Plankton Biol. Ecol. 47 (1): 65–68, 2000

plankton biology & ecology

Note

Seasonal abundance of resting spores and vegetative cells of *Chaetoceros* diatoms in Funka Bay, southern Hokkaido, Japan

HIROKI ASAMI[1] & SYUHEI BAN

Faculty of Fisheries, Hokkaido University, 3–1–1 Minato-machi, Hakodate 041–0821, Japan
[1]Present address: Hokkaido Fish Hatchery, 3–373 Kitakashiwagi, Eniwa 061–1433, Japan

Received 8 October 1998; accepted 14 July 1999

In Funka Bay located on the Pacific coast of southwestern Hokkaido (Fig. 1), *Chaetoceros sociale* and *Chaetoceros debile* are the predominant species during the spring diatom bloom (Nakata 1982; Odate 1987). Nakata (1982) noted that *C. sociale* and *C. debile* formed resting spores and sank during the latter phase of the spring diatom bloom in Funka Bay. Resting spores of *Chaetoceros* spp. were observed in aggregated detritus attached to fishing nets in the bay (Kido & Ohtani 1981). Odate & Maita (1990) found dense concentrations of *Chaetoceros* resting spores ($>10^5$ spores l^{-1}) at 50–80 m depth in late April just after the end of the spring diatom bloom in Funka Bay. These results, combined with the long survival time of *Chaetoceros* resting spores (Hollibaugh et al. 1981; Itakura et al. 1997), suggest that the resting spores are probably serving as a seed population for the bloom of *Chaetoceros* in Funka Bay in the following spring. To test this hypothesis, we investigated seasonal abundances of resting spores and vegetative cells of *C. sociale* and *C. debile* in Funka Bay throughout a year period.

Samplings were carried out from June 1985 to May 1986 at Stn 30 (41°58′N, 140°36′E; 90 m deep) near the center of Funka Bay (Fig. 1). One liter of seawater sample was taken with a series of Niskin bottles at about 5- or 10-m intervals between the surface and 90-m depth once a month except for in March when weekly samplings were carried out during the diatom blooming period. No sampling was possible in October 1985 due to stormy weather. Water samples were preserved in a 2% buffered formalin–seawater solution, and concentrated to 10–20 ml by settling in the laboratory. Subsamples (0.05–2 ml) were taken for enumeration of resting spores and vegetative cells under an inverted microscope (maximum magnification: 450×). The resting spores of these two *Chaetoceros* species can be easily distinguished from the vegetative cells by the external morphology (Kokubo 1960). Resting spores found within chains of vegetative cells were also counted together with the solitary forms. To evaluate seasonal changes in diatom densities in the water column, the abundances of both vegetative cells and resting spores at each sampling depth were integrated from the surface to 90 m and were expressed as total cell number in the water column (cells cm^{-2}). Bottom sediments were also collected with a gravity core-sampler (inner diameter: 5.5 cm) once a month, though no sediment sampling was possible in June, October and December 1985. The top 2 cm of the sediment cores was placed into plastic bags and stored in cool and dark conditions on board. In the laboratory, a part of the sediment samples (1–2 g wet weight) was suspended in 10 ml of filtered (Whatman GF/C) 2% buffered formalin–seawater solution, and 1–2 ml of the suspension was filtered through 100-μm, then 40-μm mesh to remove larger particles. The filtrates were diluted further into 10 ml of 2% buffered formalin–seawater solution. Resting spores were identified and counted in 0.1–0.5 ml subsamples under the inverted microscope. Abundance of the resting spores in bottom sediments was expressed as the number of spores per g (wet weight) of sediments. At each sampling, water temperature was determined with a DBT (MOX–BT2F, The Shin-Nippon Meteorology & Oceanography, Co., Ltd.), and salinity was determined with an inductive salinometer (Model 601 MKIII) on water samples collected with the Niskin bottles.

The annual range of variations at the surface was 2–20°C for temperature and 31.8–33.4 for salinity (Fig. 2). A thermocline developed at about 40-m depth from June to September 1985, and disappeared from November 1985 to January 1986. "Coastal Oyashio Water", characterized by low temperature-low salinity water, was found in the top 10 m in late February, and occupied the top 70 m by late March. Below 60-m depth, temperature (<8°C) and salinity (33.0–33.6) were rather stable throughout the year.

Chaetoceros sociale vegetative cells were absent in the water column from June to September 1985 (Fig. 3A). Vegetative cells occurred in November, staying at relatively low den-

Corresponding author: Hiroki Asami; email, asamih@fishexp.pref.hokkaido.jp

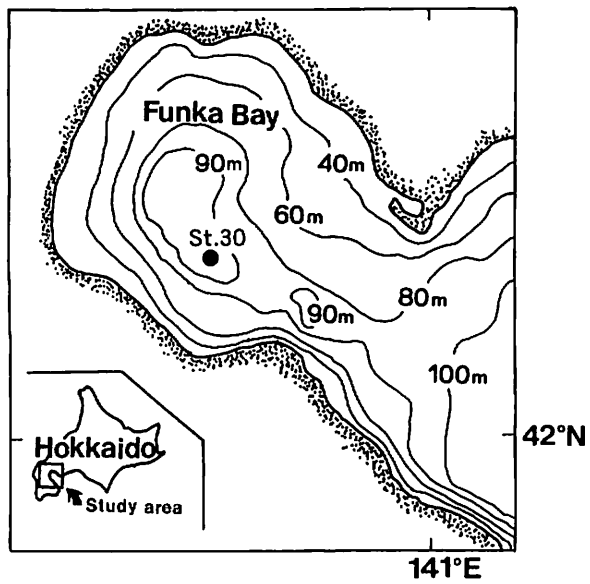

Fig. 1. Location of the sampling station (Stn 30) in Funka Bay.

sities ($<4\times10^4$ cells cm^{-2}) until February. The number of vegetative cells rapidly increased in the upper 50 m in March 1986 (see also Fig. 4A), reached the annual maximum (9.5×10^5 cells cm^{-2}) in mid-March, then decreased rapidly toward May. The resting spores of *C. sociale* sporadically occurred at relatively low densities ($<10^2$ spores cm^{-2}) in the water column, always below 80 m (Fig. 4A), from June to September 1985 when the vegetative cells disappeared from the water column. Resting spore abundance increased to 10^3 spores cm^{-2} in November, but stayed at such low abundances to through February. Spore numbers rapidly increased below 50 m after mid-March and reached the annual maximum in late March (1.4×10^5 spores cm^{-2}), when the vegetative cell numbers started to decline. After that, resting-spore abundances decreased toward May. In the bottom sediments, resting spores of *C. sociale* were observed throughout the study period, except for in August 1985 (Fig. 3A). The densities of the spores were $0.4\text{–}9\times10^3$ spores (g wet-weight sediment)$^{-1}$ from June 1985 to February 1986, temporarily decreasing in March, but

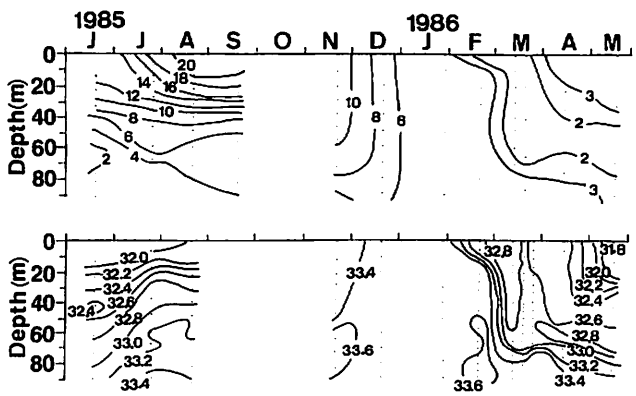

Fig. 2. Vertical profiles of water temperature and salinity at Stn 30 in Funka Bay from June 1985 to May 1986.

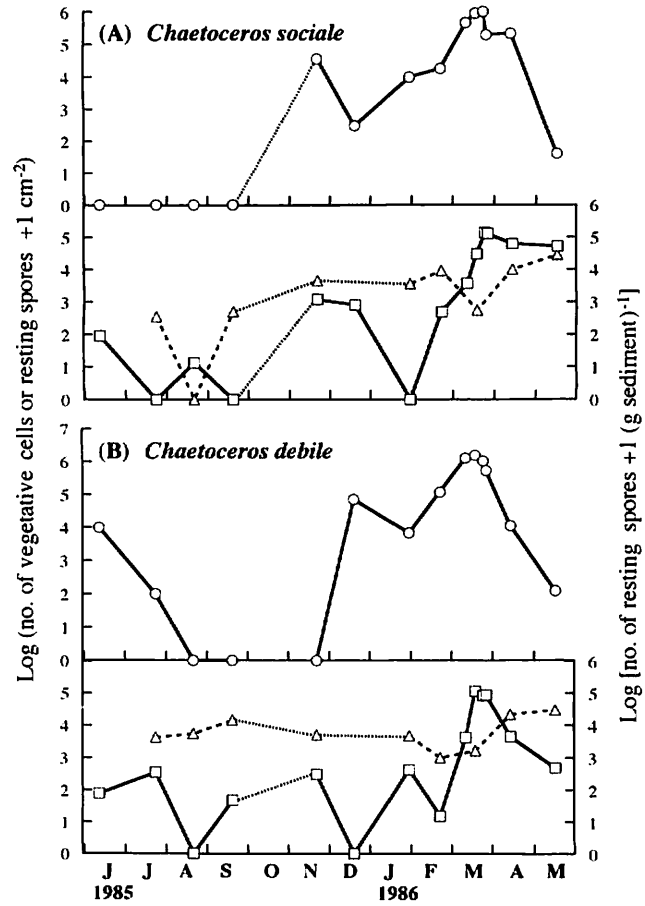

Fig. 3. Seasonal changes in the abundance of vegetative cells (circles) and resting spores (squares) in the water column (solid lines), and resting spores in the sediments (triangles and broken lines) of *Chaetoceros sociale* (**A**) and *C. debile* (**B**) at Stn 30 in Funka Bay from June 1985 to May 1986. Note that sediment samplings were not made in June, October or December 1985. Dotted lines represent discontinuous sampling periods.

rapidly increasing with the decrease in spore abundance in the water column in the next month. The annual maximum abundance, 2.8×10^4 spores (g wet-weight sediment)$^{-1}$, of the resting spores in the sediments was recorded in May 1986 when the bloom of this species had just finished.

Vegetative cells of *C. debile* disappeared from the water column during the period between August and November 1985 (Fig. 3B). In December, the vegetative cells occurred and stayed at relatively low densities ($<7\times10^4$ cells cm^{-2}) until late January 1986, before rapidly increasing in March. The annual abundance maximum (1.5×10^6 cells cm^{-2}) was in mid-March, after which vegetative cell numbers rapidly decreased. Resting spores occurred in the water column, mostly below 70 m (see also Fig. 4B), even during the period in which vegetative cells disappeared, but the densities were relatively low ($<4\times10^2$ spores cm^{-2}) (Fig. 3B). The spores stayed at these low densities until the following February, after which the spore abundances increased below 50 m and peaked (1.2×10^5 spores cm^{-2}) in mid-March before decreasing in April. In the

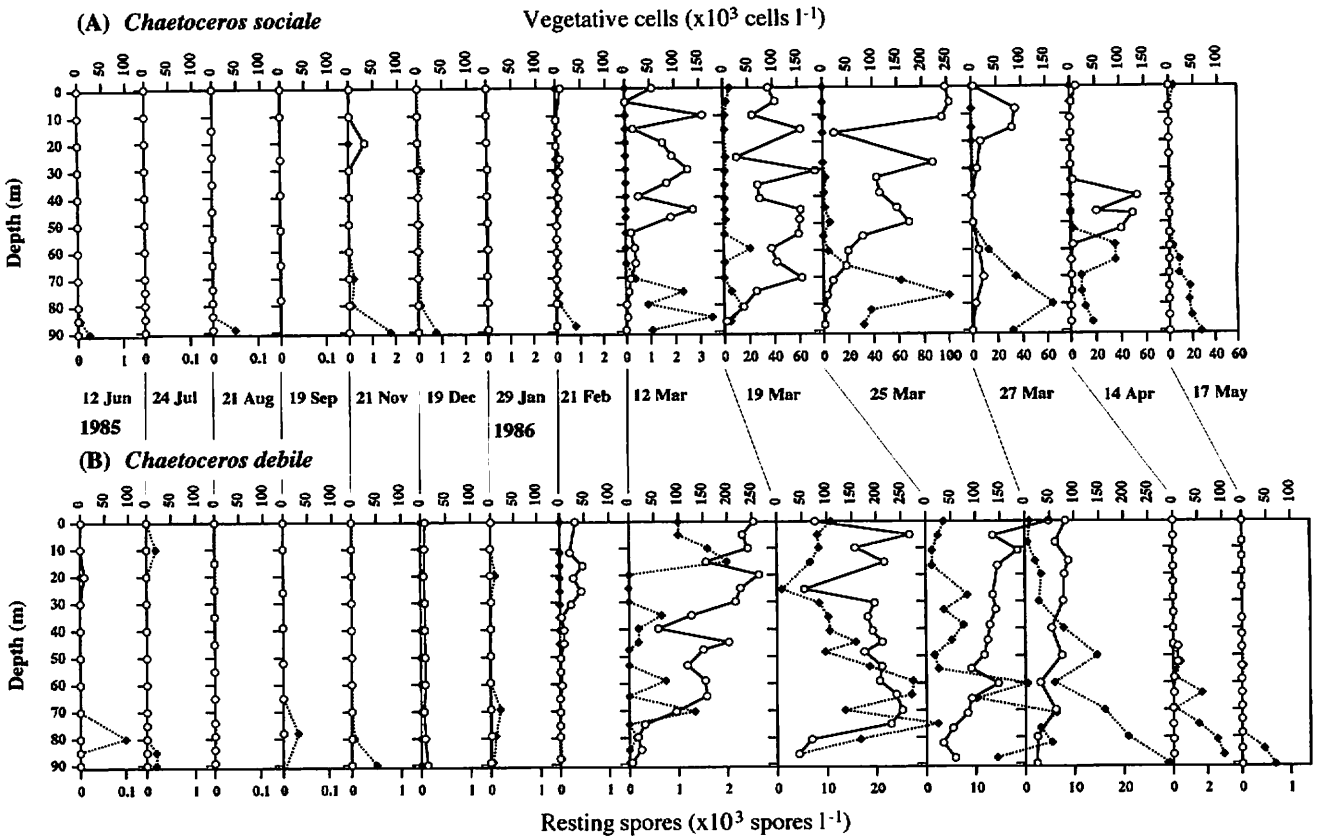

Fig. 4. Vertical distributions of vegetative cells and resting spores of *Chaetoceros sociale* (**A**) and *C. debile* (**B**) at Stn 30 in Funka Bay from June 1985 to May 1986. Note that scales of abundance for the resting spores differ during some sampling preiods.

bottom sediments, resting spores occurred throughout the study period at abundances of 10^3 to 3×10^4 spores (g wet-weight sediment)$^{-1}$. The pattern of seasonal changes in abundances of the spores was similar to that found in *C. sociale* (see Fig. 3A). The abundance of resting spores in the sediment increased with the decrease in resting spore abundance in the water column, reaching the annual maximum in May.

During the study period, blooms of *C. sociale* and *C. debile* vegetative cells occurred in March (Fig. 3). In Funka Bay during the study period, nitrate concentrations decreased with the increasing abundance of diatom vegetative cells after February and was exhausted from the water column by late March (Asami 1987). Depletion of some nutrients (especially nitrogen) has been shown to induce the formation of diatom resting spores (Davis et al. 1980; Garrison 1981; Hargraves & French 1983; Kuwata & Takahashi 1990). The depletion of nitrate may also induce the production of resting spores for both *C. sociale* and *C. debile* in Funka Bay.

In November, when the water column was well mixed vertically (see Fig. 2), the resting spores of both diatoms were slightly more abundant near the bottom, in spite of there still being enough nitrate for vegetative cell growth in the water column (Asami 1987), and the vegetative cells of these two species still being present in the water column. It is probable that repopulation of the vegetative cells is associated with resuspension of the resting spores from the bottom sediments.

Light is necessary for the germination of diatom resting spores (French & Hargraves 1980; Hollibaugh et al. 1981; Hargraves & French 1983) and in some *Chaetoceros* species resting spores do not germinate at an irradiance level of <1.3 $\mu E\,m^{-2}s^{-1}$ (Hollibaugh et al. 1981). In Funka Bay, irradiance is $<0.1\,\mu E\,m^{-2}s^{-1}$ just above the sea bottom but $>2.5\,\mu E\,m^{-2}s^{-1}$ above 40-m depth in the pre-blooming period (Saitoh, personal communication). Therefore vertical advection, which is presumed to occur during November to January in the bay due to the uniform structure of the water column (see Fig. 2), is an important mechanism not only in the transportation of resting spores from the bottom sediments into the water column but also in exposing them to light intensities sufficient for germination.

During the summer months when vegetative cells disappeared, the resting spores of *C. sociale* and *C. debile* were found in the sediments. However, the stock of resting spores was one order smaller than that found in May. Marcus (1984) demonstrated that, in copepods, temporal decreases in the abundance of resting eggs in the uppermost centimeter of the sediments was due to the translocation of eggs from the sediment surface to deeper strata, and did not necessarily reflect mortality. The decrease in resting spore abundance in the upper 2 cm of the sediment from spring to summer may be due

to physical or biological perturbation, such as storm events, current flow and/or bioturbation. In this period, the resting spores for both diatoms occurred just above the sea bottom suspended in the water column (see Fig. 4), though the densities were very low, compared with those in the sediments. Since the volume of 1-g sediments is less than 1 cm^3, the density of the resting spores in the water column is on the whole one order smaller than that of the spores in the sediments during summer months (see Fig. 3). It is not likely that this occurrence of the resting spores in the water column is related to physical perturbation because thermal stratification well developed during this season (see Fig. 2). In copepods, bioturbation has been shown to promote hatching of resting eggs in the sediments due to resuspension of the eggs from the bottom sediments through the activity of benthic animals (Marcus 1986). Such processes of bioturbation may play an important role for sporadical occurrence of resting spores resuspended from the bottom sediments during the summer period.

Resting spores rapidly accumulated on the sea bottom after mid-March, presumably because of the high sinking rate of the resting spores due to their thick frustules (Davis et al. 1980; French & Hargraves 1980; Smetacek 1985). With the progress of seasons, vegetative cells disappeared from the water column from June to September for *C. sociale*, and from August to November for *C. debile*, probably due to the adverse effect of high water temperatures. Although we did not test the viability of resting spores collected from the bottom sediments in this study, the resting spores of *C. diadema* and *C. vanheurchii* have been shown to be viable for at least 645 d under cold (2–4°C), dark and aerobic conditions (Hollibaugh et al. 1981). Itakura et al. (1997) showed that the resting spores of *Chaetoceros* spp. were more tolerant of adverse conditions than those of *Thalassiosira* spp. and *Skeletonema costatum* and could survive for ca. 500 d at 5°C. Since the water temperature near the bottom at our sampling site in Funka Bay was less than 6°C throughout the year (see Fig. 2), it is likely that *Chaetoceros* resting spores produced towards the end of the bloom remain viabile in the bottom sediments until at least the following spring. This implies that the *C. sociale* and *C. debile* populations in Funka Bay survive adverse conditions in the summer months by producing an alternate life stage, i.e. the resting spore, in the same way as *Chaetoceros* spp. in Monterey Bay (Garrison 1981), and that resting spores of *C. sociale* and *C. debile* in the bottom sediments serve as a seed population for the spring bloom of these species in Funka Bay.

Acknowledgments

We thank Drs T. Minoda, A. Kawamura and N. Shiga for their suggestions and encouragement during this study and Dr T. Ikeda for his critical reading of this manuscript. We also thank the captain and crew of T/S *Ushio-maru* of Hokkaido University for their assistance in sampling. Special thanks are due to Dr S. Saitoh, Hokkaido University, for his unpublished data of underwater irradiance.

Literature Cited

Asami, H. 1987. Seasonal distributions of diatom resting spores and spring blooms in Funka Bay. Master Thesis, Hokkaido University. (In Japanese.)

Davis, C. O., J. T. Hollibaugh, D. L. R. Seibert, W. H. Thomas & P. J. Harrison 1980. Formation of resting spores by *Leptocylindrus danicus* (Bacillariophyceae) in a controlled experimental ecosystem. *J. Phycol.* **16**: 296–302.

French, F. W. & P. E. Hargraves 1980. Physiological characteristics of plankton diatom resting spores. *Mar. Biol. Lett.* **1**: 185–195.

Garrison, D. L. 1981. Monterey Bay phytoplankton. II. Resting spore cycles in coastal diatom populations. *J. Plankton Res.* **3**: 137–156.

Hargraves, P. E. & F. W. French 1983. Diatom resting spores: significance and strategies, p. 49–68. In *Survival Strategies of the Algae* (ed. Fryxell, G.). Cambridge University Press, London.

Hollibaugh, J. T., D. L. R. Seibert & W. H. Thomas 1981. Observations on the survival and germination of resting spores of three *Chaetoceros* (Bacillariophyceae) species. *J. Phycol.* **17**: 1–9.

Itakura, S., I. Imai & K. Itoh 1997. "Seed bank" of coastal planktonic diatoms in bottom sediments of Hiroshima Bay, Seto Inland Sea, Japan. *Mar. Biol.* **128**: 497–508.

Kido, K. & K. Ohtani 1981. Preservation of particulate organic matter in the cold basin water in Funka Bay after the vernal phytoplankton bloom. *Bull. Fac. Fish. Hokkaido Univ.* **32**: 357–375.

Kokubo, S. 1960. *Fuyukeisorui (Plankton Diatoms)*, Koseisha-Koseikaku, Tokyo, 330 pp. (In Japanese.)

Kuwata, A. & M. Takahashi 1990. Life-form population responses of a marine planktonic diatom, *Chaetoceros pseudocurvisetus*, to oligotrophication in regionally upwelled water. *Mar. Biol.* **107**: 503–512.

Marcus, N.H. 1984. Recruitment of copepod nauplii into the plankton: importance of diapause eggs and benthic processes. *Mar. Ecol. Prog. Ser.* **15**: 47–54.

Marcus, N.H. 1986. Recruitment of individuals into the plankton: the importance of bioturbation. *Limnol. Oceanogr.* **31**: 206–210.

Nakata, K. 1982. Species composition of phytoplankton community of Funka Bay in the spring bloom, 1981. *Bull. Jpn. Soc. Fish. Oceanogr.* **41**: 27–32. (In Japanese with English abstract.)

Odate, T. 1987. Temporal and horizontal distribution of the diatom community during the spring bloom in Funka Bay, southern Hokkaido. *Bull. Plankton Soc. Jpn* **34**: 33–42.

Odate, T. & Y. Maita 1990. Seasonal distribution and vertical flux of resting spores of *Chaetoceros* (Bacillariophyceae) species in the neritic water of Funka Bay, Japan. *Bull. Fac. Fish. Hokkaido Univ.* **41**: 1–7.

Smetacek, V. S. 1985. Role of sinking in diatom life-history cycles: ecological evolutionary and geological significance. *Mar. Biol.* **84**: 239–251.