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Toxic effects of the dinoflagellate *Heterocapsa circularisquama* on clearance rate of the blue mussel *Mytilus galloprovincialis*

Yukihiko Matsuyama[1,*], Takuji Uchida[1], Tsuneo Honjo[2]

[1]Nansei National Fisheries Research Institute, Maruishi, Ohno, Saeki, Hiroshima 739-04, Japan
[2]Faculty of Agriculture, Kyushu University, Hakozaki, Higashi, Fukuoka 812-81, Japan

ABSTRACT: The effects of *Heterocapsa circularisquama* (Peridiniales, Dinophyceae) on the clearance rate of blue mussel *Mytilus galloprovincialis* were studied in the laboratory to clarify the mechanism involved in the toxic effects of dinoflagellates on bivalve mollusks. The clearance rate of blue mussels was significantly reduced when exposed to *H. circularisquama*, even at a low dinoflagellate cell density (50 cells ml^{-1}). Mussels also showed extreme retraction of the mantle edge and no production of feces or pseudofeces. The reduction in clearance rate did not seem to be caused by size, density, or shape of *H. circularisquama*, since no inhibitory effect was observed when mussels were exposed to other morphologically similar dinoflagellates, i.e. *Scrippsiella trochoidea* and *Heterocapsa triquetra*. A filtrate of the *H. circularisquama* culture did not repress the clearance rate of *M. galloprovincialis*. 'Naked cells' of *H. circularisquama* (without cell wall), which were prepared by centrifugation, showed no inhibitory effect on clearance rate. Therefore, it is quite likely that the source of toxicity of *H. circularisquama* is localized on the cell surface. The inhibitory effect of *H. circularisquama* was inactivated by treatment of intact cells with trypsin and sodium dodecylsulfate. These results indicate that the toxic response of *M. galloprovincialis* to *H. circularisquama* is caused by a protein-like substance involving the outer cell components. This is the first study to explain the specific inhibition process of *H. circularisquama* on the filtering activities of bivalve mollusks.

KEY WORDS: *Heterocapsa circularisquama* · *Mytilus galloprovincialis* · Clearance rate · Bivalve · Dinoflagellate · Toxic effect

INTRODUCTION

Harmful phytoplankton blooms occur worldwide, causing serious social problems and seriously affecting shellfish and aquaculture industries (Shumway 1990, Hallegraeff 1993, Anderson 1994, Honjo 1994). The paralytic shellfish poisoning (PSP) of shellfish by members of the genus *Alexandrium* has been well documented. These toxic microalgae not only bring about PSP in humans who have consumed contaminated bivalves, but also affect the physiological status of the filter-feeding bivalves themselves (Gainey & Shumway 1988, Lesser & Shumway 1993). *Gyrodinium aureolum*, which is associated with massive shellfish mortality (Mahoney et al. 1990, Heinig & Campbell 1992), causes reduction of clearance (Widdows et al. 1979) and shell growth rates (Nielsen & Strømgren 1991) and cellular damage in the gut (Widdows et al. 1979) of the blue mussel *Mytilus edulis*. Moreover, abnormal responses of both juvenile and adult bivalves exposed to *Aureococcus anophagefferense* (Gainey & Shumway 1991) and *Prorocentrum minimum* (Wikfors & Smolowitz 1995) have been reported. Thus, harmful effects of certain phytoflagellates to marine bivalve mollusks are extensive.

Heterocapsa circularisquama (Peridiniales, Dinophyceae) is a 'novel' red tide species which has caused

*E-mail: yukihiko@nnf.affrc.go.jp

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mass mortalities of bivalves in embayments of western Japan since 1988 (Yamamoto & Tanaka 1990, Horiguchi 1995, Matsuyama et al. 1995, Matsuyama et al. 1996). Incidences of red tide caused by this alga have increased recently, and the economic loss to aquaculture in terms of death of bivalves has caused industrial and social concern (Matsuyama et al. 1996). The red tide of *H. circularisquama* kills more than 12 bivalve species, but no harmful effects on wild fish populations, cultured fish, or on public health in general have been reported.

Recently, the lethal effects of *Heterocapsa circularisquama* on pearl oyster *Pinctada fucata* were demonstrated under laboratory conditions (Nagai et al. 1996). Juvenile pearl oysters exposed to 5000 to 10 000 *H. circularisquama* cells ml^{-1} showed vigorous clapping and shrinkage of their mantle edges and gills. Approximately 50% of the pearl oysters subsequently underwent cardiac arrest and died after 48 h (Nagai et al. 1996). These toxic effects on the survival and feeding behavior of pearl oysters implicate direct cytotoxicity, but details of the processes by which bivalves are affected by this alga are still unclear.

In this study, we determined the clearance rate of blue mussel *Mytilus galloprovincialis* exposed to physically and chemically treated cells of *Heterocapsa circularisquama* in order to verify the toxicity of this alga on bivalves. On the basis of the results obtained, we show that the toxicity to the bivalves involves outer components of *H. circularisquama* cells.

MATERIALS AND METHODS

Mussel preparation. Adult *Mytilus galloprovincialis* [shell height 25.7 ± 1.4 (SD) mm], which were collected from Hiroshima Bay in the Seto Inland Sea, Japan, in March 1994, were used as the assay animals in the present study. The collected mussels were maintained in running seawater at temperatures ranging from 15 to 18°C and fed cultured phytoplankters (*Isochrysis galbana*, *Skeletonema costatum*) over a 10 d period. Prior to the experiments, each individual was placed into a container containing filtered seawater (GF/F Whatman, pore size ca 0.7 µm) at 20 ± 1.5°C, and gut contents were allowed to clear over 24 h. Seawater was replenished at least once prior to the start of the experiments. Healthy individuals, displaying byssus thread production and full extension of their mantle edge, were used for the experiments.

Algal culture. *Heterocapsa circularisquama* used in the present study were isolated from the surface water of Ago Bay, Mie Prefecture in 1992. *Scrippsiella trochoidea* and *Heterocapsa triquetra* were isolated from the surface water of Hiroshima Bay in 1993. Clonal cultures of these species were obtained by repeated washings using capillary pipettes. The clonal culture of *I. galbana* was provided by Dr H. Iwasaki (Mie University). The algae were cultured in modified SWM-3 medium (Chen et al. 1969, Itoh & Imai 1987) at 22°C on a 12 h light:12 h dark cycle under illumination at 100 µE m^{-2} s^{-1} provided by cool-white fluorescent lamps. All cultures were non-axenic. Cultures in the late exponential to early stationary phase (10 to 16 d after inoculation) were used for the experiments. Measurements of algal cell size were conducted for immobilized cells (n = 30) treated with nickel chloride (final concentration ca 0.4 µg ml^{-1}) under the microscope.

Measurement of clearance rate. Clearance rate (see Omori & Ikeda 1984) was measured by an indirect method which determines the time course decrease of microalgal cell density resulting from mussel filtration. For simple and rapid measurements, microalgal cell densities were determined with a fluorometer (Turner Designs Co., CA, USA, model 110), based on the measurements of *in vivo* chlorophyll fluorescence in the cuvettes (Brand & Guillard 1980). Ambient water samples (5 ml) were transferred to a cuvette with an automatic pipette and fluorescence values of the culture were directly measured. There was no fluorescence detected in the filtered seawater (background) throughout the experiments. Clearance rates of the mussels were calculated using the following equation (Coughlan 1969):

$$CR = \ln(F_0/F_t) \cdot M/t$$

where CR is clearance rate, F_0 is the initial fluorescence value of ambient water, F_t is fluorescence after time t, M is the total volume of ambient water, and t is the time after the start of the experiments (10 to 20 min).

Mussels were placed in aerated semi-transparent plastic beakers containing 50 to 100 ml of microalgal culture diluted with filtered seawater. Stirring and aeration were not performed in order to avoid any disturbance of the filtering processes of the blue mussel. When feces and pseudofeces of a mussel were observed during the experiment, they were removed with Pasteur pipettes immediately. All experiments were carried out in triplicate. Data are expressed as means ± SD, and statistical analyses were performed with Student's t-test.

When the experiments involved low cell densities (≤500 cells ml^{-1}) of *Heterocapsa circularisquama*, cells of another algal species, *Isochrysis galbana* (50 000 cells ml^{-1}), were given in combination for the measurement of clearance rate. Clearance rate of mussels exposed to 80 000 *I. galbana* cells ml^{-1} was defined as the control in the present study. *I. galbana* is generally known to be non-toxic.

Cell treatments of *Heterocapsa circularisquama*. In order to examine the effects of an extracellular metabolite of *H. circularisquama* on *Mytilus galloprovincialis*, filtrate was prepared from a culture of *H. circularisquama* (100 000 cells ml^{-1}) by gentle filtration (<20 mm Hg), using a glass-fiber filter (GF/F). Physically treated cells of *H. circularisquama* were prepared by centrifugation and sonication. The cultures (50 000 cells ml^{-1}) were centrifuged at 2000 rpm (720 × *g*) for 5 min. After decantation, pellets were resuspended in filtered seawater at a concentration of 8000 cells ml^{-1}. Sonication was applied for about 60 s to *H. circularisquama* cultures containing 8000 cells ml^{-1}.

Heterocapsa circularisquama were chemically treated at 25°C for 2 h using 5 ml of culture containing 20 000 to 50 000 cells ml^{-1}. Chemicals [EDTA, trypsin, sodium dodecylsulfate (SDS), Triton X-100, and sodium deoxycholate] were obtained from Wako Pure Chemical Ind. Ltd (Osaka, Japan). Concentrations of each chemical employed in the present study are as follows: EDTA, 24 µg ml^{-1}; trypsin, 350 µg ml^{-1}; SDS, 10 to 50 µg ml^{-1}; Triton X-100, 7.4 µg ml^{-1}; and sodium deoxycholate, 23 µg ml^{-1}. Each chemical was used at a concentration lower than that which would inhibit the swimming ability of *H. circularisquama*. The final density of these treated cells was adjusted to 250 to 290 cells ml^{-1} in filtered seawater prior to the experiments. Chemicals which were carried over into the bioassay procedure had no effect on mussel clearance rate.

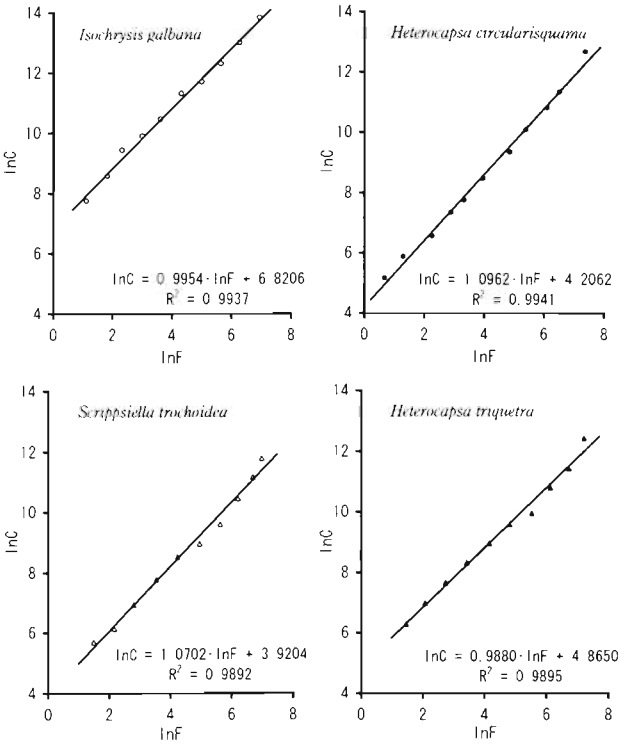

Fig. 1 Correlations between natural logarithm of cell densities (C; cells ml^{-1}) and natural logatithm of *in vivo* fluorescence (F; relative units) for the 4 microalgal species used in this study

RESULTS

Measurement of clearance rate

Relationships between cell density and fluorescence value of each microalgal species are given in Fig. 1. Linear relationships were clearly demonstrated over a wide range of cell densities and the correlation coefficients ranged from 0.9892 to 0.9941. The fluorometric technique, used in this study to determine chl *a* of the microalgae, allowed for rapid and simple measurements of the clearance rates of *Mytilus galloprovincialis* with high levels of reproducibility.

Effects of intact cells

The mean cell length of algae used was 32.8 ± 3.0 (SD) µm in *Scrippsiella trochoidea*, 25.5 ± 2.2 µm in *Heterocapsa triquetra*, 21.8 ± 2.5 µm in *H. circularisquama*, and 4.4 ± 0.8 µm in *Isochrysis galbana*.

Table 1 shows the clearance rates (ml $mussel^{-1}$ h^{-1}) of *Mytilus galloprovincialis* exposed to each of the 4 phytoflagellate species. Initial cell density was 8000 cells ml^{-1} for *Scrippsiella trochoidea*, *Heterocapsa triquetra* and *H. circularisquama*, and 80 000 cells ml^{-1} for *Isochrysis galbana*. Clearance rates for *S. trochoidea* and *H. triquetra* were almost the same as that for *I. galbana*, which is generally known as non-toxic and is used as feed for bivalves. In contrast, a remarkable reduction in clearance rate was observed in the case of *H. circularisquama*. Although the mussels exposed to *H. circularisquama* cells did not close their shells completely (during the 10 to 20 min experimental trials), their inhalant opening and exhalant siphon

Table 1. Clearance rates (ml $mussel^{-1}$ h^{-1}; mean ± SD) of *Mytilus galloprovincialis* exposed to 4 different species of microalgae. Cell densities are 80 000 cells ml^{-1} for *Isochrysis galbana* (control) and 8000 cells ml^{-1} for the other species. ns: not significant

Microalgal	Clearance rate	% control	*t*-test
Isochrysis galbana (control)	1015 ± 135	–	–
Scrippsiella trochoidea	1010 ± 95	99	ns
Heterocapsa triquetra	1008 ± 112	99	ns
Heterocapsa circularisquama	10 ± 1	0.97	$p < 0.001$

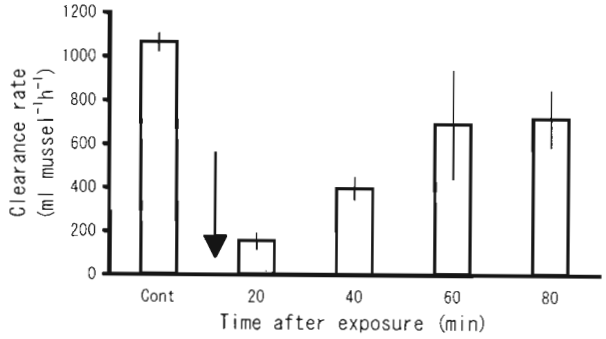

Fig. 2. Change in clearance rate of *Mytilus galloprovincialis* pre-exposed to 5000 intact *Heterocapsa circularisquama* cells ml^{-1} for about 30 min. (Control: *Isochrysis galbana*, 80000 cells ml^{-1}). Arrow denotes the replenishment of the media to 80000 cells ml^{-1} of *Isochrysis galbana*. Error bars show ±SD

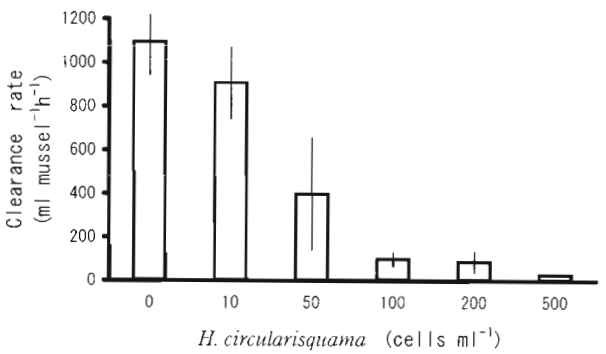

Fig. 3. Changes in clearance rate of *Mytilus galloprovincialis* exposed to various concentrations of *Heterocapsa circularisquama*. Error bars show ±SD

were obstructed due to extreme retraction of the mantle. The mussels which were exposed to 5000 *H. circularisquama* cells ml^{-1} (30 min) still exhibited refusal behavior up to 80 min after the ambient water was replenished with a medium containing *I. galbana* cells (Fig. 2).

Fig. 3. shows the relationships between cell density of *Heterocapsa circularisquama* and clearance rate of *Mytilus galloprovincialis*. Each measurement was conducted using bi-algal conditions, i.e. various concentrations of *H. circularisquama* combined with 50000 *I. galbana* cells ml^{-1}. Marked decline of the mean clearance rate was observed above a density of 50 *H. circularisquama* cells ml^{-1}. The mean clearance rate at 500 cells ml^{-1} was reduced to 2.6% of the control clearance rate. No significant ($p > 0.2$) differences were found between the mean clearance rate of the mussels exposed to 10 *H. circularisquama* cells ml^{-1} and the mean clearance rate of the controls. The mean clearance rates measured during the exposure of blue mussels to 50 and 100 *H. circularisquama* cells ml^{-1} were significantly lower ($p < 0.05$ and $p < 0.001$) than clearance rates in the controls. Thus, reduction of the clearance rate of *M. galloprovincialis* strongly depends on the cell density of *H. circularisquama*.

Effects of physical treatments

Table 2 shows the clearance rates of *Mytilus galloprovincialis* exposed to various physically treated cells of *Heterocapsa circularisquama*. The clearance rates of mussels exposed to filtrate prepared from *H. circularisquama* culture (100000 cells ml^{-1}) was 892 ml mussel^{-1} h^{-1} (Table 2). No significant ($p > 0.2$) inhibition of clearance rates or refusal behaviors, such as retraction of mantle, valve closure and pseudofeces production, were observed in mussels exposed to filtrate of *H. circularisquama*.

Most of the sonicated cells of *Heterocapsa circularisquama* retained their shape, but sonication caused cell wall shearing and loss of motility (about 90%). Cells undergoing centrifugation completely retained their shapes but lost their outer components. Clearance rates were calculated to be 590 ml mussel^{-1} h^{-1} and 1026 ml mussel^{-1} h^{-1} for sonicated and centrifugated cells, respectively. No significant ($p > 0.5$) reduction in clearance rates of mussels was observed when exposed to centrifugated cells. Mussels exposed to sonicated cells exhibited a significant ($p < 0.05$) reduction in clearance rates; however, reduction of the activity was about 50% of that in the assay using similar concentrations of intact *H. circularisquama* cells (see Table 2). Therefore, *H. circularisquama* cells, stripped of their outer components by sonication and centrifugation, exhibited little effect on clearance rates of the mussel.

Effects of chemical treatment

Clearance rate of *Mytilus galloprovincialis* exposed to *Heterocapsa circularisquama* cells treated with tryp-

Table 2. Clearance rates (ml mussel^{-1} h^{-1}; mean ± SD) of *Mytilus galloprovincialis* exposed to physically treated *Heterocapsa circularisquama* cells (control: *Isochrysis galbana*, 8000 cells ml^{-1}). Cell density of *H. circularisquama* was 8000 cells ml^{-1} for each treatment. ns: not significant

Treatment	Clearance rate	% control	*t*-test
Control	1112 ± 266	–	–
Untreated	3 ± 1	0.27	$p < 0.005$
Filtrate	892 ± 170	80	ns
Sonication	590 ± 218	53	$p < 0.05$
Centrifugation	1026 ± 231	92	ns

Table 3. Clearance rates (ml mussel^{-1} h^{-1}; mean ± SD) of *Mytilus galloprovincialis* exposed to chemically treated *Heterocapsa circularisquama* cells (control: *Isochrysis galbana*, 8000 cells ml^{-1}). Cell density of *H. circularisquama* ranged from 260 to 290 cells ml^{-1} ns: not significant

Treatment	Clearance rate	% control	*t*-test
Control	1068 ± 206	–	–
Untreated	156 ± 46	15	$p < 0.001$
EDTA	131 ± 5	12	$p < 0.005$
Trypsin	911 ± 128	85	ns

Table 4. Clearance rates (ml mussel^{-1} h^{-1}; mean ± SD) of *Mytilus galloprovincialis* exposed to chemically treated *Heterocapsa circularisquama* cells (control: *Isochrysis galbana*, 8000 cells ml^{-1}). Cell density of *H. circularisquama* ranged from 250 to 280 cells ml^{-1} ns: not significant

Treatment	Clearance rate	% control	*t*-test
Control	1188 ± 203	–	–
Untreated	230 ± 116	19	$p < 0.001$
SDS	1096 ± 69	92	ns
Sodium deoxycholate	207 ± 87	17	$p < 0.005$
Triton X-100	395 ± 147	33	$p < 0.001$

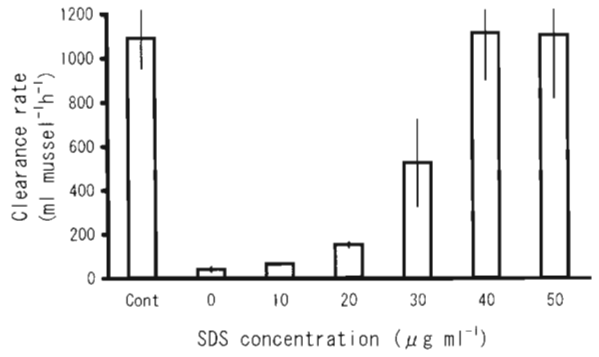

Fig. 4. Changes in clearance rate of *Mytilus galloprovincialis* exposed to *Heterocapsa circularisquama* (1000 to 1120 cells ml^{-1}) pre-treated with various concentrations of SDS. Error bars show ±SD

sin was determined to be 911 ml mussel^{-1} h^{-1}, which is 85% of that in the control (Table 3). Significant ($p < 0.005$) reduction of clearance rate was observed when the dinoflagellate was treated with EDTA. Table 4 shows clearance rates of *M. galloprovincialis* exposed to *H. circularisquama* cells treated with the surface-active chemicals. Clearance rates were 1096 ml mussel^{-1} h^{-1} for SDS (50 µg ml^{-1}), 395 ml mussel^{-1} h^{-1} for Triton X-100, and 207 ml mussel^{-1} h^{-1} for sodium deoxycholate. SDS treated cells demonstrated little inhibitory effect on the clearance rate of mussels (92% of controls). In this case, no refusal behavior was observed throughout the experiments. However, the inhibitory effect of *H. circularisquama* on clearance rates was still observed when the alga was treated with other chemicals.

Fig. 4 shows the effects of SDS concentration on clearance rates of mussels exposed to SDS-treated *Heterocapsa circularisquama* cells. Clearance rate increased with increasing SDS concentration. The difference between control and treatment clearance rates was not significant ($p > 0.5$) when algae were treated with SDS in excess of 40 µg ml^{-1}

DISCUSSION

Toxic effects of *Heterocapsa circularisquama* on *Mytilus galloprovincialis*

Mytilus galloprovincialis fed well on the 2 dinoflagellates *Scrippsiella trochoidea* and *Heterocapsa triquetra* as well as on *Isochrysis galbana*. Refusal behavior was not observed when the mussels were exposed to the cells of these species. In contrast, *Heterocapsa circularisquama* cells were strongly rejected by the mussels, with refusal behavior manifested by an extreme retraction of the mantle. Generally, filtration processes of bivalves are influenced by physical aspects of the particles: size, density, electric charge, and morphology (Ali 1970, Schulte 1975, Winter 1978, Jørgensen 1990). In the present study, however, size, density, and morphology of 3 dinoflagellates were not so different. Therefore, the inhibition of *M. galloprovincialis* clearance rate is probably not due to physical aspects of *H. circularisquama*.

The inhibitory effect of *Heterocapsa circularisquama* appears to occur even at low concentrations, that is *Mytilus galloprovincialis* showed a significantly reduced clearance rate at 50 *H. circularisquama* cells ml^{-1}. In natural populations of *Gyrodinium aureolum*, which is considered toxic to shellfish, significant inhibition of the clearance rate of *Mytilus edulis* occurs at 500 cells ml^{-1}, but not at 200 cells ml^{-1} (Widdows et al. 1979). In contrast to *H. circularisquama*, *Heterocapsa triquetra* (which has worldwide distribution) did not induce refusal behavior in *M. galloprovincialis* and did not affect the clearance rate of *M. galloprovincialis* in the present study.

Recent studies demonstrate that bivalves can selectively capture and ingest food particles. Captured particles are sorted by means of their size, shape, and chemical composition on the gill, ctenidia and labial palps of bivalves (Shumway et al. 1985). Rejected par-

ticles are filtered and ejected as pseudofeces and/or ingested at low levels (Shumway et al. 1985). *Heterocapsa circularisquama* cells were still taken up at low levels when extreme mantle retraction was evident in *Mytilus galloprovincialis*. Inhibition of the clearance rate was sustained not only during experiments but also up to 80 min after ambient water was replaced with *I. galbana* culture (Fig. 2). This type of inhibition has not been previously reported.

Characterization of chemical components

Reduction in clearance rate was significant when ≥50 intact *Heterocapsa circularisquama* cells ml^{-1} were used in the present experiment. However, no inhibitory effects on clearance rate were observed with the filtrate of dense *H. circularisquama* culture (100 000 cells ml^{-1}). Therefore, it seems likely that *H. circularisquama* cells do not produce extracellular metabolites which repress mussel clearance rates, apart from in their cell walls. A previous study (Nagai et al. 1996) also reported that death of juvenile pearl oysters was not caused by extracellular metabolites of *H. circularisquama* in the medium. Furthermore, filtrate of *H. circularisquama* has not been observed to have any toxic effect on the survival of the naked dinoflagellate *Gyrodinium instriatum*, whereas intact cells of *H. circularisquama* kill *G. instriatum* upon contact (Uchida et al. 1995).

'Naked cells' of *Heterocapsa circularisquama* prepared by centrifugation lost their inhibitory effect on mussels. Outer components (outer membrane, plates, scale, flagellum etc.) of *H. circularisquama* cells are easily removed by centrifugation and sonication. Thus, toxicity of *H. circularisquama* to blue mussels seems to be closely related to the outer components of the cells. Toxicity of *H. circularisquama* was detected neither in extracellular products nor in intracellular substances. Consequently, it is probably localized in outer components of the cell. On the basis of their observations, Nagai et al. (1996) postulated that, if a toxic substance caused the death of pearl oysters, it might be contained in the cell surface of *H. circularisquama*. This was evidently verified in the present study. However, sonicated cells which contain the whole, but physically broken-down, products of the outer components showed low inhibitory effect on the clearance rate (see Table 2). It is likely that the toxin localized on the cell surface is easily inactivated once it is released from the cell surface.

The filtration process of bivalves is sometimes influenced by chemical materials on food particles at the pre-ingestion stage. The reduction of clearance rate in bivalve mollusks exposed to *Aureococcus anophagefferense* is brought about by digestion of the extracellular coat, which releases a dopamine-mimetic compound that brings about the arrest of the lateral cilia in certain species (Gainey & Shumway 1991). Dopamine is known to inhibit filtration rates of bivalves (Jones & Richards 1993) due to reduction of ciliary activity (see Aiello 1990) and the siphon opening (Fong et al. 1993, Jones & Richards 1993). In addition, allelochemical effects of dissolved ectocrine on clearance rates of bivalves were also demonstrated for some microalgae (Ward & Targett 1989, Ward et al. 1992). Recent studies reveal that chemical interactions between microalgae and filtering activity of bivalves are generally found (Ward et al. 1992).

The toxic effect of *Heterocapsa circularisquama* on blue mussels decreased after gentle treatment of the alga with trypsin. Trypsin hydrolyzes various types of protein and polypeptides; therefore, it is probable that a protein (or polypeptide) complex on the surface of *H. circularisquama* is toxic. Toxic effects were decreased by SDS treatment in this study. SDS is known to break down protein to polypeptides. However, other surface-active chemicals which have lower protein degradation activities (sodium deoxycholate) were not as effective as SDS.

Phytoplankton have various types of species-specific substances, such as glycoprotein and carbohydrates, on the cell surface (Yokote & Honjo 1985, Yokote et al. 1985, Costas & Rodas 1994, Waite et al. 1995). These substances are known to be important in cell recognition and reception of chemical information (Hughes 1975). A polysaccharide-protein complex released extracellularly by *Heterosigma akashiwo* (= *H. carterae*, Taylor 1992) acts as an allelopathic substance which plays an important role in phytoplankton succession during *H. akashiwo* blooms (Honjo 1993). In addition, enzymes such as alkaline phosphatase and L-amino-oxydase (Palenik & Morel 1990) are present on the phytoplankton cell surface. In the present study, certain protein compounds on the surface of *H. circularisquama* cells inhibited the filtering behavior of *M. galloprovincialis*. Further investigations are necessary to clarify the interaction between phytoplankton and filter-feeding bivalves.

In addition, there is a possible concern that human consumption of bivalves which have accumulated *Heterocapsa circularisquama* may lead to illness; however no human poisoning or shellfish poisoning has ever been reported during a bloom of *H. circularisquama*. Direct HPLC analysis has detected neither PSP toxins nor DSP toxins in the cells of *H. circularisquama* (S. Sakamoto & T. Suzuki pers. comm.). On the basis of previous studies, it is concluded that *H. circularisquama* blooms do not pose a risk to human health.

Ecological importance

Mytilus galloprovincialis significantly reduced its clearance rate even at low concentrations of *Heterocapsa circularisquama* (50 cells ml^{-1}). Field observers noted that during a *H. circularisquama* bloom at densities from 50 to 200 cells ml^{-1}, pearl oysters showed obvious refusal behaviors such as clapping, valve closure and contraction of the mantle (Matsuyama et al. 1996). This may contribute to the development of red tides of this dinoflagellate since dense populations of suspension-feeding bivalves might otherwise control apparent phytoplankton growth by feeding on the ambient edible phytoplankton at high rates (Jørgensen 1990). Significant reduction of the phytoplankton standing crop by suspension-feeding bivalves has been reported in the Potomac River (Cohen et al. 1984). In fact, *H. circularisquama* red tides frequently appear in embayments in Japan where intensive commercial bivalve culture of pearl oysters, oysters, and short-neck clams is being conducted. Thus, the inhibitory effect of *H. circularisquama* on bivalve clearance rates might, itself, be of great advantage for the development of red tides.

In summary, we determined the clearance rates of blue mussel *Mytilus galloprovincialis* exposed to variously treated cells of *Heterocapsa circularisquama* and showed that significant toxic effects, as manifested in the filtration processes of this bivalve, are regulated by a protein-like substance present on the surface of *H. circularisquama* cells.

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