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Vertical distribution of *Pyrodinium bahamense* var. *compressum* (Dinophyceae) cysts in Ambon Bay and Hurun Bay, Indonesia

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Abstract: *Pyrodinium bahamense* var. *compressum* (Pbc) is one of the causative species of Paralytic Shellfish Poisoning (PSP). Incidents of PSP and red tides caused by Pbc are increasing, as well as the geographic distribution of Pbc expanding in Southeast Asia, where it has recently occurred in several areas that previously have not experienced blooms, such as Ambon Bay and Lampung Bay in Indonesia. Five factors including anthropogenic activities, natural activities, visual realization with red tide discoloration, development of techniques like RNA sequence and ELISA toxin kits, and the establishment of a regular monitoring system have been suggested to have led to the increase in reports of dinoflagellate blooms. Occurrence of dinoflagellate cysts in sediments has been used as evidence to which species of vegetative cells occurred in the water column. In this study, the vertical distribution of dinoflagellate cysts in sediments was investigated to confirm the first occurrence of Pbc cysts and to examine the floral changes of dinoflagellate cysts in Ambon Bay and Hurun Bay, Indonesia. In Ambon Bay, Pbc cysts first occurred in ca. 1850 and the cysts were continuously observed since ca. 1870. In Hurun Bay, Pbc cysts were found at 50–52 cm depth, and also at 42–44 cm depth. Cysts of Pbc first occurred at least in 1883, using depositional age calculated from the historical eruption of Mt. Krakatau. Cysts of Pbc have continuously occurred since ca. 1910, and the cyst densities rapidly increased in ca. 1960. Based on these findings, we consider that anthropogenic activities such as ballast water and transportation of shellfish seeds probably did not cause the introduction of Pbc in both bays.

Key words: dinoflagellate cysts, expansion, Indonesia, *Pyrodinium bahamense*, vertical distribution

Introduction

Paralytic Shellfish Poisoning (PSP) events and harmful blooms caused by *Pyrodinium bahamense* var. *compressum* (Böhm) Steidinger, Tester et Taylor (hereafter Pbc) have been recorded in several coastal areas in the Southeast Asian region (Azanza & Taylor 2001) since the first outbreak of a red tide reported in Papua New Guinea in 1972 (Fig. 1A). This toxic species appears to be endemic in Southeast Asia, as it occurs in the Philippines, Malaysia, Indonesia and some other countries in the region. In Indonesia, an incident of PSP caused by Pbc was first recorded in Kao Bay in 1994. Subsequently, the first PSP and/or its bloom followed in Ambon Bay, Ambon Island and Lampung Bay, south Sumatra Island (e.g. Wiadnyana et al. 1996, Widiarti et al. 2000).

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Recently, the expansion of harmful and toxic dinoflagellate blooms has drawn attention to the possible causative mechanisms for such expansions (e.g. Hallegraeff 1993). These are, 1) transportation and introduction of new populations caused by anthropogenic activities such as ballast water and hull fouling of ships and transportation of shellfish seed stocks, 2) new introduction of populations caused by natural activities such as seawater currents, 3) visual realization of a cryptogenic population as a result of red tide discoloration caused by alternation of environmental conditions, either due to natural or anthropogenic activities, 4) detection of cryptogenic populations due to the advancement of sensitive chemical methods such as probes based on RNA sequences and ELISA toxin kits, and 5) discovery of previously unobserved toxic dinoflagellates and toxin contamination by the establishment of a regular monitoring system. Among these five mechanisms, only the first two can be considered as a true geographical expansion of the organism's distribution. Several studies were recently con-

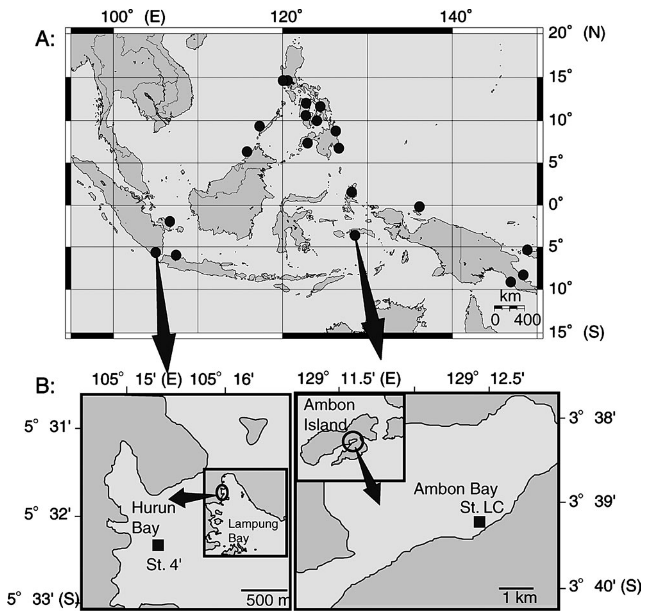

Fig. 1. Main areas of PSP incidents and blooms caused by *Pyrodinium bahamense* var. *compressum* (A), and sampling locations at St. LC in Ambon Bay in November, 1995 and St. 4′ in Hurun Bay in December, 2003 (B).

ducted to understand better the observed expansion of vegetative cell distributions from these viewpoints. For example, Lilly et al. (2002) reported that *Alexandrium catenella* in Thau Lagoon, France, was probably transported through ballast water from the Pacific Ocean, as occurred in Japanese and Australian waters, based on evidence from molecular phylogenetic and toxin analyses. On the other hand, it is also important to clarify when dinoflagellates first appeared and how their population fluctuated after the first occurrence, in order to determine the main mechanism for each case of expansion.

Approximately 200 species of modern dinoflagellates including Pbc are known to produce resting cysts. Most cysts are known to be preserved in the sediment (Head 1996). Therefore, they provide evidence of the existence of vegetative cells in the investigated areas.

Geographic distributions of dinoflagellate cysts have been investigated in several areas of Southeast Asia. Matsuoka et al. (1999) reported occurrences of Pbc cysts (paleontological name of *Polysphaeridium zoharyi** (Rossignol) Bujak et al.) in surface sediments collected from Jakarta Bay, Ujung Pandang in Sulawesi, and Larantuka in Flores, Indonesia. In the Philippines, Corrales & Crisostomo (1996) and Azanza et al. (2004) reported horizontal distributions of Pbc cysts in Manila Bay. Furio et al. (1996) and Sombrito et al. (2004) investigated vertical distributions of dinoflagellate cysts with special attention to Pbc in Manila Bay and Malampaya Sound, respectively.

In this study, the vertical distributions of dinoflagellate cysts were examined to determine the first occurrence age and fluctuation of dinoflagellate cyst abundances, especially Pbc, in Ambon Bay and Hurun Bay, Indonesia.

Materials and Methods

Study areas and sampling

Ambon Bay is located on Ambon Island, eastern Indonesia, and Hurun Bay is one of several small branch bays of Lampung Bay in the southern part of Sumatra. Two sediment cores of 64 cm and 82 cm in length were collected by an acrylic pipe of 6 cm diameter at St. LC in Ambon Bay in 1995 and at St. 4' in Hurun Bay in 2003 (Fig. 1B). Water depth of sampling locations was 26 m at St. LC and 19 m at St. 4'. The sediment cores were cut every 2 cm from top to bottom, and were used for the enumeration of dinoflagellate cysts, measurement of sedimentation rate and grain size analysis.

Cyst analysis

For measurement of the water content, each sediment sample was weighed in the wet condition. Water content rates were calculated by weighing the samples after oven-drying at 70°C for 24 hours. For analysis of dinoflagellate cysts, the palynological method (Matsuoka et al. 1989) was adopted. Each sediment sample was weighed, and distilled water added to remove the salt. The slurry was treated with hydrochloric acid (HCl: concentration 38%) to dissolve calcium carbonate, and then with hydrofluoric acid (HF: 47%) for silicate materials. The slurry was ultra-sonicated for 30 sec in order to remove extraneous matter attached to the surface cyst walls, and then sieved through two stainless screens with mesh sizes of 125 μm and 20 μm opening, respectively. The residual matter on the 20 μm mesh was made up to 10 ml by adding distilled water. A 1 ml aliquot from each of the 10 ml sample was used for the observations of dinoflagellate cysts in several batches under an inverted microscope (Olympus, IX 70). Cyst density was calculated as the number of cells per gram weight of dry sediment sample (cysts g^{-1} dry weight) after calculating the water content rate.

We use mainly paleontological names of the planktonic vegetative cells for cysts in this article. An asterisk (*) is used to indicate a paleontological name.

Sedimentation rate

The average sedimentation rate was estimated based on the ^{210}Pb dating method (Krishnaswami et al. 1971). Radioactive concentration of ^{210}Pb decreases from the top, and finally reaches a constant level, termed the supported level. The excess ^{210}Pb concentration, which is the measured concentration at each depth minus the constant level, is plotted logarithmically against each sediment depth. The decreasing trend in the excess ^{210}Pb with depth was used to calculate the average sedimentation rate (Jetter 2000). In this

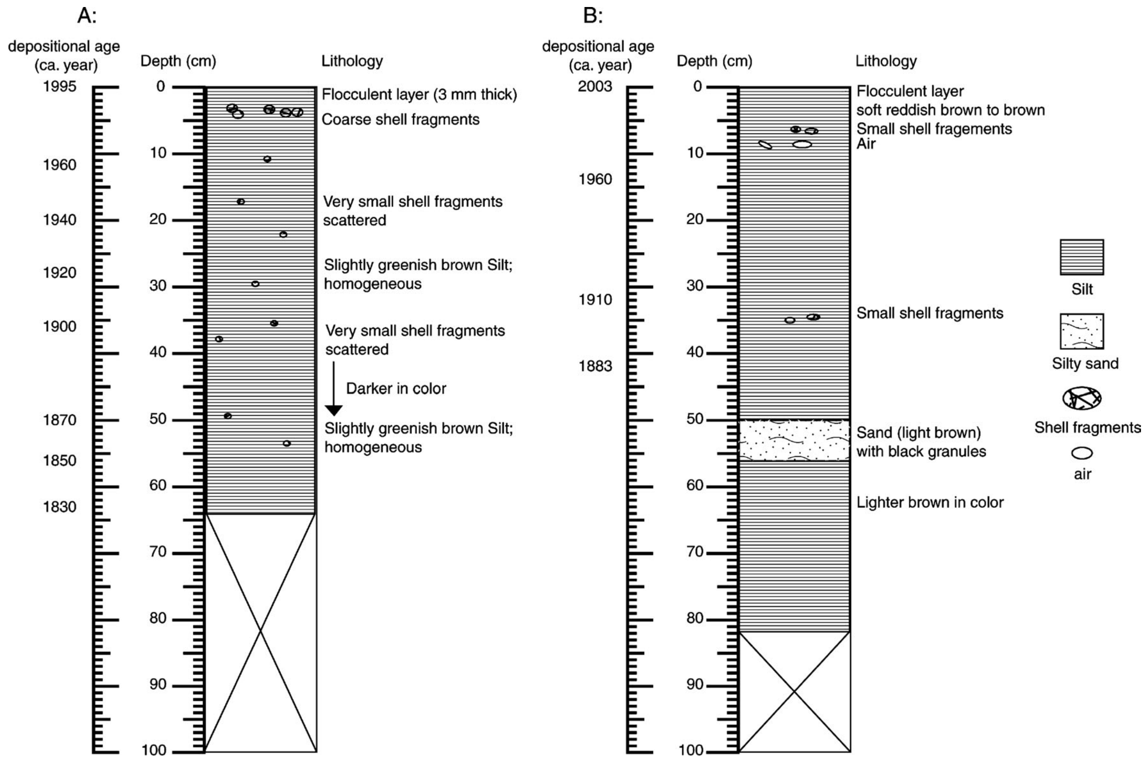

Fig. 2. Description of the cores in this study; A: Ambon Bay, B: Hurun Bay.

analysis, the following samples for ^{210}Pb concentrations were used; 2–4 cm, 10–12 cm, 20–22 cm, 30–32 cm, 44–46 cm and 60–62 cm depth from the Ambon Bay core, and 2–4 cm, 8–10 cm, 14–16 cm, 24–26 cm, 38–40 cm and 58–60 cm depth of the samples of the Hurun Bay core.

Grain size analysis

Since the grain size at specific depths from 50 cm to 56 cm in the Hurun Bay core was different from that at other depths (Fig. 2), a grain size analysis was carried out. Approximately 10 g wet sediments at 0–2 cm, 4–6 cm, 10–12 cm, 14–16 cm, 20–22 cm, 24–26 cm, 30–32 cm, 34–36 cm, 40–42 cm, 42–44 cm, 44–46 cm, 46–48 cm, 48–50 cm, 50–52 cm, 52–54 cm, 54–56 cm, 56–58 cm, 58–60 cm, 60–62 cm, 64–66 cm, 70–72 cm, 74–76 cm and 80–82 cm depth were sieved through stainless meshes of 2000 μm and 63 μm mesh size. The residual matter on each mesh was oven-dried at 70°C for 24 hours. The relative frequency of each grain size was calculated based on the dry weights.

Results

Sedimentation rate

As a result of the ^{210}Pb measurements, the average rate of sedimentation was calculated to be 0.39 cm year^{-1} in Ambon Bay and 0.27 cm year^{-1} in Hurun Bay. Consequently, the depositional age of the horizon was estimated to be ca. 1830 in Ambon Bay and ca. 1700 in Hurun Bay based on these sedimentation rates.

Vertical distribution of dinoflagellate cysts in Ambon Bay

Dinoflagellate cysts were observed in all samples, and 25 species were identified, including Pbc (Table 1, Fig. 3) in Ambon Bay. The first occurrence of Pbc cysts was 33 cysts g^{-1} and was at 56–58 cm depth, where the depositional age based on the ^{210}Pb dating method was ca. 1850 (Fig. 4). The detection limit of cysts in the core was about 4 cysts g^{-1} in this cyst analysis of the Ambon Bay core. Cysts of Pbc (Fig. 3A–D) were continuously observed above 50–52 cm depth (ca. 1870). Particularly, the cyst densities gradually increased from the 28–30 cm depth (ca. 1920). Consequently, the cyst densities above 20–22 cm depth (ca. 1940) were 2124~3477 cysts g^{-1} and this was much higher than that below 30–32 cm depth (5~277 cysts g^{-1}). The cysts of Pbc were the dominant species above 20–22 cm depth. Cysts of the genus *Gonyaulax*, consisting of *Spiniferites hyperacanthus** (Deflandre et Cookson) Sarjeant (Fig. 3E), *Spiniferites bentorii** (Rossignol) Wall et Dale, *Spiniferites mirabilis** (Rossignol) Sarjeant, and *Spiniferites bulloideus** (Deflandre et Cookson) Sarjeant decreased above 32–34 cm depth around ca. 1910, but had an average density of 1182 cysts g^{-1}. Particularly, *S. hyperacanthus** was dominant below 34–36 cm depth. *Lingulodinium machaerophorum** (Deflandre et Cookson) Wall (Fig. 3F) had an average den-

Table 1. Densities of dinoflagellate cysts in the Ambon Bay core. (cysts g^{-1})

cyst name/sediment depth (cm)	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32
Alexandrium pseudogoniaulax	6	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
*Spiniferites ramosus** (*Gonyaulax* sp.)	24	9	18	0	0	5	6	36	0	0	29	5	22	0	0	24
*Spiniferites bulloideus** (*Gonyaulax scrippsae*)	23	16	28	21	5	13	30	30	16	4	32	9	27	19	5	17
*Spiniferites mirabilis** (*Gonyaulax spinifera*)	12	9	10	5	4	18	10	20	6	0	9	0	4	9	0	0
*Spiniferites hyperacanthus** (*Gonyaulax* sp.)	304	146	243	306	252	225	418	289	250	323	322	287	407	264	261	457
*Spiniferites membranaceus** (*Gonyaulax membranacea*)	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiniferites cf. *delicatus** (*Gonyaulax* sp.)	23	0	18	5	4	9	16	10	0	0	4	0	17	5	0	12
*Spiniferites** spp.	380	486	490	595	499	465	792	593	582	626	653	762	625	782	895	930
*Spiniferites bentorii** (*Gonyaulax digitalis*)	173	57	66	114	139	87	212	102	158	128	80	120	105	180	140	89
*Lingulodinium machaerophorum** (*Lingulodinium polyedrum*)	190	111	176	137	179	120	164	330	222	159	269	160	240	109	185	348
*Operculodinium centrocarpum** (*Protoceratium reticulatum*)	22	5	4	15	13	17	26	21	11	0	22	5	9	39	13	21
*Tuberculodinium vancampoae** (*Pyrophacus steinii*)	617	404	454	381	355	319	526	427	420	339	462	365	446	407	361	336
*Polysphaeridium zoharyi** (*Pyrodinium bahamense* var. *compressum*)	3431	2182	2827	2902	2612	2817	3410	3477	2775	2124	2292	964	1615	798	679	242
Pheopolykrikos hartmannii	0	0	0	0	0	0	5	0	0	4	0	0	0	0	0	0
Scrippsiella spp.	66	40	45	66	37	21	65	39	57	24	45	34	23	54	28	20
Polykrikos kofoidii	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
*Trinovantedinium capitatum** (*Protoperidinium* sp.)	12	5	35	20	20	17	21	6	26	0	9	0	4	0	4	4
*Selenopemphix quanta** (*Protoperidinium conicum*)	30	33	32	26	23	39	6	34	0	19	28	15	23	21	13	20
Diplopelta parva	37	10	13	9	19	18	11	38	21	5	9	19	9	0	9	8
*Quinquecuspis concretas** (*Protoperidinium leonis*)	11	5	20	11	9	4	21	15	16	4	9	5	14	0	9	4
*Votadinium calbum** (*Protoperidinium oblongum*)	24	5	0	9	4	0	0	5	10	0	0	0	5	0	4	0
Protoperidinium latissinum	0	5	4	0	9	0	14	5	5	14	5	0	0	0	4	0
*Stelladinium reidii** (*Protoperidinium compressum*)	0	0	0	5	5	4	0	0	0	0	0	5	0	0	0	0
*Stelladinium abei** (*Protoperidinium* sp.)	5	0	0	0	0	4	0	15	0	0	0	0	0	0	5	4
*Stelladinium robustum** (*Protoperidinium* sp.)	11	0	4	0	0	4	5	0	10	0	0	0	0	0	0	0
*Selenopemphix nephroides** (*Protoperidinium subinerme*)	12	5	13	5	9	0	0	5	0	9	5	0	0	5	4	0
*Brigantedinium irregulare** (*Protoperidinium denticulatum*)	24	0	0	0	0	0	0	0	0	0	0	5	0	10	0	0
*Brigantedinium** spp.	197	89	90	68	63	43	106	135	117	31	108	37	64	50	27	60
*Dubridinium** sp.	0	0	0	0	0	0	0	6	0	0	0	0	5	0	0	0
total cyst density	5631	3623	4596	4699	4261	4248	5863	5637	4700	3815	4397	2797	3669	2751	2647	2596

Table 1. (cont.) (cysts g^{-1})

cyst name/sediment depth (cm)	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	48–50	50–52	52–54	54–56	56–58	58–60	60–62	62–bottom
Alexandrium pseudogoniaulax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Spiniferites ramosus** (*Gonyaulax* sp.)	0	28	0	0	16	0	13	0	0	19	0	8	0	0	27	27
*Spiniferites bulloideus** (*Gonyaulax scrippsae*)	11	19	5	8	5	20	30	20	5	38	0	0	5	4	29	14
*Spiniferites mirabilis** (*Gonyaulax spinifera*)	22	33	19	0	5	31	9	30	5	13	4	0	15	4	0	4
*Spiniferites hyperacanthus** (*Gonyaulax* sp.)	439	798	503	640	790	656	573	538	473	590	400	518	518	587	644	497
*Spiniferites membranaceus** (*Gonyaulax membranacea*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiniferites cf. *delicatus** (*Gonyaulax* sp.)	21	0	5	5	33	27	4	21	9	41	0	4	14	4	16	12
*Spiniferites** spp.	1064	955	1053	1519	774	1684	941	1789	1396	711	1276	516	1473	1580	641	499
*Spiniferites bentorii** (*Gonyaulax digitalis*)	172	139	181	332	144	242	134	194	179	108	127	88	186	201	70	56
*Lingulodinium machaerophorum** (*Lingulodinium polyedrum*)	348	167	73	123	72	71	43	68	23	20	75	91	61	44	16	4
*Operculodinium centrocarpum** (*Protoceratium reticulatum*)	32	40	9	24	10	67	22	5	19	42	18	4	14	23	23	7
*Tuberculodinium vancampoae** (*Pyrophacus steinii*)	672	581	565	578	775	877	408	695	541	555	502	350	593	479	187	237
*Polysphaeridium zoharyi** (*Pyrodinium bahamense* var. *compressum*)	144	277	74	130	44	36	9	5	19	16	0	0	33	0	0	0
Pheopolykrikos hartmannii	10	0	0	0	5	0	0	0	0	0	0	4	0	0	0	0
Scrippsiella spp.	41	27	49	32	42	35	39	26	26	34	52	23	58	39	0	11
Polykrikos kofoidii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Trinovantedinium capitatum** (*Protoperidinium* sp.)	5	0	0	9	5	5	4	5	4	14	0	4	0	0	0	0
*Selenopemphix quanta** (*Protoperidinium conicum*)	40	28	35	19	34	50	26	57	43	58	14	20	32	30	23	12
Diplopelta parva	11	9	5	9	42	0	4	20	4	29	13	0	0	8	0	0
*Quinquecuspis concretas** (*Protoperidinium leonis*)	22	21	9	4	20	6	4	10	5	4	9	0	13	8	4	7
*Votadinium calbum** (*Protoperidinium oblongum*)	0	0	0	0	5	0	0	0	0	4	0	8	0	0	0	0
Protoperidinium latissinum	6	5	0	4	0	0	0	5	4	0	0	0	0	0	0	4
*Stelladinium reidii** (*Protoperidinium compressum*)	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Stelladinium abei** (*Protoperidinium* sp.)	5	5	5	0	5	5	0	0	0	4	5	0	0	0	4	0
*Stelladinium robustum** (*Protoperidinium* sp.)	0	0	0	0	0	6	0	0	0	0	0	0	0	4	0	0
*Selenopemphix nephroides** (*Protoperidinium subinerme*)	0	9	0	0	9	10	0	0	0	0	0	4	4	0	0	0
*Brigantedinium irregulare** (*Protoperidinium denticulatum*)	0	9	0	0	0	5	4	0	0	0	4	0	0	0	4	0
*Brigantedinium** spp.	49	62	39	28	121	69	26	68	55	75	27	47	42	26	39	4
*Dubridinium** sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
total cyst density	3113	3216	2630	3465	2956	3901	2294	3555	2812	2377	2526	1687	3061	3041	1727	1400

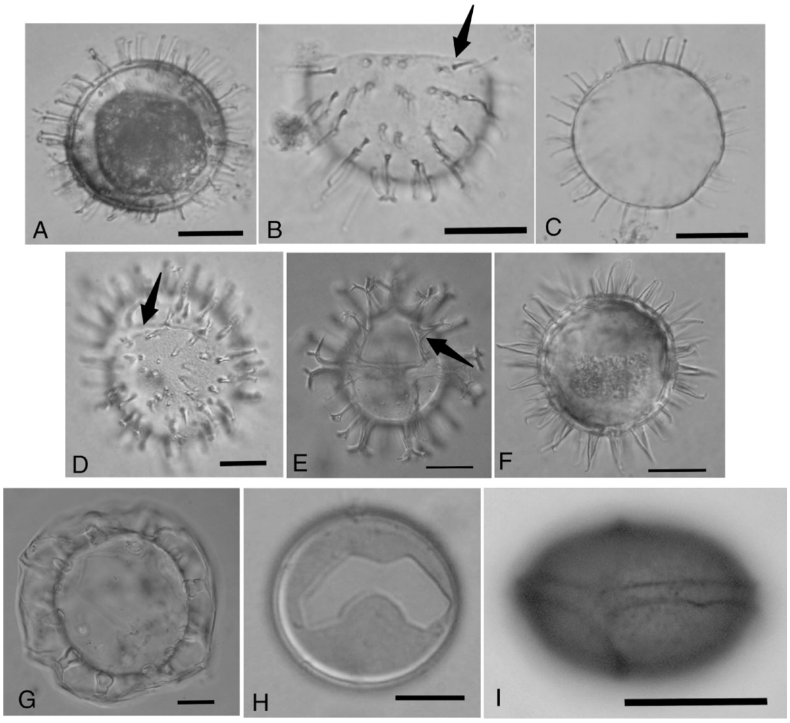

Fig. 3. Photomicrographs of dinoflagellate cysts observed in the Ambon Bay and Hurun Bay cores. A–D. Cysts of *Pyrodinium bahamense* var. *compressum* (Böhm) Steidinger, Tester et Taylor (paleontological name of *Polysphaeridium zoharyi** (Rossignol) Bujak et al.); A: cyst with protoplasm (living cyst), B: ventral view, many slender, cylindrical to tubiform processes with capitate and aculeate tips, arrow showing surface of epicystal archeopyle suture, C: polar view, showing epicystal archeopyle, D: ventral–lateral view, showing coarsely granular surface of cyst wall, arrow showing archeopyle sutures, E: *Spiniferites hyperacanthus** (Deflandre et Cookson) Sarjeant, arrow showing an intergonal process, F: *Lingulodinium machaerophorum** (Deflandre et Cookson) Wall (living cyst), G: *Tuberculodinium vancampoae** (Rossignol) Wall, H: *Brigantedinium irregulare** Matsuoka, I: *Dubridinium caperatum** Reid (scale bar: 20 μm).

sity of 201 cysts g^{-1} above 34–36 cm depth (ca. 1910), which was slightly higher than the average cyst density below 36–38 cm depth (56 cysts g^{-1}).

Vertical distribution of dinoflagellate cysts in Hurun Bay

A total of 20 species of dinoflagellate cysts, including Pbc, were identified in all samples from Hurun Bay (Table 2, Fig. 5). Detection limit of cysts in the core was about 4 cysts g^{-1} in the cyst analysis of the Hurun Bay core. Cysts of Pbc first occurred at 50–52 cm depth (6 cysts g^{-1}, depositional age based on ^{210}Pb method: ca. 1820), and then were found again at 42–44 cm depth (17 cysts g^{-1}, ca. 1850). The cysts were continuously observed above 32–34 cm depth (ca. 1880) and the density was over 282 cysts g^{-1} above 14–16 cm depth (ca. 1950), which was higher than below 16–18 cm depth (18～98 cysts g^{-1}).

There were 5～17 cyst species recorded from 56–58 cm (ca. 1800) depth to 42–44 cm depth (ca. 1850) in comparison with 11～22 cyst species at other depths of the core. Also, the cyst density, which ranged from 64～383 cysts g^{-1}, at depths from 56–58 cm to 42–44 cm depth was relatively lower than that at other depths of the core (317～1903 cysts g^{-1}). Other cysts, such as *Operculodinium centrocarpum** sensu Wall et Dale, *S. hyperacanthus** and *Selenopemphix quanta** Bradford, were observed in most samples. However, changes in the floral assemblage might not be affected by the same factors that regulate Pbc cyst occurrence.

Grain size analysis of the Hurun Bay core

In this core, silt was dominant (69.7～89.7%) at all depths, except from 56–58 cm depth to 42–44 cm depth (Fig. 6). However, the amount of silt at 58–60 cm depth (71.2%) rapidly decreased to 28.4% at 56–58 cm depth. Sand changed from 27.9% at 58–60 cm depth to 68.1% at 56–58 cm depth. Silt between 56–58 cm depth and 48–50 cm depth (ca. 1830) was low, and between 46–48 cm to 42–44 cm depth gradually increased from 23.7% to 71.2%.

Wood fragments were also observed from 46–48 cm to 42–44 cm depth, although no wood fragments occurred above 40–42 cm depth.

The depositional age based on the ^{210}Pb method is discussed in the following section, in response to the relationship between characteristic grain size and history around

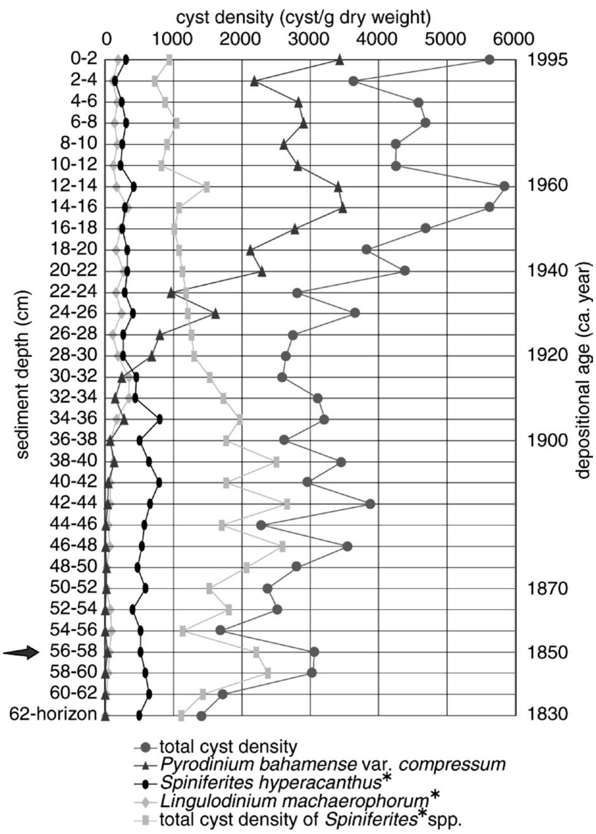

Fig. 4. Vertical distribution of dinoflagellate cysts in the Ambon Bay core. Arrow indicates where *Pyrodinium bahamense* var. *compressum* cysts first occurred at 56–58 cm depth, deposited in ca. 1850.

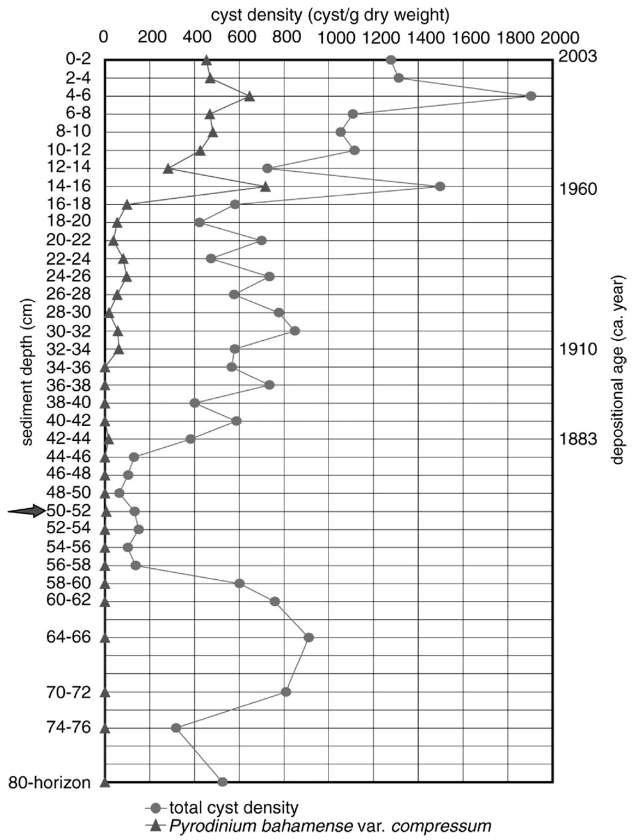

Fig. 5. Vertical distribution of dinoflagellate cysts in the Hurun Bay core. Arrow indicates where *Pyrodinium bahamense* var. *compressum* cysts first occurred at 50–52 cm depth, deposited in 1883.

Hurun Bay.

Discussion

Floral changes of dinoflagellate cysts in Ambon Bay

Pyrodinium bahamense var. *compressum* was recorded as the causative species of a PSP event in June 1994 at a village located on the shore of the inner parts of Ambon Bay where previously Pbc blooms had not been reported (Wiadnyana et al. 1996). The cell density of motile Pbc ranged from 0.4 to 1600 cells L^{-1} near the village where the PSP incident happened. However, Pbc cysts were detected at 56–58 cm depth in the sediment core where the depositional age was estimated at ca. 1850. Thereafter, Pbc cysts have continuously occurred since ca. 1870, and increased gradually from ca. 1920. Evidently, Pbc occurred in ca. 1850 and has continuously occurred since ca. 1870, before the PSP incident caused by Pbc was recorded in 1994.

Cyst densities of some species including Pbc cysts also varied. *Spiniferites** spp. decreased from ca. 1910, and particularly, *S. hyperacanthus**, which occurs in all samples, has decreased in numbers gradually since ca. 1910. *Lingulodinium machaerophorum** slightly increased around 1910. Consequently, the dominant species in the dinoflagellate cyst assemblage shifted from *S. hyperacanthus** to Pbc between ca. 1910 and ca. 1920. Such a floral change in the dinoflagellate cyst assemblage through time possibly reflects a change in environmental factors such as temperature, salinity and/or nutrients (e.g., Wall et al. 1977, Matsuoka 1999). However, in Southeast Asia, no such study on the relationship between dinoflagellate cyst assemblages and environmental factors has been carried out. Therefore, we can not indicate which environmental factors are most likely to have affected the change in the dominant cyst species from *S. hyperacanthus** to Pbc in Ambon Bay.

Immigrants to Ambon Island carried out slash-and-burn farming, and their population accounted for about 10% of the population of 107,000 people in 1930 (Palmer 2004). From the increase in agricultural activities by these people, soil erosion by farming can be presumed to have carried additional nutrients and soil (Kamp-Nielsen et al. 2002) into the coastal waters of Ambon Bay, which is surrounded by mangrove forests. The increase of Pbc cysts after ca. 1920 in Ambon Bay might be associated with environmental change caused by anthropogenic activities such as activities by immigrants.

Table 2. Densities of dinoflagellate cysts in the Hurun Bay core. (cysts g^{-1})

cyst name/sediment depth (cm)	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36
Alexandrium tamarense type	11	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alexandrium affine type	13	11	59	10	0	0	0	10	0	0	0	0	0	0	0	0	0	19
Alexandrium minutum type	22	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Lingulodinium machaerophorum** (*Lingulodinium polyedrum*)	22	0	0	0	11	0	20	11	9	0	10	0	0	0	0	0	0	0
*Spiniferites ramosus** (*Gonyaulax* sp.)	11	0	0	0	11	0	0	0	0	9	9	0	0	0	0	0	0	0
*Spiniferites bulloideus** (*Gonyaulax scrippsae*)	11	34	33	21	0	11	0	29	18	28	10	17	28	19	27	39	0	28
*Spiniferites mirabilis** (*Gonyaulax spinifera*)	0	34	0	10	0	9	0	10	11	0	30	0	9	0	0	0	0	0
*Spiniferites hyperacanthus** (*Gonyaulax* sp.)	0	24	37	10	22	53	0	21	0	9	21	0	37	10	0	19	9	9
Spiniferites cf. *delicatus** (*Gonyaulax* sp.)	0	0	0	0	0	0	0	0	11	9	0	0	0	0	0	0	0	0
*Spiniferites** spp.	0	0	0	0	0	0	11	0	0	0	10	0	0	0	0	0	0	0
*Polysphaeridium zoharyi** (*Pyrodinium bahamense* var. *compressum*)	454	470	646	468	483	426	282	718	98	55	38	83	97	55	18	58	62	0
*Tuberculodinium vancampoae** (*Pyrophacus steinii*)	34	11	0	0	0	0	0	0	0	9	0	0	0	9	0	0	0	0
*Operculodinium centrocarpum** (*Protoceratium reticulatum*)	22	13	13	19	0	19	9	50	11	9	47	9	9	19	9	10	19	9
Pheopolykrikos hartmannii	34	25	0	0	0	9	0	10	0	9	9	0	30	0	9	9	0	28
Scrippsiella spp.	39	22	114	10	0	120	39	178	29	20	91	0	159	38	9	104	19	113
Polykrikos kofoidii	0	0	0	0	0	0	0	0	0	0	0	26	19	10	28	9	38	0
*Trinovantedinium pallidifurvum** (*Protoperidinium* sp.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Trinovantedinium capitatum** (*Protoperidinium* sp.)	22	0	0	11	11	0	0	10	0	0	19	0	0	10	9	0	9	0
*Selenopemphix quanta** (*Protoperidinium conicum*)	252	303	425	234	186	139	22	0	52	19	37	10	39	26	105	45	18	19
Diplopelta parva	11	0	70	22	11	86	37	50	29	9	59	35	47	19	18	28	26	19
*Quinquecuspis concretas** (*Protoperidinium leonis*)	0	24	35	49	33	28	31	21	38	36	38	48	0	28	64	40	29	28
*Votadinium calbum** (*Protoperidinium oblongum*)	0	0	37	0	11	11	18	0	28	26	0	0	30	29	21	10	28	0
Protoperidinium latissinum	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
*Stelladinium stellatum** (*Protoperidinium compressum*)	0	11	0	10	0	0	0	0	9	0	0	0	0	0	0	0	0	0
*Stelladinium reidii** (*Protoperidinium compressum*)	0	0	11	0	0	0	0	10	0	0	0	0	0	0	0	0	0	9
*Stelladinium abei** (*Protoperidinium* sp.)	0	11	46	0	33	0	18	11	0	9	19	57	0	35	45	86	29	28
*Stelladinium robustum** (*Protoperidinium* sp.)	0	22	13	0	0	0	9	0	0	0	0	9	9	0	9	0	9	0
*Selenopemphix nephroides** (*Protoperidinium subinerme*)	0	11	11	19	11	0	18	22	9	9	10	0	0	19	30	37	9	0

Table 2. (cont.) (cysts g^{-1})

cyst name/sediment depth (cm)	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36
*Brigantedinium irregulare** (*Protoperidinium denticulatum*)	60	22	46	11	11	11	39	10	9	26	28	35	11	29	76	48	38	38
*Brigantedinium asymmetricum** (Unknown)	13	11	11	0	0	0	0	0	20	0	9	0	0	0	0	9	9	0
*Brigantedinium simplex** (*Protoperidinium conicoides*)	0	0	11	0	0	0	0	0	28	0	10	0	9	0	0	19	0	0
*Birgantedinium majusculum** (*Protoperidinium pentagonum*)	0	0	24	0	0	9	18	10	0	0	9	0	0	0	9	0	0	9
*Brigantedinium** spp.	206	218	240	172	165	173	136	308	162	106	167	116	190	202	252	250	192	198
*Dubridinium caperatum** (*Preperidinium meunierii*)	0	0	0	0	0	0	0	0	0	0	0	0	11	0	10	0	0	0
*Dubridinium** sp.	37	13	24	32	44	11	18	11	9	28	18	29	0	20	28	27	37	9
total cyst density	1276	1311	1903	1106	1053	1115	725	1497	581	422	699	472	734	576	777	848	580	565

Table 2. (cont.) (cysts g-1)

cyst name/sediment depth (cm)	36–38	38–40	40–42	42–44	44–46	46–48	48–50	50–52	52–54	54–56	56–58	58–60	60–62	64–66	70–72	74–76	80–bottom
Alexandrium tamarense types	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alexandrium affine type	0	0	0	0	0	0	0	0	0	0	6	9	0	0	0	0	0
Alexandrium minutum type	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Lingulodinium machaerophorum** (*Lingulodinium polyedrum*)	0	8	9	0	0	0	0	0	0	0	0	0	0	0	0	7	0
*Spiniferites ramosus** (*Gonyaulax* sp.)	10	0	0	0	0	0	0	0	0	0	0	9	6	10	0	7	0
*Spiniferites bulloideus** (*Gonyaulax scrippsae*)	9	10	11	25	0	0	0	0	19	0	0	9	11	10	0	0	18
*Spiniferites mirabilis** (*Gonyaulax spinifera*)	0	0	0	8	0	0	0	0	7	0	0	0	11	0	10	0	0
*Spiniferites hyperacanthus** (*Gonyaulax* sp.)	0	0	9	10	10	0	0	0	6	4	5	20	0	27	30	22	9
*Spiniferites cf. delicatus** (*Gonyaulax* sp.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
*Spiniferites** spp.	0	0	0	8	0	13	0	0	13	0	11	0	0	10	0	0	0
*Polysphaeridium zoharyi** (*Pyrodinium bahamense* var. *compressum*)	0	0	0	17	0	0	0	6	0	0	0	0	0	0	0	0	0
*Tuberculodinium vancampoae** (*Pyrophacus steinii*)	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	7	9
*Operculodinium centrocarpum** (*Protoceratium reticulatum*)	0	0	40	8	0	0	5	12	7	0	6	18	29	40	49	0	45
Pheopolykrikos hartmannii	10	19	19	0	0	0	0	6	0	0	0	0	6	37	128	0	9
Scrippsiella spp.	18	10	48	72	0	14	17	12	31	4	17	58	117	66	89	44	63
Polykrikos kofoidii	0	0	19	10	0	0	0	0	0	0	0	0	6	0	0	0	18
*Trinovantedinium pallidifurvum** (*Protoperidinium* sp.)	0	0	0	0	0	0	0	0	0	4	0	0	7	0	0	0	0
*Trinovantedinium capitatum** (*Protoperidinium* sp.)	10	0	0	0	0	0	0	12	0	0	6	0	0	0	0	0	0
*Selenopemphix quanta** (*Protoperidinium conicum*)	37	26	30	41	0	0	11	6	6	9	0	11	43	70	69	7	9
Diplopelta parva	9	18	72	10	10	7	0	0	0	4	5	20	56	106	39	22	45
*Quinquecuspis concretas** (*Protoperidinium leonis*)	46	34	19	25	15	0	0	0	0	0	16	9	18	47	49	22	45
*Votadinium calbum** (*Protoperidinium oblongum*)	26	8	9	0	0	0	0	0	0	0	0	0	22	37	10	0	0
Protoperidinium latissinum	0	0	0	0	0	0	0	0	0	0	0	20	22	0	0	0	0
*Stelladinium stellatum** (*Protoperidinium compressum*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Stelladinium reidii** (*Protoperidinium compressum*)	9	0	11	0	0	0	0	0	0	4	0	0	0	20	10	0	0
*Stelladinium abei** (*Protoperidinium* sp.)	35	17	19	0	0	8	0	6	0	10	0	0	11	43	39	0	36
*Stelladinium robustum** (*Protoperidinium* sp.)	10	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9
*Selenopemphix nephroides** (*Protoperidinium subinerme*)	18	0	39	10	0	0	0	0	0	0	0	29	11	10	10	7	18
*Brigantedinium irregulare** (*Protoperidinium denticulatum*)	81	43	37	8	0	0	0	6	0	13	6	37	17	66	20	0	27
*Brigantedinium asymmetricum** (*Unknown*)	0	10	20	8	0	7	0	0	6	9	6	0	6	0	0	0	0
*Brigantedinium simplex** (*Protoperidinium conicoides*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Birgantedinium majusculum** (*Protoperidinium pentagonum*)	0	0	0	8	0	0	0	0	0	0	0	9	0	0	0	0	0
*Brigantedinium** spp.	359	189	157	106	84	48	27	66	49	39	52	332	309	252	217	148	145
*Dubridinium caperatum** (*Preperidinium meunierii*)	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Dubridinium** sp.	47	8	0	8	10	7	5	0	0	0	0	11	49	60	30	22	18
total cyst density	733	400	587	383	129	104	64	132	151	102	136	600	758	910	808	317	524

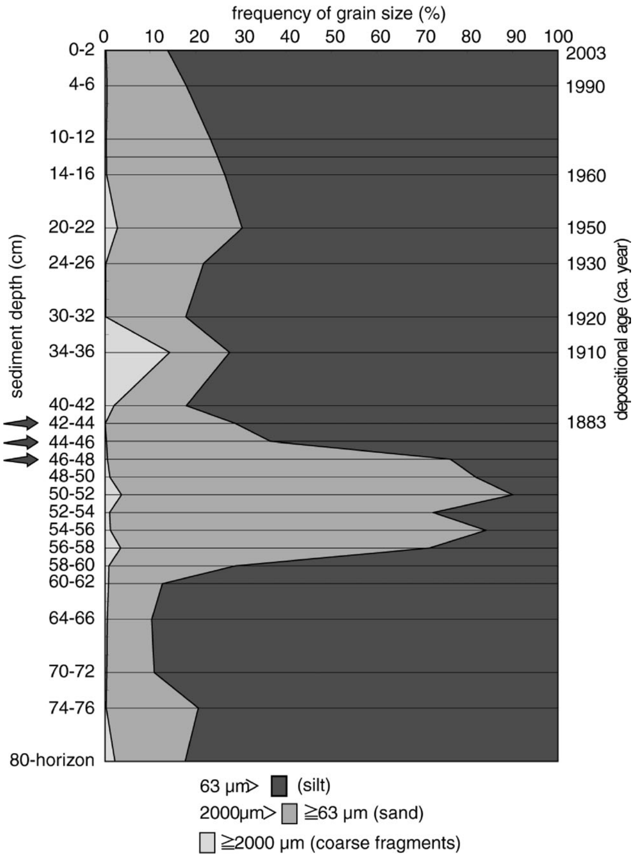

Fig. 6. Frequency of grain size in the Hurun Bay core. Sediment between 46–48 cm to 42–44 cm contained wood fragments (indicated by arrows).

Relationship between turbidite sequence and the first occurrence of Pbc cysts

The grain size at 56–58 cm depth became rapidly coarser than that at 58–60 cm depth. However, the sand fraction from 46–48 cm depth to 42–44 cm depth gradually decreased. The sediment at 44–46 cm depth and 42–44 cm depth contained a lot of small wood fragments. These sediment facies reflect a turbidite sequence probably formed by a tsunami associated with the eruption of Mt. Krakatau, located between Sumatra and Java islands, in 1883 (Decker & Hadikusumo 1961). This volcano has erupted several times after 1883, but no concomitant change in the grain size of sediment was observed in this core. Consequently, there are two sedimentation rates based on two different datum planes; one (0.35 cm year^{-1}) is based on the historical evidence by the eruption of Mt. Krakatau and another (0.27 cm year^{-1}) is based on ^{210}Pb measurement. Although the sedimentation rate given by historical evidence roughly coincided with the rate measured by ^{210}Pb dating, the depositional age at each depth was calculated by the sedimentation rate based on historical evidence, because the depositional age calculated by the ^{210}Pb method might have a larger margin of error in deeper depths.

Vegetative cells of Pbc were reported to be very abundant, with more than 89,000 cells L^{-1} during the bloom in 1999 in Hurun Bay (Widiarti et al. 2000). In the core sample collected from this bay, Pbc cysts were first detected at 50–52 cm depth, and then occurred at 42–44 cm depth, again. These intervals include a part in the turbidite sequence caused by the eruption of Mt. Krakatau in 1883. From these results, Pbc cysts can be said to have occurred around Hurun Bay since at least 1883. Consequently, using the sedimentation rate based on the eruption of Mt. Krakatau, Pbc cysts have continuously occurred since ca. 1910 (32–34 cm depth), and have rapidly increased from ca. 1960 (14–16 cm depth).

Cause of Pbc introduction

Based on the vertical distribution of dinoflagellate cysts, Pbc cysts occurred at least by ca. 1850 in Ambon Bay and by 1883 in Hurun Bay. These cysts have continuously occurred since ca. 1870 in Ambon Bay and ca. 1910 in Hurun Bay. In particular, Pbc gradually increased from ca. 1920, has become more abundant since ca. 1940 in Ambon Bay, and has rapidly increased from ca. 1960 in Hurun Bay. These data suggest that vegetative cells of Pbc occurred before the first detection of PSP and bloom events in 1994 and 1999, in Ambon Bay and Hurun Bay, respectively (Wiadnyana et al. 1996, Widiarti et al. 2000).

Ballast water is one of the anthropogenic activities and mechanisms suggested to have led to expansion of the geographical distribution of dinoflagellates (Anderson 1989). However, there was no large port to hold vessels with ballast tanks in either Ambon or Hurun bays when the Pbc cysts first occurred. Although harmful phytoplankton could be artificially transported with shellfish seeds (Anderson 1989), such transportation for Pbc has not been reported so far in these regions. Consequently, introduction of Pbc into both bays is considered not to have been caused by ballast water or transplantation with shellfish seeds, but probably by natural factors. Furthermore, Pbc cysts first occurred and increased before the first detection of Pbc vegetative cells in both Ambon Bay and Hurun Bay. Therefore, the vertical distribution of Pbc cysts suggests that the first detection of the cryptogenic population of Pbc in Ambon Bay and Hurun Bay could be attributed to visual realization, as a result of red tide discoloration and PSP in 1994 and 1999, respectively. Historical records of targeted dinoflagellate cysts can suggest clues about past occurrences, and provide information on their geographical range expansion (e.g. McMinn et al. 1997, Matsuoka et al. 2006). Investigations using the same method in other areas are required to understand the mechanism of geographical expansion for targeted dinoflagellates, in order to prevent any further invasions.

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