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Note

Resting stage cells of diatoms in deep waters in Kumano-Nada, central part of Japan

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Abstract: The abundance and species composition of viable resting stage cells of diatoms were investigated in deep waters (200, 500 and 1,000 m depths) collected at neighboring stations in Kumano-Nada, central part of Japan, in April, August and October 2006. Viable resting stage cells were enumerated by the modified extinction dilution method [most probable number (MPN) method] based on incubation. Resting stage cells were detected from all samples, except at 500 m depth in August, in a range of abundances between 200–4,415 cells L^{-1}. The resting stage cells of diatoms observed belonged to four genera of the Centrales, *Chaetoceros* spp. (*C. curvisetus*, *C. socialis* and *Chaetoceros* sp.), *Leptocylindrus danicus*, *Skeletonema* spp. and *Thalassiosira* spp., and two of the Pennales, *Cylidrotheca closterium* and *Navicula* sp., along with one centric and three pennate unidentified diatoms, in total. It is indicated, in this study, that the abundances enumerated by the MPN method could represent those of disassembled resting stage cells originally aggregated in marine snow, of which the sinking rate is reportedly great (e.g. 68 m day^{-1}). This, eventually, suggests that the abundance and species composition of resting stage cells in the deep waters reflected the states of blooms occurring just before our samplings in the surface water of the Kumano-Nada region. In this region, coastal upwelling occasionally occurs, implying that such resting stage cells sinking from the euphotic layer to deeper depths might have some chance to return to the surface and be able to act as a "seeding population" for further blooms.

Key words: coastal upwelling, deep water, diatom, Kumano-Nada, resting stage cells

Many diatoms have been reported to form resting spores or resting cells (hereafter referred to as "resting stage cells" for both types unless distinction is needed) during their life cycles (Hargraves & French 1983, Garrison 1984). Numerous studies have stressed that resting stage cells play important roles in survival under unfavorable conditions for vegetative cell living and, further, in seeding blooms in coastal waters and/or shallow neritic environments, where resting stage cells in the bottom sediments are resuspended to the euphotic layer by mixing events (McQuoid and Hobson 1996, Itakura et al. 1997), taking advantage of light, essential for their germination (Hollibaugh et al. 1981, Imai et al. 1996).

Kumano-Nada is a sea, located off the southeast of the Kii Peninsula, Japan, open to the north Pacific Ocean (Fig. 1). Due to the narrow continental shelf around this area (<20 km in width) with a very steep slope, quickly reaching to the ocean basin (around 2,000 m in depth) (Nakamura 1985), the Kumano-Nada can be characterized as a dramatically changeable environment from the neritic to oceanic zone. Furthermore, Kumano-Nada is well known as a region where coastal upwelling occasionally occurs (Takeuchi 1987). In the surface water of this region, many diatoms are distributed widely as major primary producers. By a preliminary experiment in which water samples collected from 1,000 m depth in this region were incubated under light after storing at 4°C in the dark for 2 months in the laboratory, we confirmed the growth of vegetative cells of diatoms in the samples. This indicates that their resting stage cells existed in the deep water when collected. In this study, we report the abundance and species composition of the resting stage cells of diatoms from subsurface to deeper depths in Kumano-Nada and eventually discuss the ecological role of those cells in this particular environmental region.

Water sampling was carried out at neighboring stations on April 17, 2006 (Stns. A1 and A2), on August 4, 2006 (Stn. B) and on October 23, 2006 (Stn. C), during SE 0602, SE 0614 and SE 0624 cruises of the T/S Seisui Maru of Mie University, respectively (Fig. 1). Position and bottom depth of the sampling stations in Kumano-Nada are shown in Table 1. Water samples were collected at depths of 200, 500 and 1,000 m at all stations, using rosette Niskin bottles fitted to a Niel Brown Instrument Systems CTD.

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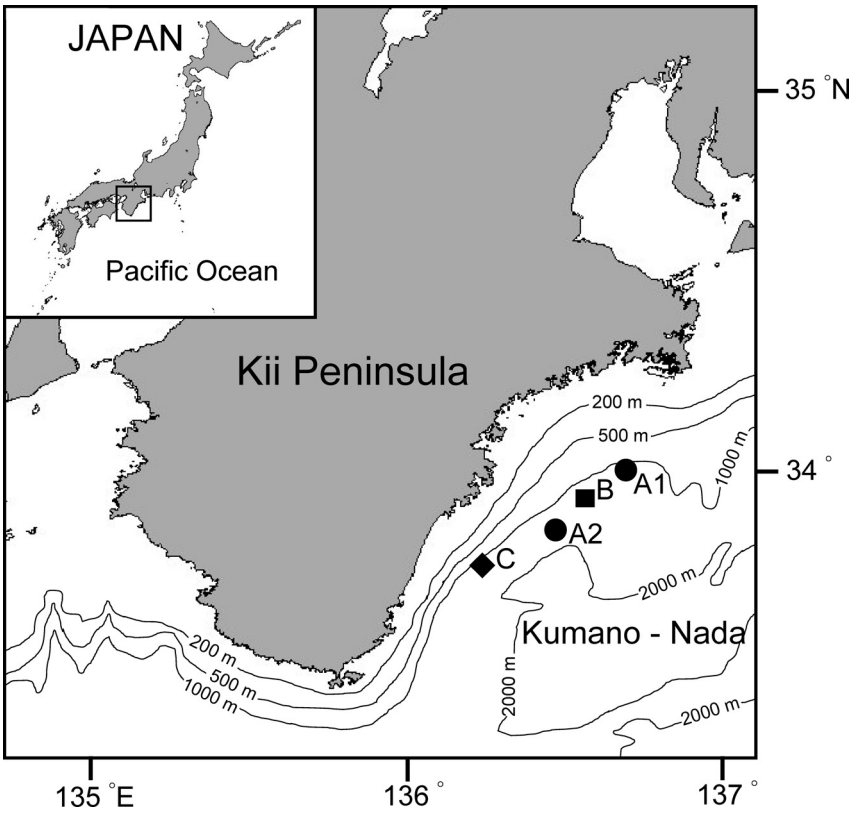

Fig. 1. Location of sampling stations in the Kumano-Nada.

Table 1. Position and bottom depth at sampling stations in Kumano-Nada.

Station	Position		Depth (m)
A1	34°00.0′N	136°42.0′E	1240
A2	33°50.8′N	136°27.8′E	1800
B	33°55.9′N	136°33.7′E	1440
C	33°45.5′N	136°12.5′E	1320

The water samples collected in April, August and October were stored at 4°C in the dark for 2, 2.5 and 1.5 months, respectively, in the laboratory. Viable resting stage cells (i.e. able to geminate or rejuvenate) of diatoms in the water samples were then enumerated following the modified extinction dilution method [most probable number (MPN) method] (Imai et al. 1984). The intact water samples (as 10^0 dilution) after shaking vigorously were subsequently diluted with autoclaved filtered seawater and dilutions of 10^{-1} and 10^{-2} concentrations of the intact water sample were obtained. Each 2 mL of the diluted samples (10^0, 10^{-1} and 10^{-2}) was inoculated in five wells on a 24-well tissue culture plate (BECTON DICKINSON). Furthermore, 0.5 mL of f/2 medium (Guillard and Ryther 1962) was added to each well for nutritional enrichment. The tissue culture plates were maintained at 20°C under illumination at 60 µmol m^{-2} s^{-1} with a 12 h light/12 h dark photoperiod. After two weeks, the number of positive wells where vegetative cells of diatoms germinated or rejuvenated were counted using an inverted microscope (NIKON TE-300). The most probable number of the resting stage cells in the deep water (MPN 20 mL^{-1}) was then calculated from a statistical table (Throndsen 1978) and was converted into the density of cells L^{-1}.

Diatoms were detected from all samples, except for one at 500 m depth at Stn. B in August, and four genera of the Centrales, *Chaetoceros* spp. (*C. curvisetus* Cleve, *C. socialis* Lauder and *Chaetoceros* sp.), *Leptocylindrus danicus* Cleve, *Skeletonema* spp. and *Thalassiosira* spp., and two genera of the Pennales, *Cylidrotheca closterium* (Ehrenberg) Reimann et Lewin and *Navicula* sp., along with one centric and three pennate unidentified diatoms, in total were observed (Fig. 2). Among these diatoms, *C. closterium* and *Navicula* sp. have not been known as species forming a resting stage cell. As mentioned above, since the water samples used in this study were stored at 4°C in the dark for 1.5–2.5 months in the laboratory before starting the MPN experiment, they could not have survived in the vegetative cell stage (if any were in the samples, when collected in the field) in the stored samples. This strongly suggests that, these diatoms, including unidentified species, also form resting stage cells in their life cycles and that those cells germinated/rejuvenated in the MPN experiment.

In April, abundances of the total resting stage cells at Stns. A1 and A2 were high (4,090 and 4,415 cells L^{-1}, respectively) at 200 m, and decreased to 500 m (1,340 and 790 cells L^{-1}, respectively) and 1,000 m (590 and 1,590 cells L^{-1}, respectively)

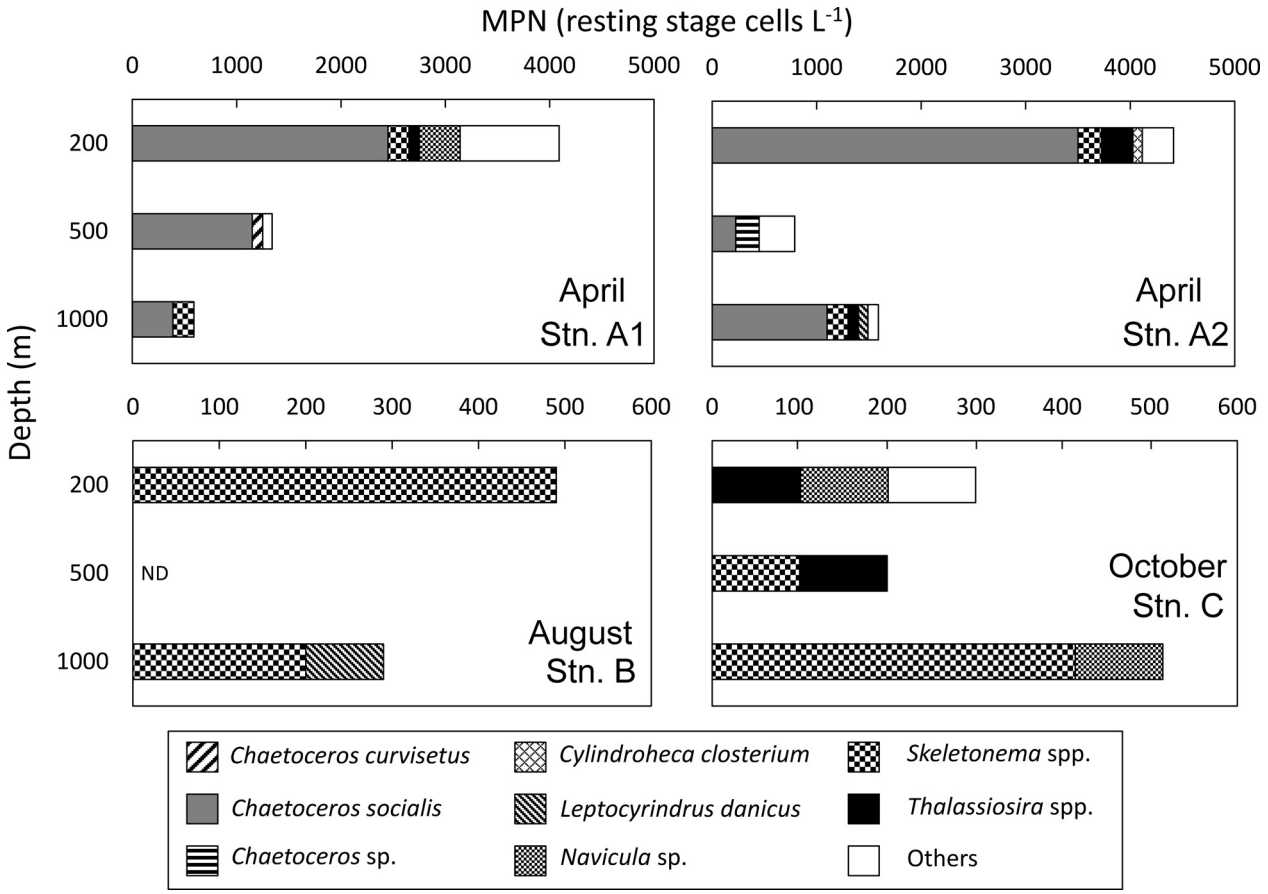

Fig. 2. Vertical distributions of abundance and species composition of viable resting stage cells of diatoms in the Kumano-Nada, enumerated by the extinction dilution method. Unidentified centric and pennate diatom species are assembled as "others". ND denotes "not detected". Note that the scales of x-axes between upper and lower panels are different.

(Fig. 2). The abundances at Stn. B in August and at Stn. C in October were lower than those at the two stations in April (Fig. 2). Even the maxima at Stn. B and at Stn. C were 490 cells L^{-1} at 200 m and 515 cells L^{-1} at 1,000 m, respectively. The abundance at 1,000 m at Stn. B was 290 cells L^{-1} and those at 200 and 500 m at Stn. C were 300 and 200 cells L^{-1}, respectively. Silver et al. (1978) indicated that resting spores of diatoms, often 100% of the resting spores in the water column, aggregate passively in marine snow. In this study, it was actually observed by direct microscopy that all of the resting spores of *C. socialis* appeared in the intact water sample at 1,000 m at Stn. A1, before shaking for the MPN experiment, aggregated in flocs (probably fragments of marine snow). Therefore, the abundances enumerated by the MPN method in this study could represent those of disassembled resting stage cells originally aggregated in marine snow.

The genus *Chaetoceros* appeared in all samples in April and *C. socialis* was always the most dominant diatom in the communities at both stations, with one exception at 500 m at Stn. A2; it contributed 60–93% at Stn. A1 and 69–79% to the total population at Stn. A2 (Fig. 2). In contrast, the genus *Chaetoceros* was not detected at Stn. B in August or at Stn. C in October. Instead, only *Skeletonema* spp. and *L. danicus* occurred at Stn. B and *Skeletonema* spp., *Thalassiosira* spp. and *Navicula* sp. dominated the diatom communities at Stn. C. Sinking rates of marine snow have been reported to be in the range of 43–95 m day^{-1} (average 68 m day^{-1}) (Shanks & Trent 1980), indicating that the resting stage cells aggregated in marine snow sink much faster than individual resting spores of which sinking rates reportedly ranged 0.57–16 m day^{-1} (French & Hargraves 1980). When the sinking rate of the marine snow is, then, assumed to be the average value (i.e. 68 m day^{-1}), it can be simply calculated that the resting stage cells sink from the surface to 1,000 m in only 14.7 days. Consequently, this suggests that the abundance and species composition of resting stage cells observed in this study (Fig. 2) reflected the states of their blooms, which occurred just before our samplings, in the surface water of the Kumano-Nada region.

The resting stage cells of diatoms require light to germinate/rejuvenate (Hollibaugh et al. 1981, Imai et al. 1996). Therefore, in the pelagic ocean, once they sink out of the euphotic layer, mortality should be their fate, suggesting that the resting stage cells in deep waters of Kumao-Nada would not be "seeding populations" for blooms but rather "abortive populations". Many of the species observed in this study, such as *C. curvisetus*, *C. socialis*, *L. danicus*, *Skeletonema* spp. and *Tha-*

lassiosira spp., are commonly distributed in coastal waters. Thus, those vegetative cells and, moreover, their resting stage cells, advected from coastal waters to the surface of the pelagic region (at least in the euphotic layer), might contribute to the initiation of annual blooms, as seed cells, in the Kumano-Nada. On the other hand, Pitcher (1990) suggested that the upwelling system of southern Benguela plays an important role to help the resting spores, distributed in the subsurface layer, to be brought back to the surface and act as seeds for their blooms. Takahashi et al. (1977) also pointed out the possibility that water mass movements, such as upwelling and vertical mixing, could return sunken phytoplankton cells to the euphotic layer in the deep inlet of British Columbia, Canada. In this context, it should be noted that coastal upwelling occasionally occurs in the Kumano-Nada region, although the scale seems to be temporally (lasting only several days) and spatially (occurring only in a few hundred meters depth) small (Takeuchi 1987). The above suggests that the resting stage cells, sinking just from the euphotic layer to deeper depths in the Kumano-Nada, might have some chance to return to the surface, when coastal upwelling occurs, and play an ecological role as a "seeding population" for further blooms.

Since the shelf slope is very steep in the Kumano-Nada, horizontal advection of coastal waters, containing resting stage cells, to the pelagic ocean should also strongly affect the distribution pattern of the resting stage cells in the region. Further studies to investigate the vertical distribution of the resting stage cells and their species composition in particular environments, such as the Kumano-Nada, in relation to hydrographic and topographic features, can contribute to a better understanding of the survival and seeding strategies of diatoms.

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