. 1.** Sampling sites in Hiroshima Bay, the Seto Inland Sea, Japan on 20 and 24 August 1998.

search boat (R/V *Aki*) belonging to Hiroshima Fisheries Experimental Station on 20 and 24 August 1998 when a bloom of *H. circularisquama* occurred in a part of the bay. Vertical profiles of temperature and salinity were measured at 15 stations (Fig. 1) with a temperature–salinity bridge and seawater was collected at the surface with a plastic bucket and from the 2-m depth layer with a Niskin bottle sampler. *Heterocapsa circularisquama* in 0.05 to 1 ml fresh seawater samples from each depth were counted under a microscope. If the concentration of *H. circularisquama* exceeded $1\times10^4$ cells $ml^{-1}$, a blood corpuscle counter was used for counting the alga. Enumeration of ciliates and copepod nauplii was carried out only in the 20 August samples. A 200-ml seawater sample was fixed with Lugol's iodine solution (final concentration: 2%), and then concentrated by settling to a volume of 1–6 ml. Ciliates and copepod nauplii in the fixed subsamples were counted in 1–2 ml of the concentrated samples using a phase contrast microscope at a magnification of ×150, and a Sedgwick–Rafter chamber. We counted only the ciliate species that had been observed to feed on *H. circularisquama* in the in-situ feeding experiments before the field investigation.

### Daily grazing impact

The daily grazing impact of the ciliate assemblage (Gr, % $d^{-1}$) was estimated at each station according to the equation:

$$Gr=\sum_i\left(\frac{I_i\cdot C_i}{\mathrm{Hc}}\right)\cdot 100,$$

where $I_i$ (cells indiv.$^{-1}$ $d^{-1}$) is the ingestion rate for ciliate species $i$, $C_i$ (indiv. $ml^{-1}$) is the abundance of ciliate species $i$, and Hc (cells $ml^{-1}$) is the concentration of *H. circularisquama*. At some stations in the field investigation, the concentration of *H. circularisquama* differed by over a factor of 10 from the algal concentration in the in-situ feeding experiments. Usually ingestion rates of ciliates are influenced by the concentration of prey algae up to a certain concentration level (e.g. Verity 1985). Hence, we estimated the grazing impact at only the stations where the concentration of *H. circularisquama* was within an order of magnitude of $10^2$ cells $ml^{-1}$.

## Results

### In-situ feeding experiments

Results from the five experiments indicated that 16 species of tintinnid ciliates and at least 3 species of aloricate ciliates have the ability to feed on *Heterocapsa circularisquama*. The average ingestion rates based on all of the positive values in the experiments ranged from 0.2 to 14.5 cells indiv.$^{-1}$ $h^{-1}$ (Table 1). The highest feeding rates were found for *Favella ehrenbergii*, and relatively larger species such as *Codonellopsis nipponica*, *Eutintinnus lususundae* and *Laboea strobila* also showed high predation rates on *H. circularisquama*. Positive ingestion rates of 7 of the ciliate species were not statistically significant, since the abundance of the ciliate species was not sufficient for a robust calculation of the ingestion rates.

### Field investigation

On 20 and 24 August 1998, in Hiroshima Bay, mean values of temperature and salinity at the surface and 2 m depth layer ranged from 25.9 to 30.1°C and from 23.6 to 30.8 psu, respectively. Various phases of the bloom of *H. circularisquama* were recognized in the bay. The mean concentration of *H. circularisquama* in both layers in the closed area between Noumi-shima and Eta-shima (Eta-shima Bay)(Fig. 1) was the highest concentration present in Hiroshima Bay on both days (Fig. 2). In particular, the values at Stn 8 on 20 August and Stn 10 on 24 August exceeded $1\times10^4$ cells $ml^{-1}$, being under red-tide conditions. In the other areas, *H. circularisquama* was found at a concentration within an order of magnitude of $10^1$ to $10^2$ cells $ml^{-1}$ on 20 August, and the concentration of *H. circularisquama* increased at almost all sites in the northern coastal areas on 24 August (Fig. 2). One week after 20 August, the concentration of *H. circularisquama* exceeded $10^3$ cells $ml^{-1}$ at almost all the coastal sites in the northern and western parts of the bay (Takayama & Nishii, unpublished data), implying that the bloom in the northern and western parts of the bay was in an early stage of development during the present investigations.

A high abundance of ciliates potentially feeding on *H. circularisquama* was observed in the northern coastal sites (Stns 2–4) of Hiroshima Bay, although the abundance was considerably lower in Eta-shima Bay (Fig. 3A). Among the ciliate assemblages, *Eutintinnus tubulosus* was numerically

**Table 1.** Ingestion rates (cells indiv.$^{-1}$ h$^{-1}$, mean±SE, *n* in parentheses) of field ciliate species in Hiroshima Bay, the Seto Inland Sea of Japan. Numbers in parentheses are total individuals of each ciliate species counted at the 10- and 30-min incubation intervals.

| Ciliates | 1997 1 Aug. | 25 Aug. | 8 Oct. | 1998 18 June | 1999 1 Oct. | Mean [range] |
|---|---|---|---|---|---|---|
| Tintinnid ciliates | | | | | | |
| *Amphorellopsis acuta* | 1.08±0.16* (537) | 0.71±0.79 (21) | | + | | 0.90 [1.08–0.71] |
| *Codonellopsis nipponica* | | | | 5.03±1.18* (349) | | 5.03 |
| *Eutintinnus lususundae* | 2.40±1.28 (16) | 4.2±1.8 (6) | | 11.42±3.00* (23) | + | 6.01 [2.40–11.42] |
| *Eutintinnus tubulosus* | | | | 0.23±0.20 (52) | | 0.23 |
| *Favella ehrenbergii* | + | + | + | + | 13.59±1.66* (101) | 14.51 [13.59–15.43] |
| | | | | | 15.43±2.11* (63) | |
| *Stenosemella ventricosa* | | | | + | + | |
| *Tintinnopsis corniger* | 0.16±0.44 (23) | | | 0.85±0.20* (365) | | 0.51 [0.16–0.85] |
| *T. butschlii* | | | | 0.16±1.16 (30) | | 0.16 |
| *T. cylindrica* | 0.40±0.47 (48) | + | | | | 0.40 |
| *T. directa* | | 0.33±0.15* (428) | | 0.95±1.41 (13) | 0.04±0.07 (234) | 0.33 [0.00–0.95] |
| | | | | | 0.00±0.03 (257) | |
| *T. radix* | | | 3.75±3.14 (3) | 6.31±2.99* (12) | + | 5.03 [3.75–6.31] |
| *T. tocantinensis* | | 1.64±1.21 (22) | | | + | 1.64 |
| *T. tubulosa* | | | 6.00±3.87 (2) | | | 6.00 |
| *T. tubulosoides* | | | | + | | |
| *Tintinnidium mucicola* | | | | 0.41±0.91 (32) | | 0.41 |
| *Leprotintinnus* sp. | | | + | | | |
| Aloricate ciliates | | | | | | |
| *Laboea strobila* | 1.83±0.66* (56) | 7.04±1.45* (19) | | | | 4.44 [1.83–7.04] |
| *Tontonia* sp. 1 | | | 0.60±0.48 (14) | | | 0.60 |
| *Tontonia* sp. 2 | | + | + | | | |

* Significant ingestion rates ($p<0.05$), calculated from the increase in the average number of ingested cells between 10 and 30 min of incubation

+: some individuals ingested *Heterocapsa circularisquama* but it was not possible to calculate the ingestion rates because no individuals occurred in the subsample after either 10- or 30-min of incubation.

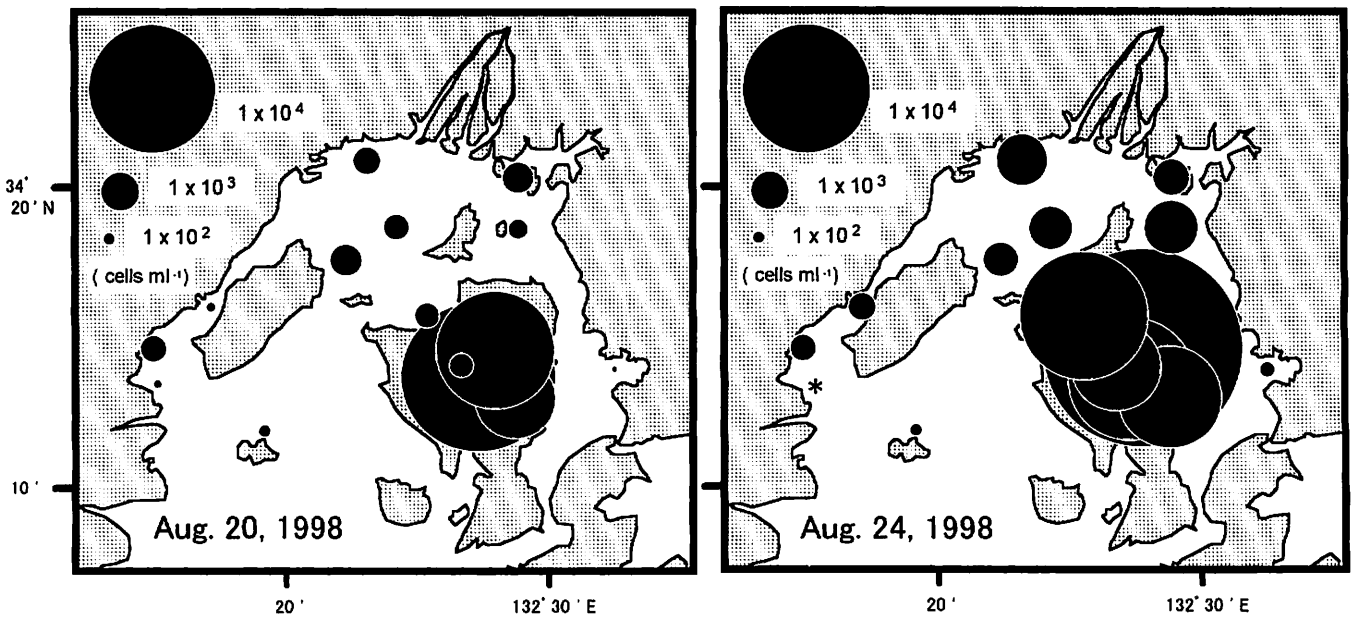


**Fig. 2.** Distribution of the concentration of *Heterocapsa circularisquama* on 20 and 24 August 1998. The values show the mean concentrations at the surface and the 2-m depth layer. Asterisk denotes no data.

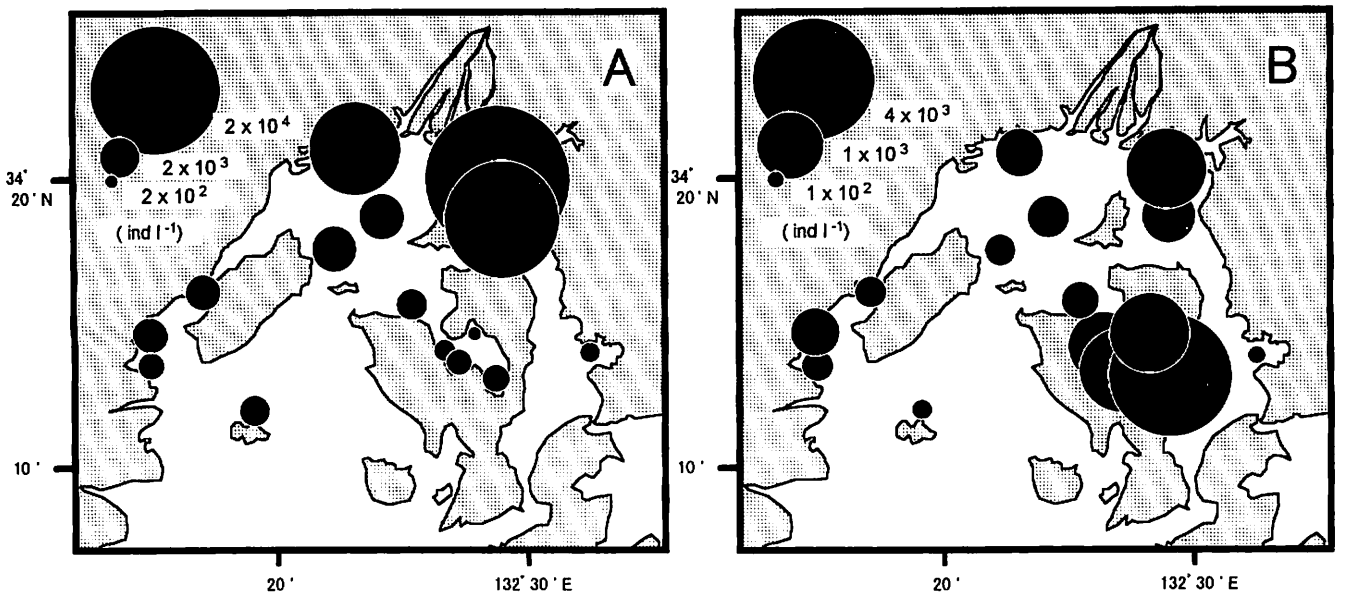


**Fig. 3.** Distribution of the densities of ciliates (**A**) and copepod nauplii (**B**). The values show the mean concentrations at the surface and the 2-m depth layer.

dominant and contributed 93–97% of the total abundance in the northern coastal sites. *Favella ehrenbergii*, which is an efficient grazer of *H. circularisquama*, occurred abundantly in the northern and western parts of Hiroshima Bay. In a contrary pattern, a high abundance of copepod nauplii was recognized in Eta-shima Bay, where the concentration of *H. circularisquama* was extremely high (Fig. 3B).

### Grazing impact

Daily grazing impact by the ciliate assemblage on the concentration of *H. circularisquama* was estimated to be from 3 to 53% at the stations where the concentration of *H. circularisquama* was within an order of magnitude of $10^2$ cells $ml^{-1}$ (Stns 2–7 and 13–15), and was relatively high at the northern and western coastal sites (Stns 2–4 and 15) (Fig. 4). Among the ciliate assemblages, the impact attributable to *E. tubulosus* and *F. ehrenbergii* contributed 69–96% of the total value, except at Stn 7.

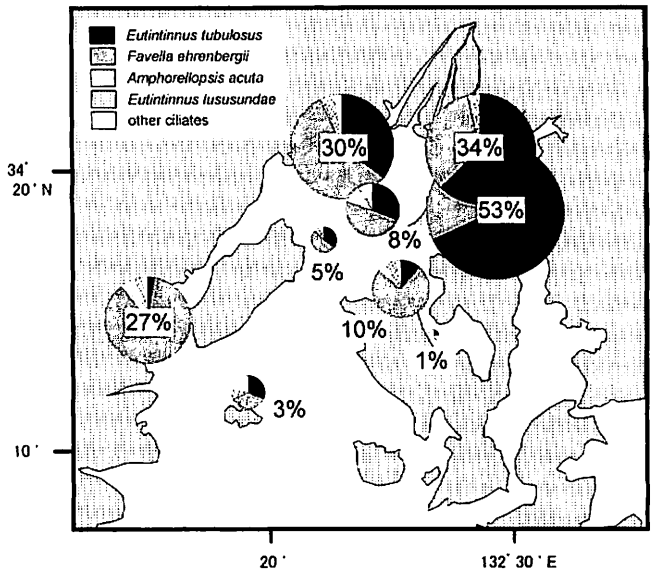


**Fig. 4.** Daily grazing impacts of the ciliate assemblage on *Heterocapsa circularisquama* on 20 August 1998. The values show daily grazing loss in the concentration of *H. circularisquama* for stations at which the concentrations of *H. circularisquama* were within an order of magnitude of $10^2$ cells $ml^{-1}$.

## Discussion

### Ingestion rates of ciliates

The ingestion rates of several ciliate species were able to be measured in the in-situ feeding experiments, indicating that toxicity of *Heterocapsa circularisquama* may not inhibit the feeding behavior of many ciliate species at concentrations around $7\times10^2$ cells $ml^{-1}$. Information on the feeding activity of ciliates on *H. circularisquama* is very limited. Ingestion rates of *Favella azorica* and *F. taraikaensis* feeding on *H. circularisquama* increased with increasing concentrations of *H. circularisquama* up to 530 cells $ml^{-1}$ and became saturated at a rate of 12.7 cells $indiv.^{-1}$ $h^{-1}$ at concentrations between 530 and 1000 cells $ml^{-1}$ (Kamiyama 1997). The ingestion rates for *F. ehrenbergii* (13.6–15.4 cells $indiv.^{-1}$ $h^{-1}$) at $6.8\times10^2$ cells $ml^{-1}$ of *H. circularisquama* that were observed in the present study are somewhat higher than those for the other *Favella* species, which may be due to the larger size of *F. ehrenbergii* compared with *F. azorica* or *F. taraikaensis*.

Feeding rates of zooplankton are influenced by the concentration of other prey species in the same seawater (Ver-

ity 1991; Strom & Loukos 1998). In the present feeding experiment, the phytoplankton assemblage inherent in the seawater probably affected the feeding activity of field ciliate species on *H. circularisquama*. Unfortunately, neither phytoplankton community nor chlorophyll-*a* concentration was investigated in the seawater used for the in-situ feeding experiments. Hence, it is difficult to evaluate the effects of the inherent phytoplankton community on the ingestion rate for each ciliate species on *H. circularisquama*.

The use of the FLA method with CMFDA dye is effective for investigating the feeding ecology of protists (Li et al. 1996) and observations under two excitation filter sets in the present study enabled us to measure the feeding activity of the field-collected ciliates on the target algal species (Kamiyama 2000). However, in the present study it was difficult to count the CMFDA-stained *H. circularisquama* cells within the food vacuoles of some individuals of agglutinated tintinnid species (e.g. *Codonellopsis nipponica*) because particles attached to the lorica interfered with the observations. Counting of CMFDA-stained *H. circularisquama* within the food vacuoles in some individuals of *F. ehrenbergii* also failed because other prey particles masked the fluorescent spots within the food vacuoles. These individuals were eliminated from the data for measurement of ingestion rates.

The heterotrophic dinoflagellates are a major group of microzooplankton, and have been focused on as a potentially important grazer group for red tide algae (Jeong 1999). In the present study, we do not have quantitative data on heterotrophic dinoflagellates having *H. circularisquama* cells in their food vacuoles but this phenomenon was not conspicuous. *Heterocapsa circularisquama* caused mortality in the mixotrophic and heterotrophic dinoflagellates *Gyrodinium instriatum* and *N. scintillans* (Uchida et al. 1995; Kamiyama 1999a). The prey–predator relationships between such dinoflagellates and *H. circularisquama* remained to be resolved.

### Relationships between *H. circularisquama* and ciliates/nauplii

The large difference between the ciliate abundances in the northern part of Hiroshima Bay and in Eta-shima Bay implies that the ciliate assemblage is highly influenced by the concentration of *H. circularisquama*. There appeared to be a threshold concentration of *H. circularisquama* (ca. 1000 cells $ml^{-1}$) at which the relationships between ciliate abundance and *H. circularisquama* concentration changed (Fig. 5), which agrees well with the level at which mortality of *F. taraikaensis* occurred in laboratory studies (Kamiyama 1997; Kamiyama & Arima 1997). Such information on the abundance of zooplankton during bloom periods of *H. circularisquama* has not previously been reported. However, harmful effects attributable to *H. circularisquama* on ciliates except for *F. taraikaensis* have also not been reported (Kamiyama & Arima 1997). It is necessary

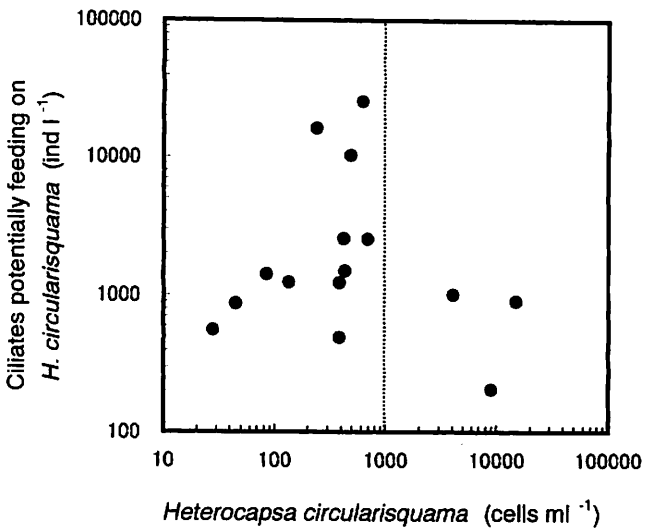


**Fig. 5.** Relationships between *Heterocapsa circularisquama* concentration and the density of ciliates potentially feeding on *H. circularisquama*. Each data set indicates the mean value at the surface and the 2-m depth layer on 20 August 1998.

to clarify the interaction between various ciliate species and *H. circularisquama* in order to further discuss the relationships between *H. circularisquama* and the ciliates observed in the present study.

Nauplii were abundant in Eta-shima Bay where the concentration of *H. circularisquama* was extremely high, which is markedly different from the distribution pattern of ciliates (Fig. 3A, B). This result implies that feeding and reproduction of copepods is not inhibited by *H. circularisquama*, even if the bloom enters the red tide stage (cell concentration $>10000$ cells $ml^{-1}$). There are no reports on feeding of copepods on *H. circularisquama*, except for the calanoid copepod *Acartia omorii* which was able to feed on *H. circularisquama* in laboratory experiments even if the concentration exceeded 10000 cells $ml^{-1}$ (Kamiyama 1999b).

To interpret the difference between abundances of ciliates and nauplii in Eta-shima Bay, we should consider the possibility that feeding activities of copepod and copepod nauplii caused the decrease in the ciliate abundance. Many reports have indicated that copepods can actively and selectively feed on ciliates (e.g. Pierce & Turner 1992; Merrell & Stoecker 1998). Predation of copepod nauplii on ciliates up to 40 $\mu$m in diameter may be as important as predation by adult copepods (Stoecker & Egloff 1987; Merrell & Stoecker 1998). Unfortunately, we have no information on the abundance of adult copepods or the species compositions and developmental stages of the nauplii in this field data.

### Grazing impact

Although there are several studies on the grazing impacts of ciliate assemblages on the phytoplankton community

(Pierce & Turner 1992), the grazing impact by field ciliate assemblages on toxic algae based on species specific ingestion rates measured under field conditions has hardly ever been estimated.

In the present investigation, the grazing impact by the ciliate assemblages was high in the northern and western coastal areas of the bay. High impacts mainly depended on the abundance of *Eutintinnus tubulosus* and *Favella ehrenbergii*. The ingestion rate of *E. tubulosus* on *H. circularisquama* was considerably lower than in the other species (Table 1) although the abundance of *E. tubulosus* was extremely high in the northern parts of the bay. Contrastingly, *F. ehrenbergii* was not a numerically dominant species but has high ingestion rates on *H. circularisquama* and can reproduce more rapidly than *H. circularisquama* if conditions for growth are appropriate (Kamiyama 1997). Hence, changes in the abundance of *F. ehrenbergii* may be an important indicator to predict whether a bloom of *H. circularisquama* will develop or not.

Regarding the precision of the grazing impacts estimated in the present study, ingestion rates measured at a fixed concentration of *H. circularisquama* in the in-situ feeding experiments were determined in all cases at concentrations in the order of magnitude of $10^2$ cells $ml^{-1}$. Only three aloricate ciliate species were identified in the in-situ feeding experiments. Hence, there is a possibility that we neglected some aloricate ciliate species that may feed on *H. circularisquama* in the field investigation, and this may have led to an underestimation of the grazing impact of ciliate assemblages. It is necessary to accumulate additional data on the feeding activities of various ciliate species under diverse conditions.

### Evaluation of the grazing impacts by field ciliate assemblages on the concentration of *Heterocapsa circularisquama*

At 9 stations (Stns 2–7 and 13–15) we predicted the concentration of *H. circularisquama* after 4 d based on data from 20 August as follows.

Assuming that the concentration of nutrients in the seawater was sufficient for the growth of *H. circularisquama*, the potential growth rate ($\mu$, division $d^{-1}$) of *H. circularisquama* was estimated based on the following equation from temperature ($T$, °C) and salinity ($S$, psu) (Yamaguchi et al. 1997),

$$\mu = -0.25767 + 0.00145 \cdot T \cdot S - 0.00005 \cdot T \cdot S^2 + 0.00009 \cdot T^2 \cdot S - 0.00003 \cdot T_3 \quad (1)$$

Hence, the specific growth rate of *H. circularisquama*, $k$ ($d^{-1}$) is

$$k = \mu \cdot \ln(2) \quad (2)$$

The specific grazing rate, $g$ ($d^{-1}$), by ciliate assemblages on *H. circularisquama* was explained by grazing impact ($G$, % $d^{-1}$) in the present study:

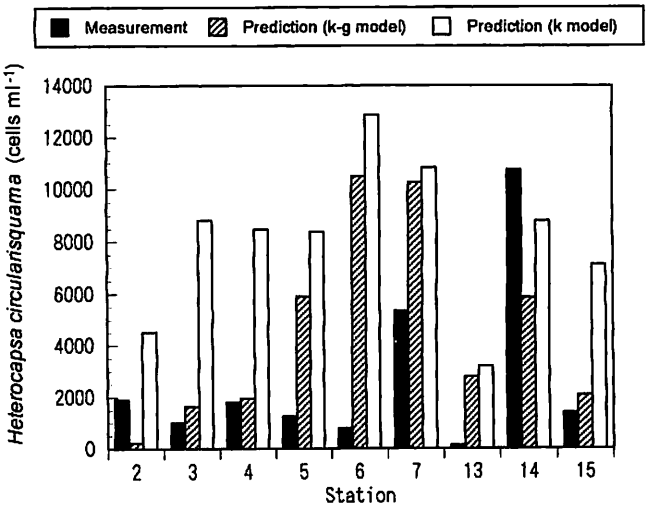


**Fig. 6.** Predicted concentrations and actual concentrations (measurement) of *Heterocapsa circularisquama* 4 d later (24 August) for stations where the original concentrations of *H. circularisquama* were within an order of magnitude of $10^2$ cells $ml^{-1}$. The predicted concentrations were calculated with the grazing parameter ($k$–$g$ model) and without the grazing parameter ($k$ model) based on the data from 20 August. The actual values are the mean concentrations at the surface and the 2-m depth layer on 24 August.

$$G = (P_0 - P_0 e^{-g}) \cdot (P_0)^{-1}$$

$$\text{Namely, } g = -\ln(1 - G) \quad (3)$$

The concentration of *H. circularisquama* on the next day ($P_{t+1}$) was predicted from the concentration on the day $t$ ($P_t$), using the exponential growth model with the equations (2) and (3):

$$P_{t+1} = P_t e^{(k-g) \cdot t} \quad (4)$$

After the initial data ($P_0$), $T$, $S$, and $G$ was used in equations (1)–(4) for each station, the calculation was repeated until $t = 3$. We assumed that the abundance of grazer ciliates and the parameters $k$ and $g$ were constant for 4 d. The concentration of *H. circularisquama* predicted after 4 d ($P_4$) was compared with the mean of the actual concentrations at the surface and 2-m depth at each station.

The concentrations of *H. circularisquama* predicted after 4 d ($k-g$ model) ranged from 54% to 192% of the actual concentration on 24 August (measurement) at each station except for at Stns 2, 5, 6 and 13 (Fig. 6). The values predicted for Stns 5, 6, and 13 were 5 to 21 times larger than the actual concentrations. Nutrient limitation inhibiting the growth of *H. circularisquama* or tidal advection diffusing *H. circularisquama* may have contributed to the low actual concentrations of *H. circularisquama* at these stations more strongly than the others. On the contrary, at Stn 2 the predicted concentration ($k-g$ model) was only 12% of the measured concentration. This may have been due to transportation of seawater masses with high concentrations of *H.*

*circularisquama* from other areas. In the northern and western coastal areas in Hiroshima Bay, the predicted values (*k*–*g* model) were usually closer to the actual measurements than the values predicted without factoring in the impact of grazing (*k* model) (Fig. 6). This suggests that information on grazing by the ciliate assemblage is necessary to correctly predict the dynamics of *H. circularisquama* blooms.

*Heterocapsa circularisquama* has characteristics advantageous for dominating the plankton community, such the ability to use various organic phosphorus compounds for growth (Yamaguchi 1999), to transform into a "temporary cyst"-like shape to ensure survival under unfavourable conditions (Uchida et al. 1996; Uchida 1999), to resist direct attacks by algicidal bacteria (Imai et al. 1999), and it has lower minimum cell quotas for nutrients than other phytoplankton (Yamaguchi 1999). These are probably essential factors that enable the formation of *H. circularisquama* red tides. However, results of the present study indicate that there is a negative factor regulating red tide formation, that information on grazer ciliates is important, and that consideration of the grazing impact by zooplankton may improve predictive models for the outbreak of *H. circularisquama* red tides. Further data on the abundance of ciliates over the course of *H. circularisquama* blooms should be accumulated as well as data on the feeding activities of ciliates. Field data on Hiroshima Bay in the present study indicates that the reproduction of copepods was not influenced by the toxic effects of *H. circularisquama* even if the bloom progressed into the red tide phase. Hence, it is necessary to clarify the role of copepods appearing in summer and autumn on the population dynamics of *H. circularisquama*.

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## Literature Cited

Chen, L. C. M., T. Edelstein & J. McLachlan 1969. *Bonnemaisonia hamifera* Hariot in nature and in culture. *J. Phycol.* **5**: 211–220.

Imai, I., S. Nakagiri & T. Maki 1999. Relationships between the harmful dinoflagellate *Heterocapsa circularisquama* and marine bacteria. *Bull. Plankton Soc. Jpn* **46**: 172–177. (In Japanese.)

Itoh, K. & I. Imai 1987. Rafido so (Raphidophyceae). p. 122–130. In *A Guide for Studies of Red Tide Organisms* (ed. Japan Fisheries Resource Conservation Association). Shuwa, Tokyo. (In Japanese.)

Jeong, H. J. 1999. The ecological roles of heterotrophic dinoflagellates in marine planktonic community. *J. Eukaryot. Microbiol.* **46**: 390–396.

Kamiyama, T. 1997. Growth and grazing responses of tintinnid ciliates feeding on the toxic dinoflagellate *Heterocapsa circularisquama*. *Mar. Biol.* **128**: 509–515.

Kamiyama, T. 1999a. Interactions between *Heterocapsa circularisquama* and zooplankton. *Bull. Plankton Soc. Jpn* **46**: 178–180. (In Japanese.)

Kamiyama, T. 1999b. Feeding ecology of marine ciliates in coastal waters (Review). *Bull. Plankton Soc. Jpn* **46**: 113–133. (In Japanese with English abstract.)

Kamiyama, T. 2000. Application of a vital staining method to measure feeding rates of field ciliate assemblages on a harmful alga. *Mar. Ecol. Prog. Ser.* **197**: 299–303.

Kamiyama, T. & S. Arima 1997. Lethal effect of the dinoflagellate *Heterocapsa circularisquama* upon the tintinnid ciliate *Favella taraikaensis*. *Mar. Ecol. Prog. Ser.* **160**: 27–33.

Li, A., D. K. Stoecker, D. W. Coats & E. J. Adam 1996. Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. *Aquat. Microb. Ecol.* **10**: 139–147.

Matsuyama, Y., A. Kimura, H. Fujii, H. Takayama & T. Uchida 1997a. Occurrence of a *Heterocapsa circularisquama* red tide and subsequent damages to shellfish in western Hiroshima Bay, Seto Inland Sea, Japan in 1995. *Bull. Nansei Natl. Fish. Res. Inst.* **30**: 189–207. (In Japanese with English abstract.)

Matsuyama, Y., K. Nagai, M. Ishimura, A. Nishimura, M. Yamaguchi, T. Uchida & T. Honjo 1996. Biological and environmental aspects of noxious dinoflagellates red tides by *Heterocapsa circularisquama* in the west Japan. p. 247–250. In *Harmful and Toxic Algal Blooms* (eds. Yasumoto, T., T. Oshima, & T. Fukuyo). Intergovernmental Oceanographic Commission of UNESCO, Paris.

Matsuyama,. Y., K. Nagai, T. Mizuguchi, M. Fujiwara, M. Ishimura, M. Yamaguchi, T. Uchida & T. Honjo 1995. Ecological features and mass mortality of pearl oysters during red tides of *Heterocapsa* sp. in Ago Bay in 1992. *Nippon Suisan Gakkaishi* **61**: 35–41. (In Japanese with English abstract.)

Matsuyama, Y., T. Uchida & T. Honjo 1997b. Toxic effects of the dinoflagellate *Heterocapsa circularisquama* on clearance rate of the blue mussel *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* **146**: 73–80.

Merrell, J. R. & D. K. Stoecker 1998. Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. *J. Plankton Res.* **20**: 289–304.

Pierce, R. W. & J. T. Turner 1992. Ecology of planktonic ciliates in marine food webs. *Rev. Aquat. Sci.* **6**: 139–181.

Rublee, P. A. & C. L. Gallegos 1989. Use of fluorescently labelled algae (FLA) to estimate microzooplankton grazing. *Mar. Ecol. Prog. Ser.* **51**: 221–227.

Strom, S. L. & H. Loukos 1998. Selective feeding by protozoa: model and experimental behaviors and their consequences for population stability. *J. Plankton Res.* **20**: 831–846.

Stoecker, D. K. & D. A. Egloff 1987. Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. *J. Exp. Mar. Biol. Ecol.* **110**: 53–68.

Uchida, T. 1999. Life cycle and other ecological features of *Heterocapsa circularisquama*. *Bull. Plankton Soc. Jpn* **46**: 169–170. (In Japanese.)

Uchida, T., M. Yamaguchi,Y. Matsuyama & T. Honjo 1995. The red-tide dinoflagellate *Heterocapsa* sp. kills *Gyrodinium instriatum* by cell contact. *Mar. Ecol. Prog. Ser.* **118**: 301–303.

Uchida, T., M. Yamaguchi,Y. Matsuyama & T. Honjo 1996. Growth interactions between a red tide dinoflagellate *Hetero-*

*capsa circularisquama* and some other phytoplankton species in culture, p. 369–372. In *Harmful and Toxic Algal Blooms* (eds. Yasumoto, T., T. Oshima, & T. Fukuyo). Intergovernmental Oceanographic Commission of UNESCO, Paris.

Verity, P. G. 1985. Grazing, respiration, excretion, and growth rates of tintinnids. *Limnol. Oceanogr.* **30**: 1268–1282.

Verity, P. G. 1991. Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. *Limnol. Oceanogr.* **36**: 729–750.

Yamaguchi, M. 1999. Growth physiology of *Heterocapsa circularisquama*. *Bull. Plankton Soc. Jpn* **46**: 171–172. (In Japanese.)

Yamaguchi, M., S. Itakura, K. Nagasaki, Y. Matsuyama, T. Uchida & I. Imai 1997. Effects of temperature and salinity on the growth of the red tide flagellates *Heterocapsa circularisquama* (Dinophyceae) and *Chattonella verrculosa* (Raphidophyceae). *J. Plankton Res.* **19**: 1167–1174.