Red Tides, ed. T. Okaichi, pp. 111–127; References 174–176.

Chapter 3
Biological Character of Red-Tide Organisms

3.2.2 Life history of raphidophycean flagellates

Ichiro IMAI

Alexandrium minutum (Halim) Balech (Fig. 3.16(16))

Cysts are bean-shaped; circular in apical view, without any surface ornament, and small, 20–30 μm in diameter. The wall probably consists of a thin periphragm and a thick endophragm which are strongly adpressed, and are colorless without any surface ornament. The archeopyle is unknown in detail.

The cyst is usually covered with a transparent gelatinous substance sometimes including fine mineral and detrial particles of diatoms, silicoflagellates and other small organisms. After gemination, the empty cyst is hardly preserved in sediment, because of its thin and fragile cyst wall. The living cyst contains many colorless food reserves of starch and oil drops, and one red pigmented body.

Taxonomic note: The cyst of *A. minutum* differs from other cysts of *Alexandrium* in being reniform in lateral view. However, it is similar to the cyst of *G. verior*.

Alexandrium tamarense (Lebour) Balech (Fig. 3.16(15))

The cyst morphology of this species is very similar to that of *A. catenella*.

Scrippsiella trochoidea (Stein) Loeblich III (Fig. 3.16(13))

Cysts are subspherical to ovoidal with many processes, small, 25–50 μm in length and 25–45 μm in width. The cyst wall consists of a thick and calcareous periphragm, and a thin and transparent endophragm, and are dark browm in color with a granular surface. Processes are calcareous, nontabular, slender, cylindrical, and solid with capitate distal ends and variable length of processes. The archeopyle is theropylic, and the archeopyle suture consists of three to four sides of paraplate. However, its location is unknown in detail, but is probably apical/anterior intercalary or anterior intercalary/precingular.

Other features: After chemical treatment with HCl, calcarous parts of cysts are removed, however, organic endophragm still remains (e.g. Matsuoka, 1999). Because of its calcareous wall and processes, the living cyst is dark brown to black in color, and usually contains a single red pigmented body.

Taxonomic note: Some other species of *Scrippsiella*, *S. crystallina*, *S. lachcrymosa*, *S. precaria*, *S. rotunda* and *S. trifida* also possess calcareous walls and processes, however the morphology of calcareous processes are different.

3.2.2 Life history of raphidophycean flagellates (Ichiro Imai)

1) Cell division

Species of raphidophycean flagellates ordinarily multiply with a longitudinal cell division. In the case of *Chattonella antiqua* (Hada) Ono, cell division takes place during darkness. Light irradiation is essential for nuclear DNA replication in *C. antiqua*, and the timing of the replication is dependent upon the timing of the onset of the last irradiation (Nemoto *et al.*, 1987). After the nuclear division, cell division commences from the anterior end of the cell. Following the

formation of new gullets from where flagella emerge, a longitudinal furrow extends about the long axis of the cell. The two daughter cells attach through only a small protoplasmic constriction at the final stage of cell division, and then they each split into two cells which finishes the cell division. *Chattonella marina* (Subrahmanyan) Hara et Chihara, shows the same manner of cell division as *C. antiqua*. Both *C. antiqua* and *C. marina* generally divide once during one period of darkness (Ono, 1988). *Heterosigma akashiwo* (Hada) Hada ex Hara et Chihara, can divide twice or more during one period of darkness (Honjo and Tabata, 1985).

2) Morphology of cysts

Currently, in marine species belonging to Raphidophyceae, natural cysts have been identified in *C. antiqua*, *C. marina*, *H. akashiwo*, and *Fibrocapsa japonica* Toriumi et Takano, from sediments collected from the Seto Inland Sea, Japan (Imai and Itoh, 1986, 1988; Yoshimatsu, 1987; Imai *et al.*, 1993a).

Figure 3.17 shows light micrographs of natural cysts of *C. antiqua* and *C. marina* found in sediments of the Seto Inland Sea. No distinct differences were noticed in morphology between the cysts of *C. antiqua* and *C. marina*, as in the case of cysts of the toxic dinoflagellate *Alexandrium tamarense* and *A. catenella* (Fukuyo *et al.*, 1982). Therefore, incubation of cysts and cultivation of germinated

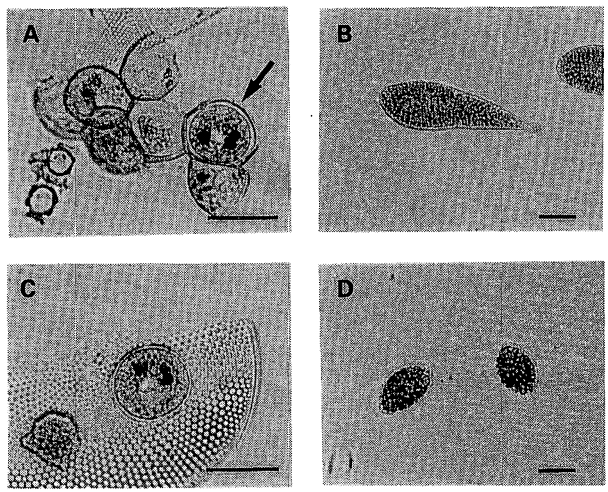

Fig. 3.17. Cysts and vegetative cells of *Chattonella antiqua* and *C. marina*. Scale bar, 30 μm (after Imai and Itoh, 1988).

A: Cyst of *C. antiqua* (indicated by an arrow)

B: Cultured vegetative cells after the germination from the cyst shown in A

C: Cyst of *C. marina* adhered to a fragment of diatom frustule

D: Cultured vegetative cells after the germination from the cyst shown in C

vegetative cells are required for the discrimination of the cysts of the two species (Imai and Itoh, 1988). The live cysts of *C. antiqua* and *C. marina* reveal the following morphological characteristics: (1) they are mostly hemispherical with a diameter of 25–35 μm and a height of 15–25 μm, (2) they usually adhere to solid surfaces such as diatom frustules, sand grains, etc., (3) they are yellow-greenish to brownish in color, (4) they have several spots of dark brown or black materials, (5) they have many chloroplasts visible with epifluorescence microscopy under blue-light excitation, (6) they are uninucleate, and (7) have no ornamentations on the surface of the cyst wall (Imai and Itoh, 1986, 1988). Solitary cysts and clusters of several cysts (occasionally more than 10) including empty ones, were observed. The cysts of *Chattonella* have a structure for future germination, and a circular opening with a diameter of about 7 μm was observed on the wall of an empty cyst after germination (Fig. 3.18).

Under adequate conditions for germination (22°C), the cysts of *Chattonella* with germinability start to germinate, and vegetative cells after germination were abundantly observed during the period from the 4th to 6th day of incubation (Imai

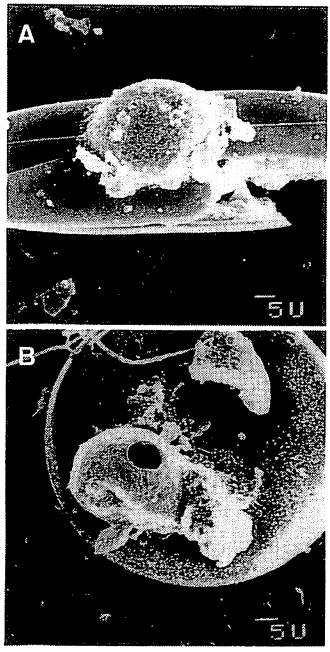

Fig. 3.18. Scanning electron micrographs of Cysts of *Chattonella* (after Imai and Itoh, 1988).

A: A cyst adhered to a pennate diatom frustule

B: An empty cyst adhered to a cantric diatom frustule with a circular opening (diameter, about 7 μm) after the germination

et al., 1984a). The cysts of *Chattonella* can germinate in the dark (Imai *et al.*, 1984a; Imai, 1995). One cell excysts from one cyst. Vegetative cells of *Chattonella* newly germinated within 24 hours, are similar in size and color to the cysts, and they enlarge later to the size of common vegetative cells and begin multiplication by cell division. The cysts are markedly smaller than vegetative cells both in *C. antiqua* and *C. marina* (Fig. 3.17).

Cysts have not been identified in other species belonging to the genus *Chattonella*. However, vegetative cells of *Chattonella ovata* Hara et Chihara were identified after several days of incubation of the bottom sediments collected from the Seto Inland Sea and stored at 11°C in the dark for several months (Imai and Itoh, 1985). Consequently, *C. ovata* presumably has a cyst stage in the life cycle for overwintering.

The cysts of *F. japonica* were identified from sediments collected in Harima-Nada, the Seto Inland Sea, by Yoshimatsu (1987). They are brownish in color and basically spherical, with a diameter of 15–20 μm. They have no spots of dark brown or black materials. Their clear, smooth walls do not possess any paratabulations, ornamentations, or mucilaginous material. The cysts of *F. japonica* were frequently observed to be adhering to diatom frustules like the cysts of *Chattonella*.

The cysts of *H. akashiwo* (Fig. 3.19) were identified from sediments collected in northern Hiroshima Bay, the Seto Inland Sea (Imai *et al.*, 1993a). The cysts are mostly spherical with a diameter of 8–12 μm and are surrounded by mucilaginous materials to which various particles such as sand grain, mud, and pieces of diatom frustule adhere. The cysts are generally solitary, but occasionally from a cluster of several cysts. They are yellow-greenish to brownish in brownish in color. The cysts have no clumps of black or dark brown materials as *Chattonella* cysts do. The cysts are generally smaller than vegetative cells. Live cysts show auto-fluorescing chloroplasts (4–11 per cyst) under observation with blue-light excitation. The cysts are uninucleate and the nuclei of cysts (3.3–5.0 μm in diameter) are smaller than those of vegetative cells. They germinate within a few days (mostly within one or two days) when incubated in filtered-autoclaved seawater at 22°C with light. They can also germinate in the dark (Imai *et al.*, 1996) as do the cysts of *Chattonella*. One cell excysts from one cyst. The cysts of *H. akashiwo* do not have the structure for future germination which *Chattonella* cysts have.

In other marine raphidophycean flagellates, Hara *et al.* (1985) observed statospore-like cells with thick walls in aged cultures of *Olisthodiscus luteus* Carter. Concerning the cysts of some other raphidophycean species from freshwater habitats, those of *Gonyostomum semen* (Ehrenb.) Diesing, and *Vacuolaria virescens* Cienkowski, have been reported (Drouet and Cohen, 1935; Spencer, 1971).

3) Conditions for cyst formation

In *C. marina*, cyst formation was observed in culture under laboratory conditions (Imai, 1989). Nitrogen limitation was effective in inducing cyst

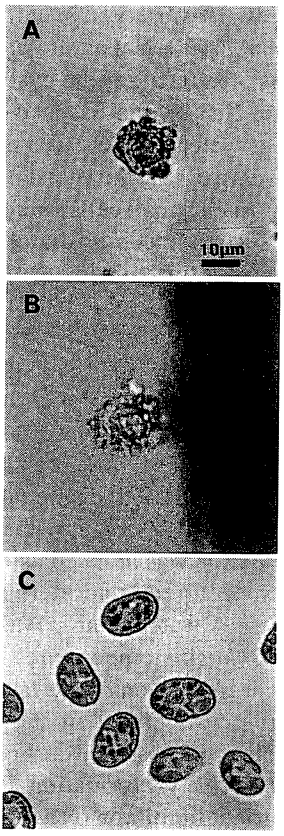

Fig. 3.19. Cysts and vegetative cells of *Heterosigma akashiwo* found in a sediment sample collected in northern Hiroshima Bay (after Imai *et al.*, 1993a).
A: A live cyst surrounded by mucilaginous material to which small detrital particles attach
B: Empty cyst after the germination of the cyst shown in A
C: Cultured vegetative cells after the germination from the cyst shown in A

formation. After the incubation in a nitrogen-limited medium at 25°C with ca. 150 μmol m^{-2} sec^{-1} on a 14 h light, 10 h dark photo-cycle, pre-encystment small cells similar to cysts in size and color were observed, and thereafter cultures were subjected to low light intensities (ca. 15 μmol m^{-2} sec^{-1} or below). Cysts were successfully formed on glass beads, added for attachment to complete cyst formation, by these treatments. The cysts formed in culture displayed morphological characteristics quite similar to those natural cysts observed in sediments. Germination of cysts produced in culture was confirmed under adequate conditions (22°C, ca. 50 μmol m^{-2} sec^{-1} on a 14 h light, 10 h dark photo-cycle) after storage at 11°C in the dark for more than 4 months. In

C. antiqua, cyst formation was also observed in culture under similar conditions to those described above (Imai, 1990; Nakamura *et al.*, 1990).

The following process is presumed for the cyst formation in *C. antiqua* and *C. marina* in the Seto Inland Sea on the basis of the above results. Initially, vegetative cells grow in surface water and thereafter experience nutrient depletion. This may act as a trigger inducing cyst formation, and pre-encystment small cells will be formed. The pre-encystment small cells sink to the sea bottom (Imai *et al.*, 1993b) and adhere to solid surfaces such as diatom frustules and sand grains, where cyst forming cells experience low light intensity, essential for the completion of cyst formation. Consequently, the combination of factors such as nutrient depletion, adherence to solid surfaces, and low light intensity (or darkness) is necessary for cyst formation. In fact, at the final stage of a *Chattonella* red tide (mainly by *C. antiqua*) observed in northern Hiroshima Bay in the late summer of 1990, it was confirmed by the direct count method using epifluorescence microscopy that many cysts are newly formed and supplied to the sea bottom there (Imai *et al.*, 1993b). Nakamura and Umemori (1991) reported that the optimum range of temperature is 21.6°–≥26.6°C in the cyst formation of *C. antiqua*.

In *H. akashiwo*, cyst formation was induced using a natural population at the final stage of a red tide which occurred in northern Hiroshima Bay in early July of 1994 (Itakura *et al.*, 1996a). A water sample was collected at 1 m above the bottom and incubated at 20°C (*in situ* temperature) in the dark with the addition of autoclaved marine sediment. A portion of the *H. akashiwo* cells changed into cysts identical to the natural cysts found in the field (Imai *et al.*, 1993a). Interestingly, smaller-sized cells were observed during the course of cyst formation, resembling those from *Chattonella* cyst formation. From the field data Itakura *et al.* (1996a) suggested that nutrient limitation (N and/or P) acts as a trigger for cyst formation. However, cyst formation has not yet been observed in culture under laboratory conditions. It is a problem for the future to clarify the factors undoubtedly inducing cyst formation in *H. akashiwo*.

The cysts of *F. japonica* were frequently observed to be adhered to diatom frustules like the cysts of *Chattonella* (Yoshimatsu, 1987). This fact suggests that cyst formation finishes after the sinking to the sea bottom, and that the process of cyst formation in *F. japonica* might be similar to that of *Chattonella*. This is an important and interesting theme to investigate in the future.

4) Life history of Chattonella

The life history of *Chattonella* had long been an enigma before the discovery of cysts. Now, the morphology of *Chattonella* (*C. antiqua* and *C. marina*) cysts has been identified and the conditions for cyst formation are also known. Yamaguchi and Imai (1994) determined the nuclear DNA contents at various stages (vegetative cells, pre-encystment small cells, cysts produced in culture, natural cysts, small cells just after the germination within 24 h) in the life history of *C. antiqua* and *C. marina* by means of an epifluorescence microscopy-based fluorometry system after staining with DNA-specific fluorochrome 4′6-diamidino-2-phenylindole (DAPI) (Yamaguchi, 1992), and found that vegetative cells of

both *C. antiqua* and *C. marina* were diploid and that their cysts were haploid, i.e., they have a diplontic life history (Fig. 3.20).

Vegetative cells of *C. antiqua* and *C. marina* ordinarily multiply by asexual binary cell fission, as mentioned before. The nucleus of the G_1 cell of *C. antiqua* was oblong, about 14 μm long by 10 μm wide, and that of the G_2 + M cells was similar in appearance but larger, about 23 μm long by 15 μm wide (Yamaguchi and Imai, 1994). Assuming the DNA content of the vegetative cells of *C. antiqua* in G_1 phase to be 2C, G_2 + M cells were 4C. Interestingly, the DNA contents of the pre-encystment small cells and cysts (both natural and artificially produced) were 1C. Therefore, the meiosis in *C. antiqua* could be indicated by the transition from the 2-4C to the 1C category in the pre-encystment small cell stage. The DNA contents of newly excysted cells, measured within 24 h of germination, revealed a DNA level from 1C to 2C. The difference 1C and 2C cells was only in nuclear size. These facts suggest that DNA diploidization occurs shortly before the cells enlarge into normal vegetative cells without cell fusion.

In *C. marina*, the nucleus of vegetative cells was very similar to *C. antiqua*, and the changes in nuclear DNA content that occur during the life cycle resembled the changes in *C. antiqua*.

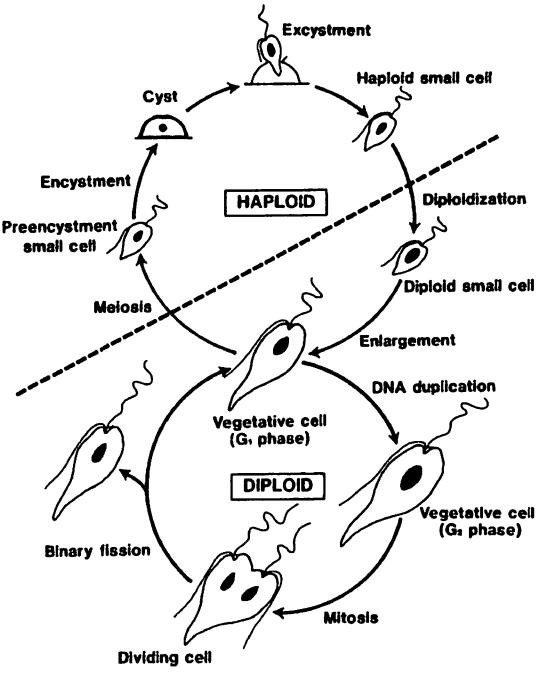

Fig. 3.20. Schematic representation of the life history of *Chattonella antiqua* and *C. marina*, based on DNA microfluorometry (after Yamaguchi and Imai, 1994).

The diplontic life cycle of *Chattonella* is similar to that of diatoms and certain brown algae. The cysts of *F. japonica* and *H. akashiwo* are smaller than the normal vegetative cells as is the case in *Chattonella*. It is accordingly suspected that *F. japonica* and *H. akashiwo* undergo the same nuclear events during their life history as *C. antiqua* and *C. marina*, although there have been no reports hitherto on the nuclear DNA contents.

5) Annual life cycle of Chattonella

Red tides of *C. antiqua* and *C. marina* are caused by the motile, planktonic stage in their life history during the summer season. Since the water temperature descends to around 10°C or below in the Seto Inland Sea during the winter season, they can not overwinter as vegetative cells (Yamaguchi *et al.*, 1991). They have a cyst stage for overwintering, and the cysts play a majar role in the total ecology of the neritic species *Chattonella* by serving several important functions, as is the case in some toxic dinoflagellates (Wall, 1971; Fukuyo *et al.*, 1982; Anderson *et al.*, 1983; Dale, 1983). Cysts settle to bottom sediments to overwinter and thereby ensure the persistent existence of the species in the same area (as in the Seto Inland Sea) and the germination of cysts provides the inoculum into overlying waters for blooms in summer.

Water temperatures seasonally fluctuate in the Seto Inland Sea. Temperatures of around 10°C or below are usual in winter and of 25°C or higher in summer. According to field observations and laboratory experiments using sediment samples collected from the Seto Inland Sea, it was confirmed that the temperature is a crucial factor affecting the physiology of cysts of *Chattonella* (Imai and Itoh, 1987; Imai *et al.*, 1989, 1991).

Figure 3.21 shows the effects of incubation temperature on the germination of mature cysts, and of storage temperature on the maturation (acquisition of the germinability) of dormant cysts in sediments (Imai *et al.*, 1989; Imai, 1990). The germination of *Chattonella* cysts was not possible at 10°C, very low at 15° and 18°C, while it increased at 20°C with maxima at 22° and 25°C, and then decreased markedly at 30°C. For maturation of immature dormant *Chattonella* cysts, low storage temperature of 11°C or below for more than four months is needed, whereas no maturation is observed at 20°C or more. Storage temperatures of 15° and 18°C are critical for the maturation. Concerning dormancy of mature cysts (loss of the germinability), the effects of temperature were furthermore investigated (Imai *et al.*, 1989). When mature cysts of *Chattonella* in sediments are stored at low temperature of 11°C or below, the germinability is maintained. They gradually lost the germinability at 15° and 18°C during storage, and did so rapidly at 20°C or more. Thus, the cysts of *Chattonella* have a temperature window for vigorous germination corresponding to the bottom temperature in early summer in the Seto Inland Sea (20°C or higher). On the other hand, immature dormant *Chattonella* cysts require a period of cold winter temperatures for more than four months as a mandatory period for maturation, indicating that the dormancy of *Chattonella* cysts can be regarded as genetically regulated spontaneous dormancy,

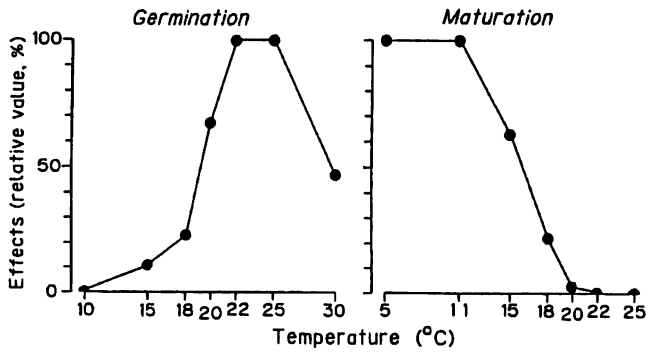

Fig. 3.21. Effects of incubation temperature on the germination of mature cysts of *Chattonella*, and of storage temperature on maturation of dormant cysts in sediments. The ordinates show the percentage of maximum value obtained in experiments (after Imai *et al.*, 1989; Imai, 1990, modified).

as is the case in some higher plant seeds.

In the case of *F. japonica*, a mandatory period of at least two or three months is needed to obtain germinability under storage temperatures of 11° and 22°C (Imai, 1990). The newly formed cysts of *H. akashiwo* demand more than two but less than three weeks as a mandatory period of dormancy at 20°C in the dark (Itakura *et al.*, 1996a).

Using freshly collected bottom sediments from Suo-Nada, the Seto Inland Sea, the seasonality of germination ability was investigated for *Chattonella* using the extinction dilution method (MPN method) (Imai *et al.*, 1984b; Imai and Itoh, 1987). The cysts of *Chattonella* revealed a marked seasonality in the germinability (Fig. 3.22). It was weak from autumn to early winter, then strengthened gradually up to a high level, which was maintained between spring and early summer, and again decreased rapidly during summer. Based on these field data and the temperature characteristics of germination and dormancy in the cysts, the annual life cycle of *Chattonella*, including vegetative and dormant phase, is summarized in Fig. 3.23 (Imai and Itoh, 1987). In the Seto Inland Sea, vegetative cells of *C. antiqua* and *C. marina* are generally observed from June to September and occasionally cause red tides, mainly in July and August. These vegetative cells originate from the germination of cysts in the bottom sediments in early summer when the bottom water temperature reaches an adequate level of around 20°C (Imai *et al.*, 1984a; Imai, 1990). They multiply asexually in summer and produce pre-encystment small cells under unfavorable conditions such as nutrient depletion. these small cells then sink to the sea bottom (Imai *et al.*, 1993b), and cyst formation is completed there after the adhesion to solid surfaces such as diatom frustules and sand grains. The cysts of *Chattonella* spend a period of spontaneous (genetically regulated) dormancy there until the following spring. They never

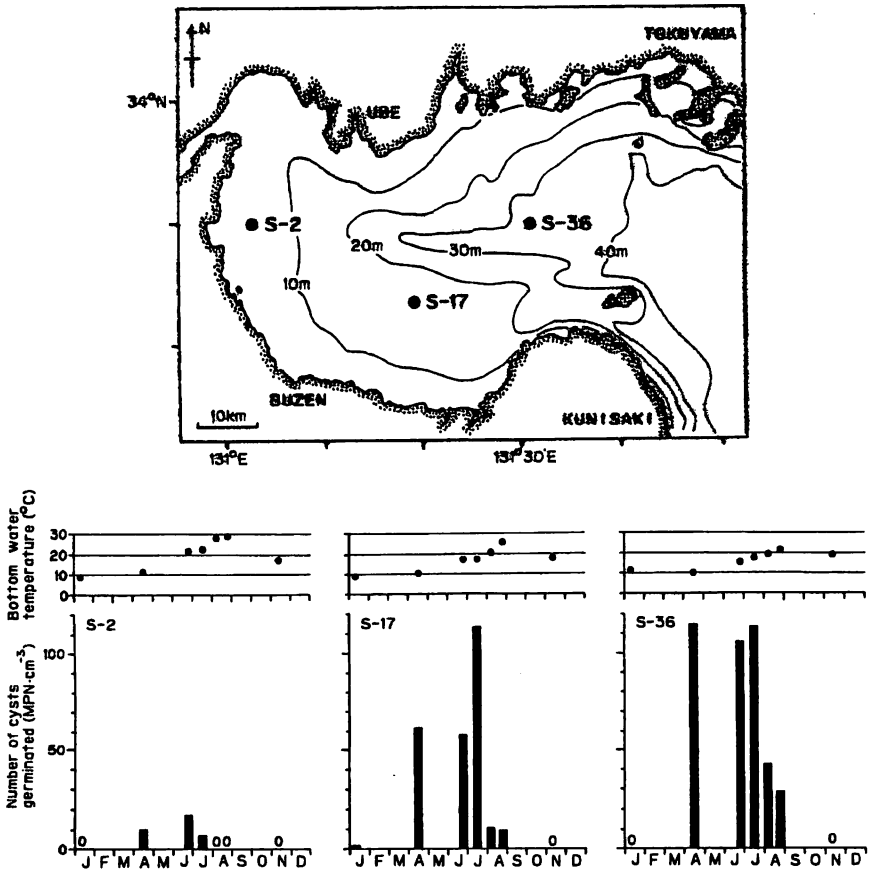

Fig. 3.22. Seasonal changes in germinability of cysts from fresh sediment samples collected at three stations in Suo-Nada (upper panel), in 1985. The changes of bottom water temperatures (1 m above the bottom) are also shown (after Imai and Itoh, 1987, modified).

germinate in autumn, even when the bottom water temperature descends to the optimal range for germination, 20° to 22°C. The maturation of cysts progresses during winter. In spring, many cysts complete the period of spontaneous dormancy and acquire the ability of germination. From spring to early summer, however, they must spend a period of post dormancy, a kind of enforced dormancy, due to the temperature being too low for germination. Vegetative cells appear in the overlying water column thereafter through the germination of cysts. Most of the cyst populations, however, remain in sediments without germination, and they are carried over to the next year, or year to year, via the secondary dormancy which is induced by high temperatures (≥22°C) during the summer season (Imai

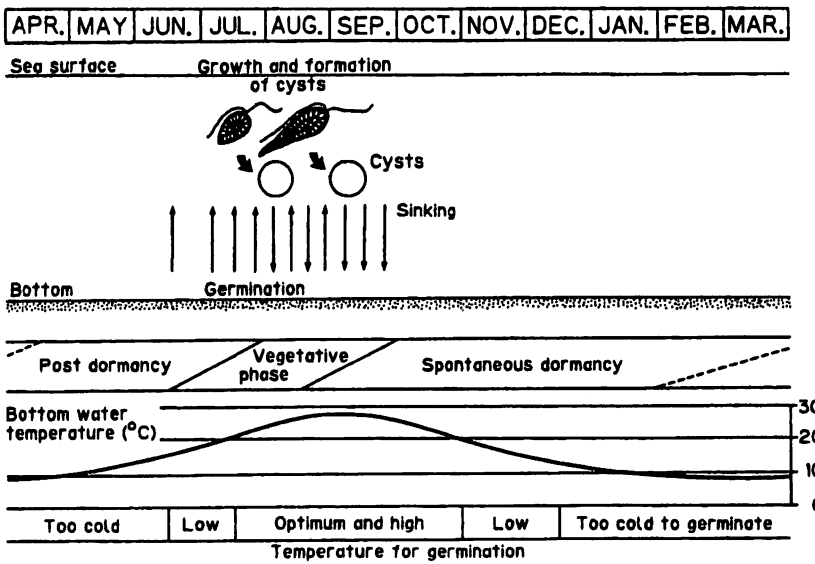

Fig. 3.23. Schematic representation of the annual life cycle of *Chattonella* in the Seto Inland Sea, including vegetative and cyst phases. The seasonal fluctuation of bottom water temperature is also shown (after Imai and Itoh, 1987, modified).

et al., 1989). Secondary dormancy has already been reported in the resting spores of the diatom *Eunotia soleirolii* (von Stosch and Fecher, 1979). The cysts of *Chattonella* thus can survive without germination for more than two years, implying that *Chattonella* can from red tides after years with no occurrences of red tides.

As *C. antiqua* and *C. marina* are observed only in the summer, they spend most of their life as cysts in sediments. In summary, the life cycle of *Chattonella* is considered to be well adapted to the seasonal temperature fluctuations in temperate coastal areas such as the Seto Inland Sea. Moreover, alternation between benthic and planktonic stages is presumably unconstrained, because most part of the Seto Inland Sea is shallower than 50 m (Imai and Itoh, 1987; Imai *et al.*, 1991).

Concerning *H. akashiwo*, benthic cysts play an important role in overwintering (Yamochi, 1989; Imai *et al.*, 1993a), although vegetative cells are detected in surface water of Osaka Bay (Yamochi, 1984) and Hiroshima Bay (Imai and Itakura, 1999) in the winter season. Red tides of *H. akashiwo* generally occur in early summer of almost every year with great regularity (Taylor and Haigh, 1993; Itakura *et al.*, 1996b). Temperature characteristics of the germination of *H. akashiwo* cysts may explain a part of the regularity of the appearance of red tides in temperate coastal waters (Imai and Itakura, 1999).

6) Cyst distribution and occurrences of red tides of Chattonella *in the Seto Inland Sea*

Live cysts of *Chattonella* (*C. antiqua* and *C. marina*) can be enumerated by the direct count technique with epifluorescence microscopy (Imai and Itoh, 1988; Imai, 1990). Figure 3.24 shows the distribution of live cysts of *Chattonella* in sediments of Suo-Nada, western Seto Inland Sea, in the springs of 1986 and 1987. Densities of cysts in sediment samples ranged from 0 to 787 cm^{-3} wet sediment (mean 125) in March of 1986, and from 0 to 490 cm^{-3} wet sediment (mean 91) in June of 1987. Spatial distributions of cysts showed similar patterns in both years, i.e. a highly accumulated area was noticed in the eastern-central part of Suo-Nada. However, the high density areas (seed beds) of *Chattonella* cysts do not always coincide with those of vegetative cells during the summer red-tide season

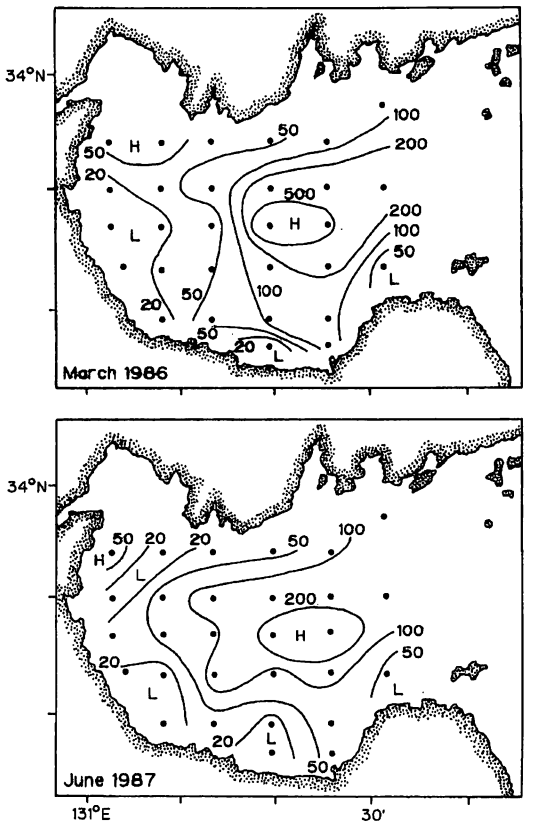

Fig. 3.24. Spatial distribution of cysts of *Chattonella* in Suo-Nada in March 1996 (upper) and in June 1987 (lower). Numerals indicate the number of cysts in cubic centimeter wet sediment. Surface sediments (top 3-cm depth) were used for enumeration (after Imai, 1990).

(Imai *et al.*, 1986; Imai, 1990). For example, in Suo-Nada, the red tides are generally found in the western-southern coastal shallow areas. The accumulation mechanisms of cysts are probably different from those of vegetative cells. In the eastern Seto Inland Sea, including Harima-Nada where severe damage to cultured yellowtail fisheries have been caused by red tides of *Chattonella*, the distribution of live cysts of *Chattonella* in sediments were studied in 1988 and 1989 (Itakura *et al.*, 1991). The cysts were widely detected in the whole area (0–1503 cysts cm^{-3} wet sediment). It was suggested that the abundance of vegetative cells in the preceding year affects the proportion of germinable cysts to the total live cysts in the following summer.

The dynamics of cysts and vegetative cells of *Chattonella* were investigated together with the environmental factors in Suo-Nada. A conceptual model of *Chattonella* red tides in Suo-Nada is presented in Fig. 3.25. The cysts of *Chattonella* presumably start to germinate from the coastal shallow area where the bottom water temperature is always relatively higher than the deeper area and reaches the optimum level (ca. 20°C) for germination in early June. The inoculated vegetative populations appear as a result of the germination of cysts in the south-western coastal shallow area in spite of the lower densities of cysts, and multiply there in surface waters. As the bottom water temperature gradually rises from the coastal shallow area to deeper area, the germination of *Chattonella* cysts presumably continues for a rather long period. In midsummer, the cysts in deeper water areas also start to germinate, and new populations start to grow in the surface waters. Accumulation of vegetative cells by winds blowing to the coastal

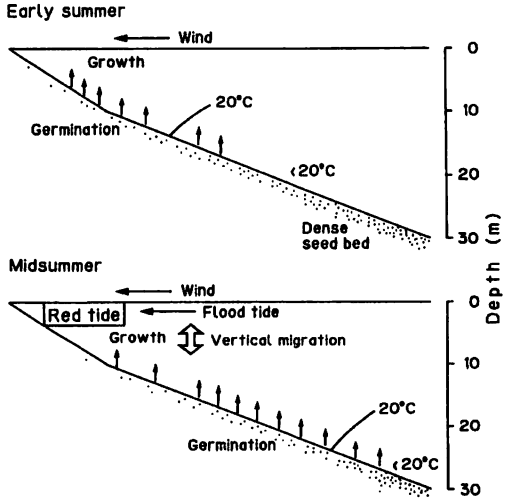

Fig. 3.25. A schematic representation of the outbreak of *Chattonella* red tide in the western coastal area of Suo-Nada, western Seto Inland Sea (after Imai *et al.*, 1986; Imai, 1990).

area from offshore in daytime probably play an important role in supporting the population density at a higher level, because motile *Chattonella* cells vertically migrate to the upper layer during daytime (Watanabe *et al.*, 1995).

The mean density (top 3-cm depth) of *Chattonella* cysts is about 100 cm^{-3} wet sediment, equivalent to about 300 cysts cm^{-2}. Even if a burst germination occurred and provided a large one-time input of vegetative populations early in the bloom, it results in an increase of only 1 cell ml^{-1} or lower in the water column of the upper 5-m layer. Accordingly, vegetative growth after inoculation by the germination of cysts is essential for the formation of *Chattonella* red tides. In the case of the *Chattonella* red tide formed in Suo-Nada in the summer of 1987, the temporal changes in the total live cysts and those with the germinability in freshly collected sediment samples (top 1-cm depth) were investigated, and it is confirmed that the number of cysts possessing the germination ability did not change markedly between late June (before the red tide) and mid July (in the middle of red tide), although the number of vegetative cells in the water column drastically increased by about two orders of magnitude from late June to mid July (Imai, 1990; Imai *et al.*, 1998). This fact implies that the number of cysts that germinated during the summer red-tide season was small, and that factors affecting the processes after germination such as vegetative growth, advection, mortality, competition, and encystment, are important for red-tide formation and sequences thereafter, as Anderson *et al.* (1983) and Imai *et al.* (1991) have suggested.

When vegetative cells of *Chattonella* appear in the water column after the germination of cysts, factors such as nutrients and competitors (mainly diatoms) may thereafter crucially affect the development of *Chattonella* populations (Imai *et al.* 1998). It is empirically known that *Chattonella* red tides have occurred when diatoms are scarce. The diatoms (mainly Centrales) seem to dominate *Chattonella* as competitors because diatoms generally have higher growth rates (Eppley, 1977; Yamaguchi, 1994). However, diatoms form resting stage cells under nutrient limitations, and those resting stage cells sink to the bottom. Consequently, the diatom cell concentrations can be reduced in the water column via resting stage cell formation and sinking, after a stratification and resultant exhaustion of nutrients in the surface layer. Because of the decrease of diatoms in the surface water, *Chattonella* can obtain a competitive advantage and thus can became dominant despite low growth rates of at most 1 division day^{-1}, after mixing events which supply nutrients to the surface layer. This "diatom resting hypothesis" can explain the occurrence of red tides by "slow-growing" *Chattonella* in the Seto Inland Sea (Imai *et al.*, 1998). Further investigations will be necessary for verifying this hypothesis.

3.2.3 Function of cyst (Yasuwo Fukuyo)

The number of cyst-forming dinoflagellates is less than 150 (Table 3.6), and this number shows that less than 10% of the extant species produce resting cysts. This low percentage may indicate that cyst formation does not have much importance in the survival strategy of dinoflagellates. However, many harmful

algae, either red-tide species or the toxin-producing species shown in Table 3.1, can be found in Table 3.7. Almost all PSP toxin-producing species belonging to the genus *Gymnodinium*, *Alexandrium* and *Pyrodinium* have a resting cyst stage. Red-tide species belonging to *Gymnodinium* and *Gonyaulax* also have resting cysts. Therefore, for clarification of the blooming and survival strategies of these harmful organisms it is necessary to consider the function of the resting cyst.

Red-tide forming harmful raphidophytes (Table 3.2) also have resting cyst stages (Imai and Itoh (1988) on *Chattonella antiqua* and *C. marina*, Imai *et al.* (1993) on *Heterosigma akashiwo*, Yoshimatsu (1987) on *Fibrocapsa japonica*). Although their cysts may be haploid instead of diploid in dinoflagellates, their ecological role discussed below is just the same.

Resting cysts formed by dinoflagellates and raphidophytes have strong resistance against extreme environmental changes and certain periods of quiescence and dormancy, which induce simultaneous germination in response to better environmental change. Therefore, the cysts have an important ecological role as the source seedlings of the recurrence, blooming, and geographical distribution expansion of harmful microalgae. Wall (1971, 1975), Hanaoka *et al.* (1972) and Steidinger (1975) had already pointed out these features more than three decades ago, but almost all of their descriptions were mere assumptions because of a lack of basic scientific data. But along with the accumulation of basic scientific knowledge concerning biology, especially the reproduction process of cyst-producing species (Table 3.7), studies were accelerated on various ecological features such as the relationships between cyst distribution and the plankton blooming area (Anderson and Wall, 1978; Fukuyo, 1982; Imai *et al.*, 1986), between cyst germination and environmental triggering factors (Anderson and Morel, 1979; Fukuyo *et al.*, 1982; Imai *et al.*, 1984; Yamochi, 1984; Endo and Nagata, 1984; Yamochi and Joh, 1986), between blooming development and cyst formation (Anderson, 1980; Anderson *et al.*, 1983; Fukuyo, 1982; Tyler *et al.*, 1982; Takeuchi, 1985). These revealed the functions of the cysts and their ecological importance in harmful algal bloomings.

The function of cysts can be considered as (1) a source of the recurrence of microalgae, (2) a vector for distribution expansion, and (3) a resistant cell against inadequate ambient conditions for survival. Other functions related to cyst formation are (4) recombination of chromosomes, and (5) termination of blooming because of a decrease of cell number through sexual fusion from two gametes to one zygote. In addition to these functions, Anderson (1982) suggested that cysts of toxic species accumulated on the surface of bottom sediment worked as (6) a toxin source for benthic shellfish which live at the deep floor and have very a limited chance of feeding toxic planktonic cells.

Field surveys and laboratory experiments to prove these functions were conducted mostly using species belonging to the genus *Alexandrium*, which contains several toxin-producing species, although some used *Peridinium* sp. (Endo and Nagata, 1980) and *Gyrodinium uncatenum* (Tyler *et al.*, 1982). For example, Fukuyo (1982) found that the period of increase and decrease of the

plankton population of *Alexandrium* in water coincided with the change of cyst population in the bottom sediment in Ofunato Bay, on the northern Pacific coast of mainland Japan, and then concluded that the plankton population came from the seed population in the bay, and not from outside the bay. In plankton samples, many planozygotes together with vegetative cells can be detected in the peak blooming season. Therefore, sexual reproduction occurs simultaneously in the period of active population growth by asexual reproduction, and the reduction of cell numbers by sexual reproduction induced a slower growth rate. Transformation of hypnozygotes from planozygotes also occurs during the blooming period and the number of resting cysts (hypnozygote) becomes highest at the end of the period (see Section 6.5). These characters of blooming were also observed in the population dynamics of *Alexandrium catenella* in Tanabe Bay, Wakayama, central Japan (Takeuchi, 1985).

Newly formed cysts drifting in waters are passively affected by water movement such as current and tide, until they settle down on the surface of the bottom sediment in a calm area. Transportation by the water movement often decides the distribution pattern and causes an expansion of the distribution. White and Lewis (1982) reported that the cyst distribution pattern of *A. tamarense* could be explained by the circling current in Fundy Bay, eastern Canada. Anderson *et al.* (1982) found the effect of various water movements on the cyst distribution of *A. tamartense* along the north-eastern coast of the United States. Tyler *et al.* (1982) also observed the distribution pattern of cysts of *Gyrodinium uncatenum*, which often caused red tides in river-mouth areas, because of water movements, such as tide and eddy, which are common to an area. In raphidophytes, the distribution pattern of cysts of *Chattonella* was observed in Harima-Nada and Suo-Nada of the Seto Inland Sea. The amount of the cysts varied by area and did not coincide with plankton distribution (Imai and Itoh, 1985; Imai *et al.*, 1984, 1986).

In addition to the natural mechanism, anthropogenic activities such as overseas trading using cargo vessels (Hallegraeff, 1998) and the transplantation of aquaculture fish and shellfish, also have an effect on the expansion of distribution. In Tasmania, Australia, several toxic species recently caused serious damage, although these species could not be found in the deeper layer of the bottom sediment. Hallegraeff *et al.* (1990) considered that the harmful species were not endemic in Tasmania and might have been carried by ships from other countries such as Japan. Then, through a detailed survey of cysts in ballast water tanks of ships coming from outside Australia revealed that some ships kept many viable cysts for a long time together with sediment sand particles. This finding is very clear evidence for the anthropogenic effect of the distribution of plankton, but the settlement of the species carried to a new area depends on the natural environment of the site. Differentiation between hidden existing groups of species and newly invaded ones is another scientific subject difficult to approach. Cyst analysis can provide information of the hidden flora, but only 10% of dinoflagellates produce resting cysts, so, the method can be applied to only a limited number of species.

We may possibly have to wait for the development of techniques, such as DNA or RNA sequence analysis and ELISA tests, to characterize the local strains of several species.

Cysts embedded in the bottom sediment endure changes of ambient temperature, salinity and oxygen concentration. Under extremely inadequate conditions cysts may slow down maturation and prolong the resting period. Endo and Nagata (1984) observed that cysts of *Peridinium* sp. cannot germinate, but can survive under aerobic conditions. This means that anaerobic conditions prevent germination of cysts (Anderson and Morell, 1979; Endo and Ngata, 1984), and there is a change to the aerobic environment, many cysts hatch out simultaneously. Light intensity and photo-period are not so influential (Anderson and Wall, 1978; Fukuyo, 1982; Endo and Nagata, 1984), and the concentration of nutrients does not affect germination, although low N and P concentrations induce the onset of the sexual process of dinoflagellates (Anderson and Lindquist, 1985; Yoshimatsu, 1985).

A most important environmental factor is temperature. Cysts of *Alexandrium tamarense* can stand low temperature conditions, and show very slow maturation. The time taken for recovering the ability to germinate after encystment is 6 weeks at 22°C, but 4 months at 5°C (Anderson, 1980; Fukuyo *et al.*, 1982). In natural conditions low temperatures prevent maturation and germination of cysts, ascending temperatures enhance the development of cell conditions (Anderson and Morell, 1979), and finally all cysts geminate in response to better conditions. Other oceanographic factors such as water movement are also important in relation to oxygen concentration and the re-suspension of cysts. Yoshimatsu (1990) found covering of cysts with sand prevented excystment and, therefore, the resuspension of cysts may be a direct triggering factor.

3.3 PHYSIOLOGICAL CHARACTERISTICS (Masayuki Mac Takahashi)

Most red-tide-forming algae are not generally prominent in natural communities. However, once they form a red tide, they become fairly abundant in water and often color the water, which is easily detectable with the naked eye. It is known that red-tide-forming algae may have some unique characteristics which may not be common in other algae. In this section, we will review how red-tide-forming algae are unique compared to other algae, particularly in their physiological characteristics.

As with many other algae, red-tide-forming algae are believed to divide actively under suitable environmental conditions for them, to deteriorating cell division if the environment becomes unsuitable, and finally reaching a dormant condition, such as forming cysts and/or resting cells. By detecting the right environment and suitable conditions via some unexplained mechanism, resting cells and cysts germinate and start to divide again. Even though the details of the life cycles of red-tide-forming algae vary greatly with different species, they basically live on vegetative growth and have repeated resting stages, according

Gyrodinium aureolum in Dutch coastal waters by particle analysis based on immuno-flow cytometry. *Neth. J. Aquat. Ecol.*, **28**, 249–254.

Vrieling, E. G., L. Peperzak, W. W. C. Gieskes and M. Veenhuis (1994b): Detection of the ichthyotoxic dinoflagellate *Gyrodinium* cf. *aureolum* and morphologically related *Gymnodinium* species using monoclonal antibodies: a specific immunological tool. *Mar. Ecol. Prog. Ser.*, **103**, 165–174.

Vrieling, E. G., R. P. T. Koeman, L. Peperzak, K. Nagasaki and Y. Ishida (1995): *Chattonella* and *Fibrocapsa* (Raphidophyceae): first observation of potentially harmful, red tide organisms in the Dutch Wadden Sea. *Neth. J. Sea Res.*, **33**, 183–191.

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Bolch, G. J. S., A. P. Negri and G. M. Hallegraeff (1999): *Gymnodinium microreticulatum* sp. nov. (Dinophyceae): a naked, microreticulate cyst producing dinoflagellate, distinct from *Gymnodinium catenatum* Graham and *Gymnodinium nolleri* Ellegaard et Moestrup.

Dale, B. (1978): Acritarchous cyst of *Peridinium faeroense* Paulsen: Implication for dinoflagellate systematics. *Palynology*, **2**, 187–193.

Dodge, J. D. (1982): Marine Dinoflagellates of the British Isles. Her Majestey's Stationery Office, London, 303 pp.

Ellegaarde, M. and Ø. Moestrup (1999): Fine structure, with emphasis on the flagellar apparatus, and morphological details of *Gymnodinium nolleri* sp. nov. (Dinophyceae), an unarmoured dinoflagellate producing a microreticulate cyst. *Phycologia*, **38**, 289–300.

Harland, R. (1977): Recent and late Quaternary (Flandrian and Devensian) dinoflagellate cysts from marine continental shelf sediments around the British Isles. *Palaeontographica, Abt. B*, **264**, 87–126.

Kokinos, J. P. and D. M. Anderson (1995): Morphological development of resting cysts in cultures of *Lingulodinium polyedrum* (=*L. machaerophorum*). *Palynology*, **19**, 143–166.

Matsuoka, K. (1967): Palaeoenvironmental study of the Saho and the Saidaiji Formations from a view point of palynology. *Bulletin of Mizunami Fossil Museum*, No. 3, 99–117.

Matsuoka, K. (1985): Archeopyle structure in modern gymnodinialean dinoflagellate cysts. *Review of Palaeobotany and Palynology*, **44**, 217–231.

Matsuoka, K. (1999): Eutrophication process recorded in dinoflagellate cyst assemblage—a case of Yokohama Port, Tokyo Bay, Japan. *The Science of the Total Environment*, **231**, 17–35.

Matsuoka, K., Y. Fukuyo and D. M. Anderson (1988): The cyst and theca of *Gonyaulax verior* Sournia (Dinophyceae) and their implication for the systematics of the genus *Gonyaulax*. *Japanese Journal of Phycology*, **36**, 311–320.

Matsuoka, K., A. McMinn and J. H. Wrenn (1997): Restudy of the holotype of *Operculodinium centrocarpum* (Deflandre & Cookson) Wall (Dinophyceae) from the Miocene of Australia, and the taxonomy of related species. *Palynology*, **21**, 19–33.

McMinn, A. (1991): Recent dinoflagellate cysts from estuaries on the central coast of New South Wales, Australia. *Micropaleontology*, **37**, 269–287.

Meunier, A. (1919): Microplankton de la Mer Flamande 3. Les Peridiniens. *Nus. Hist. Nat. Belg., Mem.*, **8**, 1–116.

Schillar, J. (1937): Dinoflagetae (Peridineae). In *Kryptogamen-Flora von Deutschland, Osterrich und der Schweiz*, 10 (Flagellatae) Abteilung, 3(2) Fasc., 4, ed. by L. Rabenhorst, Verlagsgesellschaft M.B.H., Leipzig, pp. 282–590.

Sournia, A. (1984): Classification et nomenclature de divers dinoflagelles marins (Dinophyceae), *Phycologia*, **23**, 345–355.

Wall, D. and B. Dale (1968): Modern dinoflagellate cysts and evolution of the Peridiniales. *Micropaleontology*, **14**, 265–304.

Section 3.2.2

Anderson, D. M., S. W. Chisholm and C. J. Watras (1983): Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis*. *Mar. Biol.*, **76**, 179–189.

Dale, B. (1983): Dinoflagellate resting cysts: "benthic plankton". In *Survival Strategies of Algae*.

ed. by G. A. Fryxell, Cambridge Univ. Press, Cambridge, pp. 69–136.
Drouet, F. and A. Cohen (1935): The morphology of *Gonyostomum semen* from Woods Hole, Massachusetts. *Biol. Bull. Mar. Biol. Lab., Woods Hole*, **68**, 422–439.
Eppley, R. W (1977): The growth and culture of diatoms. In *The Biology of Diatoms*, ed. by D. Werner, Blackwell, Oxford. pp. 24–64.
Fukuyo, Y., M. M. Watanabe and M. Watanabe (1982): Encystment and excystment of red-tide flagellates II. Seasonality on excystment of *Protogonyaulax tamarensis* and *P. catenella*. *Res. Rep. Natl. Inst. Environ. Stud.*, No. 30, 43–52 (in Japanese with English abstract).
Hara, Y., I. Inouye and M. Chihara (1985): Morphology and ultrastructure of *Olisthodiscus luteus* (Raphidophyceae) with special reference to the taxonomy. *Bot. Mag. Tokyo*, **98**, 251–262.
Honjo, T. and K. Tabata (1985): Growth dynamics of *Olisthodiscus luteus* in outdoor tanks with flowing coastal water and in small vessels. *Limnol. Oceanogr.*, **30**, 653–664.
Imai, I. (1989): Cyst formation of the noxious red tide flagellate *Chattonella marina* (Raphidophyceae) in culture. *Mar. Biol.*, **103**, 235–239.
Imai, I. (1990): Physiology, morphology, and ecology of cysts of *Chattonella* (Raphidophyceae), causative flagellates of noxious red tides in the Inland Sea of Japan. *Bull. Nansei Natl. Fish. Res. Inst.*, No. 23, 63–166 (in Japanese with English abstract).
Imai, I. (1995): Ecological control of *Chattonella* red tides by diatoms. *Kaiyo Monthly*, **27**, 603–612 (in Japanese).
Imai, I. and S. Itakura (1999): Importance of cysts in the population dynamics of the red tide flagellate *Heterosigma akashiwo* (Raphidophyceae). *Mar. Biol.*, **133**, 755–762.
Imai, I. and K. Itoh (1985): Distribution of dormant cells of *Chattonella* in bottom sediments of Harima-Nada, eastern Seto Inland Sea, in April, 1984. *Bull. Nansei Natl. Fish. Res. Lab.*, No. 19, 43–52 (in Japanese with English abstract).
Imai, I. and K. Itoh (1986): A preliminary note on the cysts of *Chattonella* (Raphidophyceae), red tide flagellates, found in bottom sediment in Suo-Nada, western Seto Inland Sea, Japan. *Bull. Plankton Soc. Japan*, **33**, 61–63 (in Japanese with English abstract).
Imai, I. and K. Itoh (1987): Annual life cycle of *Chattonella* spp., causative flagellates of noxious red tides in the Inland Sea of Japan. *Mar. Biol.*, **94**, 287–292.
Imai, I. and K. Itoh (1988): Cysts of *Chattonella antiqua* and *C. marina* (Raphidophyceae) in sediments of the Inland Sea of Japan. *Bull. Plankton Soc. Japan*, **35**, 35–44.
Imai, I., K. Itoh and M. Anraku (1984a): Distribution of dormant cells of *Chattonella* in Harima-Nada, eastern Seto Inland Sea, and temperature characteristics of germination. *Bull. Plankton Soc. Japan*, **31**, 35–42 (in Japanese with English abstract).
Imai, I., K. Itoh and M. Anraku (1984b): Extinction dilution method for enumeration of dormant cells of red tide organisms in marine sediments. *Bull. Plankton Soc. Japan*, **31**, 123–124.
Imai, I., K. Itoh, K. Terada and M. Kamizono (1986): Distribution of dormant cells of *Chattonella* (Raphidophyceae) and occurrence of summer red tide in Suo-Nada, western Seto Inland Sea. *Bull. Jpn. Soc. Sci. Fish*, **52**, 1665–1671 (in Japanese with English abstract).
Imai, I., K. Itoh and M. Anraku (1989): Dormancy and maturation in the cysts of *Chattonella* spp. (Raphidophyceae), red tide flagellates in the Inland Sea of Japan. In *Red tides: Biology, Environmental Science, and Toxicology*, ed. by T. Okaichi, D. M. Anderson and T. Nemoto, Elsevier, N.Y., pp. 289–292.
Imai, I., S. Itakura and K. Itoh (1991): Life cycle strategies of the red tide causing flagellates *Chattonella* (Raphidophyceae) in the Seto Inland Sea. *Mar. Poll. Bull.*, **23**, 165–170.
Imai, I., S. Itakura and K. Itoh (1993a): Cysts of the red tide flagellate *Heterosigma akashiwo*, Raphidophyceae, found in bottom sediments of northern Hiroshima Bay, Japan. *Nippon Suisan Gakkaishi*, **59**, 1669–1673.
Imai, I., S. Itakura and A. Ouchi (1993b): Occurrence of *Chattonella* red tide and cyst dynamics in sediments in northern Hiroshima Bay, the Seto Inland Sea, Japan. *Nippon Suisan Gakkaishi*, **59**, 1–6 (in Japanese with English abstract).
Imai, I., S. Itakura, M. Yamaguchi and T. Honjo (1996): Selective germination of *Heterosigma akashiwo* (Raphidophyceae) cysts in bottom sediments under low light conditions: A possible mechanism of the red tide initiation. In *Harmful and Toxic Algal Blooms*, ed. by T. Yasumoto,

Y. Oshima and Y. Fukuyo, IOC-UNESCO, Paris. pp. 197–200.

Imai, I., M. Yamaguchi and M. Watanabe (1998): Ecophysiology, life cycle, and bloom dynamics of *Chattonella* in the Seto Inland Sea, Japan. In *Physiological Ecology of Harmful Algal Blooms*, ed. by D. M. Anderson, A. D. Cembella and G. M. Hallegraeff, Springer-Verlag, Berlin. pp. 95–112.

Itakura, S., I. Imai and K. Itoh (1991): Spatial distribution of cysts of the noxious red tide flagellates *Chattonella* (Raphidophyceae) in sediments of the eastern Seto Inland Sea, Japan. *Nippon Suisan Gakkaishi*, **57**, 1079–1088 (in Japanese with English abstract).

Itakura, S., K. Nagasaki, M. Yamaguchi and I. Imai (1996a): Cyst formation in the red tide flagellate *Heterosigma akashiwo* (Raphidophyceae). *J. Plankton Res.*, **18**, 1975–1979.

Itakura, S., K. Nagasaki, M. Yamaguchi and I. Imai (1996b): Species succession between *Skeletonema* costatum and *Heterosigma akashiwo* in Hiroshima Bay, Japan, with special reference to the resting stage cells in the bottom sediments. In *Harmful and Toxic Algal Blooms*, ed. by T. Yasumoto, Y. Oshima and Y. Fukuyo, IOC-UNESCO, Paris. pp. 373–376.

Nakamura, Y. and T. Umemori (1991): Encystment of the red tide flagellate *Chattonella antiqua* (Raphidophyceae): cyst yield in batch cultures and cyst flux in the field. *Mar. Ecol. Prog. Ser.*, **78**, 273–284.

Nakamura, Y., T. Umemori, M. Watanabe, D. M. Kulis and D. M. Anderson (1990): Encystment of *Chattonella antiqua* in laboratory cultures. *J. Oceanogr. Soc. Japan*, **46**, 35–43.

Nemoto, Y., T. Kuroiwa and M. Furuya (1987): Photocontrol of nuclear DNA replication in *Chattonella antiqua* (Raphidophyceae). *Plant Cell Physiol.*, **28**, 1043–1049.

Ono, C. (1988): Cell cycles and growth rates of red tide organisms in Harima Nada area, eastern part of Seto Inland Sea, Japan. *Bull. Akashiwo Res. Inst. Kagawa Pref.*, No. 3, 1–67 (in Japanese with English abstract).

Spencer, L. B. (1971) A study of *Vacuolaria virescens* Cienkowski. *J. Phycol.*, **7**, 274–279.

von Stosch, H. A. and K. Fecher (1979): "Internal thecae" of *Eunotia soleirolii* (Bacillariophyceae): Development, structure and function as resting spores. *J. Phycol.*, **15**, 233–243.

Taylor, F. J. R. and R. Haigh (1993): The ecology of fish-killing blooms of the chloromonad flagellate *Heterosigma* in the Strait of Georgia and adjacent waters. In *Toxic Phytoplankton Blooms in the Sea*, ed. by T. J. Smayda and Y. Shimizu, Elsevier, N.Y. pp. 705–710.

Wall, D. (1971): Biological problems concerning fossilizable dinoflagellates. *Geoscience and Man*, 3, 1–15.

Watanabe, M., K. Kohata, T. Kimura, T. Takamatsu, S. Yamaguchi and T. Ioriya (1995): Generation of a *Chattonella antiqua* bloom by imposing a shallow nutricline in a mesocosm. *Limnol. Oceanogr.*, **40**, 1447–1460.

Yamaguchi, M. (1992): DNA synthesis and cell cycle in the noxious red-tide dinoflagellate *Gymnodinium nagasakiense*. *Mar. Biol.*, **112**, 191–198.

Yamaguchi, M. (1994): Physiological ecology of the red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae)—Mechanism of the red tide occurrence and its prediction. *Bull. Nansei Natl. Fish. Res. Inst.*, No. 27, 251–394 (in Japanese with English abstract).

Yamaguchi, M. and I. Imai (1994): A microfluorometric analysis of nuclear DNA at different stages in the life history of *Chattonella antiqua* and *Chattonella marina* (Raphidophyceae). *Phycologia*, **33**, 163–170.

Yamaguchi, M., I. Imai, and T. Honjo (1991): Effects of temperature, salinity and irradiance on the growth rates of the noxious red tide flagellates *Chattonella antiqua* and *C. marina* (Raphidophyceae). *Nippon Suisan Gakkaishi*, **57**, 1277–1284 (in Japanese with English abstract).

Yamochi, S. (1984): Mechanisms for outbreak of *Heterosigma akashiwo* red tide in Osaka Bay, Japan Part 3. Release of vegetative cells from bottom mud. *J. Oceanogr. Soc. Japan.*, **40**, 343–348.

Yamochi, S. (1989): Mechanisms for outbreak of *Heterosigma akashiwo* red tide in Osaka Bay, Japan. In *Red Tides: Biology, Environmental Science, and Toxicology*, ed. by T. Okaichi, D. M. Anderson and T. Nemoto, Elsevier, N.Y. pp. 253–256.

Yoshimatsu, S. (1987): The cysts of *Fibrocapsa japonica* (Raphidophyceae) found in bottom sediment in Harima-Nada, eastern Inland Sea of Japan. *Bull. Plankton Soc. Japan*, **34**, 25–31.