Journal of Shellfish Research, Vol. 20, No. 3, 1269–1272, 2001.

IMPACTS OF THE HARMFUL DINOFLAGELLATE, *HETEROCAPSA CIRCULARISQUAMA*, ON SHELLFISH AQUACULTURE IN JAPAN

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ABSTRACT In the last decade, the bloom-forming dinoflagellate *Heterocapsa circularisquama* Horiguchi has caused red tides along the Japanese coast. Because this dinoflagellate shows a detrimental effect on molluscan shellfishes such as bivalves and gastropods, almost all red tides have associated catastrophic death of farmed animals. Recent proliferations of *H. circularisquama* throughout the western Japanese coast have devastated the shellfish aquaculture industries and are a cause for concern due to the consequent economic losses.

KEY WORDS: *Heterocapsa circularisquama*, red tide, shellfish, death, toxicity

INTRODUCTION

Current proliferation of harmful algal blooms causes serious problems for public health and fisheries industries (Okaichi 1989, Smayda 1990, Shumway 1990, Hallegraeff 1993, Anderson 1994, Honjo 1994). On the Japanese coast, the novel marine dinoflagellate *Heterocapsa circularisquama* Horiguchi (Horiguchi 1995), appeared in 1988 and then rapidly expanded over the western area (Matsuyama et al. 1996). Red tides due to *H. circularisquama* have damaged shellfish aquaculture in most of the region (Yamamoto & Tanaka 1990, Yoshida & Miyamoto 1995, Matsuyama et al. 1996, Etou et al. 1998). Although *H. circularisquama* blooms mainly affect shellfisheries aquaculture, no harmful effects on wild and cultured finfish, other marine vertebrates, and public health hazards were recorded. Incidence of blooms of this species has increased recently, and the economic losses in aquaculture have been a cause for concern for the shellfisheries industry and society (Matsuyama et al. 1996). In the present article, we review the damage caused to aquaculture and the toxicity of the organism.

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DAMAGE TO SHELLFISHERIES AND TOXICITY

Red Tide and Shellfish Damage in Aquaculture Industries

The first incidence of red tide due to *H. circularisquama* and subsequent death of shellfish occurred in Uranouchi Bay, on the southern part of Shikoku Island (Fig. 1) in 1988. Red tide due to

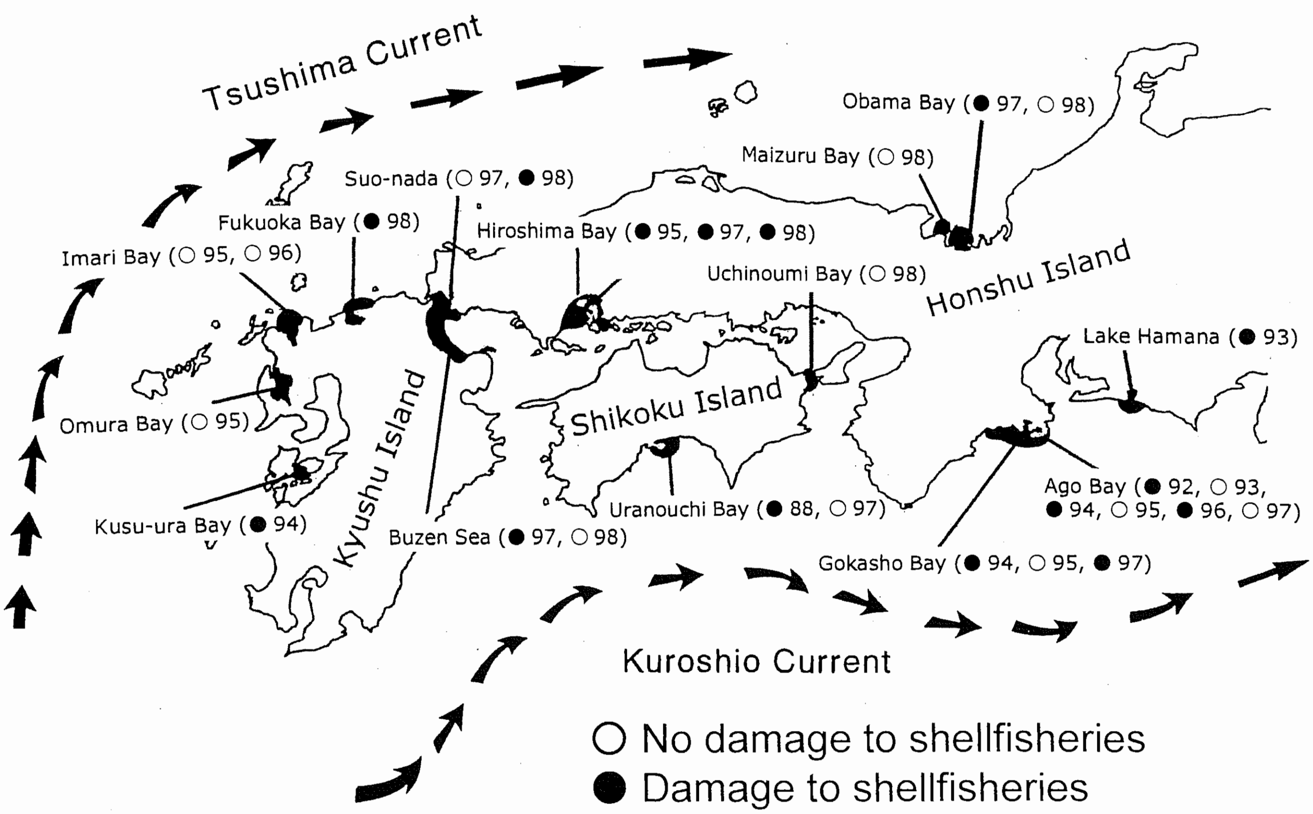

Figure 1. Records of red tide (>10^6 cells L^{-1}) due to *Heterocapsa circularisquama* and two major currents in Japan.

H. circularisquama has also been recorded at Fukuoka Bay in 1989, and at Ago Bay in 1992, resulting in mass mortality of shellfish (Fig. 2).

Until 1998, 28 cases of *H. circularisquama* red tide (maximum cell density $\geq 10^6$ cells L^{-1}), including 14 incidences leading to fisheries damage, had been recorded in 14 locations of western Japan (Fig. 1). The red tide due to *H. circularisquama* has been associated with massive killing of commercially important bivalve species: manila clam *Ruditapes philippinarum*, Pacific oyster *Crassostrea gigas*, pearl oyster *Pinctada fucata*, blue mussel *Mytilus galloprovincialis*, etc. The current proliferation of *H. circularisquama* throughout western Japan has essentially destroyed molluscan shellfish aquaculture. Economic losses of shellfish aquaculture have been estimated to be at least 93 million US dollars in the last decade (Table 1).

On the other hand, there have been no records of death of finfish and crustacean species or public health hazard due to the consumption of shellfish and other seafood products in association with the red tide of *H. circularisquama*. This type of biohazard in marine animals is markedly different from previous reports of damage caused by harmful algae responsible for PSP, DSP, ASP, NSP, ciguatera poisoning, and ichthyotoxicity.

***Behavior of Shellfish Affected by* H. circularisquama**

The effects of *H. circularisquama* on bivalve molluscs have been described in previous studies. Matsuyama et al. (1996) have observed that exposure of pearl oysters to 5–10 × 10^6 *H. circularisquama* cells L^{-1} resulted in death within several days, although the level of dissolved oxygen was not critical. The dead individuals have been characterized by a marked shrinkage of the mantle, decrease of glycogen lobe attached to the mantle, and gut discoloration. The symptoms clearly reflect the direct cytotoxic effect of *H. circularisquama* on pearl oyster physiology. Similar harmful effects on the oyster *C. gigas* and the mussel *M. galloprovincialis* have been shown during the red tides which have occurred elsewhere (Etou et al. 1998, Matsuyama et al. 1998).

Laboratory Exposure Experiment Using Cultured Material

Nagai et al. (1996) have shown that the mortality of pearl oyster spat caused by *H. circularisquama* depends on the cell density of this alga. Pearl oysters exposed to *H. circularisquama* cells at a density above 10^7 cells L^{-1} have showed vigorous contraction of the mantle and gills, clapping, sustained valve closure, paralysis, and heartbeat stoppage within 24 hours. Furthermore, the mussel

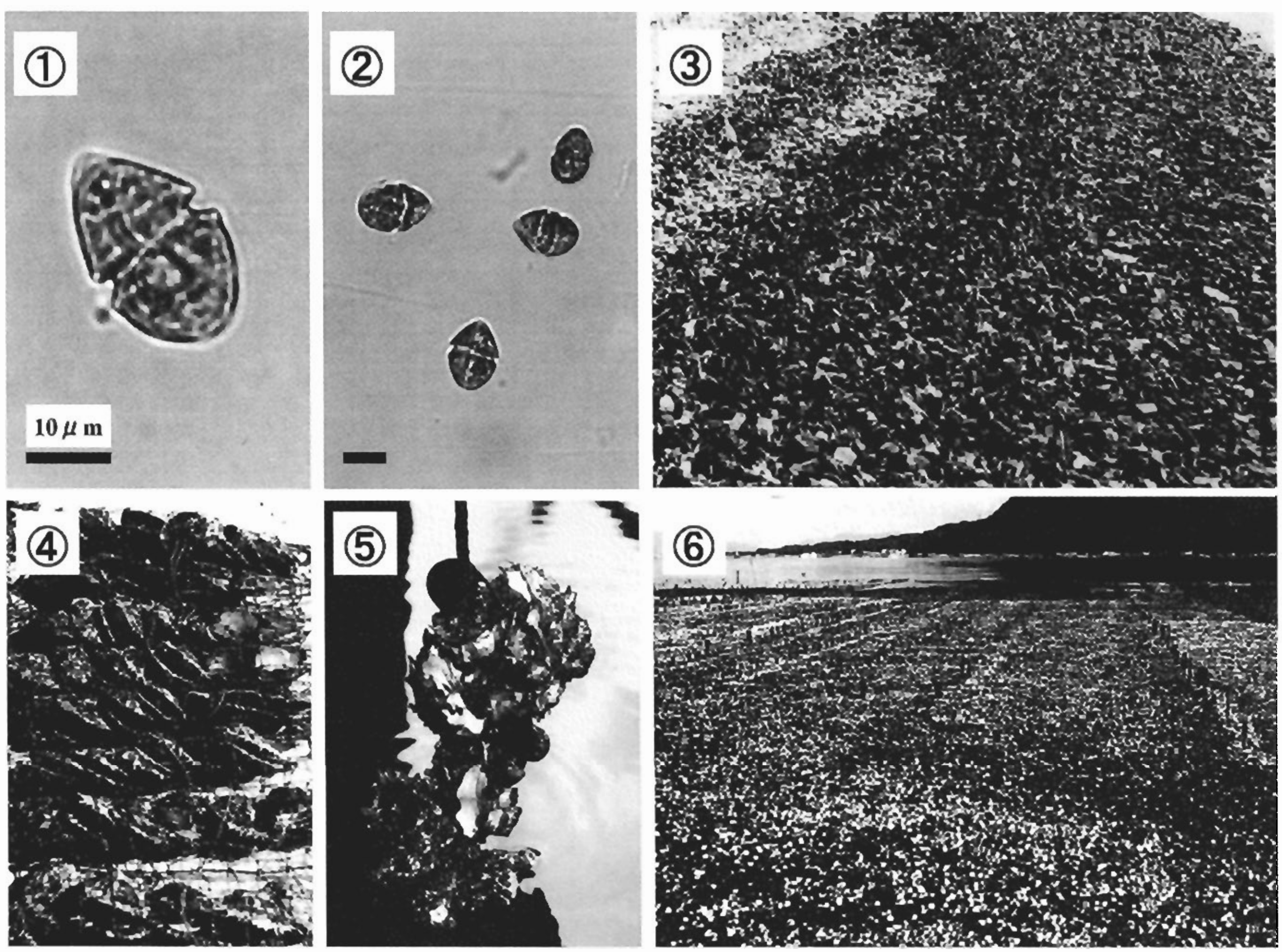

Figure 2. Photographs of *Heterocapsa circularisquama* and affected shellfish. (1) and (2): Light micrographs of *H. circularisquama;* (3) Dead shellfish: mussel *Mytilus galloprovincialis*, razor clam *Solen strictus*, manila clam *Ruditapes philippinarum* due to red tide of *H. circularisquama* (Fukuoka Bay, 1989, photograph is provided by Y. Tanaka); (4) Dead pearl oyster *Pinctada fucata* (Ago Bay, 1992); (5) Dead oyster *Crassostrea gigas* (Hiroshima Bay, 1995); (6) Farming-ground of manila clam *Ruditapes philippinarum* affected by the red tide of *H. circularisquama* (Hiroshima Bay, 1995, Photograph is provided by T. Hamasaki).

TABLE 1.

Damage to fisheries industries caused by harmful algal blooms in Japan.

Causative Agents	Periods	Amount (million $US)
Chattonella spp.	1969–1999	185
Heterocapsa circularisquama	1988–1999	92
Gymnodinium mikimotoi	1972–1999	83
Heterosigma akashiwo	1972–1999	15

M. galloprovincialis significantly reduced its feeding activity when exposed to 10^4 cells L^{-1} of *H. circularisquama*, but not in culture with 10^6 cells L^{-1} of the morphologically similar dinoflagellates *Scrippsiella trochoidea* and *Heterocapsa triquetra* (Matsuyama et al. 1997). Some harmful algae are known to be toxic to marine shellfish. The blooms associated with the unarmored dinoflagellate *Gyrodinium aureolum* and picoplankton *Aureococcus anophagefferens* referred to as "brown tide" lead to considerable failure in mussel and scallop farming (Tangen 1977, Shumway 1990). Laboratory-rearing experiments using these algae have proven considerable detrimental effects on various bivalve species. The harmful effect of *H. circularisquama* on bivalves is very specific and pronounced compared to other harmful algal species.

Harmful Effects of H. circularisquama *on Other Organisms*

Laboratory exposure experiments show that various marine animals such as bivalves, gastropods, solitary acidians, and jellyfish are affected by *H. circularisquama*, unlike vertebrates, crustaceans (lobster, shrimp, crab), starfish, and sea urchins (Table 2). On the other hand, although the occurrence of illness associated with the consumption of bivalves that accumulated *H. circularisquama* cells may be a cause for concern in humans, shellfish poisoning has never been observed in samples collected from red tide areas. Direct HPCL analysis has failed to detect PSP toxins or DSP toxins in the cells of *H. circularisquama*. No death or symptoms have been observed in 5 mice to which a cultured cell pellet of *H. circularisquama* had been injected in intraperitoneally at a rate of 10^6 cells/mouse (Table 2).

TABLE 2.

Effects of *Heterocapsa circularisquama* on various animals.

Animals	cells L^{-1}	Symptoms
Bivalves	10^4–10^5	Feeding inhibition
	$>10^6$	death
Gastropods	10^4–10^5	Unusual locomotion
	$>10^6$	death
Acidians	$>10^6$	Feeding inhibition
Jellyfish	$>10^6$	Tentacle shrinkage
Protozoa[1]	10^5–10^6	Feeding inhibition
	$>10^6$	Death
Dinoflagellates[1]	$>10^6$	Death by cell contact
Diatoms	$>10^7$	–
Copepods	$>10^7$	–
Fnfish	$>10^6$	–
Crab	$>10^7$	–
Lobster, shrimp	$>10^7$	–
Lobster, shrimp	$>10^7$	–
Gammarid	$>10^7$	–
Star fish	$>10^7$	–
Sea urchin	$>10^7$	–
Mouse[2]	–	–

–: not affected
[1] species-specific
[2] intraperitoneal injection (10^6 cells/mouse)

Characterization of Heterocapsa circularisquama *Toxicity*

The toxicity of *H. circularisquama* to bivalves has been shown to be mediated by a chemical agent. The toxic effect of *H. circularisquama* on bivalves is not due to extracellular metabolites, cell exudates, and "naked cells" prepared by sonication and centrifugation (Fig. 3A). Furthermore, trypsin and SDS (sodium dodecyl sulfate) treatments have been found to decrease drastically the toxicity of *H. circularisquama* cells (Fig. 3B). Therefore, a labile protein-like complex localized on the cell surface presumably exerts a detrimental effect on bivalves. However, purification and characterization of toxic fractions have not been successful because this agent is highly labile under neutral conditions. Recently, exposure of larval manila clam to *H. circularisquama* has demonstrated acute toxicity on their survival and development, which is associated with a significant increase of intracellular calcium concentration.

SUMMARY

Blooms of the dinoflagellate *H. circularisquama* have been associated with mass mortalities of various bivalves in western Japan since 1988. Toxicity of this species is acute and specific to

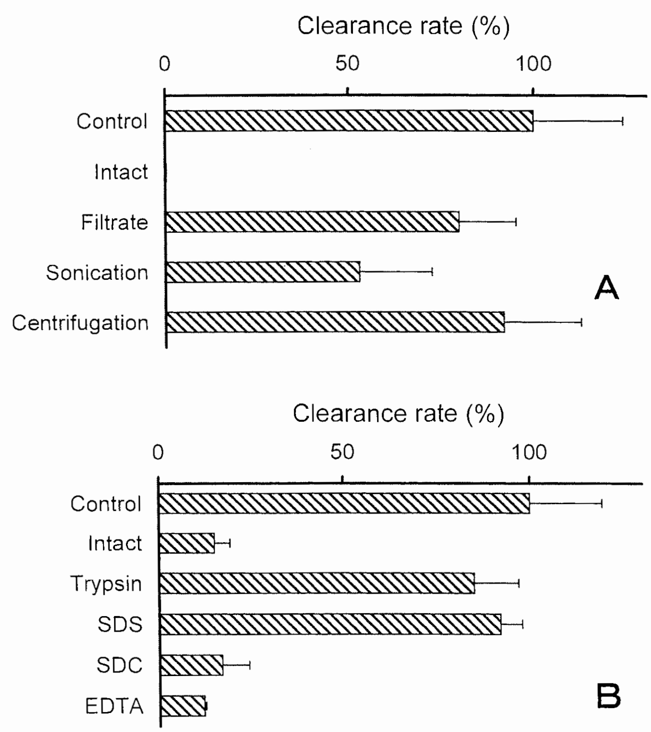

Figure 3. Relative clearance rates of *Mytilus galloprovincialis* feeding to physically (A) and chemically (B) treated *Heterocapsa circularisquama* cells (Control: *Isochrysis galbana*, 8×10^7 cells L^{-1}). Initial cell density of *H. circularisquama* ranged from 2.5–2.8×10^5 cells L^{-1}. SDS: sodium dodecylsulfate. SDC: sodium deoxycholate. DTT: dithiothreitol. Each chemical was used at a concentration lower than that which would inhibit the swimming of *H. circularisquama*. Error bars show ±S. D.

bivalves and gastropods. Unfortunately, no successful damage prevention strategy has been developed until now. Therefore, relocation and/or early harvest of organism are the only methods to reduce the shellfisheries damage. In some locations, early warning systems by local governments that are based on frequent field monitoring of *H. circularisquama* cells have successfully reduced the shellfisheries damage. *H. circularisquama* blooms do not cause finfish killing and mammals illness. However, a rapid decline of demand for products due to misinformation, rather than actual shellfish poisoning, could occur. On the other hand, toxicity of *H. circularisquama* can easily be reduced when the cells are disturbed with physical and chemical treatments (unpublished). This fact will provide crucial information for improvement of damage prevention in the future.

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