南西水研研報 (Bull. Nansei Natl. Fish. Res. Inst.), No. 31: 19–24 (1998)

Effect of Harmful Phytoplankton on the Survival of the Abalones, *Haliotis discus* and *Sulculus diversicolor*

Yukihiko Matsuyama, Yoshitsugu Koizumi, and Takuji Uchida

During harmful algal blooms (red tide) on the coast of Japan, massive mortalities of marine animals including commercially important gastropods have resulted in economic losses of wild resources and local aquaculture. In order to evaluate the detrimental effect of the algal blooms, the effects of three phytoflagellates, *Heterocapsa circularisquama, Gymnodinium mikimotoi*, and *Heterosigma akashiwo* on survival of juvenile abalone, *Haliotis discus* and *Sulculus diversicolor* were studied in the laboratory. The results demonstrate that *H. circularisquama* and *G. mikimotoi* frequently kill both abalone species within 24h at densities > 10^4 cells/ml accompanied with unusual avoidance behaviors; vigorous contraction of tentacles, escape locomotion, and paralysis of the animals. The raphidophyte species *H. akashiwo* showed lower detrimental effects compared with the two dinoflagellates.

Key words: Red tide, Dinoflagellate, Gastropod, Abalone, Mortality

The global increase of harmful algal blooms has been a concern in the last several decades. (Shumway 1990, Honjo 1994). The studies on impact of harmful algal blooms have focused on the shellfish poisoning which causes human illness after consumption of phycotoxin-contaminated organisms (Shumway 1990). In addition, harmful algal blooms impact on the physiological processes and survival of marine organisms (Shumway 1990, Honjo, 1994). These impacts lead to economic losses in the aquaculture industries, and sometimes impacts on the overall marine ecosystem (Shumway 1990, Honjo 1994).

Historically, "fish killing" by harmful red tide in Japanese coastal waters has been well documented (see Honjo 1994). However, there are few data concerning harmful effects on marine invertebrates although massive "shellfish killing" has been accompanied with red tides in Japan. Recently, a novel red tide dinoflagellate, *Heterocapsa circularisquama* caused mass mortalities of bivalves on the west coast of Japan (Yamamoto and Tanaka 1990, Matsuyama et al. 1996). In addition, harmful red tides due to *Gymnodinium mikimotoi* (= *Gymnodinium nagasakiense, Gymnodinium* sp. Type '65, see Takayama and Matsuoka, 1991) which is known as a fish killing dinoflagellate (Takayama and Adachi 1984, Honjo 1994) accompanied mass mortalities of shellfish (Shiokawa and Irie 1966, Hanai et al. 1992, Takayama 1986, Yamaguchi 1994). During *H. circularisquama* and *G. mikimotoi* red tide periods, massive mortalities of commercially important gastropods have also been found (Yamamoto et al. 1989, Yoshida and Miyamoto 1996), which impacted on local aquaculture. However, quantitative studies about lethal effects of harmful phytoflagellates on gastropods are lacking (see Shumway 1995). The present study focuses on the effects of three phytoflagellates species on two commercially important abalones, *Haliotis discus* (Kuroawabi) and *Sulculus diversicolor* (Tokobushi).

Materials and Methods

Juveniles (ten months old) of *Haliotis discus* (shell width 10.3 ± 1.1 (SD) mm, n = 44) and *Sulculus diversicolor* (shell width 15.2 ± 1.2

(SD) mm, n = 44), were provided by Ehime Prefectural Fish-farming Center. The juveniles were reared in running seawater at temperatures ranging from 13 to 26°C, and salinities from 33 to 34 S. Prior to the experiments, each individual was placed into a container and gut contents were cleared by maintaining in filtered seawater (GF/F Whatman, pore size ca. 0.7 μm) over 18h at 22°C ± 1.5°C. Seawater was replaced at least once prior to the start of the experiments. Healthy individuals which tightly attached to the container were used for the experiments.

Isolation records (place and years) of phytoflagellates used in the present study were as follows: *Heterocapsa circularisquama* (Ago Bay, central Japan in 1992), *Gymnodinium mikimotoi* (Hiroshima Bay, western Seto Inland Sea in 1991), *Heterosigma akashiwo* (Hiroshima Bay, 1992). Clonal cultures of these species were obtained by repeated washings using capillary pipettes. Each species was grown in modified SWM-3 medium (Chen et al. 1969, Itoh and Imai 1987) at 22°C on a 12h light: 12h dark cycle under illumination at 3,000 lux provided by cool-white fluorescent lamps. All cultures except for *G. mikimotoi* were not axenic. Cultures in the late exponential to early stationary phase (10 to 16 days after inoculation) were used for the experiments.

For each assay, an individual was transferred into a Petli dish (Φ 90 × 20 mm) filled with 40 ml of phytoflagellate culture (10^3, 10^4, 10^5 cells/ml). When the cell density of *G. mikimotoi* culture did not reach 10^5 cells/ml, the culture was kept in a dark for 1 hour, and then concentrated by the decantation. The treated animals were kept at 21–22°C under the low light illumination (800–1000 lux). Aeration was not adopted during the experiment. Control was set using filtered seawater without any algal cells. The culture media (100% SWM-3) without any algal cells were also used. Experiment was conducted at duplicates. Observations on the condition of abalone were done twice throughout experiment, and mortalities were confirmed by needle stimulation on foot at 24h and 48h. Individual which was not attached to Petli dish but showing slight responses by needle stimulation were treated as paralysis.

Results and Discussions

Although aeration and seawater replenishment were not conducted throughout the experiments, no control animals (filtered seawater) died within 48h after the start of experiment (Tables 1–2). In preliminary studies, exposure of 10^4 *Isochrysis galbana* cells/ml (non-toxic phytoplankton) to juvenile abalone did not resulted in death (n = 5) even through four days. We presume that survival of abalone is not interfered due to oxygen depletion in this procedure. In SWM-3 medium, no death was observed within 24h, but one individual of *Haliotis discus* died 24–48h after the start of the experiment (Table 1). The culture medium may be slightly toxic

Table 1. Effects of three phytoflagellates on the survival of *Haliotis discus*

cells/ml	Condition 24h	48h
Heterocapsa circularisquama.		
10^5	DD	DD
10^4	DD	DD
10^3	AD	PD
Gymnodinium mikimotoi		
10^5	DD	DD
10^4	DD	DD
10^3	AP	DD
Heterosigma akashiwo		
10^5	DD	DD
10^4	AD	AD
10^3	AA	AD
Filtered seawater	AA	AA
SWM-3 medium	AA	AD

A = Alive, D = Dead, P = Paralysis

to *H. discus*. Indeed, the culture medium (SWM-3) contains some heavy metals (i.e. Cu^{2+}; 1×10^{-9} M, Zn^{2+}; 3.2×10^{-6} M) which are toxic to mollusks (Akberali and Trueman, 1985) including abalone (Hunt and Anderson 1989). Therefore, it should be noted that the culture medium used in this study is not always appropriate as a basal medium of the test organisms for the long-term (≧ 48h) experiment.

***Heterocapsa circularisquama* effect** When *H. discus* and *S. diversicolor* were exposed to *Heterocapsa circularisquama*, they exhibited various avoidance behaviors (vigorous contraction of tentacles, twisting of foot, escape locomotion) within a few minutes. Most individuals tended to rush to the outside of Petli dish. These avoidance behaviors were observed even at 10^3 *H. circularisquama* cells/ml exposure. Abalones were paralyzed and lied on their back within five hours at 10^4–10^5 *H. circularisquama* cells/ml exposure. All individuals died within 24h (Tables 1–2). According to previous studies, *H. circularisquama* kills bivalve mollusks accompanied with various avoidance behaviors; valve clapping, mantles contraction, gill shrinkage, inhibition of byssus production, reduction of clearance rate and cardiac termination (Nagai et al. 1996, Matsuyama et al. 1997). However, little is known about the effect of this harmful dinoflagellate on other mollusca.

In 1992 in Ago Bay, a massive killing of pearl oyster *Pinctada fucata* were observed during a *H. circularisquama* red tide, accompanied by death of several gastropods (abalone and top shell). In addition, mass mortalities (70%) of various bivalve mollusc species and a carnivorous gastropod *Glossaulax didyma* were also observed during *H. circularisquama* red tide occurred in Kusuura Bay, 1994 (Yoshida and Miyamoto 1996). In the present study, *H. circularisquama* was found to be toxic to not only bivalve but also some gastropod species.

Table 2. Effects of three phytoflagellates on the survival of *Sulculus diversicolor*

cells/ml	Condition 24h	48h
Heterocapsa circularisquama.		
10^5	DD	DD
10^4	DD	DD
10^3	AD	AD
Gymnodinium mikimotoi		
10^5	AA	DD
10^4	AA	PD
10^3	AA	AP
Heterosigma akashiwa		
10^5	DD	DD
10^4	AA	AA
10^3	AA	AA
Filtered seawater	AA	AA
SWM-3 medium	AA	AA

A = Alive, D = Dead, P = Paralysis

Further study is necessary to clarify the toxicity on other marine mollusks.

***Gymnodinium mikimotoi* effect** *Gymnodinium mikimotoi* caused death of abalone *H. discus* and *S. diversicolor* at concentrations > 10^4 cells/ml accompanied with avoidance behaviors such as contraction of tentacles and escape locomotion as observed in *Heterocapsa circularisquama* exposure. However, death of *S. diversicolor* was not observed within 24h even at densities of 10^5 cells/ml. Thus, *S. diversicolor* exhibited high resistance against *G. mikimotoi* compared with *H. circularisquama* (Tables 1–2). The red tide due to *G. mikimotoi* has caused massive killing of the shellfish, i.e. pearl oyster, (Shiokawa and Irie, 1966), pacific oyster, *Crassostrea gigas* (Hanai et al. 1992, Park 1982), clam, (Yamaguchi 1994), blue mussel, *Mytilus edulis* (Park 1982), ark shell, *Anadrana broughtoii* (Shiokawa and Irie 1966), and octopus and cucumber (Shiokawa and Irie 1966). Indeed, several gastropods have been killed frequently in *G. mikimotoi* red tide (Yamamoto et al. 1989). Sawada and

Wada (1983) examined the *in situ* effects of *G. mikimotoi*-red tide on the survival of juvenile abalone *H. discus* under oxygenated conditions. 100% mortality was found at 6h (240,000 *G. mikimotoi* cells/ml), 8h (130,000 *G. mikimotoi* cells/ml), and 18h (12,000–54,000 *G. mikimotoi* cells/ml) after the exposure of red tide, respectively. In this case, significant death of pearl oysters *Pinctada fucata* was found above 16,000 *G. mikimotoi* cells/ml within 24h. They concluded that *H. discus* was the most sensitive mollusk to *G. mikimotoi* cells. Further, survival of cultured adult abalone *H. discus* was significantly affected by the presence of 10^2 *G. mikimotoi* cells/ml even under oxygenated condition (Honjo T. and Uki H., per. comm.). We clarified that notable mortalities of abalones were observed at $>10^4$ *G. mikimotoi* cells/ml of clonal culture. It is clear that *G. mikimotoi* has a harmful effect on the survival of abalone. In the blooms of unarmored dinoflagellate *Gyrodinium aureolum* (= *Gymnodinium* cf. *nagasakiense*) which is closely related to *G. mikimotoi* (Tangen 1977, Partensky et al. 1989), a massive killing of shellfish has been recognized (Shumway 1990). The bloom of *G. aureolum* killed various fauna including some gastropods (Southgate et al. 1984).

***Heterosigma akashiwo* effect** The present study demonstrated that death of abalone *H. discus* and *S. diversicolor* were observed at concentrations 10^5 *H. akashiwo* cells/ml exposure (Tables 1–2). Nagai et al. (1996) noted that an exposure of the juvenile pearl oyster to 10^5 *H. akashiwo* cells/ml did not show any detrimental effect. Further, the shell growth response of the mussel, *Mytilus edulis* was not inhibited by exposure to *H. akashiwo* (Nielsen and Strφngrem 1991). We presume that *H. akashiwo* does not affect survival of bivalve mollusks. However, there are possible concern that gastropods are affected by dense *H. akashiwo* red tide. Few data are available for learning the harmful effect of representative red tide organism *Chattonella, H. akashiwo*, and *Fibrocapsa japonica* on shellfish. Further study is necessary to evaluate the harmful effect of raphidophyte on marine mollusks.

In the present study, vigorous avoidance behavior and subsequent death of *H. discus* and *S. diversicolor* were observed in both *H. circularisquama* and *G. mikimotoi* exposure although the detailed mechanism of gastropod kills by these species was unclear. Thus, concern for fisheries damage due to *H. circularisquama* and *G. mikimotoi* showed not only for finfish killing but also for damage to other commercially important species including gastropods such as abalone and top shell. The outbreaks of red tide due to harmful algae frequently bring about mortallities of marine organisms and subsequently impact on the overall marine ecosystem. In European coastal water, the harmful dinoflagellate *G. aureolum* forms blooms involving massive killing of finfish and other invertebrates (Tangen 1977, Foster 1979). The killing of fauna by *G. aureolum* bloom led to unexpected succession of seagrass in Rochy shore, S. W. Ireland because primary grazers such as gastropods were eliminated from the shore (Southgate et al. 1984). Further work is necessary for understanding the impact of harmful phytoplankton on the physiological activity and survival of overall marine invertebrates and commonly structure.

Acknowledgments

We extend our appreciation to Dr. M. Maeda (Nansei National Fisheries Research Institute) for his critical reading of draft. Thanks are also due to staff of Ehime Prefectural Fish-farming Center for providing juvenile abalones. We are grateful to Prof. Sandra E. Shumway of Southampton College, Long Island University for valuable comments on manuscript. This study has been partially supported by Nansei National Fisheries Research Institute, Fisheries Agency of Japan.

REFERENCES

Akberali, H. B., and E. R. Trueman, 1985: Effects of environmental stress on marine bivalve molluscs. *Adv. Mar. Biol.*, **22**, 102–198.

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

Forster, G. R., 1979: Mortality of the bottom fauna and fish in St. Austell Bay and neighboring area. *J. Mar. Biol. Ass. U.K.*, **59**, 517–520.

Hanai, T., H. Hasegawa, M. Hasegawa, H. Noda, and K. Nonaka, 1992: The occurrence of red tide organism, *Gymnodinium nagasakiense* in Lake Hamana. *Bull. Shizuoka Pref. Fish. Exp. Stn.*, **27**, 33–40. (in Japanese)

Honjo, T., 1994: The biology and prediction of representative red tides associated with fish kills in Japan. *Rev. Fish. Sci.*, **2**, 225–253.

Hunt, J. W. and B. S. Anderson, 1989: Sublethal effects of zinc and municipal effluents on larvae of the red abalone *Haliotis rufescens*. *Mar. Biol.*, **101**, 545–552.

Itoh, K. and I. Imai, 1987: Rafido-So. In: The Japan Fisheries Resource Conservation Association (ed.). A guide for studies of red tide organisms. Shuwa, Tokyo, p. 122–130. (in Japanese)

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

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

Nagai, K., Y. Matsuyama, T. Uchida, M. Yamaguchi, M. Ishimura, A. Nishimura, S. Akamatsu, and T. Honjo, 1996: Toxicity and LD_{50} levels of the red tide dinoflagellate *Heterocapsa circularisquama* on juvenile pearl oysters. *Aquaculture*, **144**, 149–154.

Nielsen, M. V. and T. Strφmgren, 1991: Shell growth response of the mussels (*Mytilus edulis*) exposed to toxic microalgae. *Mar. Biol.*, **108**, 263–267.

Park, J. S., 1982: Studies on the characteristics of red tide and environmental conditions in Jinhae Bay. *Bull. Fish. Res. Dev. Agency*, **28**, 55–88.

Partensky, F., D. Vaulot, A. Coute, and A. Sournia, 1988: Morphological and nuclear analysis of the bloom-forming dinoflagellates *Gyrodinium* cf. *aureolum* and *Gymnodinium nagasakiense*. *J. Phycol.*, **24**, 408–415.

Sawada, S. and Y. Wada, 1983: Several examination of a *Gymnodinium* sp. type '65 red tide occurred in Uwa Sea on resistibility of fish and shellfish. *Reports on the Assessments of Red Tide Prediction*, Fishery Agency of Japan, 131–140. (in Japanese)

Shiokawa, T., and H. Irie, 1966: The hydrographic conditions and the fisheries damages by the red tide water occurred in Omura Bay in summer 1965-VI. Mass mortality of fishes resultant of red water. *Bull. Fac. Fish. Nagasaki Uni.*, **21**, 115–129. (in Japanese with English abstract)

Shumway, S. E., 1990: A review of the effects of algal blooms on shellfish and aquaculture. *J. World Aquaculture Soc.*, **21**, 65–104.

Shumway, S. E., 1995: Phycotoxin-related shellfish poisoning: Bivalve molluscs are not the only vectors. *Rev. Fish. Sci*, **3**, 1–31.

Southgate, T., K. Wilson, T. F. Cross, and A. A. Myers, 1984: Recolonization of a rocky shore in S. W. Ireland following a toxic bloom of the dinoflagellate, *Gyrodinium aureolum*. *J. Mar. Biol. Ass. U. K.*, **64**, 485–492.

Takayama, H. and R. Adachi, R., 1984: *Gymnodinium nagasakiense* sp. nov., a red-tide forming dinophyte in the adjacent waters of Japan. *Bull. Plank. Soc. Japan.*, **31**, 7–14.

Takayama, H. and K. Matsuoka, 1991: A reassessment of the specific characters of *Gymnodinium mikimotoi* Miyake et Kominami ex Oda and *Gymnodinium nagasakiense* Takayama et Adachi. *Bull. Plank. Soc. Japan.*, **38**, 53–68. (in Japanese with English abstract)

Tangen, K, 1977: Bloom of *Gyrodinium aureolum* (Dinophyceae) in north European waters, accompanied by mortality in marine organism. *Sarsia*, **62**, 123–133.

Yamamoto, C., Y. Tanaka, and K. Terai, 1989: Discussion on factor of red tide by *Gymnodinium nagasakiense* using method of exploratory data analysis. *Bull. Fukuoka Fish. Stn.*, **15**, 57–63. (in Japanese)

Yamamoto, C. and Y. Tanaka, 1990: Two species of harmful red tide plankton increased in Fukuoka Bay. *Bull.*

Fukuoka Pref. Fish. Exp. Stn., **16**, 43–44. (in Japanese)

Yamaguchi, M., 1994: Physiological ecology of the red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae). Mechanism of the red tide occurrence and its prediction. *Bull. Nansei Natl. Fish. Res. Inst.*, **27**, 251–394. (in Japanese with English abstract)

Yoshida, Y. and M. Miyamoto, 1995: Growth of *Heterocapsa circularisquama* population in Kusuura Bay and variation of diurnal motion of red tide in 1994. *Report of Kumamoto Pref. Fish. Res. Cent.*, **3**, 31–35. (in Japanese)

有害藻類がアワビ類（クロアワビおよびトコブシ）の生残に及ぼす影響

松山幸彦・小泉喜嗣・内田卓志

有害藻類の赤潮においては，商業的に重要な巻貝の大量斃死が見られ，地域の養殖業に多大な被害を及ぼしている。しかしながら，赤潮生物が巻貝に直接及ぼす影響について，培養株を用いて調べた知見はほとんど見られない。そこで，有害赤潮生物 *Heterocapsa circularisquama, Gymnodinium mikimotoi,* および *Heterosigma akashiwo* のクローン培養株を用いて，クロアワビとトコブシの稚貝の生残等に及ぼす影響を予備的に調べた。クロアワビは *H. circularisquama* および *G. mikimotoi* の暴露によって48時間以内にほとんどすべての個体が斃死した。トコブシも *H. circularisquama* の暴露によって著しく斃死したが，*G. mikimotoi* の暴露ではクロアワビと異なりやや耐性が見られた。*H. circularisquama* および *G. mikimotoi* の暴露では，試験した貝のいずれもが触角の萎縮，足筋肉のねじれ，逃避行動などの異常行動が数分以内に観察され，極めて毒性が高いことが伺われた。*H. akashiwo* の暴露では，10^5 cells/ml の濃度を除けば顕著な斃死や拒否反応はあまり観察されなかった。巻貝は二枚貝よりも赤潮に弱い可能性が示唆されたことから，今後商業的に重要な巻貝（アワビやサザエ）への影響が懸念される。

1997年 2 月15日受理（Accepted February 15, 1998）
南西海区水産研究所業績 A 第66号（Contribution No. A 66 from Nansei National Fisheries Research Institute）

松山幸彦：南西海区水産研究　〒739–0452　広島県佐伯郡大野町丸石2–17–5（Y. Matsuyama: Nansei National Fisheries Research Institute, 2–17–5 Maruishi, Ohno, Saeki, Hiroshima 739–0452, Japan）
小泉喜嗣：愛媛県水産試験場　〒798–0104　愛媛県宇和島市下波5516（Y. Koizumi: Ehime Prefectural Fisheries Experimental Station, 5516 Shitaba, Uwajima, Ehime 798–0104, Japan）
内田卓志：南西海区水産研究　〒739–0452　広島県佐伯郡大野町丸石2–17–5（T. Uchida: Nansei National Fisheries Research Institute, 2–17–5 Maruishi, Ohno, Saeki, Hiroshima 739–0452, Japan）