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Recent occurrence of toxic *Gymnodinium catenatum* Graham (Gymnodiniales, Dinophyceae) in coastal sediments of West Japan

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Abstract. In Japan, a paralytic shellfish poison (PSP) event caused by *Gymnodinium catenatum* Graham, a naked, colonial and PSP dinoflagellate, was first reported from Senzaki Bay, western part of the Sea of Japan in 1986. Since then, the geographical distribution of this species seems to have expanded through West Japan. To clarify the possibility of its migration being due to anthropogenic agency the historical occurrence of *G. catenatum* in Japanese coastal waters was examined; core sediments that preserve an historical record of this species were collected from several coastal areas where it had been observed. From the cores collected from Imari Bay, smaller spherical cysts ornamented with fine reticulated structure and with epicystal archeopyle are found which are probably different from *Gymnodinium nolleri*. Low cyst concentrations of *G. catenatum* in these cores may have resulted from such a characteristic physiological feature as chain formation of its vegetative form. The earliest occurrence of this species was recorded in sediment of Omura Bay dated to ca. 1700 A.D. This fact strongly suggests that the occurrence of *G. catenatum* in Japan is natural and that it has not been artificially introduced from different areas.

Key words: *Gymnodinium catenatum*, PSP, dinoflagellate cyst, harmful algae, recent sediment

Introduction

For understanding the expansion mechanism of harmful marine micro-algae, occurrence histories of these organisms are important and useful. Under this concept, several stratigraphical studies on harmful species have been conducted so far (e.g., Furio *et al.*, 1996; McMinn *et al.*, 1997, 2001). Mainly two different theories concerning the expansion of harmful species have been presented. According to Furio *et al.* (1996) the intensive blooms of *Pyrodinium bahamense* var. *compressum* since the late 1980's, in Manila Bay, Philippines, are probably due to oceanographic environmental changes. This is based on the fact that only a few resting cysts of *P. bahamense* var. *compressum* were recovered from sediments dated to around 1958. On the other hand, McMinn *et al.* (1997) concluded that since the cysts of *Gymnodinium catenatum* Graham from Tasmania, Australia, were first detected in sediments from around 1975, which is just before this species started producing intensive blooms, Tasmanian *G. catenatum* might have been artificially introduced from other areas.

Gymnodinium catenatum, a naked, colonial dinoflagellate, is known to be a causative species of PSP (paralytic shellfish poison). Toxic events derived from this species were first reported in Spain in 1976, and then expanded worldwide (Hallegraeff, 1993). Thereafter, the geographical occurrence of *G. catenatum* has increased, mainly in subtropical to tropical coastal waters (Viquez and Hargraves, 1995; Hodgkiss and Yang, 2001). In particular, Tasmania, Australia was first affected by PSP from this species in 1986. At that time, since this species had never been described from Australian coastal waters, Hallegraeff (1992) suggested that it might be introduced in Tasmania from other regions by ballast waters.

In Japan, a PSP event caused by *G. catenatum* was first reported from Senzaki Bay in the western part of Japan (Ikeda *et al.*, 1989). Since then, the geographical distribution of this species seems to have expanded throughout West Japan (Matsuoka and Fukuyo, 1994; Red Tide Division in Nansei National Fisheries Research Institute, 1998). Simultaneously, PSP events related to this species have also increased in West Japan. However, Matsuoka and Fukuyo (1994) mentioned that this species was reported but not described from Omura Bay, West Kyushu under the name *Gymnodinium* A_5 by Iizuka in 1968.

The purpose of this study is to clarify the historical occurrence of *G. catenatum* in Japanese coastal waters based on the fossil evidence recorded in recent sediments and to seek its possible migration mechanism.

Material and methods

In total, 11 sediment cores were collected from Omura Bay, Nagasaki Bay, Imari Bay and Ariake Sound in West Kyushu, Hiroshima Bay in the Seto Inland Sea, and Matoya Bay in Ise Bay, Central Japan, where vegetative cells and resting cysts of *Gymnodinium catenatum* have been known to occur (Figures 1, 2; Matsuoka and Fukuyo, 1994). The core samples from Omura Bay, Nagasaki Bay and Ariake Sound were collected by divers, and the samples from Imari Bay, Hiroshima Bay and Matoya Bay were taken with a handy piston corer developed by Nanba *et al.* (1998). These core samples were sliced every 1 cm or 2 cm and kept in a refrigerator.

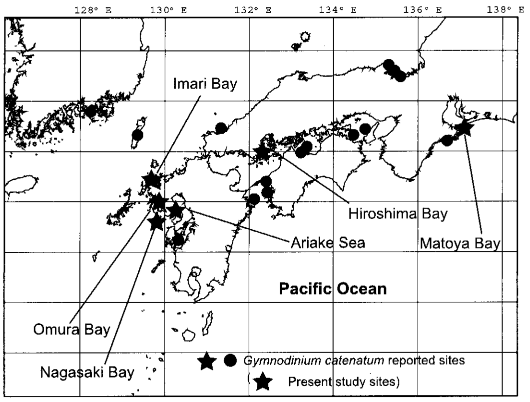

Figure 1. Map showing the reported occurrences of *Gymnodinium catenatum* (vegetative cells and cysts) (Matsuoka and Fukuyo, 1994; Red Tide Division of Nansei National Fisheries Research Institute, 1998) and the sampling locations in West Japan of the present study.

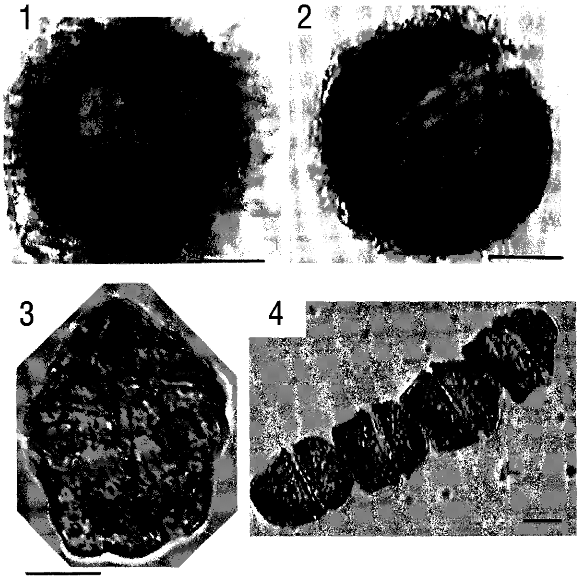

Figure 2. Morphology of *Gymnodinium catenatum* in different life stages. **1**. Living cyst filled with fresh protoplasm occurring in surface sediment. **2**. Empty cyst after germination during laboratory incubation experiment showing a large chasmic archeopyle. **3**. Planktonic cell germinated from the cyst (1). **4**. Vegetative stage making a chain composed of four cells, in a week after germination. These cells were originally the single cyst collected from Imari Bay. (scale bars: 15 μm)

Extraction of dinoflagellate cysts from the samples followed the methods of Matsuoka and Fukuyo (2000). Approximately 2 g of each sample were placed into an acid-resistant 100 ml beaker. To remove calcium carbonate, ca. 10% diluted hydrochloric acid was added, and to dissolve silicate substances ca. 35% hydrofluoric acid was used. After rinsing the samples with pure water, they were sieved through two stainless steel screens of 120 μm and 20 μm opening size. The concentrated residue on the 20 μm screen was suspended in 10 ml distilled water and kept in a vial. A 1–0.5 ml aliquot of the refined samples was provided for observation under an inverted microscope (Olympus IX70). All cysts encountered during scanning of the samples were identified and counted. For calculating the relative frequency of the cysts of *G. catenatum*, cyst concentration in each sample was initially taken as cysts per wet sediment volume, and was subsequently calculated to give final concentrations in cysts/g dry weight sediment. The data of *G. catenatum* cyst concerntrations in samples of Omura Bay and Nagasaki Bay were originally provided by Kim and Matsuoka (1998) and Matsuoka and Kim (1999) respectively. In these reports, the cyst concentration of each species was represented in wet sediment volume, however, there is no difference between the relative abundance of *G. catenatum* cysts measured in cysts per gram dry sediment and those measured in cysts per wet sediment volume.

Age assignment of core sediments

For estimating depositional ages of the core sediments, sedimentation rates of these core samples in average were measured by ^{210}Pb concentration (e.g., Jeter, 2000). Technical support for ^{210}Pb measurement in each of the core samples was provided by Teledyne Isotope Co. LTD. After establishing sedimentation rates for the core samples, the depositional ages for the analyzed samples were estimated.

Results

Sedimentation rates of core samples (Table 1)

From Omura Bay, two core samples were provided for the study; OMB-1 (sampling site: 32°54′N, 129°50′E, water depth 21 m), the core is 99 cm long, consisting of olive grey homogeneous silt with very small shell fragments and the sedimentation rate is 2.7 mm/year, and OMB-2 (sampling site: 32°50′N, 129°58′E, water depth 9 m), the core is 76 cm in length, consisting of homogeneous olive grey silt with shell fragments, and the sedimentation rate is 2.1 mm/year. 5 cm interval samples of these cores were provided for analysis. From the upper 10 cm of sediment, every 1 cm interval was analyzed.

Table 1. List of sedimentation rates of core samples provided for this study and the oldest occurrence of *Gymnodinium catenatum* cyst in each core. Each sedimentation rate was estimated by measurements of ^{210}Pb.

Location	Station No.	Sedimentation rate (mm/year)	Oldest occurrence of *Gymnodinium catenatum* Sample depth (cm)	Age (A.D.)
Omura Bay	OMB-1	2.7	90–91	ca. 1660
	OMB-2	2.1	55–56	ca. 1730
Nagasaki Bay	NGB-1	9.9	70–71	ca. 1920
	NGB-2	4.3	55–56	ca. 1850
Imari Bay	IMB-1	3.2	60–61	ca. 1810
	IMB-2	2.4	50–51	ca. 1780
Ariake Sound	ARK-3	5.8	18–19	ca. 1970
Hiroshima Bay	HRB-1	9.3	79–80	ca. 1870
	HRB-3	3.6	44–45	ca. 1920
Matoya Bay	MTB-1	5.5	30–31	ca. 1940
	MTB-2	4.1	68–69	ca. 1830

From Nagasaki Bay, two core samples were used for the study; NGB-1 (sampling site: 32°41′N, 129°49′E, water depth 26.3 m), the core is 86 cm in length, consisting of homogeneous greenish olive sandy silt to greenish grey silty sand with shell fragments, and the sedimentation rate is 9.9 mm/year, and NGB-2 (sampling site: 32°40′N, 129°48′E, water depth 25 m), the core is 60 cm long, consisting of homogeneous grayish olive sandy silt in the upper 40 cm and greenish grey silty sand with many shell fragments in the lower part, and the sedimentation rate is 4.3 mm/year. 5 cm interval samples were used for analysis.

From Imari Bay, two core samples were prepared for the study; IMB-1 (sampling site: 33°21′N, 129°45′E, water depth 32.1 m), the core is 61 cm long, comprising homogeneous greenish grey silt with small shell fragments, and the sedimentation rate is 3.2 mm/year, and IMB-2 (sampling site: 33°19′N, 129°50′E, water depth 5 m), the core is 51 cm in length, comprising homogeneous greenish grey to dark grey silt, and the sedimentation rate is 2.4 mm/year. 5 cm interval samples were provided for analysis. From the upper 10 cm of sediment, 1 cm interval samples were analyzed.

From Ariake Sound, one core sample was used for the study, ARK-3 (sampling site: 33°21′N, 129°45′E, water depth 3 m), the core is 100 cm long, consisting of homogeneous dark olive grey silt with small shell fragments, and the sedimentation rate is 5.8 mm/year. 5 cm interval samples were provided for analysis. From the upper 10 cm of sediment, samples were analyzed every 1 cm.

From Hiroshima Bay, two core samples were prepared for the study; HRB-1 (sampling site: 34°13.8′N, 132°31.6′E, water depth 19.7 m), the core is 81.5 cm long, comprising homogeneous lightly brownish grey sandy silt with shell fragments concentrated around 20 cm depth, and the sedimentation rate is 9.3 mm/year, and HRB-3 (sampling site: 34°14.3′N, 132°27.4′E, water depth 24.3 m), the core is 63 cm in length, comprising homogeneous greenish dark grey silt at the upper part with small shell fragments and semi-hard grey silt at the lower part, and the sedimentation rate is 3.6 mm/year. 5 cm interval samples were provided for analysis. From the upper 10 cm of sediment, samples were analyzed every 1 cm.

From Matoya Bay, two core samples were prepared for the study; MTB-1 (34°40′N, 136°52′E, water depth 13 m), the core is 59 cm long, consisting of olive grey sandy silt to silt with small shell fragments in the upper 20 cm and of homogeneous grey silt in the lower part, and the sedimentation rate is 5.5 mm/year, and MTB-2 (sampling site: 34°50′N, 136°53′E,

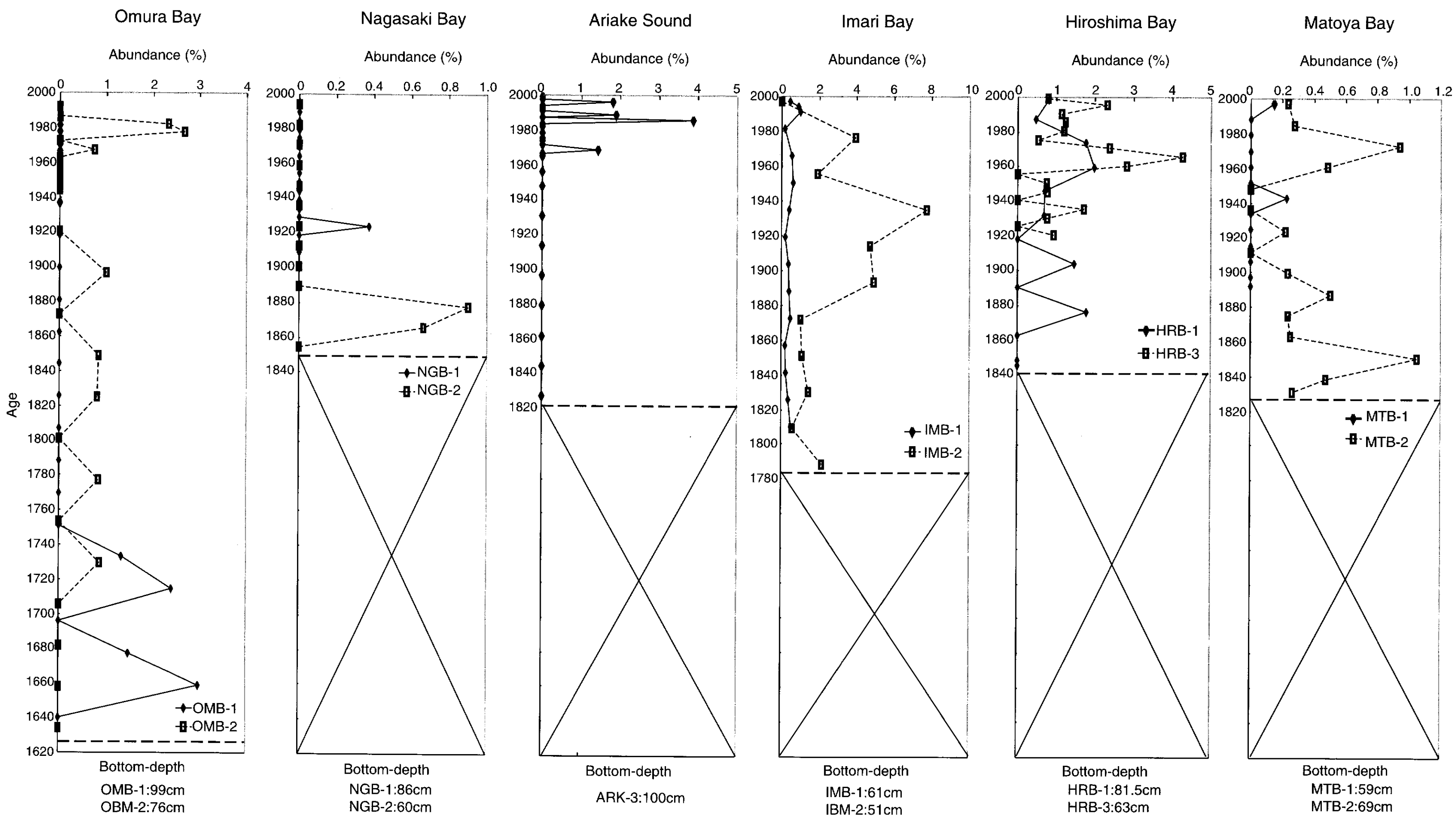

Figure 3. Relative frequency of cyst of *Gymnodinium catenatum* in total dinoflagellate cyst assemblages in core samples collected from different areas shown in Figure 1. Vertical scales are age (A.D.) normalized on the basis of sedimentation rates given by ^{210}Pb measurements of the cores in the present study.

water depth 11 m), the core is 69 cm in length, consisting of homogeneous dark grey silt with shell fragments, and the sedimentation rate is 4.1 mm/year. 5 cm interval samples were provided for analysis. From the upper 10 cm of sediment, samples were analyzed every 1 cm.

Occurrence of *G. catenatum* cysts in core sediments (Figure 3, Table 2)

In Omura Bay, based on the sedimentation rates, 2.7 mm/year at OMB-1 and 2.1 mm/year at OMB-2, the depositional age of the lowest part in both cores is approximately 350 years before 1994 A.D. In the core taken at OMB-1, cysts of *G. catenatum* occurred at 70–71 cm, 75–76 cm, 85–86 cm and 90–91 cm depths. In the core taken at OMB-2, *G. catenatum* cysts were observed at 55–56 cm and then continuously upward with low abundance (1–3% in total dinoflagellate cysts).

In Nagasaki Bay, according to a sedimentation rate of 9.9 mm/year at NGB-1, and 4.3 mm/year at NGB-2, the depositional ages of the base of the cores are ca. 90 years and ca. 140 years before 1995 A.D., respectively. In both cores, *G. catenatum* cysts occurred in very low frequency, mostly less than 1%, and the earliest occurrence was dated back to ca. 120 year at NGB-2. However, in the middle to upper part of the cores, *G. catenatum* cysts were not observed.

In Imari Bay, a sedimentation rate of 3.2 mm/year at IMB-1 and of 2.4 mm/year at IMB-2 evidenced that the lowest part of the core is approximately 190 years old at IMB-1 and approximately 210 years old at IMB-2. In the core taken at IMB-2, the relative frequency of *G. catenatum* cysts is highest (ca. 8% of total dinoflagellate cysts) in three depth ranges. In the core taken at IMB-1, the relative frequency of *G. catenatum* is very low, less than 2%, but cysts occurred continuously to the upper part of the core. The earliest record is dated back ca. 210 years.

In Ariake Sound, since the sedimentation rate for ARK-3 is 5.8 mm/year, the age of the earliest occurrence of cysts is approximately 30 years before the present time. In the core of ARK-3, the relative frequency of *G. catenatum* cysts is highest (ca. 3.8% in total dinoflagellate cysts) in the core and usually less than 2%.

In Hiroshima Bay, based on a sedimentation rate of 3.6 mm/year for HRB-1, the age of the lowest part of the core is approximately 125 years before. In the core taken at HRB-1, the relative frequency of *G. catenatum* cysts is high (ca. 2% of total dinoflagellate cysts) and usually less than 1%.

In Matoya Bay, according to the sedimentation rates of 5.5 mm/year at MTB-1 and 4.1 mm/year at MTB-2, the earliest occurrence of *G. catenatum* was recorded at ca. 1840 A.D. and 1855 A.D., respectively.

In summary, the relative frequency of *G. catenatum* cysts in analyzed samples is always very low, less than 8% even in the case of the highest abundance, in comparison with other cysts of autotrophic species, such as *Spiniferites bulloideus* and *Protoceratium reticulatum*.

Discussion

Cyst morphology of *Gymnodinium catenatum*

After the first report of the cyst of *G. catenatum* by Anderson *et al.* (1988), two morphologically similar cysts were found; non-toxic *Gymnodinium nolleri* (Ellegard and Moestrup, 1999) (Figure 4) and non-toxic *Gymnodinium microreticulatum* (Bolch *et al.*, 1999). Ellegard and Moestrup (1999) pointed out that fossilized cysts previously identified as *G. catenatum* in sediments from the North Sea by Dale *et al.* (1993) were not the cyst of *G. catenatum* but rather *G. nolleri*, because there was no record of vegetative cells of *G. catenatum* so far in the area. These three cysts, with fine reticulation on the surface, have a brownish cyst wall and only differ from each other in their cyst diameters. Bolch *et al.* (1999) clarified that the cyst of *G. microreticulatum* is the smallest, approximately 30 μm in diameter, the cyst of *G. catenatum* is the largest, approximately 50 μm in diameter, and the cyst of *G. nolleri* is intermediate, approximately 40 μm in diameter, although diameters of these cysts overlapped each other. Additionally, *G. microreticulatum* has a different, transsulcal archeopyle from the latter two, which have an epicystal archeopyle. More recently Bolch and Raynolds (2002) reexamined the morphology of microreticulate dinoflagellate cysts collected from various sites from Australia, the Black Sea, Italy, Hong Kong and Uruguay, and showed different cyst diameters for these three species; *G. catenatum* (36–62 μm), *G. nolleri* (25–40 μm), and *G. microreticulatum* (17–29 μm).

In sediments of Imari Bay, fine reticulate cysts with different diameters [40–60 μm (Figure 4-1) and 20–30 μm (Figure 4-2)] were observed. The smaller cysts have diameters in the range of *G. nolleri*, but the vegetative cell of *G. nolleri* has never been observed in plankton samples in the bay. Furthermore, the environmental conditions, in particular water temperature, between Imari Bay and the North Sea are different, more than 25°C in Imari Bay and less than 20°C in the North Sea in summer. These smaller cysts are not identical with *G. nolleri*, and probably belong to a different species. An undescribed dinoflagellate whose

Table 2. Relative frequency of *Gymnodinium catenatum* cyst in total dinoflagellate assemblages.

Omura-1 (%)/Depth (cm)	0–1	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	15–16	20–21
Gonyaulacoid	44.55	31.28	32.39	26.55	39.44	38.70	32.42	30.26	25.45	34.62	43.72	58.43
Gymnodinioid	20.79	25.59	28.64	28.32	31.99	20.87	18.72	24.10	27.68	28.21	17.75	16.85
Protoperidinioid	34.65	43.13	38.97	45.13	28.57	40.43	48.86	45.64	46.88	37.18	38.53	24.72
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Omura-2 (%)/Depth (cm)	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	15–16
Gonyaulacoid	30.71	19.86	16.28	28.00	21.43	30.60	33.13	37.58	41.40	45.22	52.59	64.46
Gymnodinioid	21.26	18.44	15.12	14.67	17.35	20.90	13.75	15.29	15.29	7.83	19.26	6.61
Protoperidinioid	48.03	61.70	68.60	57.33	61.22	48.51	53.13	47.13	43.31	46.96	28.15	28.93
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.00	0.00	2.33	2.67	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00
Nagasaki-1 (%)/Depth (cm)	0–1	5–6	10–11	15–16	20–21	25–26	30–31	35–36	40–41	45–46	50–51	55–56
Gonyaulacoid	33.75	30.08	38.68	50.98	55.41	43.28	56.80	66.53	69.39	65.95	69.00	70.24
Gymnodinioid	26.25	33.08	34.91	20.92	17.57	9.24	6.40	4.08	4.76	8.11	6.55	7.14
Protoperidinioid	40.00	36.84	26.42	28.10	27.03	47.48	36.80	29.39	25.85	25.95	24.45	22.62
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nagasaki-2 (%)/Depth (cm)	0–1	5–6	10–11	14–15	20–21	25–26	31–32	35–36	40–41	44–45	50–51	55–56
Gonyaulacoid	38.78	25.78	41.06	37.93	29.51	41.18	41.57	47.06	53.57	62.96	67.57	58.94
Gymnodinioid	17.35	29.69	19.21	6.90	4.10	9.56	2.25	9.80	1.79	1.23	1.80	11.26
Protoperidinioid	43.88	44.53	39.74	55.17	66.39	49.26	56.18	43.14	44.64	35.80	30.63	29.80
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90	0.66
Matoya-1 (%)/Depth (cm)	0–1	5–6	10–11	15–16	20–21	25–26	30–31	35–36	40–41	45–46	50–51	55–56
Gonyaulacoid	64.31	66.56	62.98	72.25	63.37	57.82	63.41	71.88	75.15	69.50	79.35	72.92
Gymnodinioid	6.17	2.92	4.39	1.98	8.34	4.51	4.26	3.31	2.10	4.42	2.45	2.11
Protoperidinioid	29.52	30.52	32.63	25.77	28.30	37.67	32.33	24.81	22.75	26.07	18.20	24.98
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.15	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00
Matoya-2 (%)/Depth (cm)	0–1	5–6	10–11	15–16	20–21	25–26	30–31	35–36	40–41	45–46	50–51	55–56
Gonyaulacoid	57.89	72.34	56.97	73.28	72.51	69.98	71.49	65.20	78.98	67.73	76.48	72.27
Gymnodinioid	2.10	0.83	1.88	1.49	1.22	1.31	1.51	1.37	0.68	1.01	2.40	1.99
Protoperidinioid	40.01	26.83	41.15	25.23	26.28	28.71	27.00	33.43	20.34	31.26	21.13	25.74
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.23	0.28	0.94	0.48	0.00	0.00	0.22	0.00	0.24	0.50	0.23	0.25
Imari-1 (%)/Depth (cm)	0–1	1–2	2–3	5–6	10–11	15–16	20–21	25–26	30–31	35–36	40–41	45–46
Gonyaulacoid	47.60	52.80	61.17	59.59	62.18	59.52	63.14	64.08	64.66	63.96	60.30	64.93
Gymnodinioid	5.67	2.97	4.45	2.85	2.42	4.51	3.21	2.21	1.76	2.72	3.92	1.97
Protoperidinioid	46.73	44.23	34.39	37.56	35.40	35.97	33.66	33.71	33.58	33.33	35.78	33.09
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.44	0.89	0.98	0.18	0.56	0.61	0.41	0.22	0.39	0.42	0.49	0.20
Imari-2 (%)/Depth (cm)	0–1	5–6	10–11	15–16	20–21	25–26	30–31	35–36	40–41	45–46	50–51	
Gonyaulacoid	31.37	46.80	45.71	48.46	52.35	54.48	62.90	64.75	63.13	70.30	65.45	
Gymnodinioid	9.41	13.30	14.76	12.86	18.84	12.65	6.14	4.85	5.24	4.65	4.30	
Protoperidinioid	59.22	39.90	39.52	38.68	28.81	32.88	30.97	30.40	31.63	25.05	30.25	
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	
Gymnodinium catenatum	0.00	3.94	1.90	7.72	4.71	4.91	1.02	1.09	1.43	0.59	2.15	
Hiroshima-1 (%)/Depth (cm)	0–1	4–5	9–10	14–15	19–20	24–25	29–30	34–35	39–40	44–45	49–50	54–55
Gonyaulacoid	36.72	37.04	29.69	24.07	28.74	27.37	18.88	22.69	21.99	23.84	23.87	20.45
Gymnodinioid	3.91	6.48	4.89	9.27	8.39	7.90	5.65	7.09	9.93	5.96	12.70	9.86
Protoperidinioid	59.37	56.48	65.41	66.67	62.87	64.74	75.47	70.22	68.07	70.20	63.43	69.69
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.78	2.32	1.13	1.24	1.19	0.53	2.37	4.26	2.83	0.00	0.75	0.76
Hiroshima-3 (%)/Depth (cm)	0–1	4–5	9–10	14–15	19–20	24–25	29–30	34–35	39–40	44–45	49–50	54–55
Gonyaulacoid	29.92	17.84	26.76	41.05	46.28	42.18	53.22	42.35	50.30	44.29	50.02	50.63
Gymnodinioid	5.51	33.34	8.33	8.62	10.20	12.24	5.64	7.29	4.61	8.39	5.37	9.26
Protoperidinioid	64.57	48.82	64.91	50.33	43.52	45.58	41.14	50.35	45.09	47.33	44.61	40.11
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.79	0.46	1.76	1.98	0.70	0.68	0.00	1.46	0.00	1.78	0.00	0.00
Ariake Sea-3 (%)/Depth (cm)	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	12–13
Gonyaulacoid	32.88	27.66	31.82	29.17	14.86	28.26	27.62	32.79	33.01	28.99	29.13	33.83
Gymnodinioid	0.00	2.13	3.64	3.33	2.70	4.35	2.86	3.28	7.77	1.45	0.00	3.01
Protoperidinioid	67.12	70.21	64.55	67.50	82.43	67.39	69.52	63.93	59.22	69.57	70.87	63.16
total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Gymnodinium catenatum	0.00	0.00	1.82	0.00	0.00	0.00	1.90	0.00	3.88	0.00	0.00	0.00

25–26	30–31	35–36	40–41	45–46	50–51	55–56	60–61	65–66	70–71	75–76	80–81	85–86	90–91	95–96
79.85	82.09	74.39	77.40	66.43	74.00	86.64	72.96	64.10	59.73	65.06	66.33	63.05	52.34	66.98
6.72	5.22	11.59	10.96	15.00	6.00	6.11	11.16	13.33	12.39	8.43	8.04	10.34	28.09	6.05
13.43	12.69	14.02	11.64	18.57	20.00	7.25	15.88	22.56	27.88	26.51	25.63	26.60	19.57	26.98
100.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	2.41	0.00	1.48	2.98	0.00

20–21	25–26	30–31	35–36	40–41	45–46	50–51	55–56	60–61	65–66	70–71	75–76
74.75	86.07	76.47	78.86	76.47	75.63	81.97	72.41	69.17	63.89	65.13	73.48
10.10	4.10	8.40	9.76	5.88	5.88	5.74	6.03	8.33	14.81	6.58	3.79
15.15	9.84	15.13	11.38	17.65	18.49	12.30	21.55	22.50	21.30	28.29	22.73
100.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
1.01	0.00	0.84	0.81	0.00	0.84	0.00	0.86	0.00	0.00	0.00	0.00

60–61	65–66	70–71	75–76	79–80	85–86
71.51	78.57	77.78	79.37	66.50	58.70
7.53	0.79	1.85	1.35	1.78	7.61
20.97	20.63	20.37	19.28	31.73	33.70
100.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
0.00	0.00	0.37	0.00	0.00	0.00

59–60
67.83
0.00
32.17
100.00
0.00

58–59
77.52
4.19
18.29
100.00
0.00

60–61	65–66	68–69
75.48	74.55	77.09
2.14	1.42	1.31
22.38	24.03	21.60
100.00	**100.00**	**100.00**
1.05	0.47	0.26

50–51	55–56	60–61
68.61	64.99	67.97
0.47	1.34	1.49
30.91	33.66	30.54
100.00	**100.00**	**100.00**
0.24	0.38	0.50

59–60	64–65	69–70	74–75	79–80
15.43	14.78	20.46	19.80	27.50
6.39	3.97	7.58	3.96	5.78
78.18	81.25	71.97	76.24	66.72
100.00	**100.00**	**100.00**	**100.00**	**100.00**
0.00	1.71	0.77	0.00	0.94

55–56
51.80
2.85
45.35
100.00
0.00

14–15	15–16	16–17	18–19	19–20	20–21	25–26	30–31	40–41	50–51	60–61	70–71	80–81	90–91	100–
33.65	40.58	37.29	38.57	71.15	47.83	56.57	59.81	63.16	63.93	60.22	67.78	71.63	64.06	72.34
3.85	5.80	8.47	5.71	3.85	7.61	8.08	1.87	1.75	0.00	1.08	0.00	0.00	1.56	2.13
62.50	53.62	54.24	55.71	25.00	44.57	35.35	38.32	35.09	36.07	38.71	32.22	28.37	34.38	25.53
100.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
0.00	0.00	0.00	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

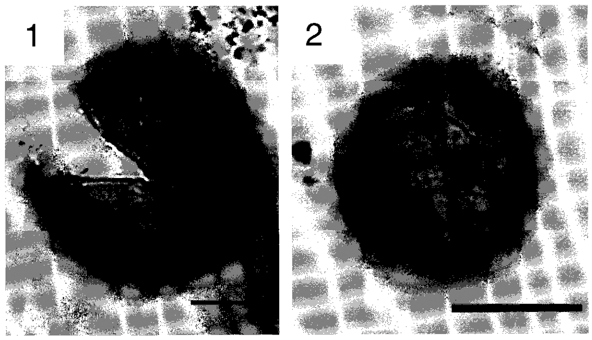

Figure 4. Reticulate cysts of two different diameters: **1**. Cyst of *Gymnodinium catenatum* Graham collected from Imari Bay, **2**. Smaller cyst of *Gymnodinium* sp. collected from Imari Bay. The morphological difference is present only in cyst diameter of these similar two reticulate cysts.

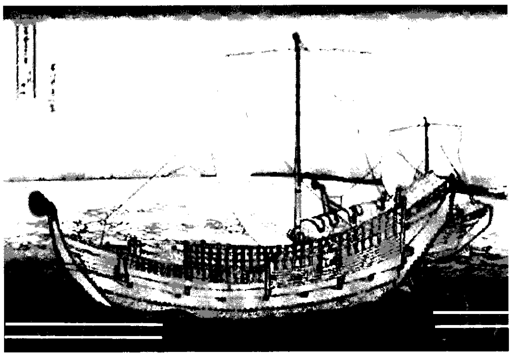

Figure 5. Typical Japanese ship in the Edo period. Most of the oldest occurrence of *Gymnodinium catenatum* in core samples provided for this study was recorded in this period. The original illustration from a Japanese stamp.

smaller cysts are ornamented with fine reticulation and which possesses an epicystal archeopyle possibly inhabits West Japan.

Low concentration of the cyst of *Gymnodinium catenatum*

Matsuoka and Fukuyo (1994) suggested that the cyst concentration of *G. catenatum* in sediments is very low in comparison with other red-tide-causing dinoflagellate species. McMinn *et al.* (1997) illustrated the vertical profile of the abundance of *G. catenatum* cysts in core sediments collected from Tasmania, Australia. In these cores, the occurrence of the cyst was always less than 2% of the total cyst population. Similarly, the relative frequency of *G. catenatum* cysts was always very low (less than 8% in the case of the highest abundance) during this study, in comparison to other cysts of autotrophic species such as *Spiniferites bulloideus* and *Protoceratium reticulatum*. *Gymnodinium catenatum* is a chain-forming species and easily forms a colony consisting of more than eight cells. This can lead to a large number of vegetative cells in surface waters. Although the cyst-forming process of this species is not yet fully understood, the following interpretation is possible: during the cyst-forming process, a single cell separating from a chain is provided in sexual reproduction. In culture conditions, cells forming a chain can survive only for a while after separating, however, they do not act as gametes in sexual reproduction. During a bloom, most of the cells of *G. catenatum* make chains and only a few single cells become gametes. Therefore, cyst formation might be very low in spite of a large population of motile cells as a result of asexual reproduction.

Gymnodinium catenatum as a natural species in Japan

In Omura Bay, the earliest occurrence of this species was recorded in sediment dated to ca. 1700 A.D. In Nagasaki Bay, *G. catenatum* appeared around ca. 1870 A.D. In Imari Bay, the earliest occurrence of *G. catenatum* was dated back to ca. 1825 A.D. The earliest recorded occurrence of this species dates back to the Edo period in Japan. At that time, the Japanese government followed a policy of national isolation, and the old Japanese ships (Figure 5) were made of wood and did not employ ballast water tanks. There is no possibility that Japanese *G. catenatum* could have been artificially introduced within ballast waters from other areas. This fact strongly suggests that the occurrence of *G. catenatum* is natural in Japan.

More than ten years before, the geographical distribution of *G. catenatum* in Japanese coastal waters was reported by Matsuoka and Fukuyo (1994). Since then, the appearance of this species has been investigated (Red Tide Division of Nansei National Fisheries Research Institute, 1998). This data shows that the areas where *G. catenatum* now appears increased during the 1990's, particularly in West Japan. Two possibilities for this apparent increase can be considered; its distribution really is expanding; or there are more records of this species, even of small numbers of cells, because increased interest in harmful algae has drawn more researchers into looking for its presence.

Our results indicate that the latter is correct. The increasing number of reports of *G. catenatum* in Japanese waters is not due to artificial spread of its distribution area, but rather to increased, intensive monitoring for harmful algae. Increased occurrences of this species are probably caused by environmental change in coastal areas, namely the progress of artificial eutrophication, change of current systems after coastal construction, and change of the coastal ecosystem, thereby creating a more favorable environment for *G. catenatum*. The reason for the recent increase of *G. catenatum* in Japanese coastal waters is different from that in Australia where this species might have been introduced from other regions by ballast waters (Hallegraeff, 1992; McMinn *et al.*, 1992).

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