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Article

What factors control the distribution of aquatic palynomorphs in surface sediments of Osaka Bay, central Japan? With special reference to the cysts of *Alexandrium catenella/pacificum* (Dinophyceae)

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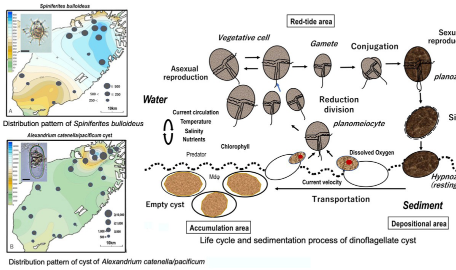
ABSTRACT



Distribution contour maps of aquatic palynomorphs in Osaka Bay were made to determine their horizontal distribution using 21 surface sediment samples. The aquatic palynomorphs consisted of planktonic autotrophic dinoflagellate cysts, raphidophyte cysts, heterotrophic dinoflagellate cysts, heterotrophic ciliate cysts, heterotrophic crustacean resting eggs, benthic foraminiferal linings, turbellarian egg capsules, as well as planktonic chlorophycean resistant cells. Most of these resistant cells are thought to have been transported via the Yodo River (*Yodogawa*), since most of them originated from freshwater areas, mainly Lake Biwa. Most of these palynomorphs were distributed mainly off the mouth of the Yodo River, while benthic foraminiferal linings were distributed mainly off Kobe Airport. A canonical correspondence analysis between each palynomorph and environmental factors including distance from the mouth of the Yodo River (Distance A), water depth, dissolved oxygen (DO), surface water temperature, salinity, transparency, grain size composition (Mdφ), chemical oxygen demand (COD), nitrogen, phosphorus, and chlorophyll was performed. The results showed that the autotrophic dinoflagellate *Spiniferites bulloideus* and foraminiferal linings were strongly positively correlated with Distance A and Mdφ but negatively correlated with DO, whereas cysts of *Alexandrium catenella/pacificum*, which often form red tides with paralytic shellfish poisoning in Osaka Bay, were positively correlated with water depth and negatively correlated with COD. Turbellarian egg capsules, heterotrophic dinoflagellate cysts, *Echinidinium* spp., and foraminiferal linings were positively correlated with salinity and transparency. However, these results do not logically explain the relationship between the distribution of these palynomorphs and environmental factors, because the distribution of aquatic palynomorphs in a narrow sea area with complex marine environments such as Osaka Bay is caused by a combination of biological factors and physical factors that act after the formation of palynomorphs.

Keywords: Aquatic palynomorph, Canonical correspondence analysis, Cyst of *Alexandrium catenella/pacificum*, Horizontal distribution of palynomorph, Osaka Bay

Introduction

Microfossils both with organic walls such as dinoflagellate cysts, pollen, and spores and with mineralized exoskeletons such as foraminifera and radiolarians are powerful tools for the reconstruction of past marine environments. The study of biogenic remains in surface sediments is essential for elucidating the fossilization process of aquatic micro-organisms. These remains are derived from a wide variety of organisms, especially microfossils with organic walls, which are collectively referred to

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as palynomorphs (Travers, 2007). Several different acidic chemicals are used to remove siliceous and calcareous particles and extract microfossils from sediments, and the residues with a certain morphology are called palynomorphs. Pollen, fern spores, dinoflagellate cysts, and other microbiological remains are representative palynomorphs because they are diverse and come from all regions of the earth. Remains of aquatic micro-organisms are called aquatic palynomorphs (Klyuvitkina and Novichkova, 2022; Matsuoka *et al.*, 2022), and dinoflagellate cysts are representative of those remains (Table 1).

For the reconstruction of paleoceanographic environments, it is fundamentally necessary to clarify the environmental factors that control their distribution (Cross *et al.*, 1966; Wall *et al.*, 1977). Studies on dinoflagellate cysts, one of most common palynomorphs, have been conducted since the late 1990s using multivariate analysis methods (e.g. Marret and de Vernal, 1997; Dale *et al.*, 2002; Esper and Zonneveld, 2002). The areas studied were open seas, coastal to deep sea transects, lagoons, and inner bays, as summarized by de Vernal *et al.* (2020).

However, a wide variety of micro-organisms inhabit aquatic environments, and some organisms with mineralized exoskeletons (e.g. benthic foraminifera) may leave behind organic membranes when treated with chemicals palynologically. Such micro-organisms, including cysts of ciliates, foraminiferal linings, crustacean resting eggs, and turbellarian egg capsules, are also classified as aquatic palynomorphs (Matsuoka *et al.*, 2022). Environmental factors controlling the distribution of aquatic palynomorphs preserved in sediments, mainly dinoflagellate cysts, have been studied globally in coastal areas of inner bays in relation to water temperature, salinity, nutrients, and distance from land. The importance of the biological processes related to production of cysts and/or resistant cells and the physical processes effected after cyst production has been pointed out (Wall *et al.*, 1977). However, the biological processes involved in the production of cysts (i.e., how they are formed) have not yet been sufficiently investigated.

In recent years, red tides and shellfish poisoning caused by the dinoflagellates *Alexandrium catenella* and *A. pacificum* have frequently been observed in coastal areas of central Japan such as Osaka Bay. Studies of the harmful red tides caused by *Alexandrium* species have been conducted (Yamamoto *et al.*, 2009). In the field of harmful algal bloom (HAB) research, the presence of resting cysts is emphasized as a seed for the next bloom formation (e.g. Anderson and Wall, 1978). *A. catenella* (Whedon and Kofoid) Balech and *A. pacificum* R.W. Litaker can form resting cysts that can be preserved in sediments (John *et al.*, 2014; Mertens *et al.*, 2020). Because the cysts of toxic and harmful species function as seeds for the next bloom, they have been studied not only from the perspective of environmental science, such as HAB studies, but also from the perspective of fisheries science (Yamaguchi *et al.*, 1996; Yamamoto *et al.*, 2006). When conducting research on resting cysts of harmful and/or toxic microalgae as seeds, one of the main issues is to elucidate the location of seedling areas and their formation mechanisms in the target marine area. In addition to elucidating the location and mechanism of formation of these cysts, it is also important to monitor the appearance of planktonic vegetative cells, especially during the red tide outbreak period. From the viewpoint of HAB studies, the timing of *A. catenella* and *A. pacificum* emergence and the growth process of vegetative cells have been actively monitored in Osaka Bay (Yamamoto *et al.*, 2017). Thus, by studying behaviors of *A. catenella*, *A. pacificum*, and the raphidophycean *Chattonella marina* (Subramanyan) Y. Hada and Chihara in Osaka Bay, we can learn both the biological process of cyst formation, in which aquatic palynomorphs are preserved in the sediment, and the physical process of their transportation and accumulation while suspended in the sea or after being deposited on the seafloor.

As in other areas, surface sediments in Osaka Bay have been found to contain various aquatic palynomorphs, including dinoflagellate cysts, acritarchs, resistant cells of green algae, foraminiferal linings, crustacean resting eggs, and turbellarian egg capsules (Matsuoka and Ishii, 2018). In this study, we aimed to understand what environmental factors control the distribution of aquatic palynomorphs preserved in the surface sediments of Osaka Bay and to clarify the distribution of both vegetative cells and cysts of *A. catenella/pacificum*, which can provide information on biological processes including formation of planozygotes. The distribution of vegetative cells and cysts of *A. catenella/pacificum*, which are ecologically different from those of planktonic and benthic aquatic palynomorphs, were compared.

Material and methods

Osaka Bay

Osaka Bay is located in the central part of Honshu, the main island of Japan, bordering a typical semi-enclosed sea. This bay is ellipsoidal in shape, oriented in a northeast-to-southwest direction, and connected to the Pacific Ocean (via the Kii Channel) to the south and the Seto Inland Sea (via Harima Nada) to the northwest by the Kitan Strait (or Tomogashima Channel) and Akashi Strait, respectively. According to the Osaka Bay Environmental Database (Kinki Regional Development Bureau, 2004), the bay is approximately 1,447 km^2 in surface area, about 30.4 m in mean water depth, and 44 billion m^3 in water volume. The central to eastern parts are rather

Table 1. List of major aquatic palynomorphs. (*: the scientific name of plankton.)

	Aquatic palynomorphs								
	Taxa	Habitat	Life mode	Mode of nutrition	Common remaining parts	Morphological types and features		Representative fossil name (example)	Reference
Procaryote	Cyanobacteria	freshwater, brackishwater, marine		photosynthetic	heterocyst, akinete sheath	elongatedly rectangular, ellepsoidal, fusiform, subspherical with tail	Anabena		van Geel 2001
Protozoa	Acritarch	mainly marine (commonly brackish water, freshwater)	probably planktonic autotrophic, heterotrophic, mixotrophic (?)	mainly photosynthetic, heterotrophic (?) mixotrophic (?)	resting cell?	sperical to subspherical type ellipsoidal type peridinioid type ovoidal type ornamented with various appendages and openings		*Baltisphaeridium** *Micrhystridium**	Downie *et al.* 1963
	Dinophyta	abundantly marine (commonly brackish, freshwater)	mainly planktonic	photosynthetic, heterotrophic (bacteria, diatoms, other phototrophic dinoflagellates, ciliates, nonnoflagellates, colorless deterial particles) mixotrophic	resting cyst	sperical to subspherical type ellipsoidal type peridinioid type ovoidal type with various surface ornamentations transverse and longitudinal furrows archeopyle		*Spiniferites* *Brigantedinium*	Wall and Dale 1968 Jacobson and Anderson 1986 Hansen 1991
	Chlorophyta	mainly freshwater, brackish water, marine	planktonic, benthic	photosynthetic	resting cell, zygospore, coenobia	spherical, ovoidal, elongatedly ovoidal, square, dome-shaped	Oedogoniaceae (*Oedpgonium*) Botryococcaceae (*Botryococcus*) Hydrodictyaceae (*Pediastrum*) Scenedesmaceae (*Scenedesmus*) *Zygnemataceae* (*Spitogyra*) *Desmidiales* (*Cosmarium*)	*Ovoidites**	van Geel 2001 Sumilovskikh *et al.* 2021
	Prasinophyta	mainly marine	planktonic	photosynthetic	resting phycoma	sperical to subspherical type with or without fenestrate walls and menbranous surface ornaments	Pterosperma Pterospermella	*Tasmanites,* *Cymatiosphaera* *Pleurozonaria*	Wall 1962 Park *et al.* 1978
	Eugranophyta	mainly freshwater, brackish to marine	planktonic	photosynthetic, heterotrophic (bacteria, smaller flagellates)	loricate	shortly ellipsoidal with short neck	*Strombomonas Trachelomonas*	*Chomotriletes* *Pseudoschizaea* *Concentricystes*	Sumilovskikh *et al.* 2019 de Schootbrugge *et al.* 2024
	Amoeboza	freshwater to brackishwater	benthic	heterotrophic (organic detritus?)	organic shell	discoidal, cup-shaped and elongated ellipsoidal with simple opning,			Meisterfeld 2002
	Foraminifera (microforaminiferal linings)	marine, brackishwater	benthic	heterotrophic (bacteria, pennate diatoms, micro-algae, other protozoans, and dead organic material)	organic inner lining	single chamber type uniserial type biserial type coiled type compound type			Stancliffe 1989 Kitazato 1981 Topping *et al.* 2006 Armstrong and Braseir 2007
	Ciliophora	marine, freshwater, brackishwater	planktonic	heterotrophic (bacteria, diatoms, dinoflagellates, microflagellates (chlorophytes, chrysophytes, pelagophytes, prasiophytes, pyrenomonadaceae), raphidophytes, other ciliates, small organic particles)	lorica	circular cylindrical inverted frustum	Strombidium Hexasterias Halodinium Radiosperma		Dolan *et al.* 2013 Reid and John 1978 Gurdebeke *et al.* 2018 Taniguchi 1975
					resting cyst	sperical type elliptical type spindle type disk-shaped type			
Fungi	Fungi	freshwater, brackishwater, marine	synbiont	heterotrophic		spore, fruiting bodies, hyphae		chlamydospore conidophore	Travers 2007 van Geel 2001
Metazoa	Crustaceae	marine, freshwater, brackishwater	planktonic heterotrophic	heterotrophic (diatoms, dinoflagellates, ciliates, fecal pellets, organic detritus)	exoskeleton	intercoxal plate tail sternite appendage, etc			van Wavern 1993
					egg envelope	discoid type folded discoid type double saucer shapd type fissured sphere type hemisphere type ellise type fusiform type double fusiform type		*Cobricosphaeridium*	van Wavern 1993 McMinn *et al.* 1992 Sherr and Sherr 1988 Uye and Takamatsu 1990
	Ostracod	marine, freshwater, brackishwater	benthic	heterotrophic	mandibles and carapace linings				Mudie *et al.* 2021
	Polychaeta	freshwater, blackishwater, marine	benthic	heterotrophic	mouth-lining parts			scolecodont	Travers 2007 Mudie *et al.* 2021
	Turbellaria	freswater Freshwater, brackishwater, marine	benthic	heterotrophic	egg capsule	stalk Group ellipsoidal type cup-shaped type spherical type non-Stalk Group ellipsoidal/cup-shaped type sp spherical type		*Palaeocystmopsis*	Mudie *et al.* 2021 Matsuoka and Ando 2021
	Tardigrada	terrestrial, freshwater, brackishwater, marine	benthic	heterotrophic (planta, algae, small invertebrates)	egg	spherical with short conical, bulbose, short tarpered, and bifurcate projection types spherical with fin-like ornament type ellepsoidal with fin like ornament type	*Richtersius Macrobiotus Paramacrobiotus Dactylobiotus*		Jankovska 1991 Sumilovskikh *et al.* 2019 Šatkauskine 2012 Guidetti *et al.* 2006
Plant	Fern spore	terrestrial							
	Pollen	terrestrial							

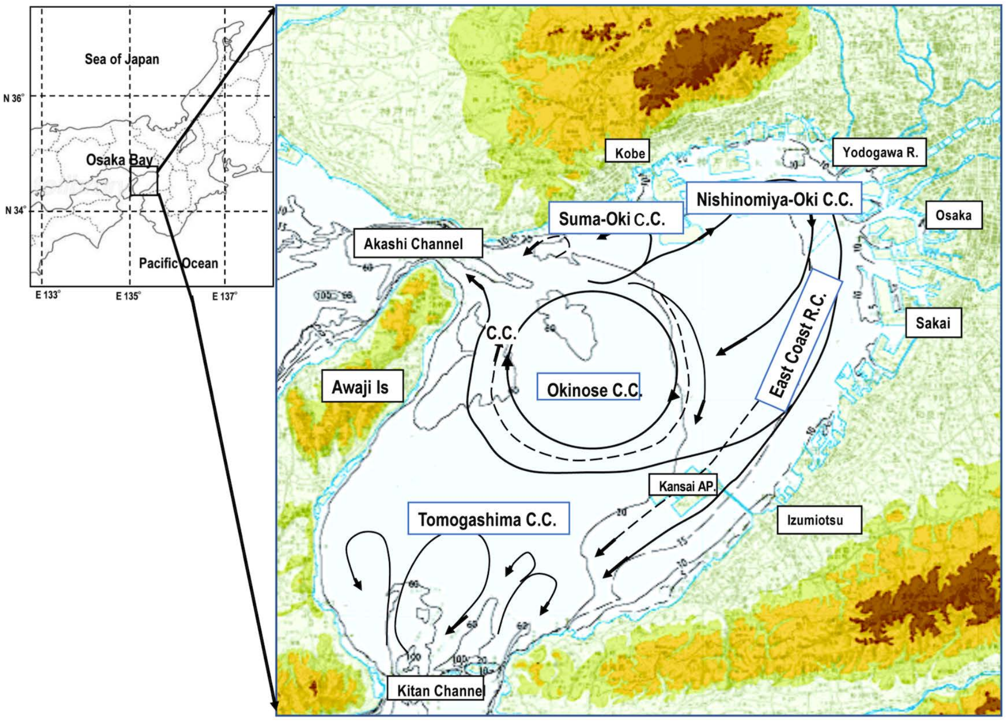


Figure 1. Topographic characteristics and current circulation patterns of Osaka Bay. The bay is connected to the Pacific Ocean (via the Kii Channel) to the south and the Seto Inland Sea (via Harima Nada) to the northwest by the Kitan Strait (or Tomogashima Channel) and Akashi Strait, respectively. Current systems are reproduced from Fujiwara *et al.* (1989).

flat in bottom topography, and sediments are muddy in the northeastern to central parts and sandy in the western parts (Yokoyama and Sano, 2015). Coarse gravelly sediments are distributed near the two connecting channels (Nagaoka *et al.*, 2004). Fujiwara *et al.* (1989) clarified that the following currents are observed in Osaka Bay: the clockwise Okinose Circulation Current in the center of the bay, the Nishinomiya-Oki Circulation Current and the following East Coast Residual Current along the northern and eastern margins of the bay, and the Suma-Oki Countercurrent off Kobe to Suma.

Several rivers flow into Osaka Bay along the northern to northeastern coast. The largest of these is the Yodo River (*Yodogawa*), which has a total annual runoff approximately an order of magnitude greater than any of the other rivers at 8.5 billion m^3/year (https://www.japanriver.or.jp/river_law/kasenzu/kasenzu_gaiyou/kinki_r/060yodo.htm). Seawater of the Pacific Ocean flows into the bay via the Kii Channel (Figure 1).

Sampling site

Samples covering almost the entire area of Osaka Bay were collected at 12 sites in 2016, 8 sites in 2017, and one site in 2018 using a TFO II gravity corer or an Ekman Burge sampler. The sites of the 2016 samples are the same as those of Matsuoka and Ishii (2018) (Table 2, Figure 2).

Method of extraction of palynomorphs

The palynomorph extraction from sediment samples was performed following Matsuoka and Fukuyo (2000). A few grams of each sample preserved in wet condition were provided for palynological processing. Siliceous and calcareous particles were removed with hydrochloric acid (36%) and hydrofluoric acid (45%) at room temperature and fractionated using a stainless steel sieve with a mesh size of 125 µm and a plankton net sieve with a mesh size of 10 µm. The organic matter remaining on the sieve with a mesh size of 10 µm was used for further observation. Another part of each sample was used for calculating moisture content. Observed numbers of aquatic palynomorphs were shown as 1 g of dry weight using moisture content.

Identification of aquatic palynomorphs

The taxonomy of palynomorphs sampled from Osaka Bay follows Matsuoka and Ishii (2018), Nieuwenhove *et al.* (2019) and Mertens *et al.* (2020). However, since subsequent studies have revealed that palynomorphs previously assigned as tintinnids contain undifferentiated cysts of turbellarian egg capsules and ciliates (Matsuoka and

Table 2. Sample locations in Osaka Bay.

Sampling Station

Station No.	Longitude (N)	Latitude (E)
St 1	34°27′30″	134°56′58″
St 2	34°25′20″	135°08′45″
St 3	34°20′16″	135°06′39″
St 4	34°20′31″	135°00′58″
St 5	34°36′46″	135°08′34″
St 6	34°38′28″	135°11′36″
St 7	34°40′30″	135°17′58″
St 8	34°38′59″	135°20′38″
St 9	34°35′16″	135°19′44″
St 10	34°33′09″	135°15′49″
St 11	34°30′33″	135°20′13″
St 12	34°19′13″	135°08′18″
OS 1	34°41′05″	135°25′43″
OS 2	34°39′59″	135°22′28″
OS 5	34°36′55″	135°17′45″
OS 14	34°31′35″	135°02′28″
OS 15	34°29′28″	135°06′28″
OS 19	34°23′49″	135°13′06″
OS 20	34°18′22″	135°29′31″
OS 23	34°38′11″	135°15′57″

Ando, 2021), in this study, we classify turbellarian egg capsules and ciliate cysts separately. Palynomorphs were observed and counted using an upright optical microscope (ASONE Digital Biological Microscope, DN-1071) at 200–400x and 1000x magnification, when necessary.

Environmental parameters

The environmental parameters of Osaka Bay were obtained from the results of the 2016–2017 shallow-water survey conducted by the Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture and stored in the Osaka Bay Environmental Database (http://kouwan.pa.kkr.mlit.go.jp/kankyo-db/link/link_env/link_env.aspx). The results of the 2016–2017 shallow water stationary survey (*Senkai Teisen Chōsa*) and public-use water quality measurements (*Kōkyō Suiiki Suishitu Sokutei Kekka*), which are also stored in the Osaka Bay Environmental Database, were used. The shallow water stationary survey was conducted once a month at 20 stations in Osaka Bay to measure transparency, water temperature, salinity, pH, dissolved oxygen (DO), chemical oxygen demand (COD), total nitrogen (TN), total phosphate (TP), and chlorophyll-a. Among the 20 stations set up in the shallow water stationary survey, 18 stations close to the present palynomorph analytical stations were selected, and the observed values for each item in February, May, August, and November 2016 were averaged to obtain the environmental item data. The distance to the sampling point was plotted on Google Earth from the point set at the mouth of the Yodo River and from the nearest coast (Table 3).

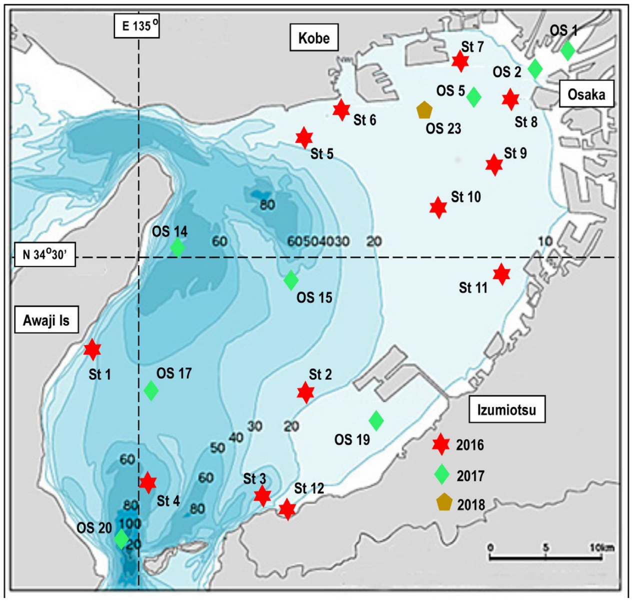


Figure 2. Sampling locations for aquatic palynomorph analysis. Different symbols indicate different sampling years as shown in the key.

Statistical analysis methods

Cluster analysis and distribution maps (contour diagrams) were prepared to characterize the distribution of the aquatic palynomorph assemblages in Osaka Bay. Microscopic observations of all sampled sites were used. The data on the occurrence of aquatic palynomorphs at the sites where environmental data were obtained were used in this analysis with Surfer ver. 26 (Golden Software) for contour diagrams and Tilia ver. 3.01 (https://www.neotomadb.org/apps/tilia) was used to create the marine palynomorph occurrence diagram.

Canonical correspondence analysis (CCA), a widely used method for direct gradient analysis, was employed using the software PAST (Hammer *et al.*, 2001) to clarify the relationship between environmental parameters and

Table 3. Environmental parameters used for statistical analysis. Original data were collected from the results of the 2016–2017 shallow water stationary survey (*Senkai Teisen Chōsa*) and public-use water quality measurements (*Kōkyō Suiiki Suishitu Sokutei Kekka*).

Station	Water depth (m)	Distance A (Km)	Mdφ (μm)	Transp (m)	SST (°C)	SAL	DO (mg/L)	COD (mg/L)	Total-ION (Mg/L)	Total-P (mg/L)	Chlorophyll-*a* (μg/L)
St 1	53	45.44	16.1	8.75	18.73375	30.9075	4.9175	7.72	72.2	9.95	30.54
St 2	18	36.36	14.9	5.425	18.49	28.07375	5.20625	12.15	116.23	17.16	72.49
St 3	43.5	45.18	17.3	5.7	18.32875	32.1925	5.06625	3.37	31.47	6.21	13.12
St 4	40	48.13	37.5	6.55	18.535	31.52125	5.34	5.34	31.23	6.96	15.96
St 5	22	18.55	15.5	6.75	18.325	31.93125	5.07125	5.24	29.64	6.14	13.5
St 6	16.3	14.5	12.2	3.45	18.7	29.2	9.35	12	51.5	3.45	47.2
St 7	14.5	6.58	12.5	ND	19.6	32.11	6.80	13.95	71.67	1.52	72.49
St 8	12.1	5.19	12.5	ND	19.025	28.205	8.635	13.7	46.9	7.45	58.5
St 9	18	11.76	14.1	5.125	18.92	32.73625	4.975	3.27	26.28	5.03	7.76
St 10	19.5	16.28	12.8	6.275	19.10125	32.8	4.90625	2.83	29.3	5.33	4.65
St 11	14	22.33	12	4.075	18.82625	32.0075	5.12125	4.31	31.22	5.78	7.36
St 12	3	44.82	11.8	4.725	17.775	31.8925	8.4	5.3	45.1	3.23	9.4
OS 1	0.5	6.09	ND	2.85	19.45	27.7	10.35	8.4	26.5	6.58	72.1
OS 2	1	4.8	ND	2.55	18.85	21.06	9.475	7.6	44.8	10.33	48.7
OS 5	17.5	6.81	ND	5.55	18.9075	32.4475	5.03875	1.1125	27.6	5.25	9.14
OS 14	54.5	34.67	ND	8.25	18.36375	31.04125	4.87375	6.16	47.67	9.66	21.32
OS 17	46.8	45.58	ND	ND	18.845	32.2625	7.62	7.2725	26.5	2.19	4.6
OS 20	52.6	52.48	ND	ND	19.21	32.255	7.57	7.0	26.5	2.38	4.5

aquatic palynomorph abundances.

Results

Classification of aquatic palynomorph assemblage

The aquatic palynomorphs identified were dinoflagellate cysts (max. 26,532 cysts/g at OS 5), acritarchs (max. 3,304 cells/g at St 11), raphidophyte cysts (max. 455 cysts/g at St 8), chlorophycean resistant cells (max. 50,255 cells/g at St 7), testate amoebae (max. 182 individuals/g at St 8), ciliate cysts and loricae (max. 1,001 cells/g at St 8), foraminiferal linings (max. 4,224 linings/g at St 5), crustacean resting eggs (max. 7,705 eggs/g at OS 5), and turbellarian egg capsules (max. 665 egg capsules/g at St 7). The density of these aquatic palynomorphs in Osaka Bay was highest around the offshore area at the mouth of the Yodo River to the inner part of the bay and generally decreased toward the southwest (Figure 3A).

The center of distribution of each aquatic palynomorph was as follows: dinoflagellate cysts off the mouth of the Yodo River, acritarchs off the mouth of the Yodo River toward the eastern coast, chlorophycean resistant cells off the mouth of the Yodo River to the deep northeast of the bay, cysts of the raphidophycean *C. marina* at the eastern part of the bay, ciliate cysts and loricae from offshore of the mouth of the Yodo River to the deep northeast of the bay, foraminiferal linings offshore to the west of Kobe Airport, crustacean resting eggs from the estuary of the Yodo River to the west of Kobe Airport, and turbellarian egg capsules in the northeastern part of the bay. Palynomorphs other than foraminiferal linings were abundantly distributed from the Yodo River estuary to the northeastern part of the bay.

Based on the cell densities at each site, a cluster analysis was conducted. As a result, the aquatic palynomorph assemblage was classified into two major groups: A and B (Figure 3B, C). OS 5, St 7, and St 8 were characterized by abundant crustacean resting eggs, foraminiferal linings, acritarchs, and chlorophyceae resistant cells, while OS

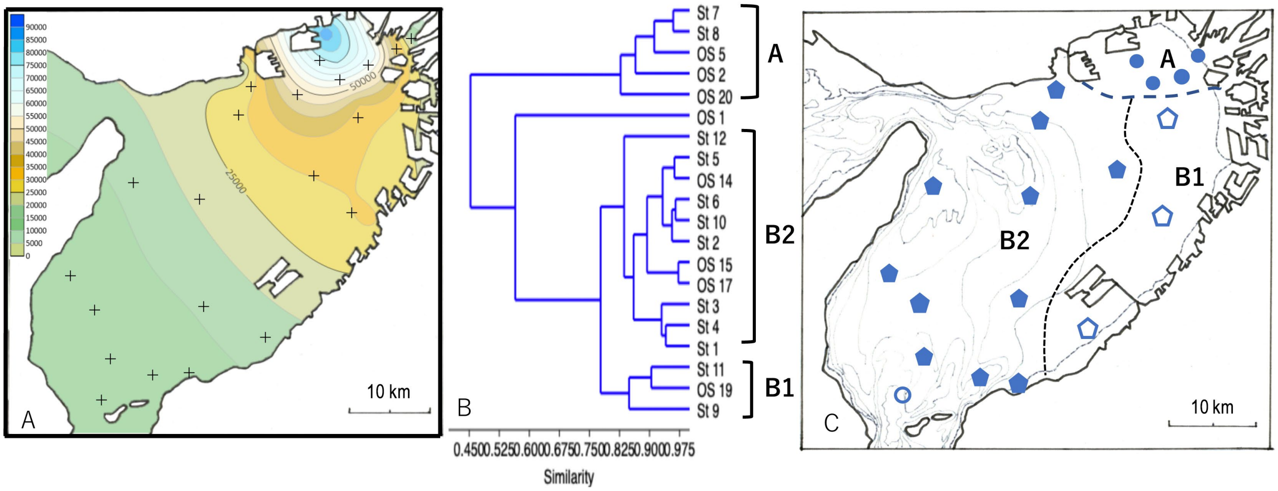


Figure 3. Cluster analysis of aquatic palynomorphs and the characteristics of their distribution. Aquatic palynomorphs are divided into two major assemblages. **A,** Abundance of all aquatic palynomorphs in Osaka Bay; **B,** Clustering of samples; **C,** Horizontal distribution of clustering data.

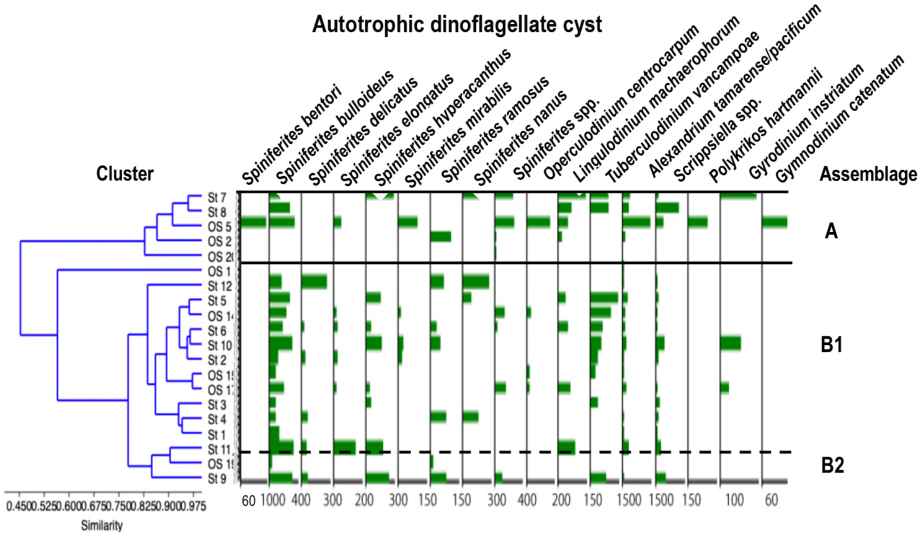


Figure 4. Diagram showing abundance of autotrophic dinoflagellate cysts.

1, OS 2, and St 7 were dominated by the chlorophycean *Staurastrum* spp. Group A was observed in the inner part of the bay near the mouth of the Yodo River, including OS 2, OS 5, OS 20, St 7 and St 8. Group B1 was distributed off the eastern coast of the bay, comprising St 9 and 11 as well as OS 19, while Group B2 occupied the central to southern parts of the bay, including St 1, St 2, St 3, St 4, St 5, St 6, St 10, and St 12, as well as OS 14 and OS 17. Additionally, OS 1 did not belong to either group (Figure 3B, C).

Group A showed high species diversity and abundance of photosynthetic dinoflagellate cysts, with *Tuberculodinium vancampoae* (M. Rossignol) D. Wall being particularly abundant. The species diversity of heterotrophic dinoflagellate cysts was also high, especially among the genera *Brigantedinium* and *Echinidinium. Staurastrum* spp. and *Monactinus biwae* (Negoro) K.Matsuoka and M.Kayama, along with abundant turbellarian egg capsules, foraminiferal linings, and crustacean resting eggs. Subgroup B1 was dominated by heterotrophic dinoflagellate cysts, including the genera *Stelladinium*, *Votadinium*, and *Dubridinium*, but the acritarch *Michystridium* and

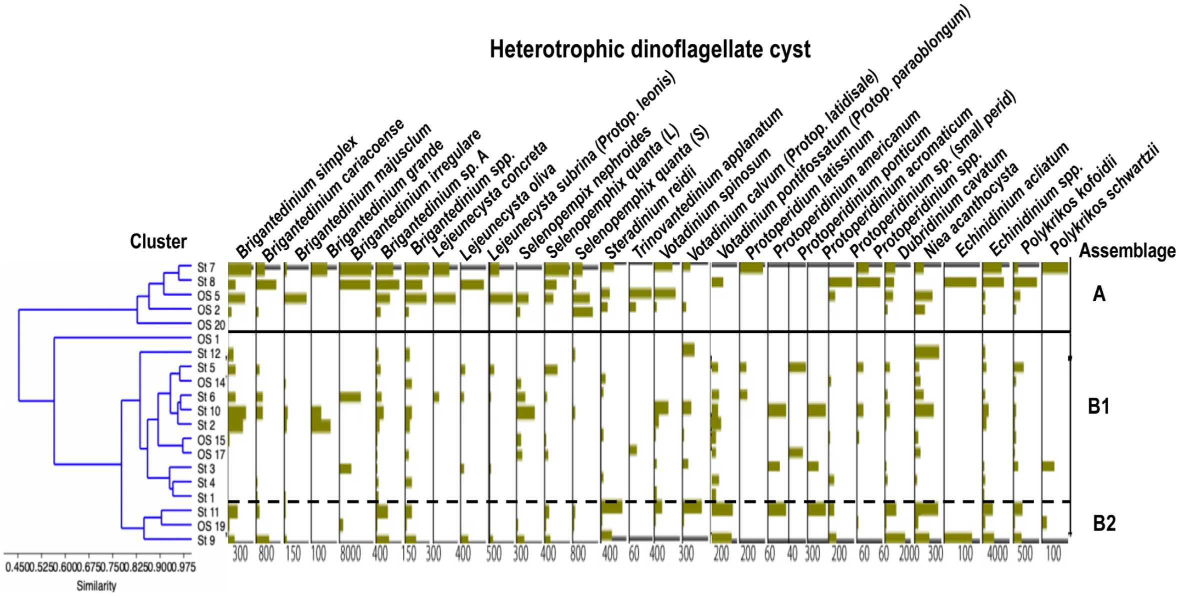


Figure 5. Diagram showing abundance of heterotrophic dinoflagellate cysts.

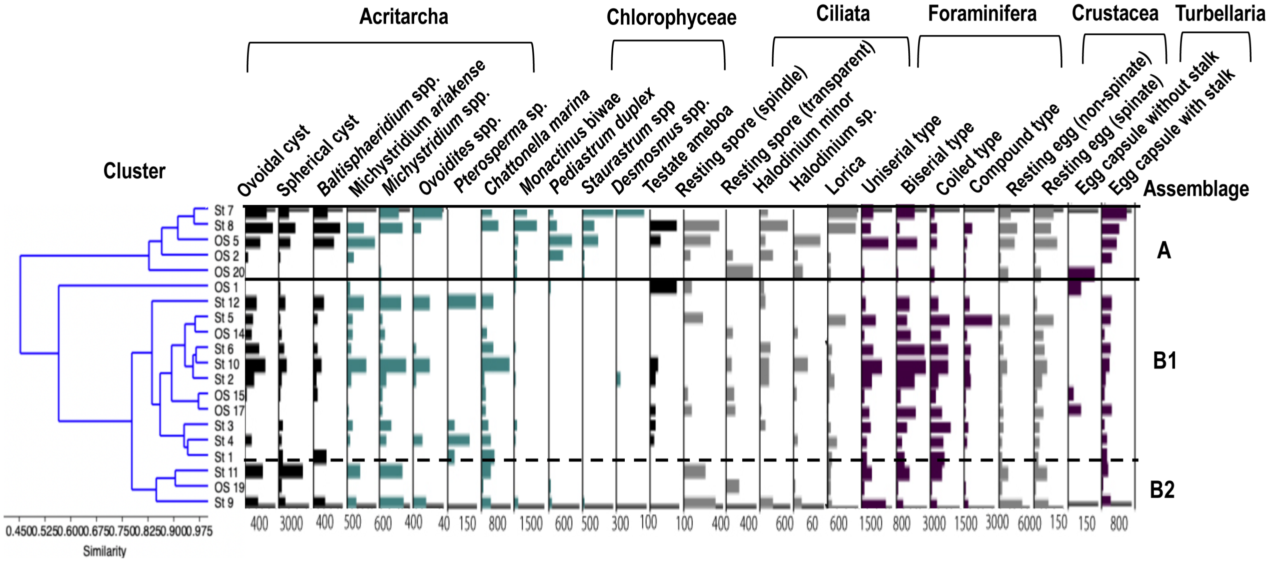


Figure 6. Diagram showing abundance of acritarchs, raphidophycean cysts of *Chattonella marina*, chlorophycean resistant cells of cysts and loricae of ciliates, foraminiferal linings, crustacean resting eggs, and turbellarian egg capsules.

the prasinophycean *Pterosperma* were also abundant. In Subgroup B2, both the species diversity and abundance of photosynthetic and heterotrophic dinoflagellate cysts were low. Additionally, there was a high abundance of coiled-type foraminiferal linings (Figures 4–6).

Palynomorphs of planktonic unicellular organisms.— This group includes dinoflagellate cysts, some acritarchs, the raphidophyte *C. marina*, cysts and loricae of ciliate and chlorophycean resistant cells. Among them, dinoflagellate cysts, mostly derived from planktonic unicellular organisms, comprised more than 20 genera and over 48 species (* denotes the scientific name of plankton, # denotes brackish water species, others are the scientific names of cysts). Among the photosynthetic species, there were over 17 species from 10 genera, such as *Spiniferites*, *Lingulodinium*, *Operculodinium*, *Tuberculodinium*, *Alexandrium**, *Scrippsiella**, *Peridinium quinquecorne* T.H.Abé*#, *Polykrikos hartmannii* W.M.Zimmermann*, *Levanderina fissa* (Levander) Moestrup, Hakanen, Gert Hansen, Daugbjerg and M.Ellegaard*, and *Gymnodinium catenatum* H.W.Graham* (including toxic species *Alexandrium catenella/pacificum*). Among the heterotrophic species, over 31 species from 10 genera such as *Brigantedinium*, *Lejeunecysta*, *Selenopemphix*, *Stelladinium*, *Votadinium*, *Protoperidinium**, *Dubridinium*, *Niea**, *Echinidinium*, and *Polykrikos** were identified (Table 4). Throughout Osaka Bay, heterotrophic cysts were more abundant in both species diversity and cyst density compared to photosynthetic cysts.

Photosynthetic dinoflagellate cysts (excluding those of Alexandrium catenella/pacificum*).*—Distribution centers of photosynthetic dinoflagellate cysts were divided between the northern and southern parts of the bay. Photosynthetic dinoflagellate cysts comprised 16,281 cysts/g off the western coast of the mouth of the Yodo River (OS 5) with over 5,000 cysts/g recorded in the area immediately distal to the mouth (St 7, St 8), but decreased in the central parts of the bay. However, there was a slight increase in the southern part of the bay (Figure 3a). The distribution centers tended to be divided between the northern and southern parts of the bay. The autotrophic dinoflagellate cyst *Spiniferites bulloideus* (Deflandre and Cookson) Sarjeant was abundant from the Yodo River mouth area to the eastern coast (max. 840 cysts/g at St 11) and the northwest (720 cysts/g at St 5) but decreased from the central to southern parts of the bay (Figure 7A).

Cysts of Alexandrium catenella/pacificum.—Cysts of *Alexandrium catenella/pacificum* were observed in all samples from Osaka Bay, with most cysts retaining cytoplasm shrunk. The maximum cell density was 14,539 cysts/g at OS 5, while the minimum density was 136 cysts/g at St 3, with an average cell density of 1,805 cysts/g. Although cell densities in sediments varied, concentrated areas were divided into three zones. In the northern part of the bay, they were abundant around OS 5 and over 2,000 cysts/g were recorded at St 5, St 7, and St 8. In the central part of the bay, they were centered around St 11 (3,248 cysts/g), while in the southern part, they were distributed around St 1 (805 cysts/g) (Figure 7B).

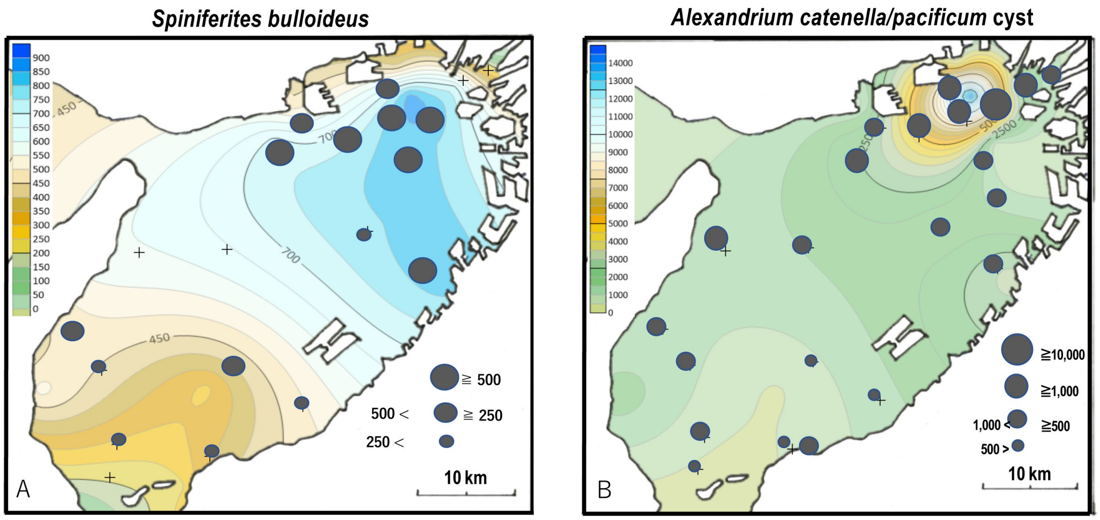
Heterotrophic dinoflagellate cysts (Figure 8).—Heterotrophic dinoflagellate cysts showed the highest cyst density off the mouth of the Yodo River (St 7), reaching 15,743 cysts/g, and exceeded 8,192 cysts/g in the eastern part of the bay (St 11). They were also abundant at over 3,000 cysts/g in the northwest part of the bay (St 5, St 6) but decreased from the central to southern parts of the bay. The distribution centers were divided between the northern and southern parts of the bay. The most abundant species among heterotrophic cysts were spherical with smooth brown surfaces belonging to the genus *Brigantedinium*. This genus includes at least *Brigantedinium simplex* (Wall) P.C. Reid, *B. cariacoense* (Wall) P.C. Reid, *B. majusclum* P.C. Reid, *B. grande* K. Matsuoka, and *B. irregulare* K. Matsuoka, but since most cysts lack and/or possess deformed archeopyles, they were collectively treated as *Brigantedinium* spp. for species identification. These cysts were most abundant from the mouth of the Yodo River to the inner bay, with a density of 7,371 cysts/g at St 8, and along the eastern coast of the bay, with 3,584 cysts/g at St 11. The areas with high cyst densities were located off the mouth of the Yodo River in the inner bay, showing the same trend for both heterotrophic dinoflagellate cysts overall and *Brigantedinium* spp.

Raphidophycean Chattonella marina *cysts* (Figure 9).—*Chattonella marina* has three morphologically distinct varieties known as var. *antiqua*, var. *marina*, and var. *ovata*; however, the simple half-spherical cysts with transparent walls formed by these varieties are mostly the same. Diatom frustules and sediment particles serving as attachment substrates for cysts were removed by hydrofluoric acid treatment during palynomorph extraction, producing empty individual or aggregate cysts with cell escape pores. *C. marina* cysts in Osaka Bay were produced at an average of 184 cysts/g, with the distribution center being from the inner to the eastern parts of the bay, and the cysts were distributed almost throughout the

Table 4. Results of the canonical correspondence analysis between major aquatic palynomorphs and environmental parameters in Osaka Bay.

Axis	Eigen value	% of contribution of inertia	% of total interia
1	0.2163	89.54	35.89
2	0.020036	8.291	3.323
3	0.0052237	3.162	0.8663
4	2.48E-05	0.01026	0.004111

Axis	Eigen value	% of contribution of inertia	% of total interia
1	0.23489	72.42	38.95
2	0.046947	14.47	7.786
3	0.034151	10.53	5.664
4	0.0057861	1.784	0.9596
5	0.002366	0.7295	0.3924
6	0.0002049	0.06317	0.03398
7	1.31E-08	4.03E-06	2.17E-06


Spiniferites bulloideus

***Alexandrium catenella/pacificum* cyst**

Figure 7. Contour maps and abundances of autotrophic dinoflagellate cysts. **A,** *Spiniferites bulloideus*; **B,** cysts of *Alexandriun catenella/pacificum*.

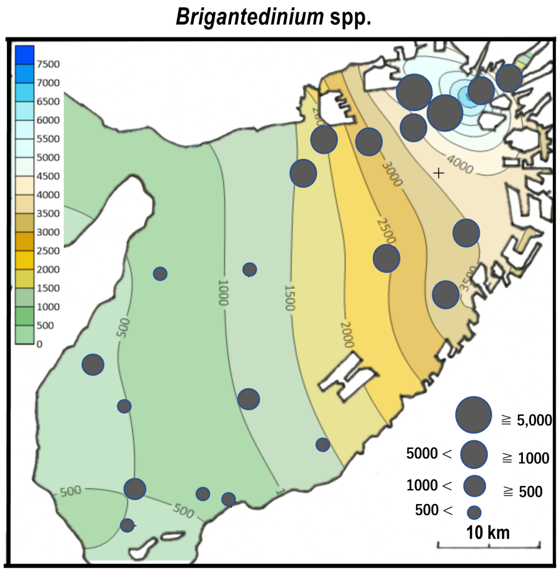

***Brigantedinium* spp.**

Figure 8. Contour map and abundance of the heterotrophic dinoflagellate cyst *Brigantedinium* spp.

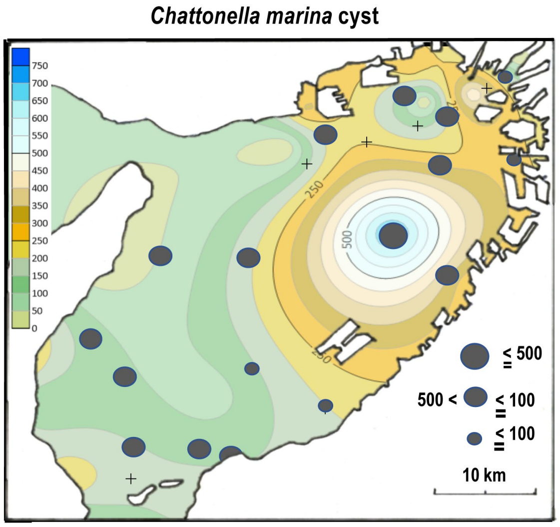

***Chattonella marina* cyst**

Figure 9. Contour map and abundance of cysts of the harmful phytoplankton *Chattonella marina* (Raphidophyceae).

entire bay. The highest cyst density of 742 cysts/g was recorded at St 10 in the eastern part of the bay, followed by 455 cysts/g at St 8 in the inner part of the bay.

Palynomorphs derived from benthic organisms

Foraminiferal linings (Figure 10A).—Foraminiferal linings had their distribution center near Kobe Airport to the west (St 5), but they were nearly homogeneously distributed throughout the bay. They were present at approximately 2,000 linings/g in the central part of the bay, decreasing towards the inner and peripheral parts of the bay. The highest value of 4,224 linings/g was recorded in the northwest part of the bay (St 5), while the minimum of 20 linings/g was observed at the mouth of the Yodo River (OS 1). The uniserial type was abundant in the inner part of the bay near the mouth of the Yodo River, the biserial type was dominant in the eastern central part of the bay, and the coiled type was abundant from the western inner bay to the central bay area, with each type having a differ-

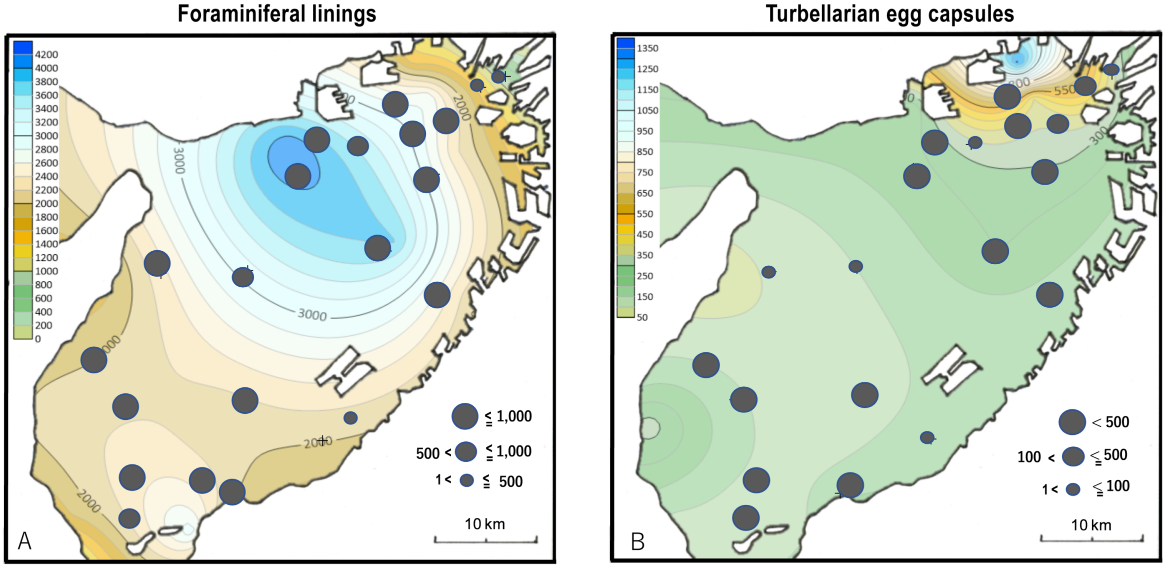


Figure 10. Contour maps and abundances of benthic and heterotrophic organisms. **A,** foraminiferal linings; **B,** turbellarian egg capsules.

ent distribution center.

Turbellarian egg capsules (Figure 10B).—Turbellarian egg capsules are relatively large palynomorphs tens of microns or greater in size. While many egg capsules have attachment stalks, some species identical to *Beringiella* lack them. The number of egg capsules detected was considerably fewer than that of heterotrophic dinoflagellate cysts or foraminiferal linings. They were most abundant in the inner part of the bay off the western coast of the mouth of the Yodo River (St 7, with a maximum capsule density of 665 capsules/g) and in the southern part of the bay off Awaji Island (OS 17, with a maximum of 329 capsules/g), while they were less abundant in the central part of the bay. The distribution centers were divided between the northern and southern parts of the bay.

Results of the cluster analysis of environmental parameters

The cluster analysis classified environmental parameters into two groups: Group I and Group II. Group I, except for St 2, was primarily located from the inner bay to the northern part of the bay (St 2, St 6, St 7, St 8, OS 1, and OS 2), while Group II was subdivided into subgroups IIa (St 5, St 9, St 10, St 11, and OS 5) and IIb (St 1, St 3, St 4, OS 14, OS 17, and OS 20). Water temperature was higher from the inner bay to the eastern part of the bay, while COD levels were higher in the inner bay and the eastern and southern parts of the bay. Salinity was higher in the eastern part of the bay. DO concentrations were highest in the peripheral areas of the bay and lowest in the inner bay. Chlorophyll-a levels were highest in the southern part of the bay. TN and TP were highest in the southern part of the bay.

Relationship between aquatic palynomorphs and environmental parameters

The relationship between aquatic palynomorphs and environmental parameters showed the following correlations: Overall marine palynomorphs exhibited positive correlations with water depth, distance from the mouth of the Yodo River (Distance A), surface water temperature, and minimum DO levels. For all dinoflagellate cysts, there were positive correlations with water depth, Distance A, grain size of sediment particles (Mdφ), surface water temperature, and minimum DO levels. Photosynthetic dinoflagellate cysts showed positive correlations with water depth, Distance A, Mdφ, transparency, minimum DO levels, and TP.

In the CCA, a matrix of aquatic palynomorph and environmental variable data (both classified by sampling sites) were presented together in ordination space (biplot) to measure the correlations between the two sets of data, in this case, to identify whether and how strongly the environmental variables determine the distribution of aquatic palynomorph. On the CCA biplot, aquatic palynomorph that have high occurrence in certain sampling sites have close distance. Environmental variables are plotted as green lines. The length of the lines demonstrates the relative importance of each environmental variable, which means the longer the green line, the greater significance with which this environmental variable determines the plot positions on the chart. The direction of the envi-

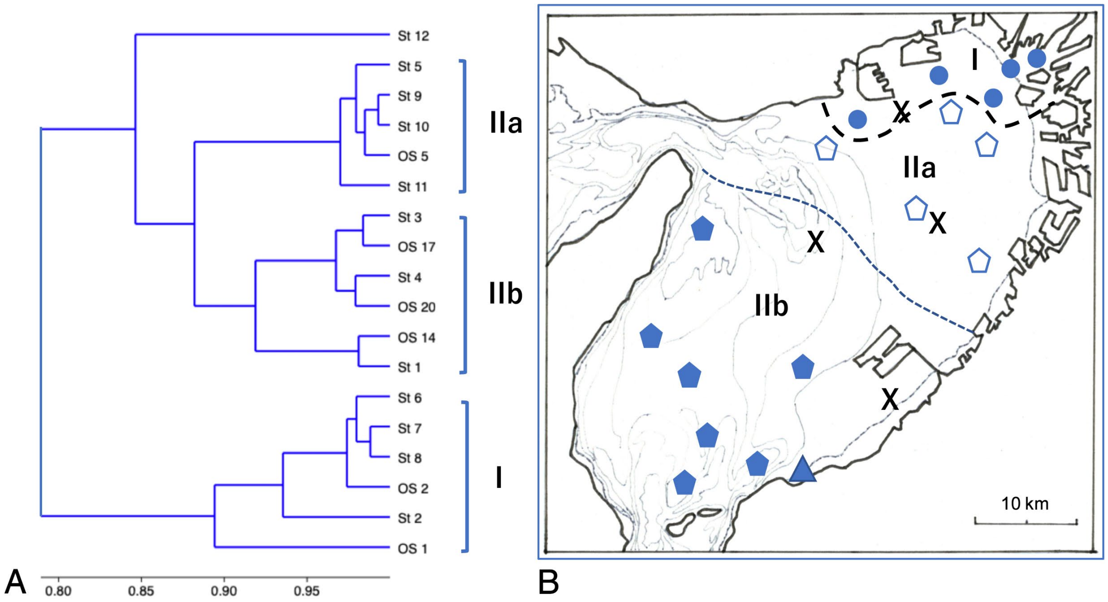


Figure 11. Clustering of environmental parameters in Osaka Bay. **A,** result of cluster analysis; **B,** distribution of these clusters. Environmental parameters are divided into two groups: Group I and Group II. Group II is subdivided into two sub-groups: Sub-group IIa and Sub-group IIb. Group I consists of OS 1, OS 2, St 2, St 6, St 7, and St 8, which are located in the innermost part of the bay. Sub-group IIa includes St 5, St 9, St 10, and St 11, which are located in the central part of the bay. Sub-group IIb is composed of St 3, OS 17, St 4, OS 20, OS 14, and St 1, which are positioned in the central to southern part of the bay.

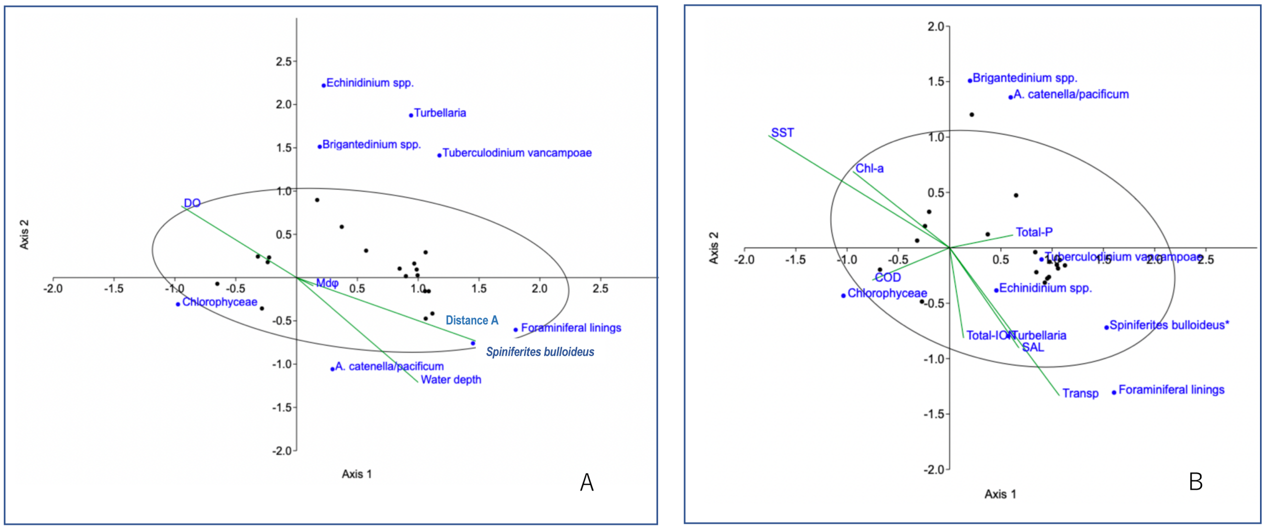


Figure 12. Results of canonical correspondence analysis showing the relationships between major aquatic palynomorphs and physical (**A**) and biological (**B**) environmental parameters. Abbreviations: Chlo-a, chlorophyll-a; COD, chemical oxygen demand; Distance A, distance from the mouth of Yodogawa; DO, dissolved oxygen; Mdφ, grain size composition; Total-ION, total nitrogen; Total-P, total phosphate; SAL, salinity; SST, sea surface temperature; Transp, transparency.

ronmental variables (green lines) is determined by their correlation coefficients (eigenvalue) against the two axes. Plots distributed on the same direction have positive correlation to these environmental variables. The correlation level between the environmental variables and each plot (both aquatic palynomorph and sampling site are applicable) is in inverse proportion to the distance between them.

Environmental variables which may determine the distribution of aquatic palynomorphs in Osaka Bay were divided into two groups. The first group comprised Distance A, water depth, DO and Mdφ, and the second group comprised sea surface temperature, salinity, water transparency, COD, TN, TP, and Chlorophyll-a. As shown in Figure 12A, in which the Axis 1 explains over 89.5% of the variance, *Spiniferites bulloideus* and foraminiferal linings showed strongly positive relationships with Distance A and Mdφ and negative relationships with DO. *A. catenella/pacificum* showed a somewhat positive relationship with water depth. Other taxa did not show significant relationships with the physical parameters. For the biological parameters shown in Figure 12B, in which the Axis 1 explains 72.4% of the variance, Chlorophyceaen palynomorphs showed positive relationships with COD, turbellarian egg capsules, and heterotrophic dinoflagellate cysts; while *Echinidinium* spp. and foraminiferal linings showed positive relationships with salinity and transparency; however, other taxa did not show any relationships with biological parameters (Table 4).

Discussion

Characteristics of the aquatic palynomorph assemblage in Osaka Bay

The grain size composition of surface sediments in Osaka Bay was analyzed by Nagaoka *et al.* (2004) and Yokoyama and Sano (2015). Nagaoka *et al.* (2004) showed that the mud component comprises more than 75% of the sediment distribution from the inner to the eastern part of the bay, and in the inner part of the bay, it comprises nearly 100%. In the central, western, and southern parts of the bay, the sand component increases. Nagaoka *et al.* (2004) and Yokoyama and Sano (2015) agree that the silty clay component increases in the inner part of the bay and the sandy component increases from the center to the western part of the bay. However, Nagaoka *et al.* (2004) found gravelly sand mixed with gravel in the southern part of the bay near the Kitan Strait. The area where muddy particles are abundant in the bottom sediment is largely consistent with the deep part of the bay corresponding to the Group A aquatic palynomorph assemblage and the eastern part of the bay corresponding to the Group B1 palynomorph sub-assemblage (Figures 3–6). This suggests that aquatic palynomorph distribution depends on grain size, which is consistent with the results of previous studies focusing on dinoflagellate cysts (Wall *et al.*, 1977; Dale, 1996; Baula *et al.*, 2011; Heikkila *et al.*, 2014; de Vernal *et al.*, 2020). The palynomorphs analyzed in this study also contain relatively large turbellarian egg capsules (equivalent to the grain size of medium- to coarse-grained sands), but as a total palynomorph assemblage, they tend to be abundant in areas where muddy sediments predominate.

Distribution characteristics of aquatic palynomorphs in Osaka Bay

According to Fujiwara *et al.* (1989), Osaka Bay has six major currents (Figure 1). The topographic Okinose Circulation Current moves from the western to central part of the bay with a maximum velocity of 24 cm/s. The East Coast Residential Current is a topographic type density current that flows southward from the mouth of the Yodo River to the east coast via the west offshore of Izumiotsu with a maximum velocity of 5 cm/s. The Sumaoki Countercurrent flows westward with a maximum velocity of 15 cm/s. The Nishinomiyaoki Circulation Current is a density current that flows eastward from offshore of Kobe after branching off from the Sumaoki Countercurrent with a maximum velocity of 7 cm/s. The Tomogashima Countercurrent flows from the Kitan Strait into the southern part of Osaka Bay with a maximum velocity of 14 cm/s.

The grain size composition of the sediments is fine-grained in the area where the Nishinomiyaoki Circulation Current and the East Coast Residential Current develop, and coarse-grained in the area influenced by the Okinose Circulation Current and the Tomogashima Countercurrent (Nagaoka *et al.*, 2004). The deep part of the bay where the Group A aquatic palynomorph assemblage is located, including the Nishinomiya breakwater, is located in an area where the current velocity is reduced to 7 cm/s, which is one of the reasons for the accumulation of fine-grained sediments. Therefore, the aquatic palynomorph assemblages in Osaka Bay are controlled by physical factors such as current movement and velocity.

Photosynthetic dinoflagellate cysts.—*Spiniferites bulloideus* and cysts of *Alexandrium catenella/pacificum* belonging to Gonyaulacales were selected as representative photosynthetic dinoflagellate species. *S. bulloideus* appeared mainly in the eastern part of the bay, its density decreased toward the western and southern parts of the bay, and it did not appear off the mouth of the Yodo River (Figure 7A). This indicates that *S. bulloideus* did not germinate and become empty cysts in situ after deposition but rather that the germinated empty cysts were transported by the current and accumulated and deposited at a different location.

Cysts of *A. catenella/pacificum* were distributed differently from other photosynthetic and heterotrophic dinoflagellate cysts (Figure 7B). These cysts occurred in all samples but were more abundant in the inner part of the bay and less abundant in the southern part of the bay. Cysts of *A. catenella* are known to form motile zygotes (planozygotes) by conjugation of gametes at the end of reproduction (i.e., red tide) and then become dormant and settle on the seafloor (Matsuoka and Takeuchi, 1995). Cysts of *A. catenella/pacificum* appeared in all samples, and their sample density did not differ significantly from other dinoflagellate cysts throughout the whole Osaka Bay area except for St OS 5. The cyst wall of *A. catenella/pacificum* is thinner than that of other photosynthetic dinoflagellate cysts, and thus the cysts often do not retain their original shape after germination (Mizushima and Matsuoka, 2004).

Although some of the cysts of *A. catenella/pacificum* observed in this study had some contracted cytoplasm, most were living cysts filled with cytoplasm. This indicates that the cysts had developed not long after forming as hypnozygotes. The distributions of vegetative cells and cysts of *A. catenella* and *A. pacificum* are discussed below with reference to the results of the studies mentioned above.

The horizontal distribution of cysts of *A. catenella/pacificum* in this study differs from that reported by Yamamoto *et al.* (2009). The distribution of cysts of *A. catenella/pacificum* in October 2006 and in May and October 2007 was abundant from the inner bay to the eastern offshore area (100–499 cysts/cm^3, converted to 330–1,000 cysts/g of dry sediment, assuming the dry density of the sediment was 0.3 g/cm^3) (Yamamoto *et al.*, 2009). The distribution of *A. tamarense* (= *Alexandrium catenella*) in October 2006 was confirmed in almost all areas of Osaka Bay (100–499 cysts/cm^3, 330–1,646 cysts/g of dry sediment). In contrast, *A. tamarennse* (= *Alexandrium catenella*) produced cysts at a maximum density of 5,683 cysts/cm^3 (187,053 cysts/g) from the eastern part of the inner bay to the eastern offshore area in May 2007, immediately after a red tide in the offshore area in April 2007. Six months later, in October, the density was still 3,275 cysts/cm^3 (10,807 cysts/g). This may be due to differences in the methods used to extract cysts from the sediments. Yamamoto *et al.* (2009) adopted a fluorescent staining method (Yamaguchi *et al.*, 1995), while we used a palynological method using chemicals.

Yamamoto *et al.* (2017) reported on the dynamics of vegetative cells of *A. catenella* in Osaka Bay with results of continuous surveys since 2002. According to these surveys, *A. catenella* red tides occurred at the mouth of the Yodo River and off Ashiya in 2007, 2013, 2014, and 2016, and in the eastern coastal area of the bay in 2008 and 2011. In 2016, they also occurred in the southeast bay (Yamamoto *et al.*, 2017: figure 3). Judging from the sedimentation rate of 0.12–0.58 cm/y in Osaka Bay estimated by Matsumoto and Yokota (1978), the top 2 cm of surface sediment collected in the present study represents several to 20 years. This spans the period studied by Yamamoto *et al.* (2017), which suggested the dynamics of the vegetative cells of *A. catenella*. Yamamoto *et al.* (2017) showed that the vegetative cells of *A. catenella* were either absent or very low (10 cells/L) in the offshore area except in the eastern and southern parts of the bay. However, the presence of cysts of *A. catenella/pacificum* filled with cytoplasm in these areas is important when considering the distribution of other palynomorphs. The fact that the production of planktonic marine palynomorphs is positively correlated with Distance A strongly suggests that these palynomorphs were transported and deposited by currents after their formation.

However, the fact that cysts of *A. catenella/pacificum* were produced in considerable quantities in the southern part of the bay suggests that the vegetative cells of *A. catenella* reproduced and formed cysts in this area as well. The Fisheries Technology Institute of the Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries has been monitoring *A. catenella* in Sumoto Port in the western part of Osaka Bay following the early spring bloom in the inner part of the bay. The results show that *A. catenella* proliferates in early spring from late March to April, as in the inner part of the bay, with a density of 11,500 cells/L reported in April 2017 (https://www.seto.or.jp/kenkyu/wp-content/uploads/2023/06/2017_04.pdf) . Since this cell density is at the same level as the red tide condition of *A. catenella* occurring in the inner part of the bay, it is inferred that a large number of cysts formed in the waters around Sumoto Port at that time. The fact that the distribution of cysts of *A. catenella/pacificum* in Osaka Bay is different from that of other photosynthetic dinoflagellate cysts such as *S. bulloideus*, mainly in the northern part of the bay, suggests that *A. catenella/pacificum* bloomed to red tide conditions and spread throughout the bay. In other words, in cysts of *A. catenella/pacificum* the distribution of planktonic vegetative cells, which is one of the fundamental factors controlling the distribution of photosynthetic dinoflagellate cysts, is different from those of other photosynthetic dinoflagellate cysts. As shown in Figure 12, cysts of *A. catenella/pacificum* are not correlated with any of the environmental factors considered in the present study, because these cysts are located far from Axis 1 and are not included in the 95% confidence ellipse. The cysts of *A. catenella/pacificum* may therefore be controlled by other unknown environmental parameters.

Cysts of *Chattonella marina*

Itakura *et al.* (1991) investigated the distribution of *C. marina* cysts preserved in surface sediments in the eastern Seto Inland Sea, including Osaka Bay, and found that cysts were preserved in almost the entire area. Especially in the eastern and southeastern parts of Osaka Bay, Itakura *et al.* (1991) reported that wet sediment samples collected in 1988 and 1989 yielded 250–499 cysts/cm^3 (max. 833–1,663 cysts/g). The formation of *C. marina* cysts occurs when nutrients are depleted in the vegetative cell stage. Somatic cell division does not continue, the cells become smaller, and after settling at the seafloor, they attach to diatom shells and/or sand grains (Imai and Itoh, 1988). The original shape of *C. marina* cysts is spherical, but it becomes hemispherical due to attachment of the cysts to the substrate (Imai, 1990).

During the 1980s, *C. marina* (= *Chattonella antiqua*) red tides occurred frequently throughout the Seto Inland Sea, including Osaka Bay, whereas *A. catenella* and other species were rare. The abundance of *C. marina* cysts in this study was lower than that reported by Itakura *et al.* (1991) and extremely small compared to that of cysts of *A. catenella/pacificum*, as shown in Figure 7B. Red tides of *C. marina* did not occur in Osaka Bay from 2016 to 2018. On the other hand, red tides of *A. catenella* have occurred frequently since the 2000s (Osaka Bay red tide information: https://www.knsk-osaka.jp/suisan/gijutsu/akashio/index.html). The low production of *C. marina* cysts in the present study is presumably a reflection of the appearance of planktonic vegetative cells. The distribution of *C. marina* cysts is biased toward the eastern part of the bay, which is the same trend as that observed in the survey by Itakura *et al.* (1991). This suggests that *C. marina* cysts may have accumulated in this area and migrated there after their formation.

Resistant cells of chlorophyceae

The palynomorph *Staurastrum* spp. and chlorophycean green alga *Pediastrum biwae (=Monactinus biwae)* are found in Osaka Bay, but they originate from the freshwater Lake Biwa–Yodo River system (Ishigami *et al.*, 2024). Therefore, the distribution of *Staurastrum* spp. is not controlled by the seawater environment of Osaka Bay but responds to the behavior of freshwater from the Yodo River. Hongo and Nakajo (2003) used the *P. biwae* preserved in the surface sediments of Osaka Bay as a tracer of fine particles of riverine origin and determined that water from the Yodo River spread about 10 km from the mouth based on their production. The distribution of *Staurastrum* spp. (the majority of which are *S. dorsidentiferum*) in this study differs from that of other palynomorphs, and *Staurastrum* spp. are abundant in the Yodo River estuary and the eastern coast of Osaka Bay like *P. biwae*.

Heterotrophic dinoflagellate cysts

Brigantedinium spp. were the most abundant heterotrophic dinoflagellate cysts, occurring as more than 5,000 cysts/g in the inner part of the bay and 3,000 cysts/g off the eastern coast, with a decrease from the inner to the southern part. This trend was similar for *Echinidinium* spp. and *Polykrikos kofoidii*, although they were less abundant. *Brigantedinium* spp., *Echinidinium* spp., and *P. kofoidii* showed strong positive correlations with COD and Chlorophyll-a, consistent with areas of rich organic matter and phytoplankton abundance, which are thought to be proxies for the plankton that feed on them. Previous studies have suggested that heterotrophic dinoflagellates feed on diatoms and other small phytoplankton (Jacobson and Anderson, 1996; Matsuoka *et al.*, 2000) and that they are abundant in eutrophic waters where these organisms are abundant. Such areas are dominated by upwelling currents (Wall *et al.*, 1977; Dale *et al.*, 2002; Holzwarth *et al.*, 2007; Radi *et al.*, 2007; Bouimetarhan *et al.*, 2009; Bringué *et al.*, 2018) and areas with high anthropogenic nutrient loads (Matsuoka, 1999; Matsuoka *et al.*, 2000; Pospelova *et al.*, 2004, 2005). The innermost part of Osaka Bay is an area with high anthropogenic eutrophication due to the densely populated hinterland of Osaka, Amagasaki, Nishinomiya, Ashiya, and Kobe, and it thus seems likely that heterotrophic cysts and crustacean resting eggs are abundant in this area.

Foraminiferal linings

Foraminiferal linings, which represent benthic protozoa, were distributed almost homogeneously throughout the bay, although the center of distribution was located west of Kobe Airport. However, different types of linings had different centers of distribution. Uniserial type linings were abundant in the Yodo River estuary in the inner part of the bay, biserial type linings were abundant in the central eastern part of the bay, and coiled type linings were abundant from the western to central parts of the bay. Foraminiferal linings were positively correlated with transparency as a whole and negatively correlated with Chlorophyll-a, indicating that the respective foraminifera inhabit waters with relatively good water quality (Armstrong *et al.*, 2007).

Turbellarian egg capsules

The benthic metazoan turbellarians produce egg capsules as they reproduce. The egg capsules are large aquatic palynomorphs whose remains are several tens of micrometers long and composed of scleroproteins (Matsuoka and Ando, 2022). Most egg capsules have an adherent pattern, but some species of the genus *Beringiella* lack it. The number of egg capsules detected in the sediments was lower than that of heterotrophic dinoflagellate cysts and foraminiferal linings. The highest density of egg capsules was found in the inner part of the bay off the west coast of the mouth of the Yodo River (St 7, maximum density of 665 egg capsules/g) and in the southern part of the bay off Awaji Island (St OS 17, maximum density of 329 capsules/g), while they were less abundant in the central part of the bay, and the distribution was mainly divided between the northern and southern parts of the bay. The distribution of the turbellarian taxa was positively correlated with Distance A, water depth, salinity, and minimum DO, and negatively correlated with COD, water temperature, and chlorophyll-a. However, little information is available on the ecological characteristics of turbellarian egg capsules in marine environments, and at present no material is available to evaluate the low number of egg capsules. Kumar (2023) recently reported the establishment of a morpho-species (*Katora arabica* Kumar) based on the superficial morphological characteristics of palynomorphs, including bovid egg capsules. However, observations and discussions on the morphology and ecology of each egg capsule, such as detailed examination of the filaments and the appendages observed on their tips and the presence of similar morphologies of egg capsules in freshwater and marine waters, have not been sufficiently addressed. Accepting an artificial taxonomic category while the relationship between egg capsules and their parental organisms remains unclear is likely to lead to confusion in the future.

Characteristics and controlling factors of the horizontal distribution of aquatic palynomorphs in surface sediments of Osaka Bay

The distribution of dinoflagellate cysts in Osaka Bay was examined by statistical analysis to clarify what environmental factors control the distribution of dinoflagellate cysts. Davey and Rogers (1975) studied two traverses in southwestern Africa using water turbulence, distance from the shore, availability and proximity to understand the distribution of dinoflagellate cysts. Wall *et al.*, (1977) analyzed dinoflagellate cyst assemblages in sediments from closed inner bays to deep pelagic waters, mainly in the Atlantic Ocean, and found that the distribution of dinoflagellate cysts was determined by biological (e.g. the members of plankton communities) and physical (e.g. hydrodynamic composition) factors. Dinoflagellate plankton assemblages are strongly affected by nutrients and water temperature and dinoflagellate cysts are well preserved in sediments with a 50–60% mud content. Subsequently, dinoflagellate cysts have been utilized as proxies for reconstruction of paleoceanography using the modern analog concept for analyzing dinoflagellate cyst assemblages preserved in surface sediments from various oceanic regions. De Vernal *et al.* (2020) summarized the results of studies on this topic published since Wall *et al.* (1977), explaining that sea surface water temperature is the fundamental factor controlling dinoflagellate cyst assemblages on a global scale but that dinoflagellate cyst assemblages in each region vary depending on the species that dominate in each region. In general, phytoplankton species diversity is higher in tropical regions and lower in polar regions, possibly due to a more stable light environment than water temperature in tropical regions (e.g. Thomas *et al.*, 2012).

However, dinoflagellate cyst assemblages that respond to global-scale environmental changes do not exist in narrow coastal zones such as Osaka Bay. The aquatic palynomorphs consisting of resistant cells, resting cysts, resting eggs, linings, and egg capsules produced by various organisms in response to the local environment are not deposited on site, but rather are transported and deposited by currents. In other words, the sites of formation of aquatic palynomorphs and their accumulation are different. Therefore, it is reasonable to assume that the environmental factors that regulate the distribution of marine palynomorphs, including dinoflagellate cysts, are a combination of biological factors related to cyst formation and physical factors related to migration and deposition after cysts have formed. However, previous studies to clarify the environmental factors controlling the distribution of marine palynomorphs have considered these factors together. In this study, we divided these factors into biological environmental factors consisting of water temperature, salinity, transparency, COD, Chlorophyll-a, Total N, and Total P, and physical environmental factors as Distance A, water depth, Mdφ, and DO.

Figure 12A shows the relationship between physical environmental factors and aquatic palynomorphs of chlorophycean resistant cells, foraminiferal linings, turbellarian egg capsules, the dinoflagellate cysts *S. bulloideus*, *T. vancampoae*, *Brigantedinium* spp., and *Echinidinium* spp., and cysts of *A. catenella/pacificum.* The accumulated significance of Axis 1 and Axis 2 is high, as they can explain more than 97% of the total variance. The negative relationship between foraminiferal linings and DO is consistent, because these organisms prefer a hypoxic environment. The cysts of *A. catenella/pacificum* are negatively related to water depth, indicating that these cysts are deposited in shallow depths near the mouth of the Yodo River. The negative relationship of chlorophyceaen resistant cells with Distance A and the positive relationship with water depth reflect the decrease of this organism as distance from the Yodo River estuary increases. *Brigantedinium* spp. and *Echinidinium* spp. and turbellarian egg capsules have no clear relationship with the environmental factors considered here. Other environmental factors may be controlling these organisms, but this is unknown at this time.

Figure 12B shows the relationship between biological environmental factors and aquatic palynomorphs consisting of foraminiferal linings, turbellarian egg capsules, the dinoflagellate cysts *S. bulloideus*, *T. vancampoae*, *Brigantedinium* spp., and *Echinidinium* spp., and cysts of the dinoflagellate *A. catenella/pacificum*. The accumulated significance of this two-dimensional display is lower than that between physical environmental factors and aquatic

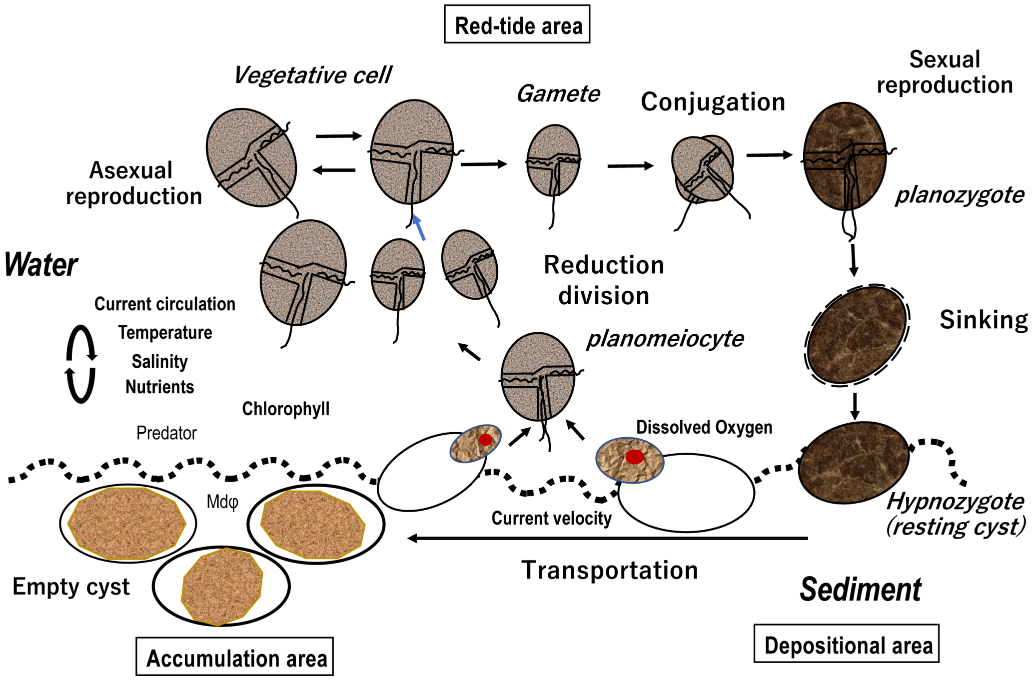


Figure 13. Schematic diagram showing the life cycle of the toxic dinoflagellate *Alexandrium catenella*. The environmental parameters sea surface temperature, salinity, and nutrients may affect asexual and sexual reproduction, cyst germination, and current movement. On the other hand, current movements and velocity may affect lateral transportation from depositional area to accumulation area.

palynomorphs but still explained near 87% of the total variance. Only chlorophycean resistant cells show a positive correlation with COD, but it is difficult to explain this ecologically. None of the aquatic palynomorphs show a strong relationship with biological environmental factors (Figure 12B). This suggests that these factors do not control the distribution of aquatic palynomorphs but are probably closely related to their parental organisms.

There are no studies that have clarified the factors controlling the distribution of aquatic palynomorphs in wide areas from coastal to open ocean regions. However, from the point of view of the study of dinoflagellate cysts, biological factors such as water temperature and salinity seem to be important (Dale *et al.*, 2002; Radi *et al.*, 2007; Pospelova and Kim, 2010; Garcia-Moreiras *et al.*, 2023; Li *et al.*, 2023). In relatively small coastal bays such as Osaka Bay, the environmental factors controlling the distribution of aquatic palynomorphs are not biological factors such as water temperature, salinity, and nutrients, which are directly related to the growth of parent organisms, but physical factors involved in the transport and deposition of aquatic palynomorphs after their formation. The environmental factors controlling the distribution of aquatic palynomorphs are not biological factors which are directly related to the growth of the parent organisms (Figure 13).

In conclusion, most aquatic palynomorphs of planktonic origin in Osaka Bay showed accumulation centers near the mouth of the Yodo River. After being formed, these planktonic palynomorphs were moved by tidal currents and deposited in locations away from where they were formed. On the other hand, benthic palynomorphs such as foraminiferal linings showed different areas of their accumulation where they inhabited from other aquatic palynomorphs. This suggests that palynomorphs generated by planktonic organisms may be transported after production by currents, which is likely one important factor controlling their distribution in sediments.

Issues to be resolved when handling each aquatic palynomorph

Among the dinoflagellate cysts in the present study, those of *A. catenella/pacificum* were the most abundant, but most of the cysts contained cytoplasm, which indicates that they were deposited immediately after formation. This is a major difference from other dinoflagellate cysts, and it occupied a characteristic position in the results of our multivariate analysis. Therefore, it is important to consider the characteristics of the assemblage bearing in mind that the conditions under which dormant spores and dormant eggs are preserved in the sediment depend on the chemical composition of the walls of cysts, resistant cells, resting eggs, egg capsules, and linings of each palynomorph (Versteegh *et al.*, 2012) and the environmental conditions of the sediment (Zonneveld *et al.*, 2008). The alternation and diagenesis of these palynomorphs after their production, such as movement by ocean currents, and their preservation as fossils in the sediment must also be considered.

The foraminiferal linings are larger (>74 μm) than reported in previous marine environmental analyses (~63 μm: Fossil Research Group, 2001; Kikui *et al.*, 2021; Schönfeld *et al.*, 2012). At present, there are only a few studies that have clarified the relationships between foraminiferal linings and the species from which originated, but this is an important issue that should be clarified in the future. The number of abundance of foraminiferal linings should then be evaluated in terms of the number of foraminiferal species.

The relationships between dormant eggs and the parental copepods should be clarified by focusing on the morphological and biochemical characteristics, especially the shape and morphology of the spines and the microstructure of the egg surfaces in future aquatic palynomorph studies.

Ciliates and benthic foraminifera, the organisms mainly comprising aquatic palynomorphs, are typically unicellular, and therefore, the number of linings and cysts can be treated as equal to that of the parent organism. However, some metazoans, such as copepods, may produce multiple dormant eggs from a single female. Therefore, if the dormant eggs of metazoans are to be treated as aquatic palynomorphs, it may be necessary to weight the number of dormant eggs detected.

It is necessary to define the morphological characteristics of the turbellarian egg capsules. In particular, an egg capsule with a string or stalk may have lost its attachment apparatus.

Supplemental materials

Table S1, abundance of aquatic palynomorphs preserved in surface sediments of Osaka Bay, Japan; **Figure S1,** dinoflagellate cyst occurrences in surface sediments of Osaka Bay. A, *Lejeunecysta oliva* (Reid) Turon et Londeix (= cyst of *Protoperidinium* sp.), OS5-1 O35/3; B, *Quinquecuspis concretum* (P.C. Reid) Head (= cyst of *Protoperidinium leonis*), OS5-1 M43/2; C, *Votadinium calvum* P.C. Reid (= cyst of *Protoperidinium quadrioblongum*, 0S5-1 G46/1; D: *Dubridinium caperatum* P.C. Reid, OS5-1 031/1; E, *Steradinium reidii* Bradford, St 8 R45/1; F, *Selenopenphix quanta* (Bradford) Matsuoka (Cyst of *Protoperidinium conicum*), St 8 G32/2; G, *Brigantedinium simplex* (Wall) P.C. Reid (= cyst of (=*Protoperidinium conicoides*; St 8 H46/1; H, *Brigantedinium cariacoense* (Wall) P.C. Reid (= cyst of *Protoperidinium avelanum*), OS5-1 D41/2; I, cyst of *Niea acanthocysta* (Kawani, Iwataki et Matsuoka) T.Liu, K.N. Mertens et H. Gu, St 8 R33/1; J, *Echinidinium transparentum* Zonneveld (= cyst of *Protoperidinium* sp.), St 6 D46/4; K, cyst of *Polykrikos schwartzii* Bütschli, OS5-2 M26/4; L, cyst of *Alexandrium catenella/pacificum*, 0S5-1 K35/2. Scale bar: 20 μm; **Figure S2,** other palynomorphs. A, Turbellarian egg capsule, slender form with stalk, OS5-1 C25/3; B, Turbellarian egg capsule, ellipsoidal form with stalk, OS5-1 V41/4; C, cyst of *Chattonella marina* (Subrahmanyan) Y.Hara & Chihara, Raphidophyceae, St 8 Q48/3; D, copepod resting egg, spinate form, St 8 F49/1; E, copepod resting egg, spinate form, OS5-1 N37/1; F, copepod resting egg, spinate form, St 8 O31/2; G, copepod resting egg, non-spinate form, OS 5-1 D41/2; H, foraminiferal lining, uniserial type, OS 5-1 N30/1; I, foraminiferal lining, coiled type, St 6 R47/2; J, foraminiferal lining, biserial type, St 6 T34/2; K. unknown form, OS5-1 M47/1.

Conclusion

Dinoflagellate cysts were positively correlated with the distance from a reference point established in the inner bay area. This reflects the behavior of dinoflagellate cysts as sediment particles after their formation rather than the habitat of their respective motile forms.

The horizontal distribution of cysts of *Alexandrium catenella/pacificum* was different from that of other photosynthetic planktonic palynomorphs. These were distributed not only in the areas affected by red tide in the inner and eastern areas of the bay but throughout the entire bay. This suggests that the cysts of *A. catenella/pacificum* are not only transported by currents after the formation but are also proliferating near the sampling points and that cyst formation is taking place in the red tide areas in Osaka Bay.

When considering the paleoecology of aquatic palynomorphs, not only the habitat of the parent organisms, but also their transport after production and alteration by diagenetic processes until they are fixed as fossils in the sediment should be fully considered.

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References

Anderson, D. M. and Wall, D., 1978: Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. *Journal of Phycology*, vol. 14. p. 224–234.

Armstrong, H. A. and Brasier, M. D., 2007: *Microfossils* (2nd edition), viii + 296 p. Blackwell publishing Ltd., London.

Cross, A. T., Thompson, G. G. and Zaitzeff, J. B., 1966: Source and distribution of palynomorphs in bottom sediments, southern part of Gulf of California. *Marine Geology*, vol. 4, p. 467–524.

Dolan, J. R., 2013: Introduction to tintinnids. *In*, Dolan, J. R., Montagnes, D. J. S., Agatha, S., Coats, D. W. and Stoecker, D. K. *eds.*, The Biology and Ecology of Tinntinid Ciliates, p. 1–16. John Wiley and Sons, Hoboken.

Downie, C., Evitt, W. R. and Sarjeant, W. A. S., 1963: Dinoflagellates, hystrichospheres, and the classification of the acritarchs. Stanford University Publications, *Geosciences*, vol. 7. p. 1–16.

Fossil Research Group, 2001: *Fossil Research Methods—From Collecting to the Latest Analytical Methods*, 388 p. Kyoritsu Shuppan Co. Ltd., Tokyo. (*in Japanese*)

Fujiwara, T., Higo, T. and Takasugi, Y., 1989: Constant currents, tidal currents and eddies in Osaka Bay. *Proceedings of Coastal Engineering, JSCE*, vol. 36, p. 209–213. (*in Japanese*)

Guidetti, R., Altiero, T. and Hansen, J. G., 2006: A new species of freshwater tardigrades from Disko Island (Greenland) increases as unsolved paradox in tardigrade systematics. *Hydrobiologia*, vol. 558, p. 69–79.

Gurdebeke, P. R., Mertens, K. N., Takano, Y., Yamaguchi, A., Bogus, K., Dunthorn, M., *et al.*, 2018: The affiliation of *Hexasterias problematica* and *Halodinium verrucatum* sp. nov. to ciliate cysts based on molecular phylogeny and cyst wall composition. *European Journal of Protistology*, vol. 66, p. 115–135.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D., 2001: PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, vol. 4, p. 1–9.

Hansen, P. J., 1991: Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagical food web. *Marine Ecology Progress Series*, vol. 73, p. 253–261.

Hongo, M. and Nakajo, T., 2003: Fine-grained sediments dispersion in the eastern part of Osaka Bay, based on distribution of remains of *Pediastrum biwae* (fresh water green alga) from the Yodogawa River. *Journal of Sedimentological Society of Japan*, no. 56, p. 17–26.

Imai, I., 1990: Physiology, morphology, and ecology of cysts of *Chattonella* (Raphidophyte), causative flagellates of noxious red tides in the Inland Sea of Japan. *Bulletin of Nansei National Fisheries Research Institute*, no. 23, p. 63–166.

Ishigami, M., Ichise, S. and Ohtsuka, T., 2024: *The Plankton in Lake Biwa—Photographs and Video*, 214 p. Bunri-Kaku, Kyoto. (*in Japanese*)

Jacobson, D. M. and Anderson, D. M., 1986: Thecate heterotrophic dinoflagellates: feeding behavior and mechanism. *Journal of Phycology*, vol. 22, p. 249–258.

Jankovská, V., 1991: Unbekannte objekte in pollenpraparaten—Tardigrada. *In*, KovarEder, J. *ed.*, *Palaeovegetational development in Europe and regions relevant to its palaeoXoristic evolution*, p. 19–23, Naturhistorisches Museum, Wien.

John, U., Litaker, R. W., Montresor, M., Murray, S., Brosnahan, M. L. and Anderson, D. M., 2014: Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: The Introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist*, vol. 165, p. 779–804.

Kikui, S., Abe, O., Yokoi, D. and Morimoto, M., 2021: A statistical evaluation for the community structure of the modern benthic foraminifer. *Scientific Report, Faculty of Education, Gifu University (Natural Science)*, vol. 45, p. 49–55.

Kinki Regional Development Bureau, 2004: *Database of Osaka Bay* [online]. [Cited 7 November 2024]. Available from: http://kouwan.pa.kkr.mlit.go.jp/kankyo-db/link/link_env/link_env.aspx.

Kitazato, H., 1981: Observation of behavior and mode of benthic foraminifera in laboratory. *Bulletin of Earth Science, Shizuoka University*, vol. 6, p. 61–71. (*in Japanese with English abstract*)

Klyuvitkina, T. S. and Novichkova, E. A., 2022: Laboratory Processing in the Aquatic Palynomorph Analysis: Problems and Solutions. *Oceanology*, vol. 62, p. 270–277.

Kumar, A., 2023: New form taxa of non-pollen palynomorphs (NPP) from southern Red Sea coastal sediments of Saudi Arabia. *Geophytology*, vol. 53, p. 1–24.

Matsumoto, E. and Yokota, S., 1978: Accumulation rate and heavy metal pollution in Osaka Bay sediments. *Journal of Oceanography*, vol. 34, p. 108–155.

Matsuoka, K. and Ando, T., 2021: Review—Turbellarian egg capsule as one type of aquatic palynomorph; reconsideration of Tintinnomorph. *Laguna*, no. 28, p. 15–35.

Matsuoka, K., Cho, H.-J. and Jacobson, D. M., 2000: Observations of the feeding behavior and growth rates of the heterotrophic dinoflagellate *Polykrikos kofoidii* (Polykrikaceae, Dinophyceae). *Phycologia*, vol. 39, p. 82–86.

Matsuoka, K. and Fukuyo, Y., 2000: *Technical Guide for Modern Dinoflagellate Cyst Study*. i+ 29 p., WESTPAC-HAB/WESTPAC/IOC, Japan Society for the Promotion of Science, Tokyo.

Matsuoka, K. and Takeuchi, T., 1995: Productivity of vegetative cells, planozygotes and resting cysts of dinoflagellate *Alexandrium catenella* (Whedon et Kofoid) Balech based on field observation. *Fossils (the Palaeontological Society of Japan)*, no. 59, p. 32–46. (*in Japanese with English abstract*)

McMinn, A., Bolch, C. and Hallegraeff, G., 1992: *Cobicosphaeridium* Harland and Sarjeant: Dinoflagellate cyst or copepod egg? *Micropaleontology*, vol. 38, p. 315–316.

Meisterfeld, R., 2002: Order Arcellinida Kent, 1880. *In*, Lee, J. J., Leedale, G. F., Bradbury, P. *eds.*, *An Illustrated Guide to the Protozoa*. *Second Edition*, 2, p. 827–860. Society of Protozoologists, Allan Press, Lawrence.

Mizushima, K. and Matsuoka, K., 2004: Vertical distribution and germination ability of *Alexandrium* spp. cysts (Dinophyceae) in the sediments collected from Kure Bay of the Seto Inland Sea, Japan. *Phycological Research*, vol. 52, p. 408–413.

Mudie, P. J., Marret, F., Gurdbeke, P. R., Hartman, J. D. and Reid, P. C., 2021: Marine dinocysts, acritarchs and less well-known NPP: tintinnids, ostracod and foraminiferal linings, copepod and worm remains. *In*, Marret, F., O'Keefe, J., Osterloff, P., Pound, M. and Shumilovskikh, L. *eds.*, *Applications of Non-Pollen Palynomorphs: from Palaeoenvironmental Reconstructions to Biostratigraphy*. Geological Society, London, Special Publications, 511(1): 159. doi.org/10.1144/SP511-2020-55.

Park, M., Boalch, G. T., Jowett, R. and Harbour, D. S., 1978: The genus *Peterosperma* (Prasinophyceae): species with a single equatorial ala. *Journal of marine biological association, U.K.*, vol. 58, p. 239–276.

Reid, P. C. and John, W. G., 1978: Resting cysts if the Ciliate Class Polyhymenophorea: phylogenetic implications. *Journal of Protozoology*, vol. 30, p. 710–713.

Šatkauskine, I., 2012: Tardigrades (Tardigrada) in Baltic States. *Biologija*, vol. 58, p. 245–255.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S., Spezzaferri,

S. *et al.*, 2012: The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—Towards a standardized protocol for soft-bottom benthic foraminiferal monitoring studies. *Marine Micropaleontology*, vols. 94–95, p. 1–13.

Sherr, E. and Sherr, B., 1988: Role of microbes in pelagic food webs: a revised concept. *Limnology and Oceanography*, vol. 33, p. 187–197.

Stancliffe, R. P. W., 1987: Microforaminiferal linings: their classification, biostratigraphy and paleoecology, with special reference to specimens from British Oxfordian sediments. *Micropaleontology*, vol. 35, p. 337–352.

Taniguchi, A., 1975: A role and position of zooplankton in marine ecosystem. *In*, Motoda, S. *ed.*, *Marine Science Basic Series 6*, p. 119–235, Tokai University Press, Tokyo. (*in Japanese*)

Thomas, M. K., Kremer, C. T., Klausmeier, C. A. and Litchman, E., 2012: A global pattern of thermal adaptation in marine phytoplankton. *Science*, vol. 338, 1085.

Topping, J. N., Murray, J. W. and Pond, D. Z. W., 2006: Sewage effects on the food source and diet of benthic foraminifera living in anoxic sediment: a microcosm experiment. *Journal of Experimental Marine Biology and Ecology*, vol. 329, p 239–250.

Traverse, A., 1988: *Paleopalynology*, xiii + 600 p. Allen and Unwin, Winchester.

Uye, S. and Takamatsu, K., 1990: Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters. *Marine Ecology Progress Series*, vol. 59, p. 97–107.

Van de Schootbrugge, B., Koutsodendris, A., Taylor, W., Weston, F., Wellman, C. and Shorther, P. K., 2024: Recognition of an extended record of euglenoid cysts: Implications for the end-Triassic mass extinction. *Review of Palaeobotany and Palynology*, vol. 322, 105043.

Van Geel, B., 2001: Non-pollen palynomorphs. *In*, Smol, J. P., Birks, H. J. B. and Last, W. M. *eds.*, *Tracking Environmental Change Using Lake Sediments. Terrestrial, Algal, and Siliceous Indicators*, 3, p. 99–119. Kluwer, Dordrecht.

Van Waveren, I. M., 1993: Planktonic organic matter in surficial sediments of the Banda Sea (Indonesia)—a palynological approach, Universiteit Utrecht. *Geologica Ultraiectina*, no. 104, p. 1–242.

Versteegh, G. J. M., Blokker, P., Bogus, K. A., Harding, I. C., Lewis, J., Oltmanns, S. *et al.*, 2012: Infra red spectroscopy, flash pyrolysis, thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) of cultured and sediment-derived *Lingulodinium polyedrum* (Dinoflagellata) cyst walls. *Organic Geochemistry*, vol. 43, p. 92–102.

Wall, D., 1962: Evidence from Recent plankton regarding the biological affinities of *Tasmanites* Newton 1875 and *Leiosphaeridia* Eisenack 1958. *Geological Magazine*, vol. 99, p. 353–362.

Wall, D. and Dale, B., 1968: Modern dinoflagellate cysts and evolution of the Peridinilales. *Micropaleontology*, vol. 14, p. 265–304.

Wall, D., Dale, B., Lohmann, G. P. and Smith, W. K., 1977: The environmental and climatic distribution of dinoflagellate cysts in modern marine sediments from regions in the North and South Atlantic Oceans and adjacent seas. *Marine Micropaleontology*, vol. 2, p. 121–200.

Yamaguchi, M., Itakura, S., Nagasaki, K. and Imai, I., 1996: Distribution and abundance of resting cysts of the toxic dinoflagellate *Alexandrium tamarense* and *A. catenella* in sediments of the eastern Seto Inland Sea, Japan. *In*, Yasumoto, T., Oshima, Y. and Fukuyo, Y. *eds.*, *Harmful and Toxic Algal Blooms*, p. 177–180. Intergovernmental Oceanographic Commission of UNESCO, Paris.

Yamamoto, K., 2004: Occurrence of paralytic shellfish toxins in the spring of 2002 in east side of Osaka Bay. *Bulletin of Osaka Fisheries Station*, no. 16, p. 1–8.

Yamamoto, K., Nabeshima, Y., Yamaguchi, M. and Itakura, S., 2009: Distribution and abundance of resting cysts of the toxic dinoflagellates *Alexandrium tamarense* and *A. catenella* in 2006 and 2007 in Osaka Bay. *Bulletin of the Japanese Society of Fisheries Oceanography*, no. 73, p. 57–66.

Yamamoto, K., Nakajima, M. and Imai, I., 2017: Expansion of blooming in the toxic dinoflagellate *Alexandrium tamarense* and environmental fluctuation analyzed from long-term monitoring data in Osaka Bay, eastern Seto Inland Sea, Japan. *Bulletin of the plankton Society of Japan*, vol. 64, p. 11–21.

Zonneveld, K. A. F., Versteegh, G. and Kodrans-Nsiah, M., 2008: Preservation and organic chemistry of Late Cenozoic organic-walled dinoflagellate cysts: A review. *Marine Micropaleontology*, vol. 86, p. 179–197.

Author contribution

KM initiated the study and extracted and observed palynomorphs from surface sediments of Osaka Bay, analyzed the aquatic palynomorph assemblages, MWLT conducted statistical analysis of the aquatic palynomorphs. All authors contributed to the writing the paper.