Vol. 118: 301–303, 1995 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published March 9

NOTE

The red-tide dinoflagellate *Heterocapsa* sp. kills *Gyrodinium instriatum* by cell contact

Takuji Uchida, Mineo Yamaguchi, Yukihiko Matsuyama, Tsuneo Honjo

Red Tide Research Division, Nansei National Fisheries Research Institute, Ohno, Saeki, Hiroshima 739-04, Japan

ABSTRACT: The effects of the red-tide dinoflagellate *Heterocapsa* sp. on *Gyrodinium instriatum* were studied using laboratory cultures. Growth of *G. instriatum* was strongly suppressed when cultured together with *Heterocapsa* sp. Microscopical observations revealed that *G. instriatum* cells were immobilized by cell contact with *Heterocapsa* sp. The time necessary for the immobilization of *G. instriatum* cells decreased with increasing cell densities of *Heterocapsa* sp., which was related to the increasing chance of contact between the 2 organisms. Immobilized *G. instriatum* cells became abnormal in shape and finally lysed. *H. triquetra* had no effect on the motility of *G. instriatum*. This is the first report of inhibition of phytoplankton growth by cell contact.

KEY WORDS: Killer phytoplankton · *Heterocapsa* sp. · Cell contact · Immobilization · Growth inhibition · *Gyrodinium instriatum*

Red tides of the dinoflagellate *Heterocapsa* sp. caused heavy damages to bivalves in the inner bays of Japan (Yamamoto & Tanaka 1990, Y. Matsuyama et al. unpubl.). The bloom of this species occurred from August to December in Ago Bay. It developed to red tides in summer and autumn, with a maximum cell density of more than 80 000 cells ml^{-1} in August (Matsuyama et al. unpubl.). Ecological and physiological studies have been conducted to elucidate the cause and mechanism of red tides of this species. In the course of investigating the relation between *Heterocapsa* sp. and other flagellates, it was found that *Heterocapsa* sp. immobilized cells of *Gyrodinium instriatum* by cell contact, resulting in the death of the *G. instriatum* cells. There have been many reports on interactions between phytoplankton species being mediated by chemical compounds secreted into the environment (Maestrini & Bonin 1981, Rice 1984) and on grazer-prey relationships such as phagotrophy, myzocytosis and pallium feeding (Elbrächter 1991). We report here a new type of inhibition involving *Heterocapsa* sp. and *G. instriatum*.

Materials and methods. The strains of *Gyrodinium instriatum* Freudenthal et Lee (Fig. 1A), *Heterocapsa* sp. (Fig. 1D), and *H. triquetra* (Ehrenberg) Stein used in these experiments were isolated from seawater samples collected from Uranouchi Bay in April 1993, from Ago Bay in December 1992, and from Hiroshima Bay in April 1991, respectively. A morphological study of *Heterocapsa* sp. has been made for the cultured materials. This

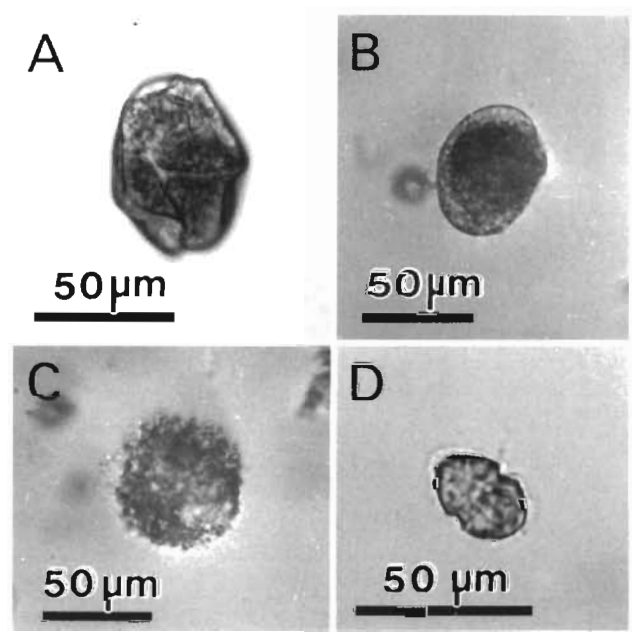

Fig. 1. Photomicrographs of *Gyrodinium instriatum* and *Heterocapsa* sp. (A) Normal motile cell of *G. instriatum*. (B) Cell of *G. instriatum* immobilized by *Heterocapsa* sp. The cell became elliptical and lost flagella, girdle, and sulcus. (C) *G. instriatum* cell lysed as a result of contact with *Heterocapsa* sp. (D) Motile cell of *Heterocapsa* sp. (fixed with 1% glutaraldehyde)

species is light yellow-brown in color and spindle shaped. Cells have a typical dinoflagellate morphology with sulcal and girdle grooves. Cells are 18 to 30 µm in length and 12 to 22 µm in width. Epitheca is slightly pointed. According to T. Horiguchi (pers. comm.), the cells of *Heterocapsa* sp. have scales on their surface, and this species is closely related to *H. illdefina* (= *Cachonina illdefina*) described by Herman & Sweeney (1976) and Morrill & Loeblich (1981). Further study is necessary to decide the taxonomical status of this species.

Clonal cultures of *Gyrodinium instriatum*, *Heterocapsa* sp. and *H. triquetra* were obtained by repeated washings using capillary pipettes. The cultures thus obtained were subjected to sterility tests using $ST10^{-1}$ medium (Ishida et al. 1986). For each of the cultures, no bacterial growth was observed more than 1 mo after inoculation. Modified SWM-3 (Itoh & Imai 1987) was used as a culture medium throughout the experiments. Growth experiments using bialgal cultures were conducted in 50 ml Erlenmeyer flasks with 25 ml of medium. Cells in the logarithmic phase of growth were inoculated at densities of about 100 cells ml^{-1} for *G. instriatum* and 200 to 300 cells ml^{-1} for *Heterocapsa* sp. Cultures were maintained at 20°C on a 12 h light:12 h dark cycle; illumination was provided by cool-white fluorescent lamps at 120 to 150 µmol photon m^{-2} s^{-1}. Growth was measured at 2 to 5 d intervals by counting cells in 0.01 to 0.1 ml culture samples using a Sedgwick-Rafter slide. When cell densities were greater than 20 000 cells ml^{-1}, the samples were diluted 10 to 20 times with autoclaved seawater. The cell conditions of the 2 organisms in the bialgal cultures were observed using an inverted microscope. When necessary, 1 ml of the culture was aseptically transferred to a well of a 24-well plate (Sumitomo Bakelite Co., Ltd) to undergo detailed microscopic observations.

Short-term effects of *Heterocapsa* sp. on the motility of *Gyrodinium instriatum* were studied at room temperature (22 to 24°C). A total of 0.5 ml of *Heterocapsa* sp. culture (monoalgal) with 10 000 to 50 000 cells ml^{-1} were added to each well of a 24-well plate. Three wells were used for each concentration of *Heterocapsa* sp. cells. Then, 20 cells of *G. instriatum*, in log-phase growth, were isolated by capillary pipette and inoculated into the wells. As controls, the *Heterocapsa* sp. culture was replaced either with filtrate from a *Heterocapsa* sp. culture or with fresh culture medium. Three wells were used for each control. The filtrate was prepared by mild filtration of the *Heterocapsa* sp. culture through membrane filters of 0.2 µm pore size using disposable filter holders (Schleicher & Schuell). Using an inverted microscope, the time course of the occurrence of immotile *G. instriatum* cells was followed immediately after inoculation. In a similar fashion, *H. triquetra* was tested for its ability to immobilize *G. instriatum* cells.

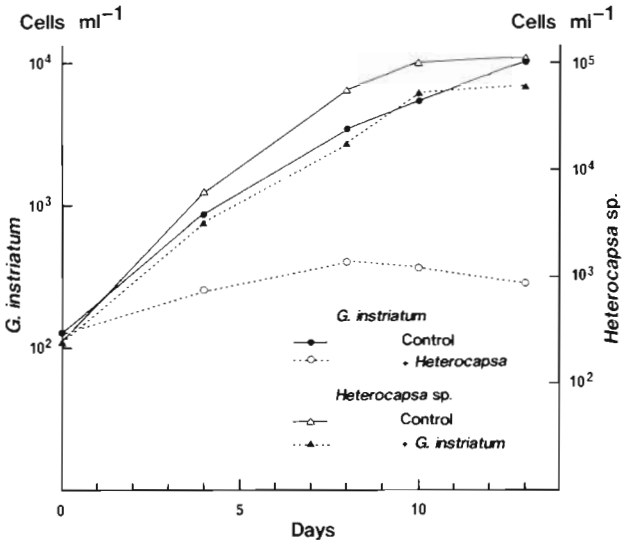

Fig. 2. Growth of *Gyrodinium instriatum* and *Heterocapsa* sp. when cultured alone (●, ▲ respectively) or together (○, △ respectively)

Results and discussion. Fig. 2 shows the growth of *Gyrodinium instriatum* and *Heterocapsa* sp. cultured alone or together. It is clear that the growth of *G. instriatum* was strongly suppressed when cultured with *Heterocapsa* sp. The growth of *G. instriatum* was very limited up to 8 d after inoculation, then cell densities decreased gradually, whereas the cells in the control continued to grow during the experiment. There was no such remarkable difference in growth of *Heterocapsa* sp. between bialgal and unialgal (control) cultures, although growth in the bialgal culture seemed to be slightly suppressed.

The condition of *Gyrodinium instriatum* and *Heterocapsa* sp. cells in the bialgal culture was observed using an inverted microscope. The observations indicated that *G. instriatum* cells became immotile immediately after contact with *Heterocapsa* sp. The immotile *G. instriatum* cells became elliptical and lost their flagella, girdles, and sulci (Fig. 1B) and finally lysed (Fig. 1C). In some cases, it was observed that immobilized *G. instriatum* cells recovered their motility. However, these cells soon became immotile again after repeated contact with *Heterocapsa* sp. Immobilization of *G. instriatum* cells did not always occur upon contact with *Heterocapsa* sp. Perhaps there is a specific region on *G. instriatum* cells that is sensitive to the attack of *Heterocapsa* sp. Finally, the motility of *Heterocapsa* sp. cells was not affected by contact with *G. instriatum* cells.

Fig. 3 shows the decrease in the number of motile cells of *Gyrodinium instriatum* at a density of 23 400 cells ml^{-1} of *Heterocapsa* sp. Most *G. instriatum*

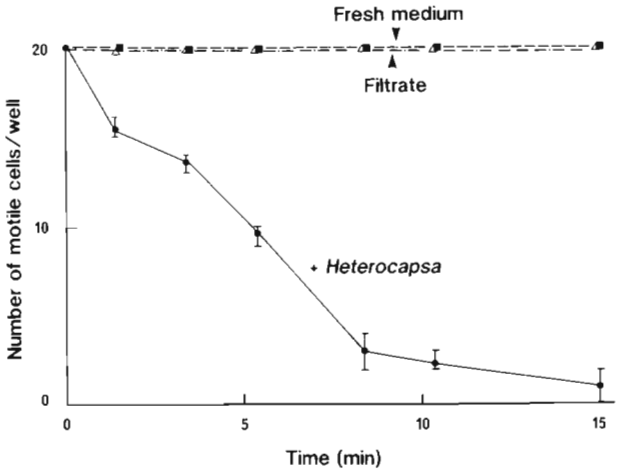

Fig. 3. Time course of the decrease in the number of motile cells of *Gyrodinium instriatum* upon exposure to *Heterocapsa* sp. at 23 400 cells ml^{-1}. Mean value (•) and the range (vertical bars) are shown for each measurement (n = 3). (■) Fresh medium; (▵) filtrate

cells became immotile within 15 min. Immobilization was not observed in the fresh medium or the filtrate. The time necessary for immobilization of half of the *G. instriatum* cells (10 cells) was 15 min at a density of 10 200 cells ml^{-1} of *Heterocapsa* sp., 5 min at 23 400 cells ml^{-1}, and 2 min at 49 000 cells ml^{-1}. It is evident that the immobilization of *G. instriatum* was more rapid at higher cell densities of *Heterocapsa* sp. This is probably due to the fact that higher cell densities of *Heterocapsa* sp. increased the chance of contact between the 2 organisms. The ability of *Heterocapsa* sp. to immobilize *G. instriatum* cells did not change significantly during the experiments. The cells of *Heterocapsa* sp. used in the present experiments were the 50th to 100th generation after isolation from seawater (maintained for 6 to 10 mo in laboratory cultures).

Experiments were conducted to determine if *Heterocapsa triquetra*, a common species, produces a similar effect on *Gyrodinium instriatum*. Results indicate that the motility of *G. instriatum* was not affected by the presence of 90 000 cells ml^{-1} of *H. triquetra*.

Allelopathy between phytoplankton species has been limited to cases mediated by chemical compounds secreted by certain species into the environment (Maestrini & Bonin 1981, Rice 1984). In this context, the inhibitory effect of *Heterocapsa* sp. on *Gyrodinium instriatum* is noteworthy for 2 reasons. One is that the inhibition was realized by cell contact between the 2 organisms. The other is that the immobilization of *G. instriatum* occurred immediately after the contact and finally resulted in the death of the cells. This type of inhibition has never been reported for phytoplankton species until now.

The mechanisms of immobilization are unknown. It is possible that an inhibitory substance was attached to the *Gyrodinium instriatum* cells by contact with *Heterocapsa* sp. Another possible explanation is that the cells of *G. instriatum* were damaged mechanically by contact with *Heterocapsa* sp. Perhaps *Heterocapsa* sp. has an unusual organelle that enables it to immobilize *G. instriatum* cells. If *Heterocapsa* sp. also immobilizes other phytoplankters, it may be a strategy for keeping itself dominant in the phytoplankton community. The newly found type of inhibition by *Heterocapsa* sp. revealed in the present study offers a unique phenomenon for further ecological and physiological studies.

Acknowledgements. We thank the members of the Fisheries Research Institute of Mie and the members of the Kochi Prefectural Fisheries Experimental Station for providing us seawater samples for the isolation of the phytoplankton species used in the experiments. We also thank Dr T Horiguchi of Hokkaido University for the identification of the genus of *Heterocapsa* sp. Thanks are due to Dr I. Imai of Kyoto University for comments on the experiments and to Dr T Kamiyama of the same institute for providing us with the culture of *H. triquetra* used in the experiments.

LITERATURE CITED

Elbrächter, M. (1991). Food uptake mechanisms in phagotrophic dinoflagellates and classification. In: Patterson, D. J., Larsen, J. (eds.) The biology of free-living heterotrophic flagellates. Systematics Association by Clarendon Press, Oxford, p. 303–312

Herman, E. M., Sweeney, B. M. (1976). *Cachonina illdefina* sp. Nov. (Dinophyceae): chloroplast tubules and degeneration of the pyrenoid. J. Phycol. 12: 198–205

Ishida, Y., Eguchi, M., Kadota, H. (1986). Existence of obligately oligotrophic bacteria as a dominant population in the South China Sea and the West Pacific Ocean. Mar. Ecol. Prog. Ser. 30: 197–203

Itoh, K., Imai, I. (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)

Maestrini, S. Y., Bonin, D. J. (1981). Allelopathic relationships between phytoplankton species. In: Platt, T. (ed.) Physiological bases of phytoplankton ecology. Can. Bull. Fish. Aquat. Sci. 210: 323–338

Morrill, L. C., Loeblich, A. R. III (1981). A survey for body scales in dinoflagellates and a revision of *Cachonina* and *Heterocapsa* (Pyrrophyta). J. Plankton Res. 3: 53–65

Rice, E. L. (1984). Allelopathy. Academic Press, London

Yamamoto, C., Tanaka, Y (1990). Two species of harmful red tide plankton increased in Fukuoka Bay. Bull. Fukuoka Fish. Exp. Stn. 16: 43–44 (in Japanese)

This note was submitted to the editor

Manuscript first received: July 18, 1994
Revised version accepted: November 11, 1994