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HORIZONS

Temporal changes in the ciliate assemblage and consecutive estimates of their grazing effect during the course of a *Heterocapsa circularisquama* bloom

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Temporal changes in ciliate assemblages during the course of a bloom of the harmful microalga Heterocapsa circularisquama (Dinophyceae) were investigated and consecutive estimates of species-specific maximum grazing losses were analyzed from August to September 1998 at a site in western Hiroshima Bay, the Seto Inland Sea of Japan. Temporal increases of the H. circularisquama mean concentration in the water column were observed twice (25–29 August and 7–10 September) with the maximum concentration (ca. 4000 cells mL^{-1}) being recorded on 25 August. The main ciliate genera during the bloom were Favella, Tontonia, Eutintinnus, Tintinnopsis and Amphorellopsis. Increases of Favella and Tontonia were observed when the concentration of H. circularisquama ranged from 260 to 1170 cells mL^{-1}. Total maximum grazing loss estimated from the abundance and ingestion rate of each ciliate species on H. circularisquama ranged from 1 to 75% standing stock removed d^{-1} of the H. circularisquama concentration. High grazing losses mainly due to the genera Favella and Tontonia occurred during the period when the H. circularisquama concentration was decreasing. These results suggest that grazing by ciliate assemblages can influence the population dynamics of H. circularisquama despite the potentially toxic nature of the phytoplankter.

INTRODUCTION

Since being first recorded in 1988, blooms of the harmful dinoflagellate *Heterocapsa circularisquama* Horiguchi have spread throughout the coastal waters of western Japan and caused serious damage to the aquaculture of pearl and Pacific oysters and to the short-necked clam fishery (Matsuyama, 2003). Recently data on the physiological and ecological characteristics of *H. circularisquama* (Matsuyama, 2003) and interactions with bacteria and viruses infecting this alga have been summarized (Nagasaki *et al.*, 2000; Tarutani *et al.*, 2001).

Ciliates are regarded as important potential grazers of *H. circularisquama*, since this alga [cell dimension: 23.9 μm × 17.3 μm (Horiguchi, 1995)] is within the size range of nanoplankton on which ciliates can effectively feed (Kamiyama, 1999). The potential growth rate of ciliates in laboratory experiments [generally, 1–2 divisions day^{-1} (Pierce and Turner, 1992)] is equivalent to or exceeds that of *H. circularisquama* [1.3 division day^{-1} (Yamaguchi *et al.*, 1997)]. Kamiyama (Kamiyama, 1997) reported that two species of tintinnid ciliates *Favella azorica* and *Favella taraikaensis* actively feed on *H. circularisquama* in laboratory

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experiments when concentrations of this alga are <1000 cells mL^{-1}. Furthermore, Kamiyama *et al.* (Kamiyama *et al.*, 2001) measured the feeding activities of natural ciliate species on *H. circularisquama* in field samples using the fluorescently labeled algae (FLA) method with the vital fluorescent dye CMFDA (5-chloromethylfluorescein diacetate) (Li *et al.*, 1996; Kamiyama, 2000), reporting ingestion rates of *H. circularisquama* by 15 species of ciliate. In that study, the distribution of ciliate assemblages was investigated in an area where a *H. circularisquama* bloom had occurred. Based on the abundance of each ciliate species and its measured ingestion rates, Kamiyama *et al.* (Kamiyama *et al.*, 2001) evaluated the grazing loss by the ciliate assemblage on the cell density of *H. circularisquama*. Their paper was the first report of an estimation of the grazing losses of *H. circularisquama* populations by zooplankton; however, the survey was limited to one investigation and only samples from the early stage (concentration <1000 cells mL^{-1}) of a *H. circularisquama* bloom were assessed.

These previous data indicate that it is important to accumulate information on not only the physical and chemical environmental factors influencing the growth of *H. circularisquama* but also on grazing effects of zooplankton during bloom events. This is required in order to clarify the factors controlling the occurrence of *H. circularisquama* blooms and predict outbreaks of dense blooms (red tides). However, to our knowledge, even basic information on changes in ciliate assemblages during blooms of *H. circularisquama* has not been reported yet.

In the present study, temporal changes in the ciliate assemblage and consecutive estimates of their grazing over the course of a *H. circularisquama* bloom were elucidated based on data from intensive field sampling.

METHOD

Field sampling

Hiroshima Bay is a semi-enclosed bay and 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. In this bay a variety of harmful algal blooms, notably *Heterosigma akashiwo*, *Karenia* (formerly Gymnodinium) *mikimotoi* and *H. circularisquama* often occur in early summer to autumn. Intensive seawater samplings were carried out at a fixed station (6-m depth) in the Ohno Strait, western Hiroshima Bay (Fig. 1) from 22 August, 1998, to 20 September, 1998, during the course of a *H. circularisquama* bloom.

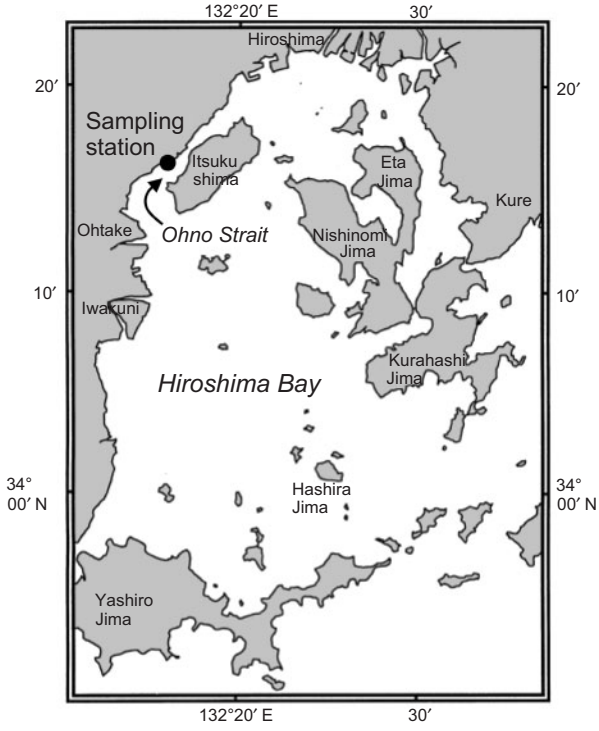

Fig. 1. Location of the sampling station in Ohno Strait, Hiroshima Bay, the Seto Inland Sea of Japan.

Examining plankton succession using only one fixed station for sampling has drawbacks in that water mass at the fixed station is sometimes affected by short-term water advection due to coastal currents and eddies. Our sampling site was located in the Ohno Strait, one of the most enclosed areas in Hiroshima Bay. Kawanisi (Kawanisi, 1999) estimated the water exchange rates at eighteen locations in Hiroshima Bay, based on the data obtained from field observations by applying the Princeton Ocean Model (POM) of Blumberg and Mellor (Blumberg and Mellor, 1987). According to this estimation, our sampling site shows the lowest water exchange rate (79% of the water mass remained at the same site over a 14-day simulation) in Hiroshima Bay (Fig. 2). Also, high densities of *H. circularisquama* were observed in Ohno Strait on 27 August 1998 (Fig. 2) (unpublished data from Hiroshima Fisheries Experimental Station), further indicating the low exchange rate of seawater in this area. Hence, the data obtained from a fixed sampling site in the present study is considered to be representative of the trend of *H. circularisquama* blooms and the other plankton in the Ohno Strait.

Water-column samples were collected within 2 h before or after the time of high tide (Fig. 3) using either

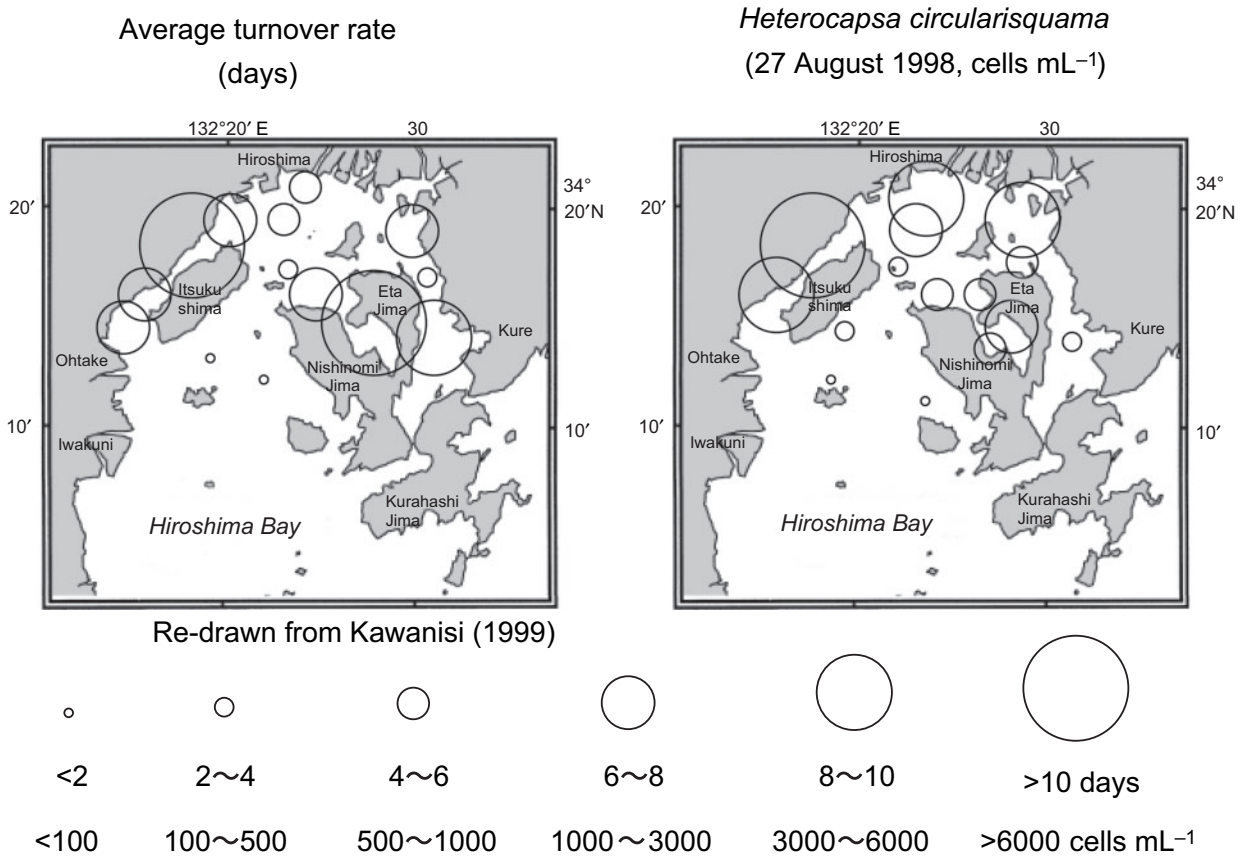

Fig. 2. Horizontal distributions of average turnover rates in surface seawater and mean concentrations of *Heterocapsa circularisquama* in surface, 2 m and 5 m depth water in Hiroshima Bay. Data are cited from Kawanisi (Kawanisi, 1999) and a report in Hiroshima Fisheries Experimental Station (unpublished).

of the following two methods. As one method, a pressure-resistant tube (inner diameter 18 mm × length 5 m) was vertically lowered into the water to 1 m above the bottom at the sampling site and then the upper exit of the tube was plugged, and the tube was lifted up (Matsuyama, 2003). Seawater within the tube was collected as a single water-column sample. Alternatively, seawater samples were collected at the surface with a plastic bucket and at the 2 and 4-m depth layer with a Niskin bottle sampler and then aliquots of the samples were pooled in a bottle as a single water-column sample. Water temperature and salinity were determined at each sampling time.

Observations and enumeration of plankton

Cells of phytoplankton in freshly collected seawater or concentrated seawater sample as described below were counted in 3–5 replicates with a Sedgwick-Rafter chamber under a microscope on the day of sampling without fixation of samples. If the concentration of *H. circularisquama* was below 1 cell mL^{-1}, a 400-mL sample of the seawater was concentrated to 2 mL on a cellulose-acetate membrane filter (pore size 5 or 10 μm, Millipore or Advantec) under gravity pressure (Itakura *et al.*, 1990). An aliquot of the concentrated sample was then used for enumeration of *H. circularisquama*. For microzooplankton enumeration, a 280-mL to 500-mL seawater sample was fixed with Lugol's iodine solution (final concentration 2%) and then concentrated by settling to a volume of 1–2 mL. In some instances, the samples concentrated on the filter were fixed with Lugol's iodine and glutaraldehyde solution (final concentration 0.2% and 2.5%, respectively) (Jensen, 1998), although there is a possibility that the abundance of aloricate ciliates in the concentrated samples using filters was underestimated because this group is very fragile to mechanical and chemical stress (Gifford and Caron, 2000). Ciliates and metazoans with a body width of less than 200 μm in the fixed subsamples were counted using a phase contrast microscope at a magnification of 150× and a Sedgwick-Rafter chamber.

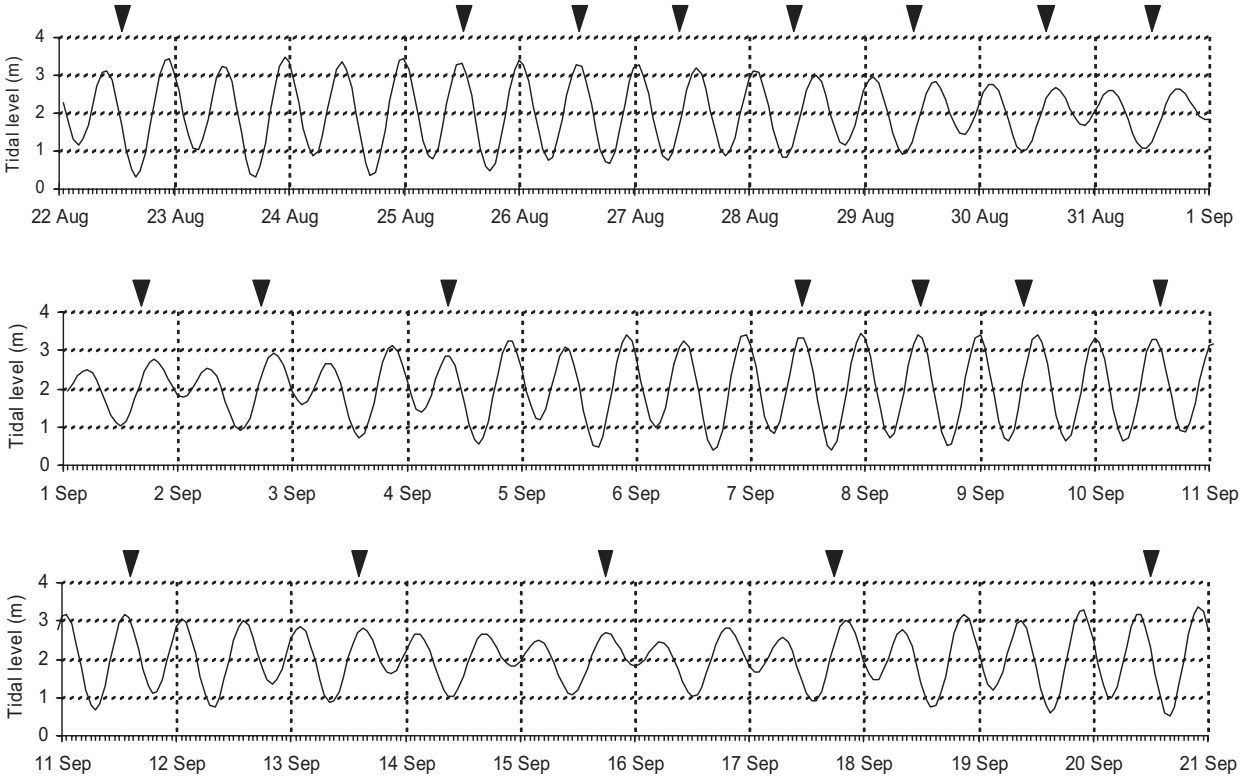

Fig. 3. Sampling time in relation to the tidal cycle during the sampling period from 22 August to 20 September 1998.

Estimation of grazing loss by ciliates

Based on the ciliate species that can feed on *H. circularisquama* reported in Kamiyama *et al.*'s (Kamiyama *et al.*, 2001), the grazing loss of *H. circularisquama* (GL, % standard stock removed day^{-1}) was estimated each day using the equation:

$$\mathrm{GL} = \frac{100}{Hc}\sum_{\mathrm{i}}(I_{\mathrm{i}} \cdot C_{\mathrm{i}}) \qquad (1)$$

where I_{i} (cells individual^{-1} day^{-1}) is the ingestion rate of the ciliate species i documented in Table I, C_{i} (individuals mL^{-1}) is the abundance of ciliate species i, and *Hc* (cells mL^{-1}) is the concentration of *H. circularisquama* from the present study. The ingestion rate of each ciliate species except for *Favella* spp. was cited from Kamiyama *et al.*'s (Kamiyama *et al.*, 2001) data, which was measured using the FLA method at concentrations ranging from 640 cells mL^{-1} to 780 cells mL^{-1} of *H. circularisquama* under 20–25°C and constant light conditions. These ingestion rates were extrapolated for other prey densities. As for *Favella* spp., the relationships between ingestion rates ($I_{Favella}$, cells individual^{-1} h^{-1}) of two *Favella* species (*F. taraikaensis* and *F. azorica*) and *H. circularisquama* concentration (*Hc*, cells mL^{-1}), $I_{Favella} = 0.026 \times Hc$–1.19 ($60 \leq Hc \leq 530$), $I_{Favella} = 12.47$, ($530 < Hc$) (Kamiyama, 1997) were also applied for the rates of other Favella species. Also, the grazing rate of *Tontonia* sp. in Kamiyama *et al.* (Kamiyama *et al.*, 2001) was used for those of all *Tontonia* species in the present study with similar cell dimensions.

The grazing loss (G, day^{-1}) in the present study is also explained by the specific grazing rate, g (day^{-1}), by ciliate assemblages on *H. circularisquama*:

$$\mathrm{G} = \frac{P_0 - P_0 e^{-g}}{P_0} \qquad (2)$$

where P_0 is the initial concentration of *H. circularisquama*. Since G =GL/100, *g* was calculated using the *GL* in (1):

$$g = -\ln\left(1 - \frac{\mathrm{GL}}{100}\right) \qquad (3)$$

RESULTS

Heterocapsa circularisquama and other phytoplankton

At the start of the investigation (22 August 1998), the mean concentration of *H. circularisquama* in the water

Table I: Ingestion rates of ciliate species on Heterocapsa circularisquama

Ciliate species	Ingestion rate (cells individual^{-1} h^{-1})
Amphorellopsis acuta	0.90
Codonellopsis nipponica	5.03
Eutintinnus lususundae	6.01
Eutintinnus tubulosus	0.23
Favella spp.	0–12.5[a]
Tintinnopsis corniger	0.51
Tintinnopsis butschlii	0.16
Tintinnopsis cylindrica	0.40
Tintinnopsis directa	0.33
Tintinnopsis radix	5.03
Tintinnopsis tocantinensis	1.64
Tintinnopsis tubulosa	6.00
Tintinnidium mucicola	0.41
Laboea strobila	4.44
Tontonia sp.	0.60

Species specific ingestion rates except for *Favella* spp. are cited from data in Kamiyama *et al.* (Kamiyama *et al.*, 2001), which were estimated from direct uptake rates of *Heterocapsa circularisquama* stained with a vital fluorescent dye during 20 min at concentrations ranging from 640 cells mL^{-1} and 780 cells mL^{-1}of *Heterocapsa circularisquama* under conditions of 20–25°C of temperature and 30 µmol photon m^{-2} s^{-1} of light.
[a]The ingestion rate of *Favella* spp. was estimated from the equation that fitted the relationships between *Heterocapsa circularisquama* concentration and ingestion rates of *Favella azorica* and *Favella taraikaensis*, reported in Kamiyama (Kamiyama, 1997).

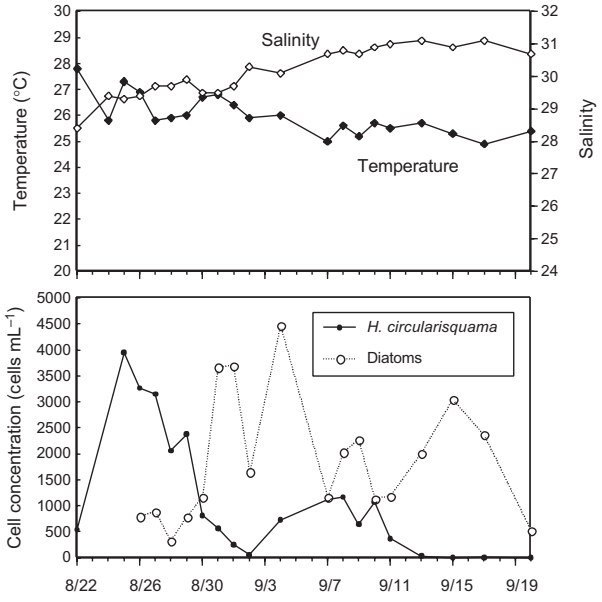

Fig. 4. Temporal changes in the temperature, salinity and mean densities of *Heterocapsa circularisquama* and diatoms in the water column at the sampling station from 22 August to 20 September 1998.

column was 540 cells mL^{-1} (Fig. 4). A dense bloom of *H. circularisquama* (red tide) occurred from 25 August to 11 September in the Ohno Strait. The maximum cell concentration was 3950 cells mL^{-1} at the sampling site on 25 August. Seawater appeared to be colored dark brown around the sampling site and then cell concentration fluctuated between 10 and 1000 cells mL^{-1} and finally decreased to below 1 cell mL^{-1} on 20 September. Water temperatures and salinities during the outbreak of the red tide of *H. circularisquama* ranged from 24.9 to 27.8°C and from 28.4 to 31.1, respectively.

Heterocapsa circularisquama was consistently the dominant dinoflagellate species and other dinoflagellate species (*Prorocentrum triestinum*, *Prorocentrum micans*, *Protoperidinium steinii*, *Gymnodinium* sp., *Gyrodinium dominans* and *Ceratium furca*) co-occurred at densities of 0.1–170 cells mL^{-1} during the course of sampling. Diatoms (*Skeletonema costatum*, *Chaetoceros* spp., *Thalassiosira* spp., *Thalassionema* spp., *Thalassiothrix* sp., *Pseudo-nitzschia* spp., *Leptocylindrus danicus*, *Rhizosolenia fragilissima*) were observed at concentrations of 317–4474 cells mL^{-1} during the investigation period (Fig. 4). Concentration of diatoms was relatively low (<1000 cell mL^{-1}) until 30 August, but then fluctuated in the order of magnitude of 10^3 cells mL^{-1} until 17 September. Generally, this group showed a tendency to increase when *H. circularisquama* decreased.

Ciliate community and other microzooplankton

As for the ciliate assemblage, 9 genera and 20 species of tintinnid ciliates and 4 genera of aloricate ciliates were observed during the course of the bloom (Fig. 5). The genera *Tintinnopsis*, *Favella*, *Eutintinnus*, *Amphorellopsis* and *Tontonia*, which have the ability to feed on *H. circularisquama*, mostly dominated the total ciliate assemblage, accounting for 40–96% of the total ciliate assemblage. High abundances of the genera *Favella* and *Tontonia* were observed at *H. circularisquama* concentrations of 260–1170 cells mL^{-1} between 30 August and 1 September and between 8 and 13 September during the investigation (Fig. 6). Their maximum abundances were 1150 individuals L^{-1} and 8040 individuals L^{-1}. The ciliate species other than the genus *Favella* were not counted after 13 September. The genus *Favella* mostly consisted of *Favella ehrenbergii* and *F. azorica* (mean contribution of their abundance during all periods: 79% and 14%, respectively), and the former species dominated in total abundance of the genus *Favella* on almost all occasions. *Tintinnopsis* and *Amphorellopsis* were relatively stable during the course of the bloom. Abundance of the genus *Eutintinnus* seemed to increase when the *H. circularisquama* concentration was around 10^2 cells mL^{-1}.

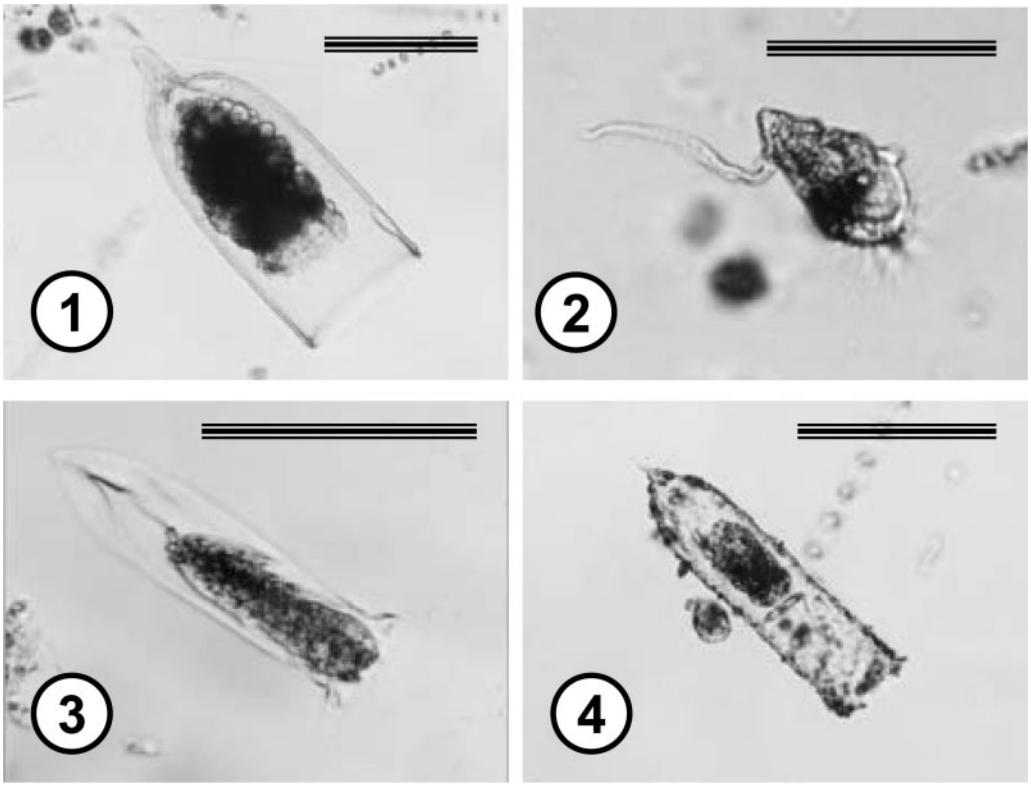

Fig. 5. Main ciliate species (①, *Favella ehrenbergii*; ②, *Tontonia* sp.; ③, *Amphorellopsis acuta*; ④, *Tintinnopsis cylindrica*) observed during the *Heterocapsa circularisquama* bloom. The photographs ①, ③ and ④ were taken from samples fixed with Lugol's iodine-glutaraldehyde solution, and ② was taken from a fresh sample. All ciliates were collected on 7 September 1998. The scale bars = 100 μm. A colour version of this figure is available online as Supplementary Material.

Copepod nauplii and appendicularians (*Oikopleura* spp.) were numerically the most abundant other members of the microzooplankton. Abundance of copepod nauplii ranged from 210 to 860 individuals L^{-1} and were generally stable during the bloom period. Abundance of *Oikopleura* spp. was under 90 individuals L^{-1} but temporally increased to be 190–340 individuals L^{-1} during 30–31 August

Species-specific and consecutive maximum grazing loss by ciliates on *Heterocapsa circularisquama*

Maximum estimated daily grazing losses by the ciliates with the ability to feed on *H. circularisquama* ranged from 5 to 75% standing stock removed day^{-1} of the *H. circularisquama* concentration when it was around 10^2 cells mL^{-1}. High losses of more than 60% standing stock removed day^{-1} were recorded on 31 August and 11 September (Fig. 7), and the specific grazing rates were 1.1 and 1.4 day^{-1}, respectively. These high values were mostly due to the grazing of the genera *Favella* and *Tontonia*. The estimated grazing loss by ciliate assemblages on the *H. circularisquama* concentration was only 1–3% standing stock removed day^{-1} at the algal concentrations of more than 2000 cells mL^{-1} (25–29 August).

DISCUSSION

Harmful algae do not exist in isolation in natural ecosystems and food–web interactions have profound effects on the population dynamics of harmful algal species in nature (GEOHAB, 2001). In general, harmful algal bloom (HAB) species are toxic to their potential grazers and thus have been shown to reduce the grazing activity by upper trophic organisms (Uye and Takamatsu, 1990; Hansen, 1995; Matsuyama *et al.*, 1997; Turner and Tester, 1997; Kim *et al.*, 2000). Food–web interactions are sometimes advantageous for the proliferation of HAB species (Uye and Takamatsu, 1990; Turner and Tester, 1997). However, recent studies have revealed that population dynamics of HAB species are significantly affected by grazing of zooplankton: copepods (Teegarden and Cembella, 1996), ciliates (Nakamura *et al.*, 1996; Kamiyama *et al.*, 2001), heterotrophic dinoflagellates (Nakamura *et al.*, 1995; Matsuyama *et al.*, 1999) and by algicidal activities of bacteria (Yoshinaga *et al.*, 1995) and viruses (Nagasaki *et al.*, 2000; Tarutani *et al.*, 2000). Field and laboratory studies on food–web interactions are essential to understand the adaptive strategies allowing the development and continuation of HAB.

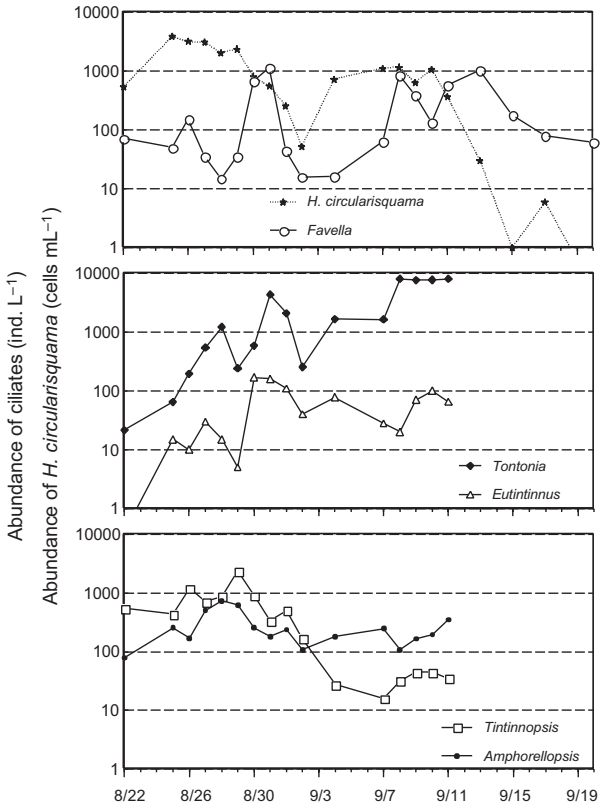

Fig. 6. Temporal changes in the mean water column abundances of five ciliate genera (*Favella*, *Tontonia*, *Eutintinnus*, *Tintinnopsis* and *Amphorellopsis*) and *Heterocapsa circularisquama* during the course of the bloom. Ciliate data after 11 September were not taken except for the genus *Favella*.

Heterocapsa circularisquama is a HAB species and dense blooms of this alga have been shown to cause the collapse of populations of some ciliate species (Kamiyama and Arima, 1997; Kamiyama *et al.*, 2001). However, in the present study, diverse species of ciliates were observed even when the concentration of *H. circularisquama* reached ca. 4000 cells mL^{-1}. High abundances of the genera *Favella* and *Tontonia* were observed during 30 August to 1 September and 8–13 September. In particular, *Tontonia* spp. increased exponentially from 20 to 1220 individuals L^{-1} when *H. circularisquama* was more than 1000 cells mL^{-1}. This implies that a high concentration of *H. circularisquama* did not inhibit the active growth and under such conditions the population of this aloricate ciliate was able to increase. As for the other ciliate species (the genera *Eutintinnus*, *Tintinnopsis* and *Amphorellopsis*), the relationships between their abundances and the *H. circularisquama* concentration are not clear but it seems that these ciliates can co-occur without being adversely affected by the effects of *H. circularisquama*.

High ciliate grazing mainly due to the genera *Favella* and *Tontonia* was estimated on 31 August and on 11 September. The high grazing loss by *Favella* spp. was due to the high potential ingestion rate on *H. circularisquama*. In this article *F. ehrenbergii* was the predominant species in the genus *Favella* but the ingestion rates of *Favella* spp. were cited from data for *F. taraikaensis* and *F. azorica*. Since the ingestion data of *F. ehrenbergii* measured with the FLA method [13.6–15.4 cells individual^{-1} h^{-1} (Kamiyama *et al.*, 2001)] was similar to the value cited in the present study at the same concentration of *H. circularisquama* (12.5 cells individual^{-1} h^{-1}), applying the ingestion rates of the other *Favella* species to *F. ehrenbergii* is considered not to affect the results significantly. The high grazing loss by *Tontonia* spp. was due to their high abundance. It is interesting that the estimated high grazing losses by ciliates occurred during the period of decrease of the *H. circularisquama* concentration. The specific grazing rates by the ciliate assemblage during the two periods (1.1 and 1.4 day^{-1}) exceeded the potential specific growth rate (0.65–0.76 day^{-1}) of *H. circularisquama* estimated from the temperature and salinity conditions (Yamaguchi *et al.*, 1997) during the bloom period. Grazing loss by the ciliate assemblage is probably one of causes of decreases of *H. circularisquama* during these periods.

We could not obtain information on abundances of the ciliate species other than the genus *Favella* after 13 September. Furthermore, although the abundance of *Favella* spp. remained at a high level during this period, the ingestion rates of *Favella* spp. could not be estimated because under less than 45 cells mL^{-1} of *H. circularisquama*, it is not possible to fit the relationships between feeding rates of *Favella* spp. and concentrations of *H. circularisquama* (Kamiyama, 1997). It is therefore difficult to evaluate whether or not the ciliate grazing loss was the main cause of the rapid decrease of *H. circularisquama* concentration observed from 13 September to 20 September However, the grazing pressure by the ciliate assemblage is considered to be one of the principal factors for the rapid decrease of *H. circularisquama*. Also, the various other factors such as temporal intrusion of a different community of phytoplankton into the seawater in the study site, interactions with other phytoplankton (Uchida *et al.*, 1996), bacterial attack and virus infection (Nagasaki *et al.*, 2000; Tarutani *et al.*, 2001) are potentially associated with the rapid decrease of *H. circularisquama*.

Ingestion rates of ciliates reported from Kamiyama *et al.* (Kamiyama *et al.*, 2001) were measured during 30 min incubations only under a light condition (30 μmol photon m^{-2} s^{-1}). In this study these data except for *Favella* spp. were extrapolated to ingestion rates per 24 h to evaluate the daily grazing loss by ciliates on *H. circularisquama*. Strom (Strom, 2001) showed that ingestion rates of ciliates under illuminated conditions were several times higher than those under dark conditions. Also light has

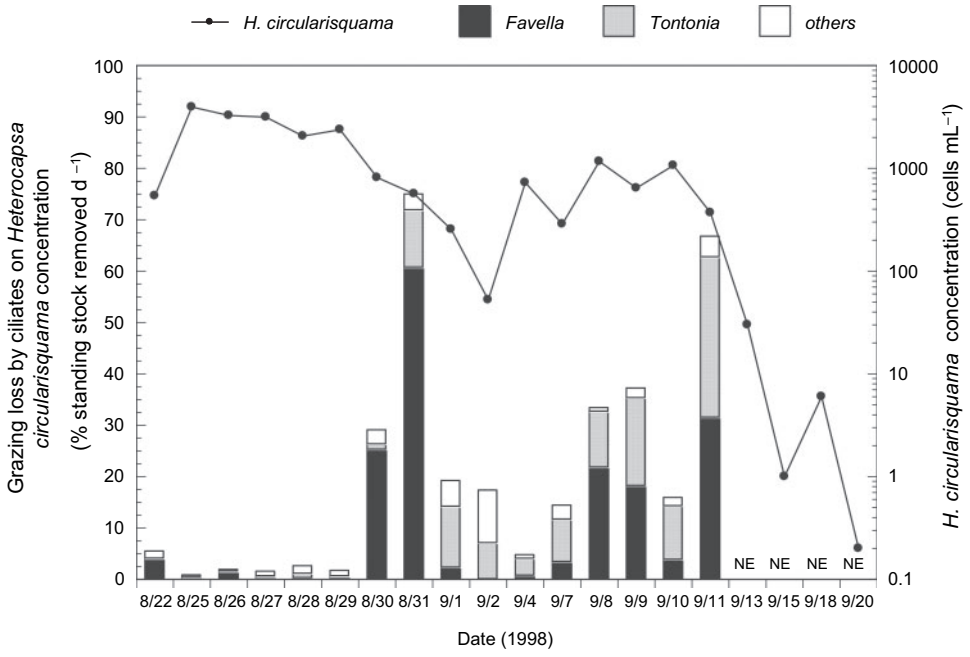

Fig. 7. Consecutive maximum estimated daily grazing loss of *Heterocapsa circularisquama* (% standing stock removed day^{-1}) by the ciliate assemblage during the sampling period. The species-specific grazing rates were estimated from the abundance of each ciliate species and its ingestion rate on *Heterocapsa circularisquama* (Table I, Kamiyama *et al.*, 2001). NE, not estimated.

been shown to strongly stimulate grazing of the toxic dinoflagellate *Pfiesteria piscicida* (heterotrophic form) feeding on nanoflagellates (Feinstein *et al.*, 2001). Hence, total grazing loss by the ciliate assemblage may be overestimated. Kamiyama *et al.* (Kamiyama *et al.*, unpublished data) found that the maximum hourly ingestion rate of *F. taraikaensis* estimated during 15–25 min incubations under light conditions only was 1.5 times higher than indicated by data collected from incubations over 24 h under light–dark conditions. Assuming the same overestimation rate for ingestion rates in the present study, the maximum grazing loss is corrected to be 70% standing stock removed day^{-1} of the *H. circularisquama* concentration.

Another problem for this estimation is the validity of applying the ingestion rates measured at the concentrations ranging from 640 cells mL^{-1} to 780 cells mL^{-1} of *H. circularisquama* (Kamiyama *et al.*, 2001) to various algal densities. Generally, the feeding rates of ciliates increase with the increasing prey concentration up to a certain level before saturation and the response to the prey concentration is often fitted to the rectangular hyperbolic equation (Jeong *et al.*, 2003). The ingestion rates of ciliates except for *Favella* spp. were measured under the prey condition where the ingestion rates are saturated (Kamiyama *et al.*, 2001). Hence, there is a possibility of an overestimation at the concentration of less than 100 cells mL^{-1} of *H. circularisquama* on 2 September, and even at concentrations in the order of 10^2 cells mL^{-1} some of the ingestion rates may be overestimated because ingestion rates seem to strongly depend on the *H. circularisquama* concentration during this range of prey concentration. Over 1000 cells mL^{-1} of *H. circularisquama*, there may not be serious problems with the estimations because the high mortality of ciliates reported for *F. taraikaensis* at concentrations of more than 4000 cells mL^{-1} of *H. circularisquama* (Kamiyama, 1997) was not seen in the present study.

This is the first article on the temporal change in ciliate abundance during the course of a *H. circularisquama* bloom. The results in the present study suggest that grazing by ciliates assemblage can influence the population dynamics of *H. circularisquama* (especially, in the order of magnitude of 10^2 cells mL^{-1}), although it is acknowledged that there are some assumptions for the estimation of the grazing loss that require further investigation. Further information on the abundance and probable role of grazer plankton such as ciliates is essential to improve predictive models of the development and decline of *H. circularisquama* blooms.

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