TECHNICAL GUIDE FOR

MODERN DINOFLAGELLATE CYST STUDY

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PREFACE

For the last few decades, harmful algal blooms have given a large economic loss to fisheries industry and serious poisoning problem to public health. The main causative organisms of these blooms are dinoflagellates. The study on modern dinoflagellate cysts as well as planktonic cells has been strongly recommended for understanding the mechanism implicated in the recurrence and geographic expansion of the harmful dinoflagellate blooms.

This technical guide was originally prepared by Y. Fukuyo, the latter author, for the First WESTPAC Red Tide Workshop held in Bangkok, Thailand in 1985, and then published by Matsuoka et al. (1989) with several addenda and amendments to use one of the resource materials for the Workshop on Biology of Dinoflagellates held at the First International Symposium on Red Tide at Takamatsu in 1987. Afterwards this guide was revised several times at different training workshops dealing with the modern dinoflagellate cyst study such as IOC-DANIDA Training Course on the Taxonomy and Biology on Harmful Marine Microalgae and WESTPAC/IOC Training Course on Ecology and Physiology of Harmful Algae. In 1995 UNESCO published a book entitled "Manual on Harmful Marine Microalgae" (Hallegraeff et al., 1995), which contains the chapter "Cyst Methodologies" by A.M. Anderson and authors of this guidebook, Y. Fukuyo, and K. Matsuoka. Although the chapter included several technical descriptions such as sample collection, processing and cyst quantification, detailed description of various techniques and several helpful figures and tables were unfortunately excluded due to limitation of pages. Especially the most important guiding tool for species identification, i.e., photographs of various cyst species, was not able to be included.

Therefore this guidebook is prepared to supplement the manual by adding stepwise description of procedures of several important techniques and providing photographs of various cysts. This guidebook is consists of nine sections, i.e., Introduction, Sampling, Fixation and Preservation, Cleaning and Concentration, Isolation of cysts, Culture, Morphological characteristics, Identification trees, Photograph tables and References.

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1. INTRODUCTION

Some dinoflagellates produce two different types of non-motile cells called as a temporary cyst and a resting cyst in their life cycle. The resting cysts can survive in harsh environmental condition and stay in certain periods of quiescence and dormancy, which induce simultaneous germination in response to better environmental change. Therefore, the resting cysts have an important ecological role as the source seedlings of the recurrent blooming and expansion of geographical distribution. As of now more than 80 marine and 15 freshwater species of modern dinoflagellates are known to produce resting cysts (Table 1). Although the number of the cyst producing species is small comparing to the total number of extant dinoflagellates (more than 2000), the former contains many harmful species, i.e., more than 16 species have been known to cause a red tide and seven species to be toxic. Thus, the study on modern dinoflagellate cysts is one of important subjects to understand blooming mechanism of harmful algae.

1.1 Definition of cyst

The term "cyst" is generally adopted for a non-motile cell that lacks flagella and an ability of movement. Two types of cysts are often observed in natural environment and laboratory cultures.

***Temporary cyst*:** A non-motile cell that lost flagella is called "temporary cyst". The loss of flagella is one of external appearance changes induced by sudden and vigorous shock to physiological condition of cell. For example, non-motile dinoflagellates are often observed in fresh plankton samples collected by net towing. Mechanical shocks such as strong water movement and dense compaction of cells caused by plankton net towing make environmental condition unfavorable for plankton cells. They cut flagella accidentally, and some armored species shed even their thecal plates. Cell contents round off inside a smooth and transparent wall. Loss of mobility also occurs by long exposure of stressed condition. Non-motile cells observed on the bottom of culture vessels, especially in cultures after logarithmic growth phase, are produced by depletion of nutrients and/or high biomass. These non-motile cells can re-establish motile population within a short time after recovery of environmental condition. This quick recovery ability is one of the important features of temporary cysts.

A non-motile cell produced in the process of asexual cell division is also called a temporary cyst. Dinoflagellates have several different manners in their asexual cell division. One of the manners possesses a stage of two newly divided non-motile cells in one pellicle. For example,

Alexandrium pseudogonyaulax cannot divide into two cells asexually in planktonic condition, and cell division always occurs in a pellicle, i.e. a temporary cyst (Kita et al. 1985).

***Resting cyst*:** A resting cyst is a resting zygotes formed in the process of sexual reproduction (Fig. 1). Planktonic zygotes formed by fusion of gametes are often found in natural plankton population mainly during active blooming period. Planktonic zygotes may swim for several days and then transform into non-motile zygotes (resting cysts). They may float in waters and eventually sink on the sea / lake floor after encystment. Cysts seem to require a mandatory resting period (2 weeks to 5 months depending on species) before they re-establish motile populations under favorable conditions. During dormancy, cysts sunk on exposed open floor may be transported to place where water movement is slow in the much same way as fine sands or mud particles. Under a certain condition with low oxygen and low temperature, they are known to be viable in sediments for at least 6 years. Temperature change, exposure to light, and floating up by water turbulence are thought as external triggering factors for induction of germination, but internal mechanism, i.e., biological clock, also controls the germination.

In this manual the term "cyst" means only "resting cyst". There are two different forms of resting cysts in surface sediments: living cyst and empty cyst. The living cyst with fresh protoplasm can germinate under favorable condition, while the empty cyst is defined as a cyst left after the release of protoplasm through a distinct opening, the archeopyle. The cyst wall of modern dinoflagellates includes two different phragma; organic sporo-pollenin and calcareous material. Since the organic sporo-pollenin wall is highly resistant to chemically and biologically adverse ambient condition, this provides the specific resistant features of the resting cyst.

1.2 Significance of cyst in dinoflagellate blooms

The function of cysts can be considered as (1) a source of the recurrence of blooms, (2) a vector for expansion of geographical distribution, 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 decrease of cell number through sexual fusion from two gametes to one zygote. In addition to these functions, Anderson et al. (1982) suggested that cysts of toxic species accumulated on the surface of bottom sediment worked as (6) a toxin source for benthic shellfish that live at the deep floor and have very a limited chance of feeding toxic planktonic cells. The cysts trapped and accumulated in sediments also provide useful

information not only on the presence of certain harmful species at certain time period, but also on plankton assemblage occurred for a particular geological period.

In order to clarify the role of cysts, it is essential to investigate the abundance and distribution of cysts. For this purpose there are several types of approach. The approaches may be modified depending on objectives of study, available equipment, timing of survey implementation during blooming season, and so on.

2. SAMPLING

2.1 Planning for cyst sample collection

Two methods have been adopted for collecting surface sediments: by using a corer and a sediment trap. The sampling method should be selected according to the purpose of the cyst study (Figs. 3 & 4). If it is to know when and how many cysts of a certain species are produced, the sediment trap method should be used. If it is to document the presence of certain species or the change of cyst assemblages, the core method is preferable. A dredger and a grab bucket are not usefule, because they often lose the light fluffy materials, which contain a lot of newly formed cysts, deposited at the surface of sediment.

2.2. Core sampler method

The sample naturally contains large amount of mud and other particles. Cleaning and concentration of cysts are the processes needed to isolate them from the raw sample. The cyst assemblages are assumed to reflect a long term of phytoplankton succession.

Gravity corers such as a Phleger corer (Phleger bottom sampler) and a piston corer are most desirable in order to collect thin surface layer of bottom sediments that include many fresh living cysts. A light-weight core sampler (TFO gravity corer; Fig. 5A) is handy and most convenient for investigation of shallow near-shore and inner bay areas, where only small boat can approach. To get a longer sediment core, a light-weight piston core sampler is also useful (HPC sampler; Nanba et al., 1998).

The procedure of collecting bottom sediments by TFO gravity corer is following:

Step 1 Preparation of TFO gravity corer:

1-1. Insert a polycarbonate transparent tube in the corer body.

1-2. Attach a 1-10kg lead weight on the corer. The weight should be selected according to the nature of sediments and the water depth, for example, 1-2 kg weight for mud and 5-10 kg

weight for sand and deeper stations.

Step 2 Collection of sample:

2-1. Tie one end of a rope to the bottom of the corer and the other end to a boat.

2-2. Throw the corer and let it sink down freely to the bottom.

2-3. Pull the corer smoothly back on board, and keep the bottom-up position not to loose the collected sediment core in the tube of the corer.

2-4. Take the tip of the corer out gently, and then insert immediately a rubber stopper tightly to the bottom end of the inner tube.

2-5. Take the inner tube off by holding it up in the vertical position and cover the upper end tightly with a plastic sheet and a rubber band to prevent the leaking and evaporation of the surface water. In addition to them, it is desirable to cover the whole tube by tin foil to shut out sunlight.

2-6. Vertically place the inner tube in a cooler box to keep sediment in the dark and cool condition and to prevent mixing of the sediment layer.

For sampling at an area with coarser sediment, often the core sampler does not work effectively. In such case, application of grabs such as Smith McIntyre or Ekman Berg type sampler is eligible. But careful handling not to loose surface soft layer is always necessary. The tube of 10-15 cm long could be inserted to separate surface sediment of the sample collected by the grab. If possible, it is recommended to take the short core sample with keeping the grab at the surface of water (if the grab is pulled up on board, surface sediment often leaks with water). After collecting the subsample from the grab, follow the Step 2-5 to 2-6 above.

2.3 Sediment trap method

Sediment trap method is designed to catch cysts floating and sinking in water column before they settle on the sea floor, and provide information on the plankton community that is reflected by the cyst assemblage in the sediment trap. However, samples collected by the trap are recommended to use only for qualitative analysis or for limited purposes such as sexual process study, not to use for quantitative analysis of cysts formed during certain period. There are two major reasons; 1) There is no widely-accepted standardized design of sediment trap and procedures for setting. As water movement affect to the behavior of sinking cysts, shape of the trap and its position in the water column also give quantitative difference of cysts. 2) Materials re-suspended from the bottom may settle in the trap, and it makes the interpretation of sedimentation data complicate.

Some researchers designed traps for certain purposes. For example, Kamiya et al. (unpublished data) set a relatively large trap consisting of three polyethylene bottle (20*l*) for collecting a considerable amount of cysts of *Alexandrium tamarense* for the analysis of toxicity (Fig. 6A). Takeuchi (1985) made a small trap composed of a polyethylene bottle (2*l*), a buoy, and a weight to get newly produced cysts of *A. catenella*. Matsuoka et al. (unpublished data) also use a small device of a sediment trap at near shore area to collect dinoflagellate cysts and diatoms (Fig. 6B).

2.4 Germinating cell trap/sampler

The new device named "Germinating cell trap/sampler" has been designed by Ishikawa et al. (1995) for measurement of germination rates of dinoflagellate cysts in surface sediments (Fig. 7). The instrument is originally designed for collection of emerging aquatic insects (Tsuda 1962). It consists of two main parts; an inverted trapezoidal vessel covering a defined area of sediment surface and a pump unit connected by a hose. This vessel is walled with filtering mesh to prevent invasion of plankton.

3. FIXATION AND PRESERVATION OF RAW SAMPLES

In order to stock raw natural samples before laboratory analysis and/or cyst culture, it is advisable to keep the temperature of the stocker lower than that of bottom sediment of the sampling area at the time of collection. Furthermore, to exclude light stimulus, the samples should be covered with tin foil. The most desirable preservation method to prevent germination is the introduction of nitrogen gas into sample vials, because an anoxic condition prevents germination of cysts.

If the culture of cysts is not in the research plan, the raw sample should be fixed as soon as possible to avoid the change of composition (ratio of living and empty cysts) by excystment. Even for long-term preservation, neutralized formalin or glutaraldehyde can be used as fixing agent. Addition of neutralized formalin (10% against the volume of sample) or glutaraldehyde (30% against the volume of sample) to supernatant for fixation of sample in core-tube. If the sample does not contain much organic substances, the amount of the preservative can be reduced. For refined sample after cleaning (see following section), neutralized formalin (3% against the volume of sample) or glutaraldehyde (10% against the volume of sample) is advisable.

4. CLEANING AND CONCENTRATION OF CYSTS FROM SEDIMENT SAMPLES

Two different processing methods have been adopted for cleaning and concentrating cysts from raw natural materials: sieving technique without chemicals and palynological technique using several chemicals. The choice of techniques is depending on the purpose of the study. When cyst culture is needed for establishing the cyst-theca relationship, the sieving technique should be adopted. But for the cyst assemblage analysis, the palynological technique is better than the sieving technique, because the larger amount of samples can be observed in the former technique.

Usually sediment between surface and 2 cm depth (top 2 cm) is used for analysis. The top 2 cm layer contains newly formed living cysts and newly germinated empty cysts, and the layer provides information on recent occurrence of cyst-producing species. In order to compare cyst abundance among areas, number of cysts in the top 2 cm is often referred. But sedimentation rate among sites are variable, and therefore the top 2cm of sediment does not always contain the cyst produced in the same time-interval. For example, at oligotrophic area the sedimentation rate is less than 0.25 cm per year, and top 2 cm sediment is made in more than 8 years. But at eutrophic area with river water, the rate often exceed more than 1 cm per year, and only 2 years is necessary for sedimentation of 2 cm. For the analysis of cyst production at various sites, the rate should be taken in consideration.

4.1 Sieving procedure (Fig. 8)

Step 1 Prepare a series of sieves of various mesh-sizes with 250μm being the upper, 125μm in the middle and 20μm being the lowest sieve. (For large size cyst, a sieve of 38μm mesh-size is also useful, as fine clay particles, which fairly disturb microscopic observation, could be eliminated)

Step 2 Put a mark on the core tube at 2 cm position from the bottom by a permanent marker.

Step 3 Remove the plastic cover from the tube and take the overlying seawater in the tube into a 50*ml* vial by a pipette.

Step 4 Remove the rubber stopper from the bottom of the tube.

Step 5 Push out the mud in the tube slowly into a beaker by blowing at the upper end of the tube the upper surface of the mud reaches the 2 cm's mark. It is sometimes effective to push the sediment out of the tube from the bottom end using a rubber stopper cut to be slightly smaller than the inside diameter of the core tube.

Step 6 Put the remaining sediment in the tube into the same vial used in the Step 3.

Step 7 Rinse the inner surface of the tube with small amount of filtered seawater by using wash bottle. Pour the rinse water into the same vial.

Step 8 Mix all contents in the vial and pour onto the upper sieve. Rinse the vial with small

amount of filtered seawater, if needed, in order to transfer all the content onto the sieve. (Note that it makes processing time short to use an ultrasonic probe or bath to desegregate sediments before sieving. But it is also advisable that the microwave may give unusual effect to he physiological condition of cysts.)

Step 9 Wash the sediment on the upper sieve carefully with filtered seawater by using wash bottle. Cysts and fine particles will pass through the 250μm and 125μm sieves and accumulate on the 20μm sieve.

Step 10 Transfer all the residues on the 20μm sieve to a petri-dish.

Step 11 Separate the lighter particles, which contain cysts, from other heavy sands in the residue by squirting filtered seawater from the wash bottle. The water is squirted at one side of the petri-dish in such a way that the residue is surrounded by the swirling motion of water. Cysts and other light-weighted particles will suspend in the circulating water, while heavy sand particles remain at the bottom of the center of the petri-dish.

11a Alternatively, transfer all residues on 20μm to a watch glass. Make water-eddy in the watch glass for separating cysts and lightweight particles from heavier sand grains (panning in the geological sense). After this process, cysts and other fine particles except heavier grains are concentrated at the center of the watch glass.

Step 12 Gently pour the supernatant water with cysts suspended onto the 20μm sieve. The heavy sand particles are left on the petri-dish.

12a Suck up the cysts and light-weighted particles and transfer them into a test tube with a syringe.

Step 13 Repeat the washing, if needed.

13a Repeat the panning for several times, if needed.

Step 14 Transfer the residue on the 20μm sieve into a 20*ml* vial and rinse the sieve with small amount of filtered seawater in order to get all particles. Make up the solution in the vial to 10*ml* using filtered seawater. This is the refined sample ready for observation.

4.2 Fluorescent dye staining method

Some gonyaulacoid and calciodinellid cysts show autofluorescence both in protoplasm and in cyst wall (Elbrechter 1994; Matsuoka 1989). By applying these characteristics, Yamaguchi et al. (1995) tried various kinds of dye and choose primuline as the best fluorescent stain for enumeration of resting cysts of *Alexandrium* spp. in natural sediments. The basic procedure of this technique is as follows;

1. After fixing dinoflagellate cysts with glutaraldehyde, stain dinoflagellate cysts with which is the most preferable agent for cysts.
2. Observe and count cysts under a fluorescence microscope by using such different filter combinations as blue light (B), green light (G), and ultra violet (UV) excitations.

This method is easy to process and useful for counting cysts in sediments. However it also has a limitation as well as other preparation technique. The cyst wall of some protoperidinioid and gymnodinioid species such as *Gymnodinium catenatum* does not show fluorescence, even after staining using primuline, it is difficult to observe them under this optics (Matsuoka 1989). Another disadvantage is in the observation of complex morphology. For the identification of spinate cysts of *Gonyaulax*, *Protoceratium* and *Pyrodinium*, the fluorescent microscopy is not sufficient because of its lower resolution.

4.3 Palynological technique (Fig. 9)

The palynological technique introduced in this section is mainly based on regular palynological processing using hazardous chemicals. Therefore safety should be cared during the processing in laboratory. Strong and continuous ventilation is necessary. It is recommended to use protective measures such as rubber gloves, goggles, and an apron.

Step 1 Put a small amount of sediment, for example, upper 2cm sediment (see 4.1 step 6) into a 50*ml* polyethylene beaker. About 1-2 gram wet weight sediment is appropriate for the following processing.

Step 2 Add 15-20 *ml* distilled water in the beaker, and mix with the sediment. Then keep it on a table for a while until all sediment particle sink down to bottom. After particles sink completely, supernatant water should be decanted slowly and carefully not to loose light weight cysts. Repeat this washing processing two to three times to remove salt.

Step 3 Add 10% hydrochloric acid of adequate amount to remove calcium carbonate which compose calcareous nannoplankton, foraminiferous and others in the sample. Keep the beaker for one day in a draft. The wall and ornaments of calcareous cysts such as *Scrippsiella trochoidea* and *Ensiculifera carinata* are also dissolved by the acid, but a inner organic phragma remains intact.

Step 4 Wash with distilled water to remove the acid in the same way as the Step 2.

Step 5 Add 1% potassium hydroxide (KOH) solution of adequate amount into the beaker and

heat it at approximately 70 °C in a water bath for three minutes. It is advisable to maintain the water temperature constantly and keep the exposure time carefully. In higher temperature and longer exposure, the relatively thin cyst phragma of *Protoperidinium* and *Alexandrium* sometimes disappears.

Step 6 Wash with distilled water to remove the alkaline in the same way as the Step 2.

Step 7 Add conc.-(25%-30%) hydrofluoric acid (HF) of adequate amount into the beaker by polyethylene pipette of 10 *ml* volume to remove silicate materials such as sand, diatoms, silicoflagellates and others. Heat it in the water bath at approximately 70 °C for two to three hours. Since solution and gas of hydrofluoric acid are very dangerous and toxic, this processing should be conducted in a draft chamber with such safety wears as rubber or vinyl gloves and gas masks. The residue solution containing hydrofluoric acid should be neutralized with calcium carbonate.

Step 8 Wash with distilled water to remove the acid in the same way as the Step 2.

Step 9 When cellulose substances such as plant tissue are found in the sample abundantly, acetylation may be useful to remove them. If such materials are invisible, the acetylation is not necessary. The procedure is as follows:

9a Take out a certain volume of the treated sediment into 10 *ml* polyethylene centrifuge tube, and add 5 *ml* glacial acetic acid (CH_3COOH) in the tube by pipette. Mix contents in the tube well.

9b Centrifuge the tube (approx. 3000 rpm, 20 sec.) and take out the supernatant by a pipette. After taking away the chemical, add the Erdtman's solution composed of nine parts of acetic anhydride [$(CH_3CO)_2O$] and one part concentrated sulfuric acid [H_2SO_4] in the tube and heat it to approximately 70°C in a water bath.

9c After taking away the Erdtman's solution by centrifuge in the same way as previous, add 5 *ml* glacial acetic acid by a pipette in the tube again.

9d Wash the residue with distilled water in the same way as 9b.

Step 10 Prepare a set of two sieves of mesh-sizes with 125μm being the upper and 20μm being the lower.

Step 11 Transfer the sample after processing the step 7 or 9 onto the upper sieve.

Step 12 Wash the sample on the upper sieve thoroughly. The cysts and other organic particles such as spores and pollen grains will pass through the 125μm sieves and accumulate on the 20μm sieve.

Step 13 Take all residues on the 20μm sieve into the 10*ml* vial and make up the solution 10*ml*

with distilled water. This is the refined sample ready for the next study.

4.4 Preparation of permanent slides (Fig. 10)

The refined samples containing not only dinoflagellate cysts but also various kinds of palynomorphs can be preserved as a permanent slide with adequate mounting medium. These slides have the advantage for later use, when re-observation is needed. There are two different media useful for permanent slides; glycerin jelly and bioplastics. Bioplastics is more stable than glycerin jelly for long preservation. The procedure in using bioplastic is shown below;

Step 1 Put a drop of polyvinyl alcohol solution on the cover slip (18x24mm or 18x40mm).

Step 2 Take a small portion of the refined sample and put it on the cover slip.

Step 3 Mix the sample well with the polyvinyl alcohol and extend it over the slip by using needle.

Step 4 Waiting until it dry up completely on a hot plate or under the room temperature.

Step 5 Take a drop of bioplastic solution onto the dry slip. Turn it over immediately and then put it on a slide glass.

Step 6 Leave it horizontally until completely it dry.

5. ISOLATION OF A SINGLE CYST

The isolation of a single cyst is the most fundamental and important technique prior to culture establishment and detailed morphological observation with photograph taking. The steps to isolate a single cyst is as follows (Fig. 11):

Step 1 Prepare a needle (insect pin) attached at a tip of a glass tube is used for cleaning materials in the background around the cysts. Also a pasteur pipette with capillary tip (50-100μm in diameter) at one end and with approximately 60 cm long silicone tube attached to the opposite end is used for sucking up a cyst.

Step 2 Take out a small amount (about 0.5*ml*) of the cyst-concentrated sample and transfer it onto a counting chamber slide.

Step 3 Spread the sample evenly on the slide by adding about 1ml filtered seawater.

Step 4 Search under the microscope for the expected cysts.

Step 5 Once the cyst is found, clear off debris around the cyst by using the needlet.

Step 6 Use the pasteur pipette to suck up the cyst.

Step 7 Transfer the cyst into the medium for culture in the culture chamber. For morphological observation, the cyst is introduced into a droplet of water on a slide.

6. CYST CULTURE

Three types of culture vessel; i.e. glass tube, culture chamber and single-cell chamber (Fig. 11) are used for several different purposes (see also Fig. 4). In the case of unialgal culture using glass tube, "f/10" is useful as a medium. This medium is made by adding 1 ml of the medium "f" (Table 3) to 10 ml of sterilized filtered seawater. For culture chamber and single-cell chamber, sterile filtered seawater is preferable without adding any nutrient. Table 2 also shows the chemical composition of several media used for cyst cultures. (*Note the culture is not clonal, as cysts are diploid cell.)

For the cultures to study on the cyst-motile form relationship and to establish clonal cultures via unialgal cultures, a 24-cell well culture chamber such as CORNING Cell Wells (No. 25820) is preferable. The procedure of the culture is as follows:

Step 1 Pour 1*ml* of sterilized filtered seawater to each of the 24 wells of the culture chamber.

Step 2 Inoculate one cyst into each well.

Step 3 Seal the chamber with para-film to prevent the evaporation.

Step 4 Keep the chamber in an incubator at a constant temperature between 10^0C and 30^0C with approximately 100 μmol photons/m^2/s with appropriate light-dark photo period. These culture conditions should be decided depends on physiological and ecological characters of the species.

Step 5 Observe the germination from the cyst and growth of germinated cells every day in 2 or 3 weeks after the inoculation under the inverted microscope.

Step 6 When the germinated motile cells divide into more than 10 cells, isolate one cell and observe the morphological characters under the microscope for species identification. Observation of the character of the empty cyst is also indispensable. For clonal culture establishment, one cell isolation and inoculation into a new well is basic procedure.

7. IDENTIFICATION OF CYST

There are two different taxonomic systems for dinoflagellate cysts: one based on paleontology

and the other on planktology. After finding fossil dinoflagellates in 1838 by Ehrenberg, these organisms have been classified in their morphological features. Paleontologists have developed its own classification system. In 1960s paleontologists (e.g. Wall and Dale 1968) found extant modern cysts with same morphology as fossils in plankton and on the surface sediment. They have same morphological characteristics as fossils, and some of them are proved to be one of the stages in the life cycle of modern dinoflagellates. These modern dinoflagellate cysts have been also classified and described under the paleontological system. It is curious situation that the same organism has two different scientific names. As taxonomic systems for classification is different, it is not unusual to have different species name scientifically. But practically it causes confusion for non-taxonomists, as different names are used for description of same organisms. It need more than decades to solve the unpleasant situation.

7.1 Descriptive terminology of dinoflagellate cysts

As described above, taxonomy and identification technique of modern dinoflagellates cyst are mostly developed by scientists who have deep knowledge on fossil cysts. They adopted terminology commonly used for fossil cysts. Actually there is no new scientific term in modern planktology for the description of modern cysts. Therefore it is strongly recommended to be familiar to the terms for fossil cysts.

The important morphological characteristics for identification of cysts are the shape of the cyst body and its ornamentation, wall structure and color, and the type of aperture or archeopyle through which germinating cells leave from the cyst. The archeopyle type is a very useful character in determining the higher classification ranks (genus and family) of the cyst species. However, since cysts possess no archeopyle before excystment, it is impossible to use this feature for routine identification. In comparison to the morphology of motile forms, cysts usually have a simple, mostly spherical to peridinioid shape. As a result, identification of cysts based on a single morphological characteristic is not always reliable, and combination of several characteristics such as the type of archeopyle, morphology of ornaments, wall structure, wall color, and paratabulation are indispensable.

7.1-1 Morphology of the cyst body

Cysts are classified into three major groups based on the position of the cyst formed in the planozygote (Figs. 12 & 13).

The proximate cyst is formed directly beneath the theca of the planozygote and therefore its volume occupies approximately one half to one-third of the original zygote. Some cysts of this type

have a characteristic ornamentation that possibly reflects the position and shape of plate, cingulum, sulcus and other structures such as apical groove of the motile forms. Proximate cyst bodies vary from spherical to peridinioid and sometimes have several projections on the surface.

The chorate cyst is characterized by various kinds of ornaments rising from the cyst surface. These ornaments functionally support the cell wall of the planozygote from the cyst surface and are morphologically very variable. The process formation during maturation from the planozygote toward the hypnozygote (resting cyst) has been observed in *Lingulodinium polyedrum* (Stein) Dodge by Kokinos and Anderson (1995). Generally, the cyst body of this type is spherical, subspherical or ovoidal. The volume of the cyst cavity is reduced comparing to the planozygote, mostly less than one-third of the original.

The cavate cyst consists of more than two walls clearly separated, and usually possesses a cavity partly or entirely around the cyst body. Therefore, the outline of the cyst is variable. The volume of the cyst containing the protoplasm is much reduced during the maturation from planozygote to hypnozygote (resting cyst), and the inner cyst body is probably less than one-fifth of the original in volume. In general, the outlines of modern dinoflagellate cysts are relatively simple in comparison with fossil forms; that is, they are mainly spherical, subspherical, ovoidal, ellipsoidal, or peridinioid. Some brackish water cysts, however, vary in shape from simply spherical through peridinioid to cursiform. It is notable that even in a single species, environmental parameters such as salinity, temperature and nutrient can produce remarkable variation in the cyst shape. This is well investigated for *Spiniferites cursiformis* Wall et Dale and *Tectatodinium pellitum* Wall et Dale found in Quaternary sediments of the Black Sea (Wall et al., 1973).

7.1-2 Wall structure and color

The wall of modern cysts consists of one, two or three layers composed mostly of biopolymer, chemically similar to the sporopollenin of spores and pollen grains in higher plants, however rarely of calcium carbonate e.g. *Scrippsiella*. The cyst wall can be composed of up to three or four layers (Evitt, 1985); autophragm, periphragm, mesophragm, and endophragm (Fig. 12). The color of the cyst wall is also variable, mostly transparent, light yellow, pale brown, brown, or dark brown. Cysts partly composed of calcium carbonate such as *Scrippsiella trochoidea* are dark brown to black from live specimen.

7.1-3 Morphology of surface ornamentation

For the description of surface ornaments of cysts, the terminology for pollen grains and spores

is adopted. Some terms frequently used for modern cysts are shown in Figs. 14 & 15.

7.1-4 Archeopyle

The term "archeopyle" is defined by Evitt (1963) as an excystment opening formed at the germination stage of dinoflagellate cysts. In dinoflagellate cysts, apical, intercalary, precingular, and hypocystal archeopyle types and combinations of these series have been recognized. However, it is not possible to use this definition for cysts of gymnodinialean species that are not covered with typical thecal plates at the motile stage. Matsuoka (1985a) proposed new descriptive terms for the archeopyle of modern dinoflagellate cysts in both naked and thecate dinoflagellates, and divided them into saphopylic, theropylic and cryptopylic archeopyles (Fig. 15).

Saphopylic archeopyle: Archeopyle sutures correspond to paraplate boundaries and the operculum. Part of the cyst wall corresponding to the archeopyle is always detached from the cyst body. The archeopyle type can be subdivided into either apical, intercalary, precingular, or a combination from one or more plate series. Most modern cysts belonging to Peridiniales and Gonyaulacales have this archeopyle type.

Theropylic archeopyle: Archeopyle sutures follow paraplate boundaries. The operculum is usually attached to the cyst. This caused by the incomplete development of archeopyle sutures on the cyst body. This archeopyle can also be subdivided into several types based on the position of the sutures. Matsuoka et al. (1989) showed a provisional subdivision for this archeopyle, but further careful examination is needed to confirm this observation. Modern cysts produced by diplopsalid and calciodinellid species have this archeopyle type.

Cryptopylic archeopyle: Archeopyle suture does not reflect any plate boundary. The operculum is detached or free from the cyst caused by the absence of thecal plates in motile forms. Some modern gymnodinialian and gonyaulacacean cysts have this archeopyle type. On the basis of the shape of opening, this archeopyle type is classified into two forms, either chasmic (slit-like opening) or tremic (hole-like opening) (Matsuoka, 1985a). It is notable that some modern cysts do not show any distinct excystment aperture after germination, probably because of the fragile nature of the cyst wall. This archeopyle type includes most cysts of *Alexandrium* species, *Peridinium faeroense* Paulsen, and *Ensiculifera imariense* Kobayashi et Matsuoka.

7.2 Description of harmful marine dinoflagellate cysts

Species described below sometimes cause harmful events such as toxin contamination in shellfish and red tide discoloration with fish mass mortality.

7.2-1 Prorocentrales cysts

Only two species have been reported to produce cysts in the Prorocentrales. They are *P. lima* and *P. marinum* (Faust, 1990; 1993). Although Faust did not discuss anything on the morphological similarity and difference, there is possibility that these two species are same. If they are same, number of cyst producing species in the Prorocentrales become only one. Their cysts are morphologically simple, spherical and similar to each other. As there have been no records in modern sediments, these cysts probably are not preservable.

7.2-2 Dinophysiales cysts

Sexuality of *Dinophysis* cf. *acuminata* has been documented on the basis of the presence of planozygotes possessing two trailing flagella by McLachlan (1993), but there is no evidence concerning hypnozygotes or resting cysts for this species. Two other species, *D. acuta* and *D. tripos* have been observed by Moita and Sampayo (1993) to produce resting cysts. These cysts have not yet fully been studied for their morphology including wall and archeopyle structures, and have never been recorded from modern sediments.

7.2-3 Gymnodiniales cysts (Pl. 1)

Shape of the cyst body is mostly spherical to ovoidal and sometimes ellipsoidal, with or without spinate or reticulate ornaments on the surface. The cyst wall is organic and pale brown, brown, or rarely reddish brown in color; mostly composed of a single layer and sometimes two layers. The archeopyle type is cryptopylic, chasmic or tremic.

Species producing cysts

Gymnodinium breve Hulburt

Gymnodinium catenatum Graham (Pl. 2)

Gyrodinium instriatum Freudenthal et Lee (Pl. 3)

Gyrodinium impudicum Frage et Bravo (Pl. 4)

Cochlodinium sp. cf *C. polykrikoides* Margalef (Pl. 5)

Pheopolykrikos hartmannii (Zimmermann) Matsuoka et Fukuyo (Pl. 6)

Polykrikos kofoidii Chatton - *Polykrikos schwartzii* Butschlii complex (Pl. 7)

7.2-4 Gonyaulacales cysts (Pl. 8 & Pl. 15)

Shape of the cyst body is basically spherical to ellipsoidal and rarely discoidal, and with or

without process-like ornaments. The cyst wall is organic, colorless and sometimes transparent; rarely composed of a single or usually two layers. The archeopyle type is mostly saphopylic, or precingular, but sometimes epicystal or combination. In *Alexandrium* spp. and *Gonyaulax verior* cysts, no typical archeopyle is formed.

Species producing cysts

Lingulodinium polyedrum (Stein) Dodge (Pl. 9)

Gonyaulax scrippsae Kofoid (Pl. 10)

Gonyaulax spinifera (Claparede et Lachmann) Diesing complex (Pl. 11)

Gonyaulax verior Sournia (Pl. 12)

Protoceratium reticulatum (Claparede et Lachimann) Butschli (Pl. 13)

Pyrodinium bahamense Plate var. *compressum* (Bohm) Steidinger, Tester et Taylor (Pl. 14)

Alexandrium affine (Inoue et Fukuyo) Balech (Pl. 16)

Alexandrium andersoni Balech

Alexandrium catenella (Whedon et Kofoid) Balech

Alexandrium minutum Halim (Pl. 17)

Alexandrium pseudogoniaulax (Biecheler) Balech

Alexandrium tamarense (Lebour) Balech (Pl. 18)

7.2-5 Peridiniales cysts (Pl. 19, 20 & 21)

Shape of the cyst body is mainly spherical, ellipsoidal, peridinioid, and rarely discoidal, mainly without process-like ornaments. The cyst wall is mainly organic and mostly brown in color, rarely transparent, and sometimes calcareous; mainly composed of a single and rarely two layers. The archeopyle type is saphopylic of the intercalary and sometimes theropylic type, or apical, intercalary, epicystal and combination types.

Species producing cysts

Scrippsiella trochoidea (Stein) Loeblich, III (Pl. 22)

Cachonina hallii (Freudenthal et Lee) Dodge

Heterocapsa triquetra (Ehrenberg) Stein

Peridinium hangoei Schiller

Peridinium cunningtonii (Lemmermann) Lemmermann

8. RELATIONSHIP BETWEEN CYST ABUNDANCE IN SEDIMENT AND CYST PRODUCTION IN WATER

Does the abundance of cysts in sediment reflect the cyst productivity and total production at the site of sampling? To answer this question, we have to bear in mind that the cyst assemblages in sediment are made as a result of production, accumulation and dispersal by water movement and biological disturbance.

After sexual fusion, newly formed planozygotes float as plankton for several days and then loose their mobility by transformation into hypnozygotes (cysts). During this short period, they are passively brought to area where water movement is weak and slow. It means that cysts rarely sink down at the place where they are made, because almost all coastal area have water movement by tides and currents. Even after the settlement, cysts may float accidentally by strong water movement and are brought somewhere again. Activity of benthic organisms is also one of the factors that change the cyst distribution, especially their vertical distribution. Benthos may eat the cysts, but cannot digest them. Cysts may be brought to deeper part. Some of the settled cysts may germinate after dormant period, but the germination rate varies depending on environmental condition. Low oxygen concentration in water and sediment may disturb germination.

It is completely impossible to monitor all these factors that give chance of distributional change of the cysts. But if we can observe some parameters such as size distribution of sediment grain particle, number of benthos and their active depth, and dissolved oxygen amount in sediment, we can interpret and understand cyst distribution in better way.

Another important factor we have to consider is difficulty in standardization of sample amount. As described in the Section 4.1, 2 cm core sample from surface is often used for analysis. But this amount is decided just for the shake of convenience. If it is 1 cm, separation of sediment from the core may be difficult because of softness of surface sediment. If it is 3 cm, amount of sediment is too much for sieving. Therefore 2 cm sample in the core taken by the TFO corer (see Section2.2), which is about 2 cm^3 or 2 *ml* in volume, is a kind of standard for analysis, as long as we use the corer. The important thing is that the 2 cm sediment in the core is not 2 cm sediment in natural environment. There must be inevitable compaction of sediment in the core and the rate of compaction varies place by place. Not only the compaction, sedimentation rate should be considered also. As the rate vary every places, necessary period for the accumulation of the 2 cm sediment also vary at each place.

Because of difficulty in standardization of sample amount, it looks like a matter of preference to adopt a unit for expression of cyst amount; i.e., a unit mass (gram of either dry weight or wet weight; e.g. Erard-le Denn et al. 1993) or volume (*ml*, e.g. Yamaguchi et al. 1995). Water content at the interface of sea water and bottom sediment is usually constant at approximately 90% in the case of mud-dominated sediments. Based on this fact, the cyst abundance based on wet sediment volume

was adopted by Matsuoka (1999), Matsuoka et al. (1989) and others (e.g. Yamaguchi et al. 1995) to compare cyst concentrations among locations of similar sediment type. However, the cyst concentrations estimated by this method are incorrect owing to varying water contents at different core depth. The discussion by Dale (2000) focused on this point. To compare cyst concentrations in samples of different water contents, the results should be expressed as cysts per gram dry weight of sediments.

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*: In Japanese

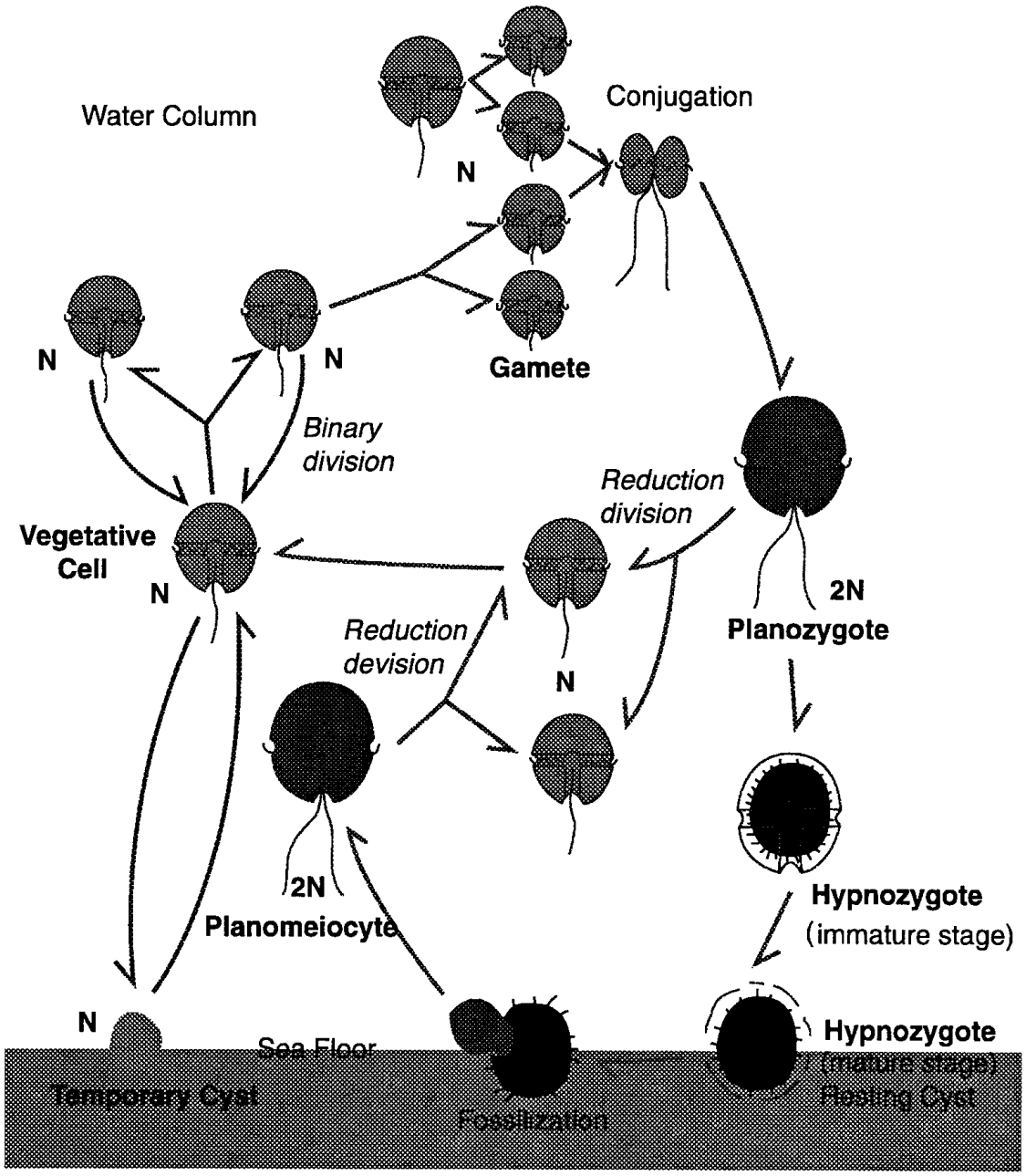


Fig. 1 Life cycle of dinoflagellates

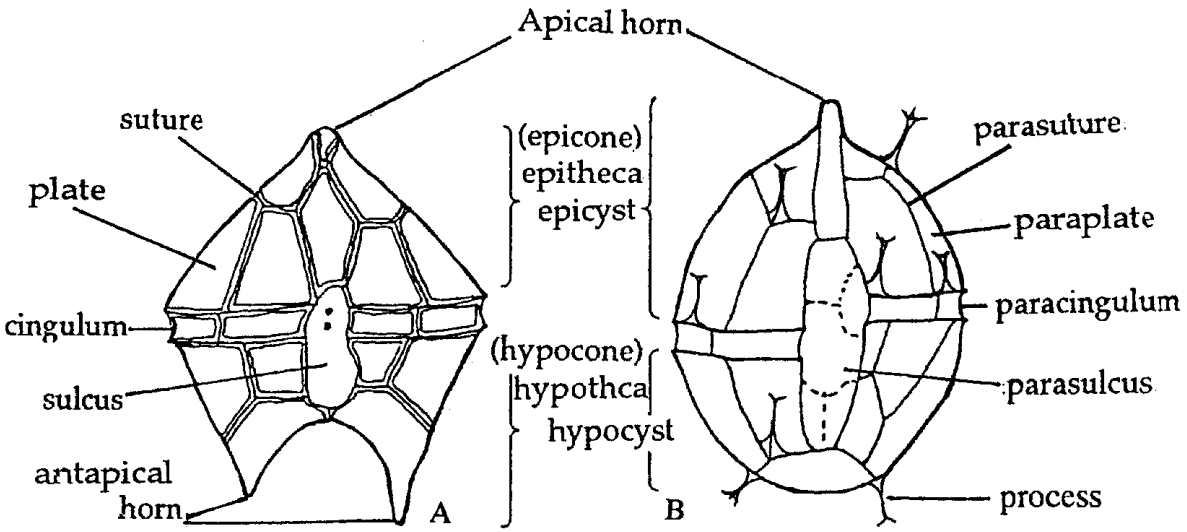


Fig. 2 Morphological comparison between thecae (A) and cyst (B), and descriptive terms for thecae and cyst.

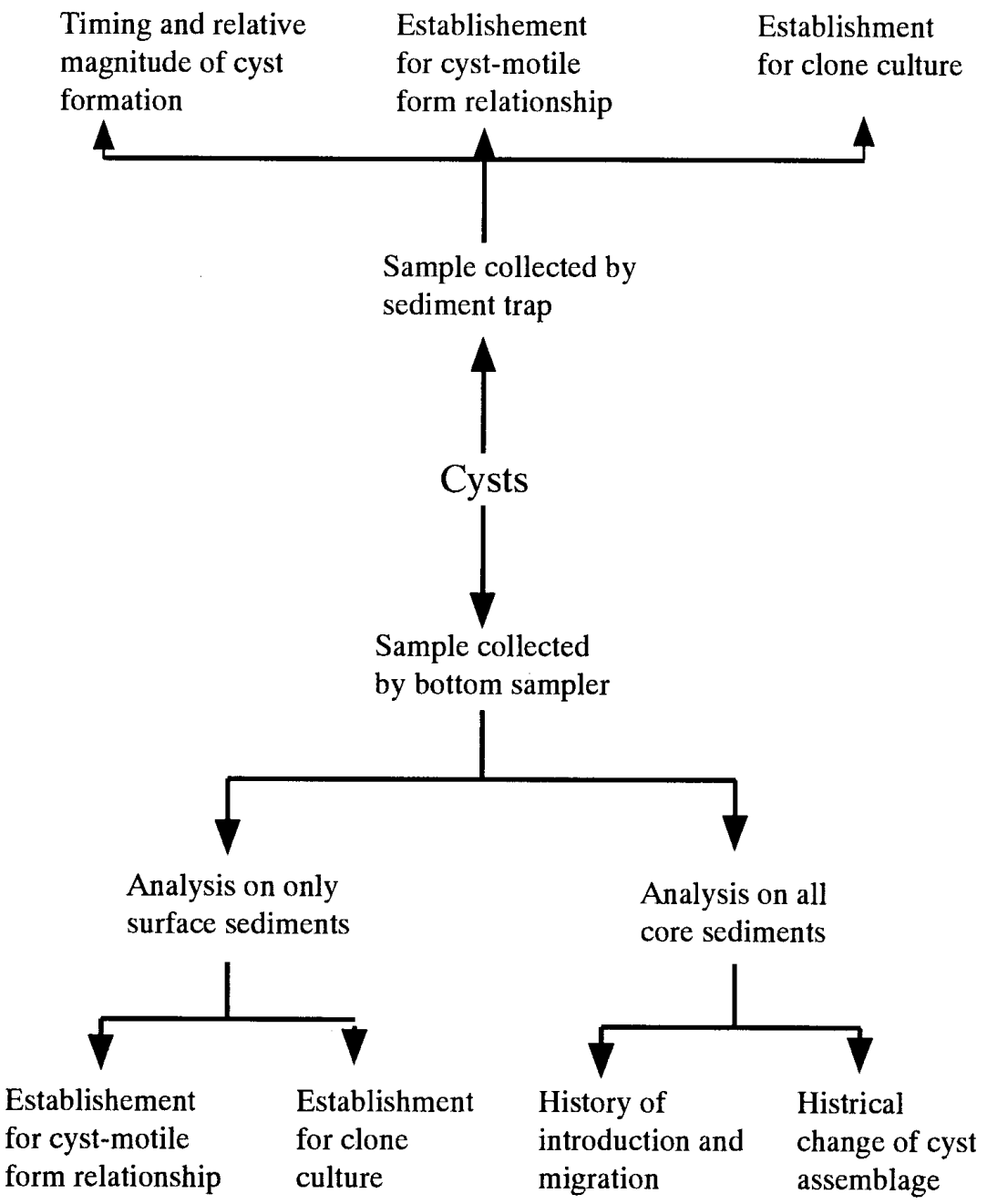


Fig. 3 Purpose of the modern dinoflagellate cyst study.

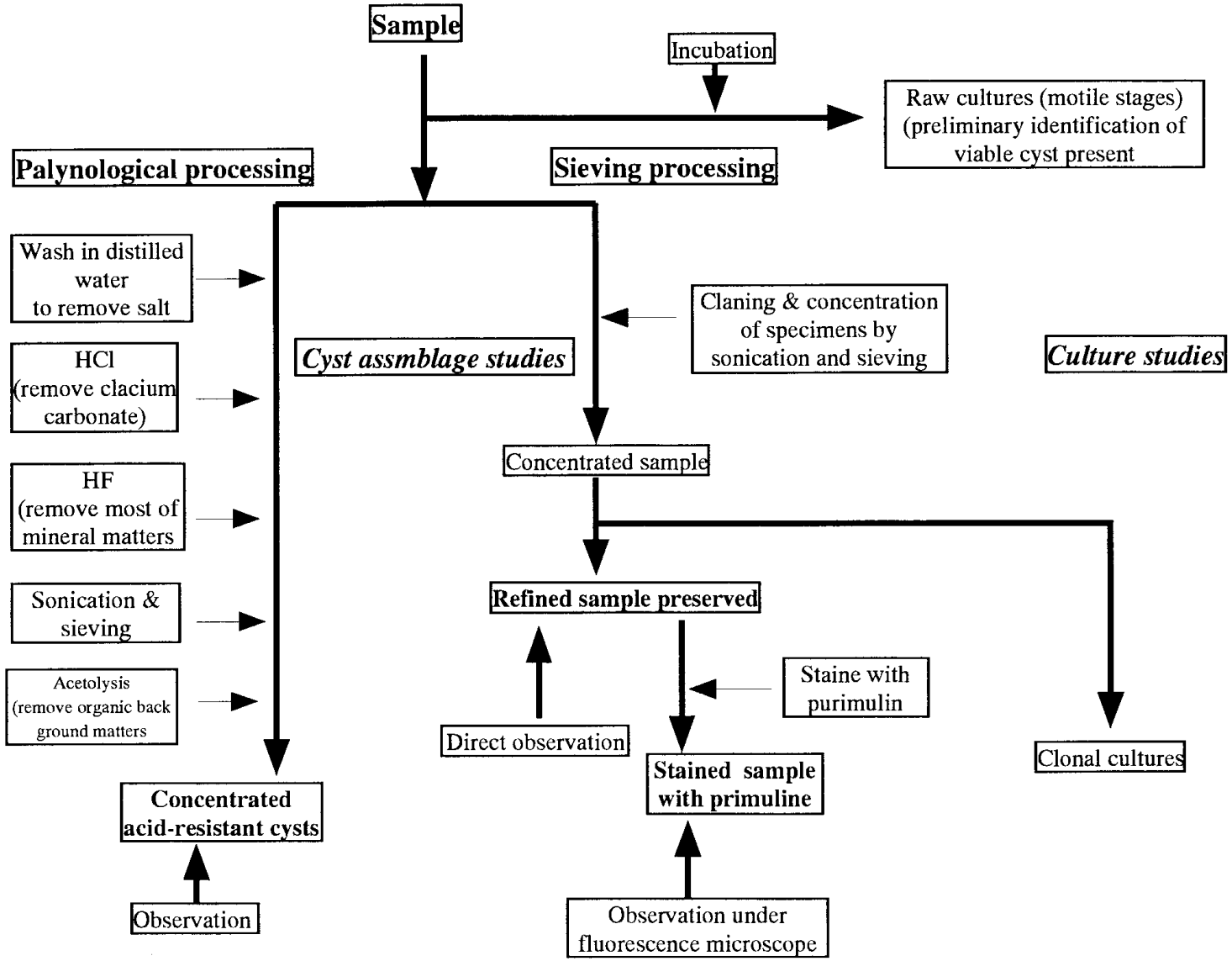
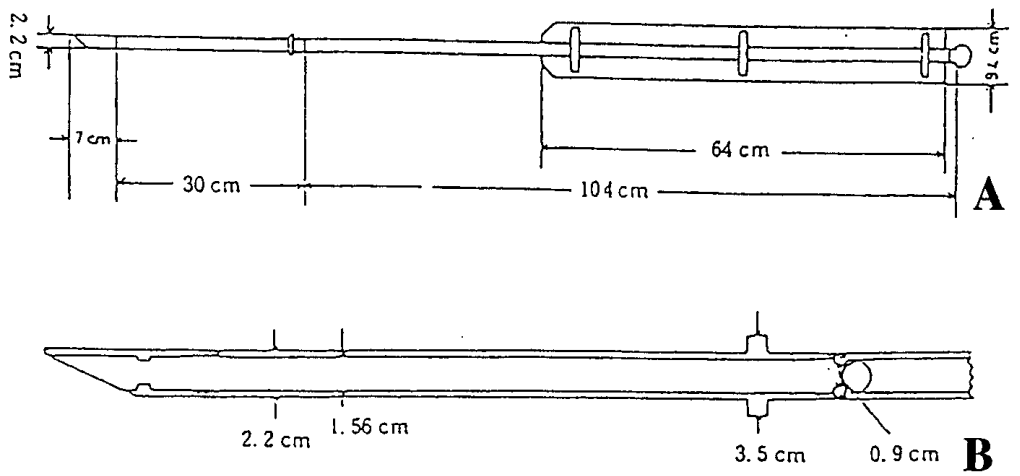
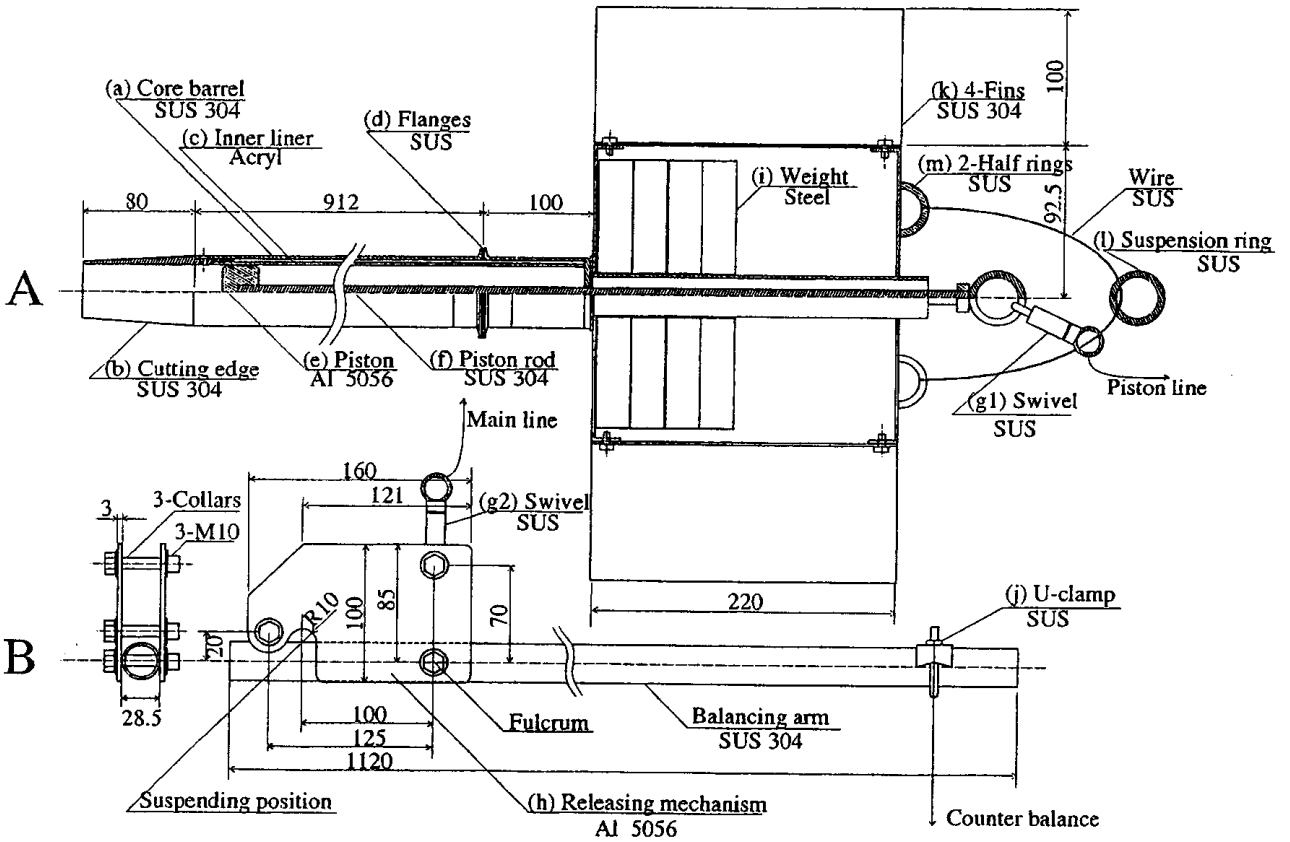


Fig. 4 Techniquues used to prepare sediment samples for modern dinoflagellate cyst studies (Dale 1983 with modification)



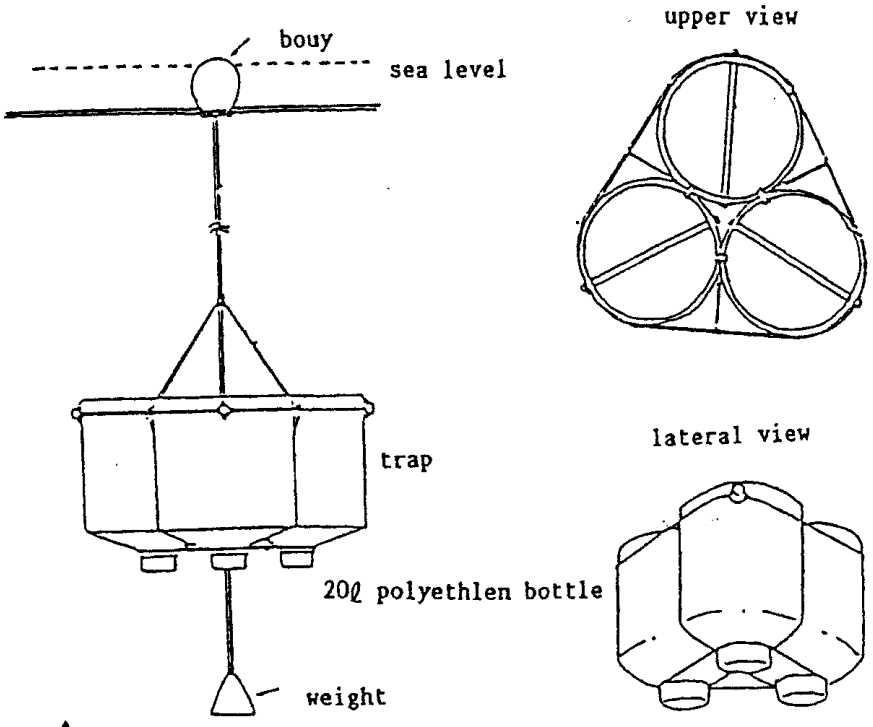
TFO core sampler (A); enlargment of the top of the corer (B)



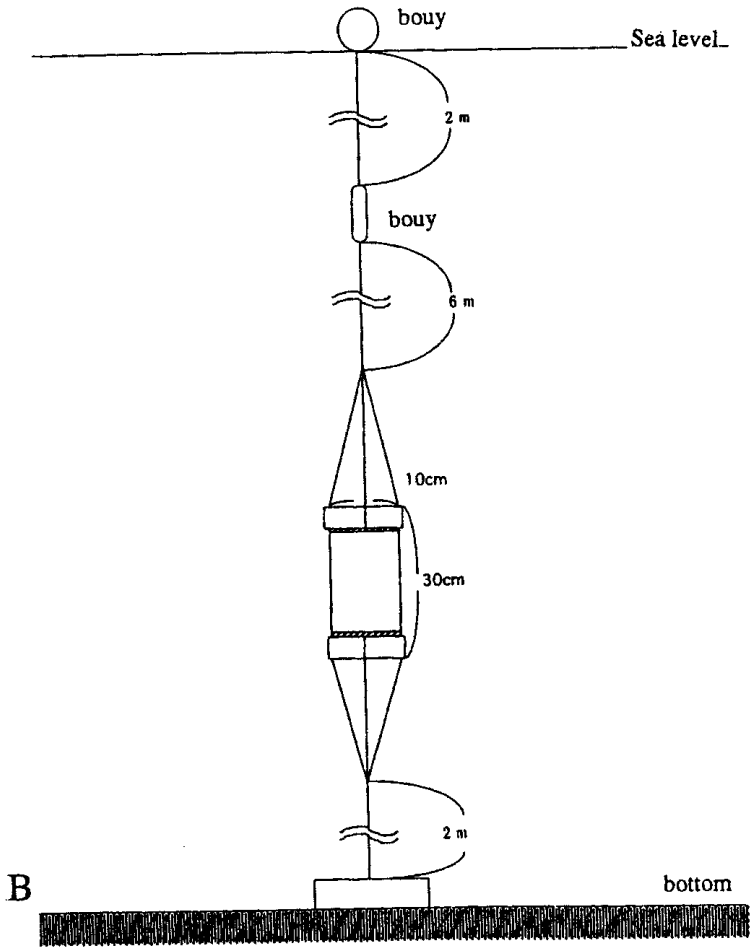
HPC (Light-weight Piston Core) sampler)Nanba et al., 1998)

A-main unit; B-releasing unit. Lengths are in mm. Abbreviations for the materials used are given according to the JIS.

Fig. 5 Sampling devices

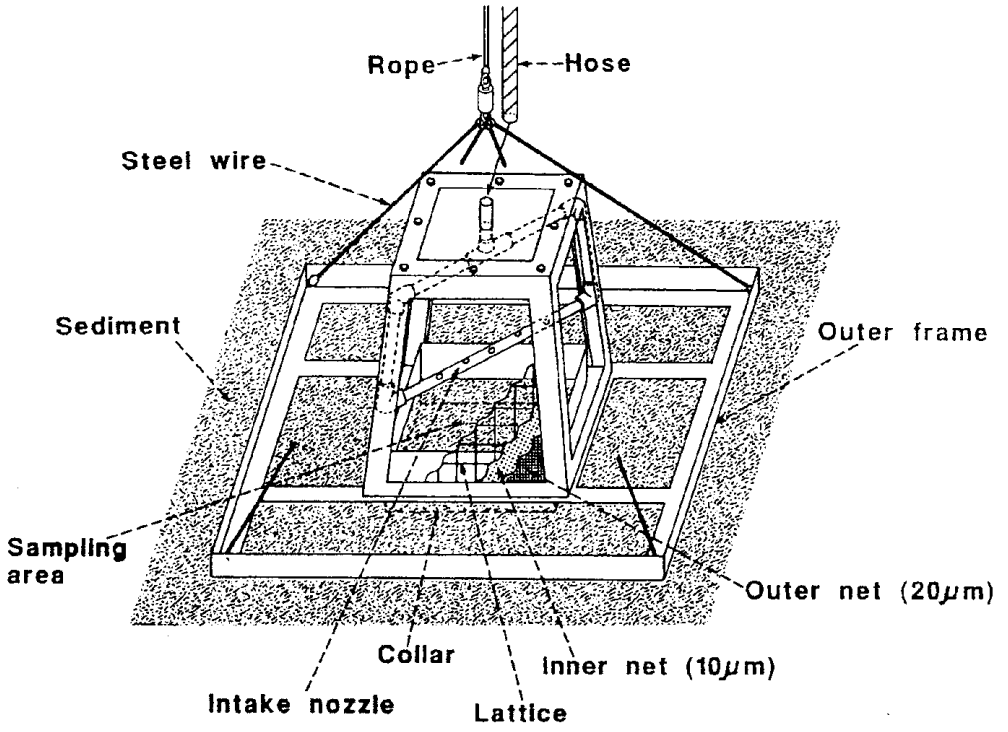


Large sediment trap adopted for collecting living cysts (Kamiya et al. unpublished)



Small sediment trap (Matsuoka et al. unpublished)

Fig. 6 Sediment trap



Design of the germinating cell trap/sampler to collect germinating cells of dinoflagellates *in*

Fig. 7 Germination cell trap/sampler (Ishikawa et al. 1995)

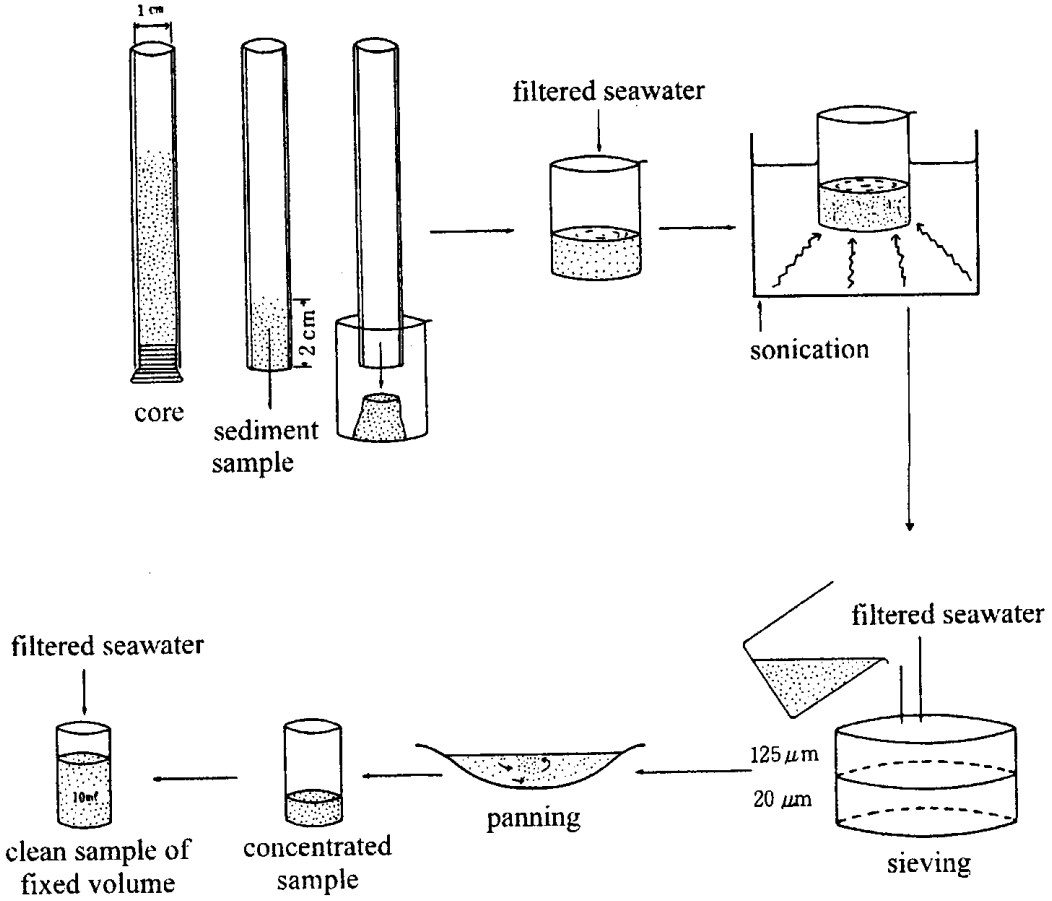


Fig. 8. Procedure of sieving technique

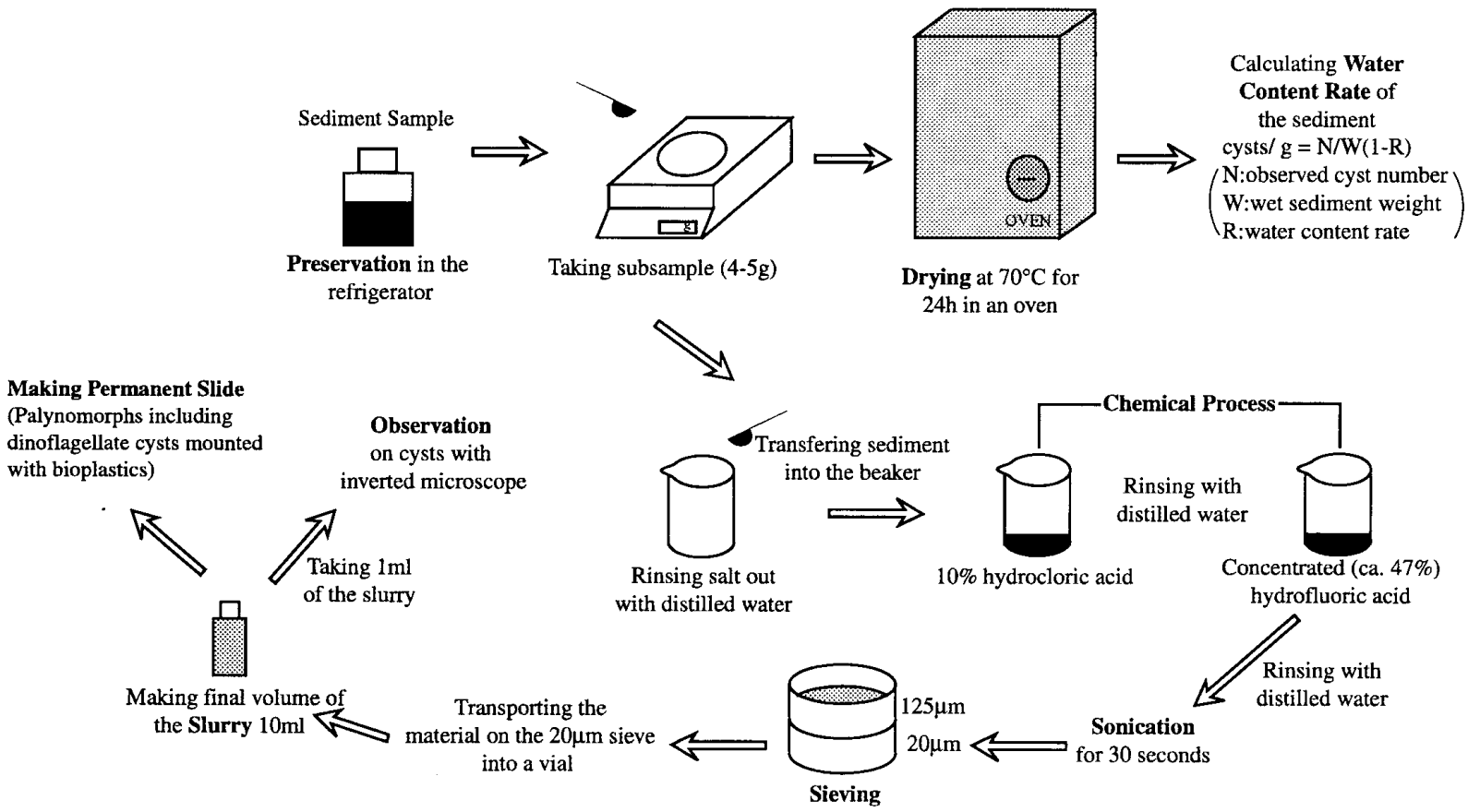
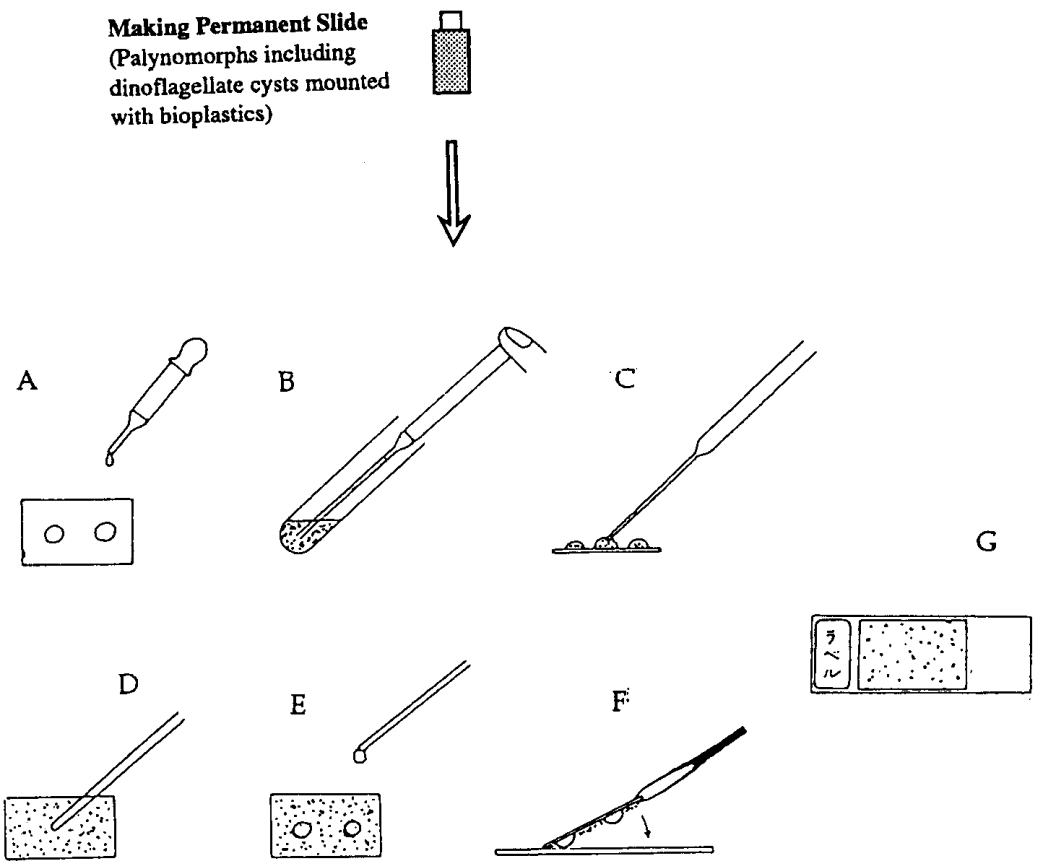


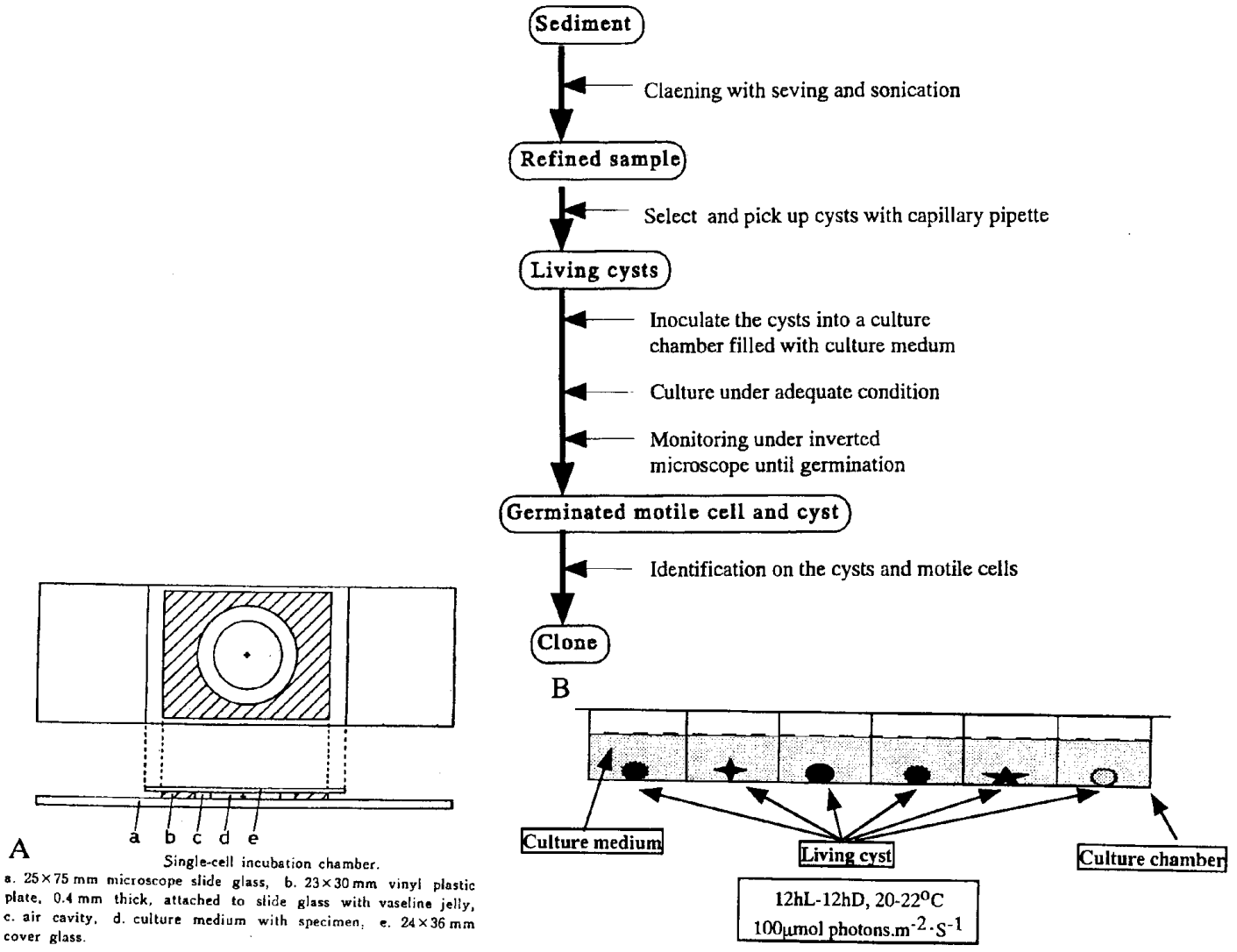
Fig. 9 Procedure of palynological technique



Procedure for making a permanent slide by using bioplastics (Shimazaki 1979).
A; Step 1, B; Stape 2, C; Step 3, D; Step 4, E, F; Step 5, G; Step 6

Fig.10 Procedure for making a permanent slide

Procedure of cyst culture



Single-cell incubation chamber.
a. 25×75 mm microscope slide glass, b. 23×30 mm vinyl plastic plate, 0.4 mm thick, attached to slide glass with vaseline jelly, c. air cavity, d. culture medium with specimen, e. 24×36 mm cover glass.

Fig.11 Procedure of cyst culture

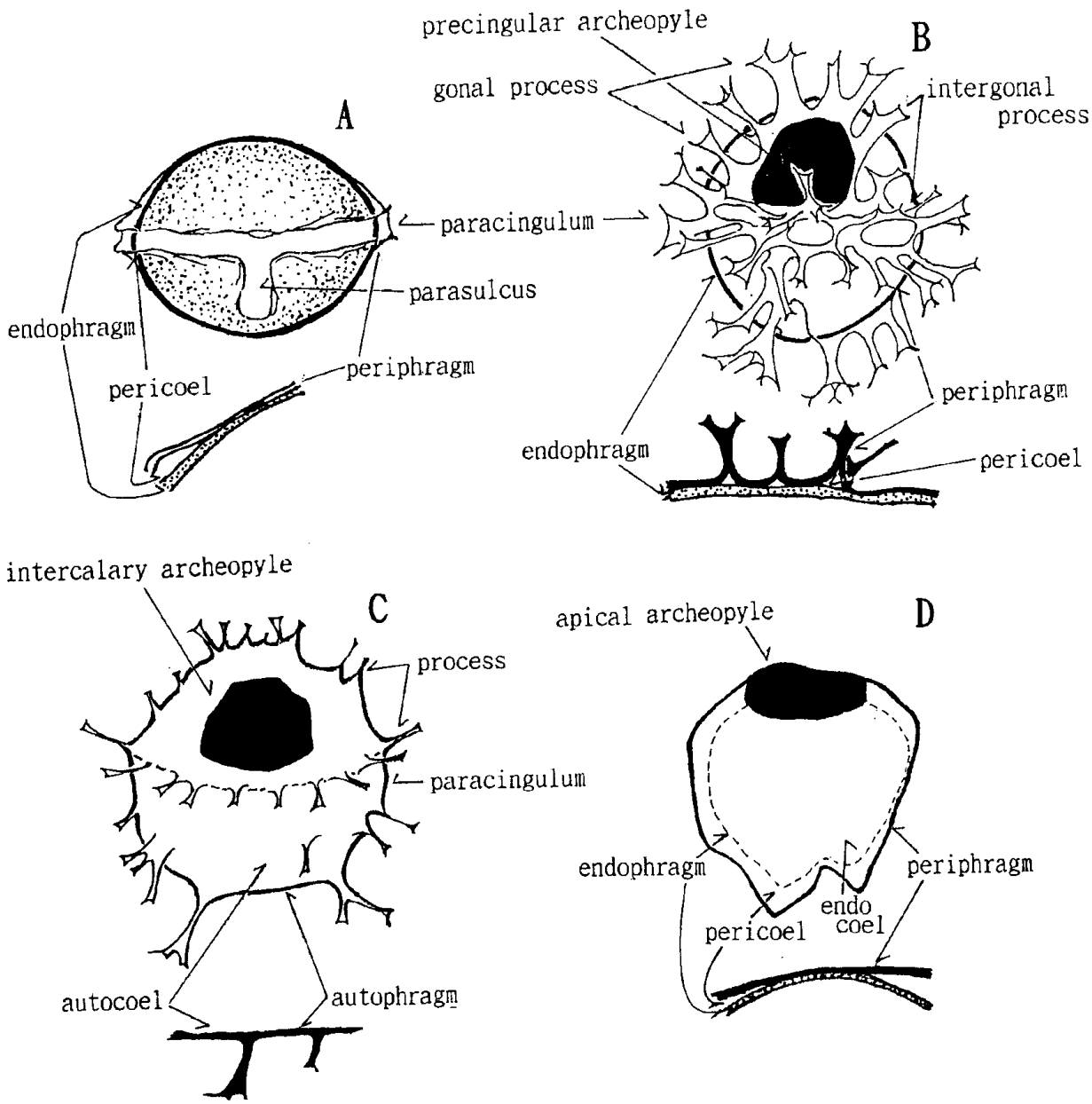


Fig.12 Wall structure of modern cysts and descriptive terms

A: proximate cyst with two layers, B: chorate cyst with two layers and processes
C: proximochorate cyst with a single layer, D: proximate cyst with two layers

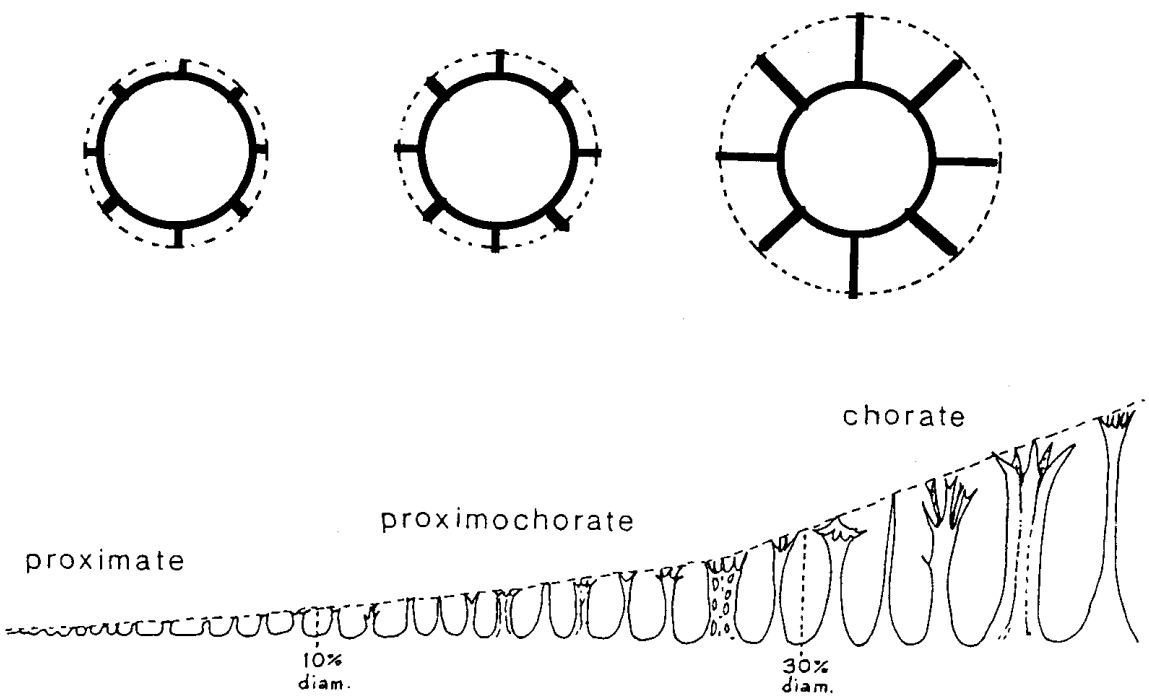


Fig.13 Major cyst shapes; proximate, proximochorate and chorate cyst (Sarjeant 1984 with modinifation).

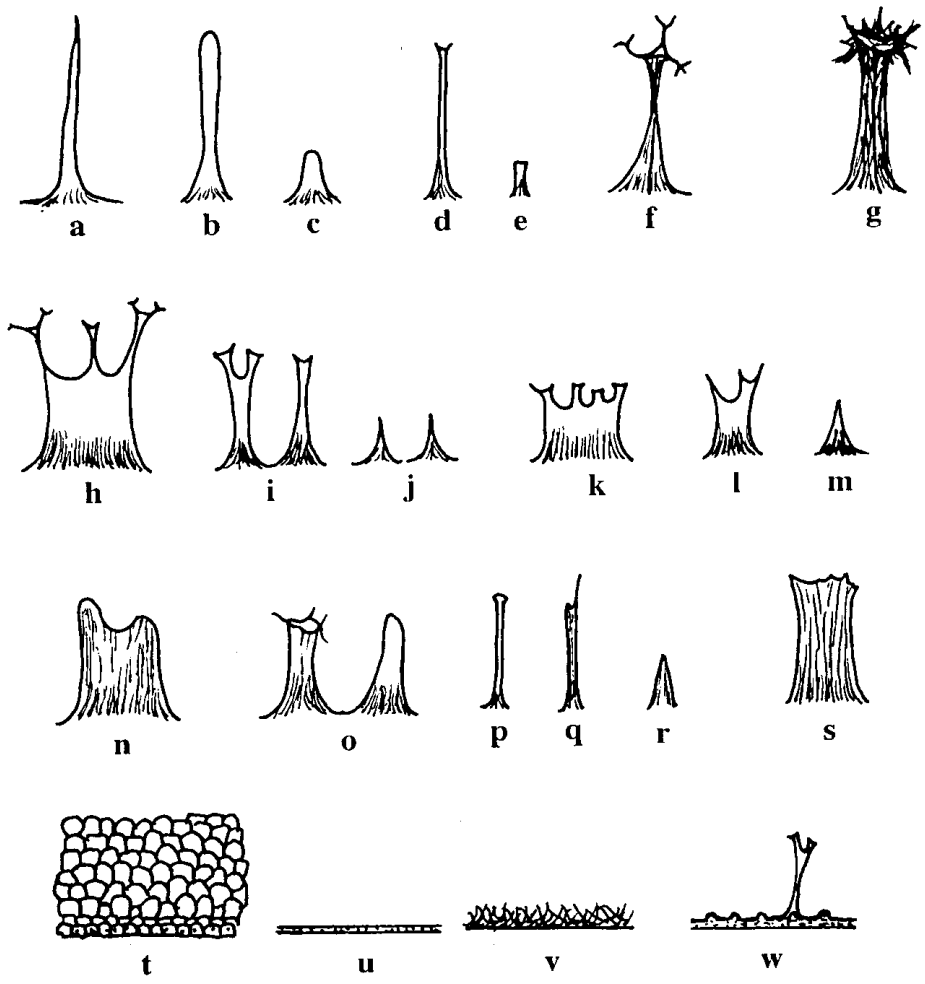


Figure 14 Descriptive terms for cyst wall and processes of modern dinoflagellate cyst.

A: acuminate, *Lingulodinium mahcaerophorum*, B: bulbous, *Lingulodinium mahcaerophorum*, C: evexate, *Lingulodinium mahcaerophorum*, D: capitate, *Operculodinium centrocarpum* sensu Wall et Dale (= cyst of *Protoceratium reticulatum*), E: cylindrical, *Operculodinium centrocarpum* sensu Wall et Dale (= cyst of *Protoceratium reticulatum*), F: trifurcate, *Spiniferites* spp., G: orthogonal, *Polykrikos kofoidii*, H: vallate, *Spiniferites mirabilis*, I: oblate, *Polysphaeridium zohariyi* (= cyst of *Pyrodinium bahamense*), J: conical, *Selenopemphix quanta*, K: hystricate, *Spiniferites mirabilis*, L: antleriform, *Spiniferites mirabilis*, M: conical with striations at the proximal base, *Pheopolykrikos hartmanii*, N: machicolate, *Cochlodiniium* cf. *polykrikoides*, O: patulate and evexate, *Protoperidinium* sp., P: capitate,, *Islandinium minutum* var. *cezare* Q: cylindrical, *Scrippsiella trochidea* (calcareous spinate cyst), R: conical, *Scrippsiella* sp., *S:* membranous, T: microreticulate, *Gymnodinium catenatum*, U: psilate,, *Alexandrium tamarense*, V: fibrous, *Bitectatodinium tepikiense*, W: scabrate, *Polysphaeridium zoharyi* (= cyst of *Pyrodinium bahamense*)

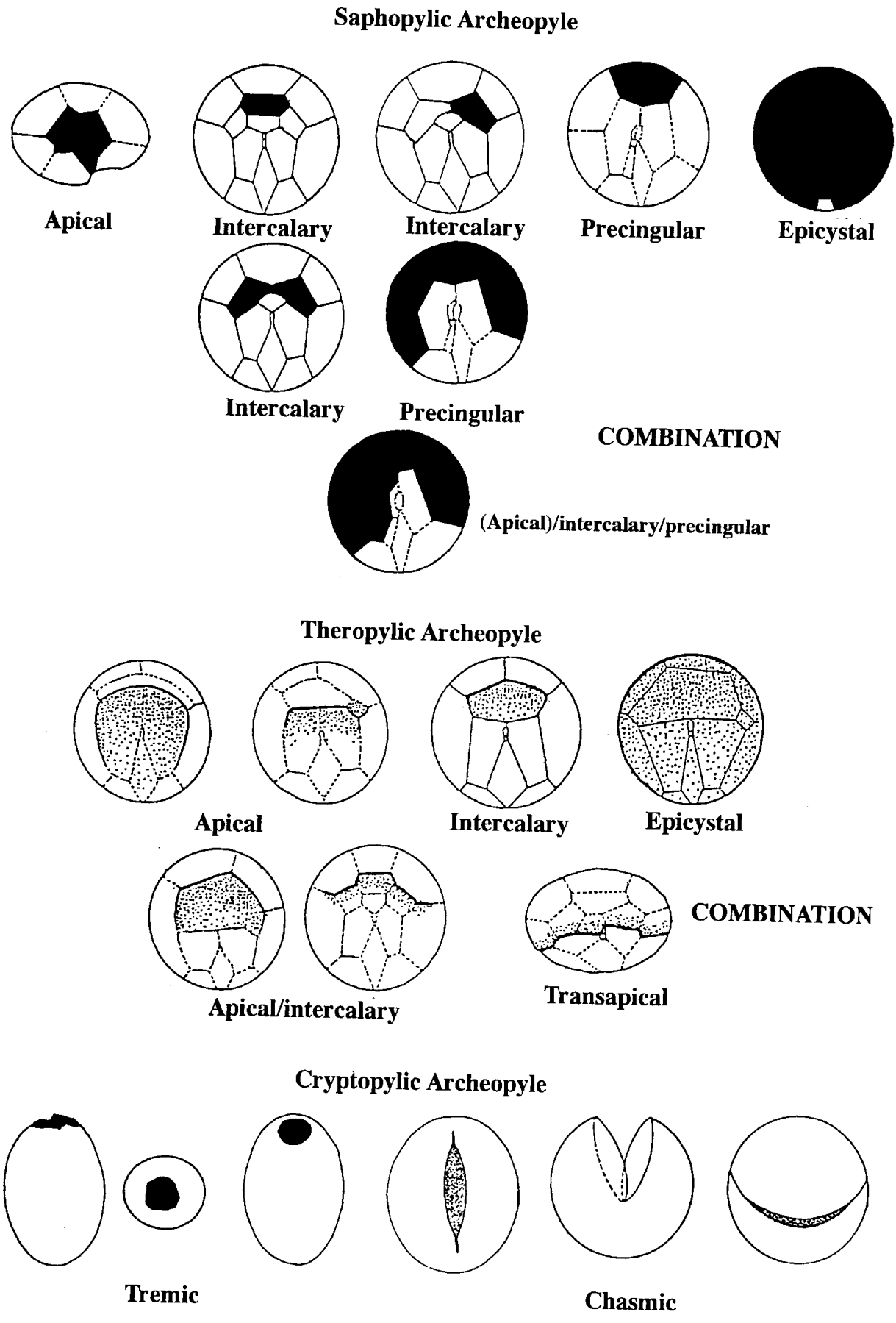


Fig.15 Archeopyle types in modern dinoflagellate cysts.

Figure 16 Cysts of harmful dinoflagellates

A: *Polykrikos schwartzii* Butschli – *P. kofoidii* Chatton complex B: *Gyrodinium instriatum* Freudenthal et Lee C: *Pheopolykrikos hartmanii* (Zimmermann) Matsuoka et FukuyoD: *Gymnodinium catenatum* Graham E: *Alexandrium minutum* Halim F: *Alexandrium affine* (Inoue et Fukuyo) Balech G: *Alexandrium tamarense* (Lebour) Balech H: *Alexandrium pseudogoniaulax* (Biecheler) Horiguchi, I: *Pyrodinium bahamense* Plate var. *compressum* (Bohm) Steidinger, Tester et Taylor J: *Gonyaulax scrippsae* Kofoid K: *Gonyaulax spinifera* (Claparede et Lachmann) complex (= *Spiniferites ramosus* (Ehrenberg) Mantell) L: *Gonyaulax verior* Sournia M: *Lingulodinium polyedrum* (Stein) Dodge N: Protoce*ratium reticulatum* (Claparede et Lachmann) Butschli O: *Scripssiella trochoidea* (Stein) Loeblich III

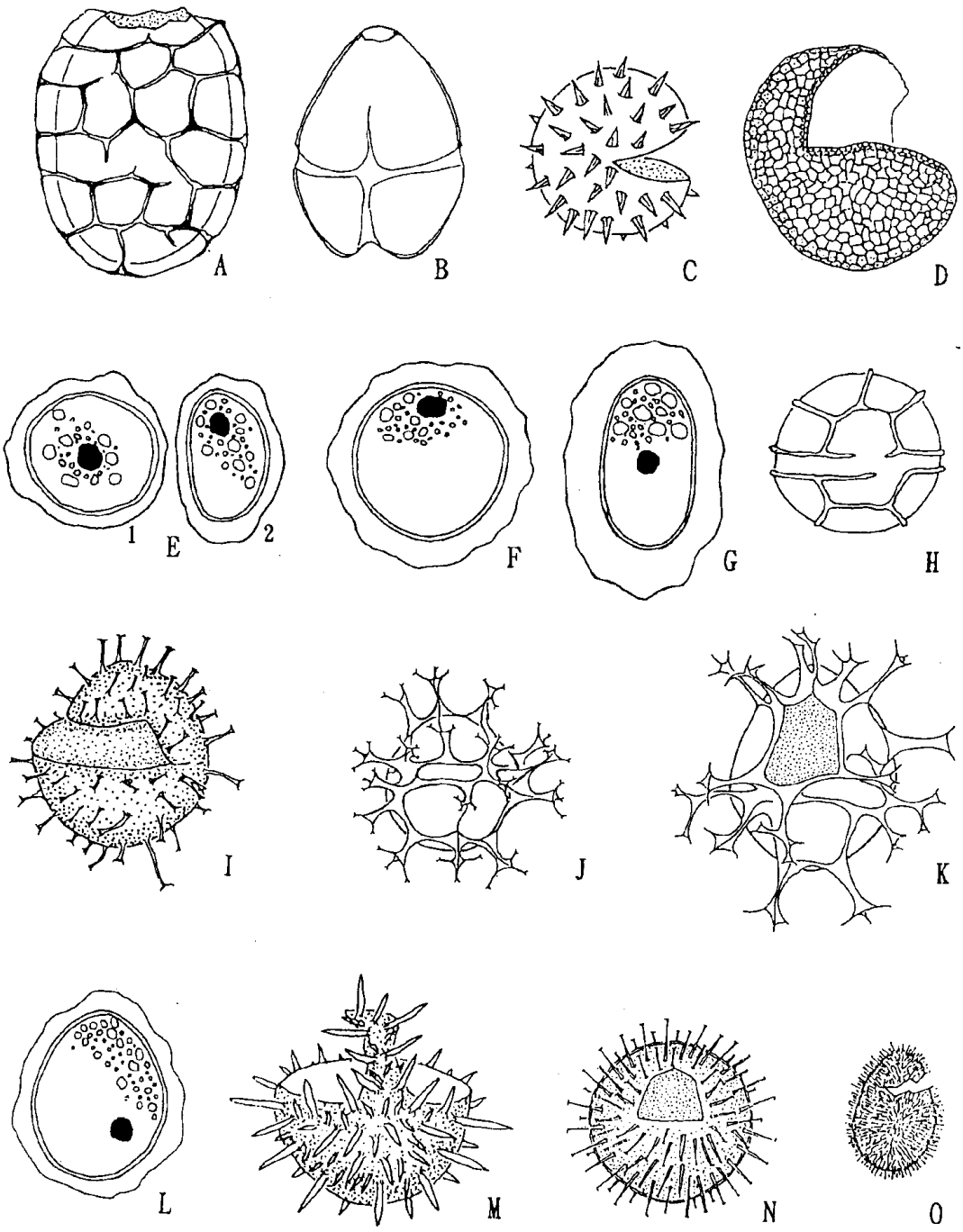


Fig.16

Fig. 17-1

Dinoflagellate cyst

Covered with ornaments

Covered without ornaments
Fig. 17-2

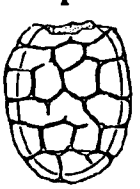
Cysts possessing brownish wall

Cysts possessing colorless wall
Fig. 17-5

Cysts composed of calcareous wall
Fig. 17-7

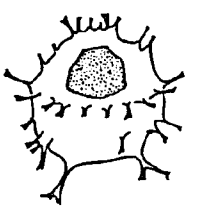
Cyst ellipsoidal and with rod-like or shelf-like processes

***Polykrikos kofoidii / schwartzii* complex**



Cyst with hexagonal outline

Protoperidinium divaricatum
"Xandarodinium variabile"



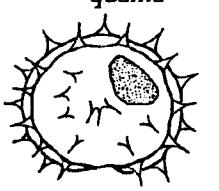
Cyst with peridinioid outline

Protoperidinium claudicans
"Votadinium spinosum"

***Protoperidinium* sp.**
"Trinovantedinium pallidifurvum"

Cyst compressed antero-posteriorly and with spinate processes

Protoperidinium conicum
"Selenopemphix quanta"



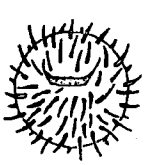
Cyst spherical and with numerous acuminate processes

Cyst with spinate processes bearing some striations on the proximal base

Pheopolykrikos hartmannii

Cyst with large spinate processes

Diplopelta parva



Cysts without ornaments

Cysts possessing coloreless wall

Cysts possessing brownish wall
Fig. 17-3

Cyst with smooth surface and sometimes covered with gelatinous materials

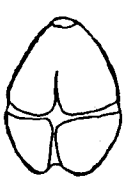
Cyst with parasutural structure

Alexandrium pseudogoniaulax

Cyst with unclear archeopyle

Cyst ovoidal with tremic archeopyle at the apical end and sometimes bearing paracingulum and parasulcus

Gyrodinium instriatum

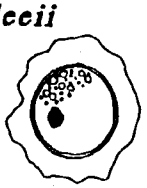


Cysts ovoidal

Gonyaulax verior

Cyst spherical

Alexandrium andersonii
Alexandrium affine
Alexandrium leeii

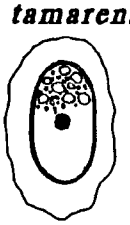


Cysts subspherical to ellipsodal

Alexandrium tamiyavanichi

Cysts ellipsoidal

Alexandrium catenella
Alexandrium tamarense



Cysts bean-like shape at lateral view

Alexandrium minutum

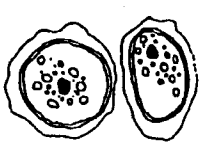
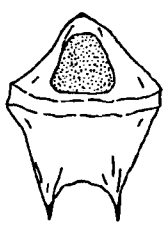


Fig. 17-3

Cysts possessing brownish wall

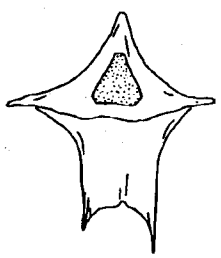
Cysts with peridinioid outline

Protoperidinium leonis
'Quinqucuspis concreta"
Protoperidinium oblongum
"Votadinium carvum"
Protoperidinium latissinum



Cysts with seastar-like outline

Protoperidinium compressum
"Stelladinium reidii"
Protoperidinium* cf. *compressum
"Stelladinium abei"



Cysts broadly elliptical with apical-antapical compression

Protoperidinium subinerme
"Selenopemphix nephroides"

Cysts spherical

Cysts with fine reticulate structure

Gymnodinium catenatum
Gymnodinium microreticulatum
Gymnodinium nolleri

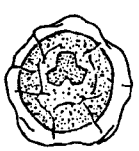
Cysts with two-layered wall

Cysts with a single layered wall

Fig. 17-4

Archeopyle corresponding to 2' to 4' paraplates

Protoperidinium americanum



Archeopyle theropylic-epicystal

Zygabikodinium lenticulatum

"Dubridinium caperatum"

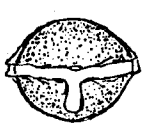


Fig. 17-4

Cysts with a single layered wall

Archeopyle corresponding to 2a paraplate

Archeopyle theropylic-epicystal

Diplopsalis lenticula

Archeopyle theropylic type with zigzag slit

Diplopelta abei

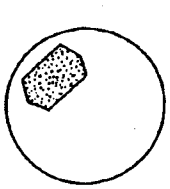
Archeopyle hexagonal laterally expanded

Protoperidinium avellanum
"Brigantedinium caracoense"

Protoperidinium denticulatum
"Brigantedinium irregulare"

Protoperidinium thorianum

***Protoperidinium* sp.**
"Brigantedinium grande"



Archeopyle hexagonal (apparently trapezoidal) comprising four long sides and two short sides

Protoperidinium conicoides
"Brigantedinium simplex"

***Protoperidinium* sp.**
"Brigantedinium majusculum"

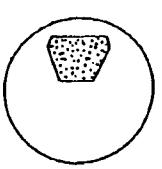


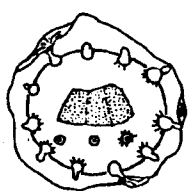
Fig. 17-5

Cysts possessing colorless wall

Cysts discoidal with large barrel-shaped processes

Pyrophacus steinii

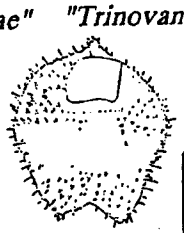
"Tuberculodinium vancampoae"



Cysts peridinioid covered with numerous short spines

***Protoperidinium* sp.**

"Trinovantedinium capitatum"

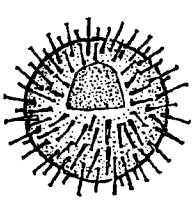


Cysts spherical with many processes distributed in random apparently

Cysts with gonal and/or intergonal furcate processes

Cysts spherical with many processes bearing capitate distal ends

Protoceratium reticulatum



Cysts spherical with many hollow and bullbouse processes

Lingulodinium polyedrum

"Lingulodinium machaerophorum"

Cysts spherical to ovoidal

Fig. 17-6

Cysts elongate

***Gonyaulax spinifera* complex**

"Spiniferites elongatus"
"Spiniferites frigidus"

Fig. 17-6

Cysts spherical to ovoidal

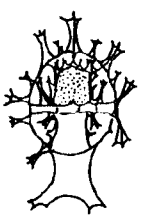
Cysts with gonal processes only

Cysts with gonal and intergonal processes

Membranous parasutural septa on the antapical area

***Gonyaulax spinifera* complex**

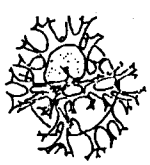
"Spiniferites mirabilis"



Solid processes only

***Gonyaulax spinifera* complex**

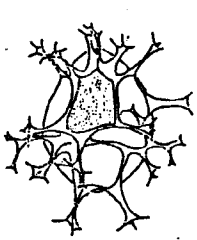
"Spiniferites hyperacanthus"



Cyst large (>40µm) with granular surface

Gonyaulax spinifera

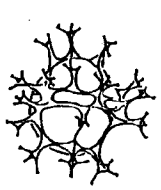
"Spiniferites ramosus"



Cyst small (<40µm) with smooth surface

Gonyaulax scrippsae

"Spiniferites bulloideus"



Cyst small (<40µm) with hollow process on the antapical area and smooth surface

***Gonyaulax* sp.**

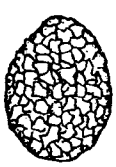
"Spiniferites cf. *delicatus"*

Fig. 17-7

Cysts composed of calcareous wall

Cyst elongatedly ovoidal with brick-like ornaments

Scrippsiella crystallina



Cyst ellipsoidal to ovoidal with numerous needle-like processes

Scrippsiella precaria
Scrippsiella rotunda
Scrippsiella trochoidea

Cyst spherical with numerus truncated cone-like ornaments

Ensiculifera carinata

Table I List of species producing resting cysts in modern marine dinoflagellates

Species	Reference
Prorocentrales	
Prorocentrum lima	Faust (1990)
Prorocentrum marinum	Faust (1993)
Prorocentrum pyrenoideum	Bursa (1959)
Dinophysiales	
Dinophysis acuta	Moita et Sampayo (1993)
Dinophysis tripos	Moita et Sampayo (1993)
Gymnodiniales	
Amphidinium carterae	Cao Vien 1967
Cochlodinium sp.	Fukuyo (1982)
Cochlodinium sp.	Matsuoka (1985a, 1987a)
Gymnodinium catenatum *	Anderson *et al.* (1988), Matsuoka (1987a), Hallegraeff *et al.* (1989)
Gymnodinim nolleri	Ellegaard et Oshima (1998)
Gymnodinium microreticulatum	Bolch, Negri et Hallegraeff (1999)
Gymnodinium sp. 1	Bolch et Hallegraeff (1990)
Gyrodinium impudicum	Kobayashi, Kojima, Itakura, Imai et Matsuoka (2000)
Gyrodinium instriatum	Wall et Dale (1968a), Fukuyo (1982), Matsuoka (1985a) Kojima et Kobayashi (1992)
Gyrodinium resplendens	Dale (1983)
Gyrodinium uncatenum	Tyler *et al.* (1982)
Gyrodinium sp. 1	Bolch et Hallegraeff (1990)
Pheopolykrikos hartmannii	Fukuyo (1982), Matsuoka et Fukuyo (1986),
Polykrikos kofoidii	Matsuoka (1985a), Morey-Gains et Ruse (1980), Fukuyo et Matsuoka (1983)
Polykrikos schwartzii	Wall et Dale (1968a), Matsuoka (1985a)
Woloszynskia sp. 1	Bolch et Hallegraeff (1990)
Katodinium fungiforme	Spero et Moree (1981)
Pfiesteria piscimorte	Burkholder et al. (1992)
Gonyaulacales	
Gonyaulax digitalis	Wall et Dale (1968a)
Gonyaulax scrippsae	Wall et Dale (1968a), Matsuoka (1984b)
Gonyaulax spinifera *	Wall et Dale (1968a)
Gonyaulax cf. *spinifera*	Dale (1983)
Gonyaulax verior	Matsuoka *et al.* (1988)
Gonyaulax sp.	Dobell et Taylor (1981)
*Lingulodinium polyedraum**	Wall et Dale (1968a), Kobayashi *et al.* (1981), Lewis (1988)
Protoceratium reticulatum	Wall et Dale (1968a)
*Alexandrium affine**	Fukuyo *et al.* (1985), Hallegraeff *et al.* (1991)
*Alexandrium andersonii**	Montresor, Zingone et Sarno (1998)
*Alexandrium catenella**	Yoshimatsu (1981), Fukuyo (1985)
Alexandrium cohorticula	Fukuyo *et al.* (1988)
Alexandrium fundyense	Hawryluk et al. (1992)
Alexandrium globosum	Dale (1977b)
Alexandrium hiranoi	Kita *et al.* (1985)
Alexandrium leei	Fukuyo *et al.* (1988)
Alexandrium lusitanicum	Blanca et al. (1991)
Alxeandrium margalefi	Hallegraeff et al. (1991); as *A.* sp.
*Alexandrium minutum**	Bolch *et al.* (1991)

*Alexandrium monilatum**	Walker et Steidinger (1979)
Alexandrium osterfeldii	Braarud (1945)
Alexandrium pervianum	Fukuyo *et al.* (unpublished data)
Alexandrium pseudogonyaulax	Montresor *et al.* (1993a)
*Alexandrium tamarense**	Dale (1977b), Fukuyo (1985)
Helgolandium subglobosum	Von Stosch (1969b)
Fragilidium heterolobum	Steidinger (1975)
Pyrodinium bahamense var. *bahamense*	Wall et Dale (1969)
Pyrodinium bahamense var. *compressum**	Steidinger *et al.* (1980), Matsuoka *et al.* (1989), Corrales *et al.* (1995)
Pyrophacus horologium	Wall et Dale (1971)
Pyrophacus steinii	Wall et Dale (1971), Matsuoka (1985b)

Peridiniales

Scrippsiella crystallina	Lewis (1991), Ishikawa et Taniguchi (1993)
Scrippsiella lachrymosa	Lewis(1991)
Scrippsiella minima	Gao *et al.* (1989)
Scrippsiella pentagonica	Akselman et Keupp (1990)
Scrippsiella precaria	Montresor et Zingone (1988), Ishikawa et Taniguchi (1993), Kobayashi *et al.* (1994)
Scrippsiella ramonii	Montresor (1995)
Scrippsiella rotunda	Lewis(1991), Ishikawa et Taniguchi (1993)
Scrippsiella trifida	Lewis(1991)
*Scrippsiella trochoidea**	Wall et al. (1970), Bolch et Hallegraeff (1990), Lewis (1991)
Scrippsiella sweeneyae	Wall et Dale (1968b)
Ensiculifera cf. *mexicana*	Wall et Dale (1968b)
Ensiculifera carinata	Matsuoka *et al.* (1990)
Ensiculifera imariensis	Kobayashi et Matsuoka (1995)
Pentapharsodinium dalei	Dale (1977a, 1978), Lewis(1991)
Pentapharsodinium tyrrhenicum	Montresor *et al.* (1993b)
Peridinium hangoei	Iwasaki (1969), Lewis *et al.* (1984)
Cachonina hallii	Von Stosch (1969a)
Coolia monotis	Faust (1992)
Hetrocapsa triquetra	Braarud et Pappas (1951)
Protoperidinium acromaticum	Popovsky et Pfiester (1990)
Protoperidinium americanum	Lewis et Dodge (1987)
Protoperidinium avellanum	Wall et Dale (1968a), Matsuoka (1984a), Lewis *et al.* (1984)
Protoperidinium antarcticum	Akselman (1987)
Protoperidinium brochii	Blanco (1989)
Protoperidinium claudicans	Wall et Dale (1968a), Akselman (1987)
Protoperidinium compressum	Wall et Dale (1968a)
Protoperidinium conicoides	Wall et Dale (1968a), Akselman (1987)
Protoperidinium conicum	Wall et Dale (1968a), Fukuyo (1980), Kobayashi et Matsuoka (1984), Bolch et Hallegraeff (1990)
Protoperidinium denticulatum	Wall et Dale (1968a)
Protoperidinium divaricatum	Matsuoka *et al.* (1982)
Protoperidinium excentricum	Wall et Dale (1968a), Lewis *et al.* (1984), Akselman (1987)
Protoperidinium expansum	Hallegraeff et Bolch (1992)
Protoperidinium grandii	Meunier (1910)
Protoperidinium latissinum	Wall et Dale (1968a)
Protoperidinium leonis	Wall et Dale (1968a)
Protoperidinium minutum	Wall et Dale (1968a), Fukuyo *et al.* (1977)
Protoperidinium monospinum	Zonneveld et Dale (1994)

Protoperidinium nudum	Wall et Dale (1968a)
Protoperidinium oblongum	Wall et Dale (1968a), Akselman (1987), Bolch et Hallegraeff (1990)
Protoperidinium obtsum	Akselman (1987)
Protoperidinium pentagonum	Wall et Dale (1968a), Matsuoka (1982), Lewis *et al.* (1984), Inoue (1990)
Protoperidinium punctulatum	Wall et Dale (1968a)
Protoperidinium subinerme	Wall et Dale (1968a)
Protoperidinium thorianum	Lewis *et al.* (1984)
Protoperidinium thulesense	Dodge (1985)
Protoperidinium cf. *divergens*	Dale (1983)
Protoperidinium sp.	Dale (1983)
Diplopelta parva	Matsuoka (1988), Bolch et Hallegraeff (1990)
Diplopelta symmetrica	Dale *et al.* (1993)
Diplopsalis lenticula	Wall et Dale (1968a)
Diplopsalis lebourae	Matsuoka (1988)
Diplopsalopis orbicularis	Wall et Dale (1968a), Matsuoka (1988)
Diplopsalopsis latipeltata	Dale *et al.* (1993)
Gotoius abei	Matsuoka (1988)
Oblea rotunda	Lewis (1990)
Zygabikodinium lenticulatum	Wall et Dale (1968a), Akselman (1987), Matsuoka (1988), Bolch et Hallegraeff (1990)

*:harmful species

Table 2 Dormancy periods in cyst-producing dinoflagellates

Marine species	**Dormancy periods**
Gymnodinium catenatum	Two weeks (Blackburn et al. 1989)
Pyrodinium bahamense var. *compressum*	2.5 to 3.5 months (Corrales et al. 1995)
Pyrodinium bahamense var. *bahamense*	6 weeks (Wall & Dale 1969)
Alexandrium catenella	10 days (Yoshimatsu 1992)
Alexandrium tamarense	4 months under 16 ° C after 5 ° C and 1 month under 22 ° C (Anderson 1980) 6 months under the natural condition (Fukuyo et al. 1982)
Alexandrium hiranoi	Several days (Kita et al. 1993)
Pyrophacus steinii	Several weeks (Pholpunthin et al 1998)
Scrippsiella trochoidea	ca. 25 days (Binder & Anderson, 1987)
Freswater species	
Ceratium cornutum	Several weeks under lowere temperature (von Stosch 1964)
Ceratium hirundinella	6 weeks after sporulation (Huber & Nipkow 1923)
Woloszynskia apiculata	Several months without light and in 6 ° C (von Stosch 1973)
Peridiniu bipes	Several months in 20 ° C (Kido et al. 1985)
Peridinium cunningtonii	3-4 months (Sako et. al, 1985)

Table 3 Medium for cyst cultures with their chemical composition. Most of them are also useful for the culture of motile forms.

Guillard & Ryther's "f" (1962)

NaNO3	150 mg
NaHPO4 2H2O	10 mg
Fe-EDTA	9.39 mg
Filtered Sea water	1*l*
Trace metal:-	
CuSO4 5H2O	0.0196 mg
ZnSO4 7H2O	0.044 mg
CoCl2 6H2O	0.020 mg
MnCl2 4H2O	0.360 mg
Na2MoO4 2H2O	0.0126 mg

Iwasaki's SWII (1961)

KNO3	7.2mg
KH2PO4	0.45mg
H3H7Na2O65H2O	1.05mg
Fe(as EDTA)	50μg
Vitamin B12	0.2μg
Tris(hydroxymethyl) aminomethane	50mg
Sea water	100ml

Fóyn (1934)

NaNO3	10mg
NaHPO4 12H2O	2mg
Soil extract*	5ml
Sea water	100ml

*Soil extract; [Soil 1kg + Distillated water 1000ml] boiled for 60 min., left in dark and cool place for two days and then filtered. [Filtered soil extract 600ml + Distillated water 400ml] for original

Provasoli-Pinter III* (1953)

$Ca(NO_3)_2\ 4H_2O$	10.0mg
$MgSO_4\ 7H_2O$	2.0mg
K_2HPO_4	2.0mg
Glucose	50.0mg
Trypticase*	50.0mg
Distillated water	100ml
pH	6.5

Trypticase is rovided by Baltimore Biological Laboratory.

*Autoclaved under nomal pressure.

Provasoli-Pinter IV* (1953)

KNO3	5.0mg
K2HPO4	25.0mg
MgSO4 7H2O	10.0mg
CaCO3	10.0mg
CaCl2	4.0mg
FeCl3	0.2mg
ZnCl2	0.08mg
MnCl2	0.08mg
Ma2MoO4	0.05mg
CoCl2	0.001mg
CuCl2	0.0001mg
Sodium Glutamate	100mg
Glycine	70mg
DL-Alanine	70mg
Glucose	50mg
Distillated water	100ml
pH	6.2

*: Autoclaved under nomal pressure.

ESM

NaNO3	10mg
K2HPO4	2mg
Vitamin B12	1µg
Biotin	1µg
Thiamine HCl	100µg
Fe-EDTA	259µg
Mn-EDTA	332µg
Tris(hydroxymethyl) aminomethane	1g
Soil extract*	50ml
Sea water	950ml
pH	8.0

Table 4 Biological names of modern dinoflagellate cysts

Names based on motile form	Names in paleontology
Gonyaulacales	
Gonyaulax digitalis (Pouchet)	*Spiniferites bentori* (Rossignol)
Gonyaulax scrippsae Kofoid	*Spiniferites bulloideus* (Defl.et Cook.)
Gonyaulax spinifera (Clap. et Lach.) complex	*Spiniferites ramosus (*Ehrenberg)
	Ataxiodinium choanum Reid
	Spiniferites elongatus Reid
	Spiniferites mirabilis (Rossignol)
	Spiniferites hyperacanthus (Defl. et Cook.)
	Bitectatodinium tepikiense Wilson
	Tectatodinium pellitum Wall
	Nematospharopsis labyrinthus (Ostenfeld)
Lingulodinium polyedrum (Stein)	*Lingulodinium machaerohorum* (Defl. et Cook.)
Protoceratium reticulatum (Claparede et Lachmann)	*"Operculodinium centrocarpum"* (Defl. et Cook.)
Alexandrium pseudogoniaulax (Biecheler)	*"Impagidinium"* sp.
Pyrodinium bahamense var. *bahamense* (Plate)	*Polysphaeridium zoharyi* (Wall)
Pyrodinium bahamense var. *compressum* *(Bohm)	*Polysphaeridium zoharyi* (Wall)
Pyrophacus steinii (Schiller)	*Tuberculodinium vancampoae* (Rossignol)
Peridiniales	
*Pentapharsodinium faeroense (*Paulsen)	*Mychystridium bifurcatum* Williams (MS)
Protoperidinium avellanum (Meunier)	*Brigantedinium cariacoensis* (Wall)
Protoperidinium claudicans (Paulsen)	*Votadinium spinosum* Reid
Protoperidinium compressum (Abe)	*Stelladinium stellatum*(Wall)
	Stelladinium reidii Bradford
Protoperidinium conicoides (Paulsen)	*Brigantedinium simplex* (Wall)
Protoperidinium conicum (Gran)	*Selenopemphix quanta* (Bradford)
Protoperidinium denticulatum (Gran et Braarud)	*Brigantedinium* sp.
Protoperidinium divaricatum (Meunier)	*Xandarodinium variabile* Bujak

Protoperidinium leonis (Pavilard)
Protoperidinium nudum (Meunier)
Protoperidinium oblongum (Aurivillius)
Protoperidinium pentagonum (Gran)
Protoperidinium punctulatum (Paulsen)
Protoperidinium subinerme (Paulsen)
Zygabikodinium lenticulatum (Paulsen)
Quinqucuspis concreta (Reid)
Selenopemphix quanta (Bradford)
Votadinium carvum Reid
Brigantedinium sp.
Brigantedinium sp.
Selenopemphix nephroides Benedeck
Dubridinium caperatum Reid

Table 5 Key for the modern dinoflagellate cysts based on shape of cyst body

1 Cordate in dorso-ventral view	----- *Protoperidinium oblongum*
	---- *Protoperidinium claudicans*
	(Votadinium)
1 Peridinioid in dorso-ventral view and compressed antero-posteriorly	----- *Protoperidinium conicum*
	----- *Protoperidinium nudum*
	----- *Protoperidinium subinerme*
	(Selenopemphix)
1 Roundly polygonal with hollow processes distally closed	----- *Protoperidinium divaricatum*
	(Xandarodinium)
1 Subspherical to ovoidal with well-developed parasuture	----- Gonyaulacaceae
	(Impagidinium)
1 Subspherical endophragm (inner body) with membranous periphragm	---*Gonyaulax spinifera* complex
	(Ataxiodinium)
1 Discoidal with many short barrel-shaped processes	----- *Pyrophacus steinii*
	(Tuberculodinium)
1 Ellipsoidal with coarsely reticulate ornaments on surface	----- *Polykrikos schwartzi*i
1 Ellipsoidal with shelf-like or hollow processes	----- *Polykrikos kofoid*ii
1 Ovoidal and transparent phragma, sometimes with mucilaginous material	----- *Gyrodinium instriatum*
	----- *Gonyaulax verior*
1 Ellipsoidal and transparent phragma, sometimes with mucilaginous material	---*Alexandrium catenella*
	----- *Alexandrium tamarense*
1 Spherical and transparent phragma, sometimes with mucilaginous material	----- *Alexandrium leei*
	----- *Alexandrium pervianum*
	---- *Alexandrium pseudogoniaulax*
	----- *Diplopsalopsis orbicularis*
1 Spherical to ovoidal	------**2**
1 Spherical to ovoidal with processes densly distributed	------**3**
1 Peridinioid (pentagonal to stellar) in dorso-ventral view	------**4**
1 Spherical to ellipsoidal with well developed paratutures and processes	------ **5**
2 Ovoidal with two phragma well adpressed (simple precingular archeopyle)	---*Gonyaulax spinifer* complex
	(Tectatodinium)

2 Subsphaerical with two phragma well adpressed (combination precingular archeopyle)

--- *Gonyaulax spinifera* compex

(Bitectatodinium)

2 Spherical to subspherical with a single brouwn layer (Intercalary archeopyle) —*Protoperidinium avellana*

--- *Protoperidinium denticulatum*

--- *Protoperidinium punctulatum*

--- *Gotoius abei*

(Brigantedinium)

2 Subspherical, antero-posteriolly compressed with paracingular and parasulcus

--- *Zygabikodinium lenticulatum*

(Dubridinium)

2 Spherical with finely reticulate surface ----- *Gymnodinium catenatum*

3 Evexate to bulbous processes closed distally ----- *Lingulodinium polyedra*

(Lingulodinium)

3 Slender capitate processes closed distally ----- *Protoceratium reticulatum*

(Operculodinium)

3 Short denticulate to patulate processes ----- *Pyrodinium bahamense*

(Polysphaeridinium)

3 Calcareous wall with acuminate to conical processes ----- *Scrippsiella trochoidea*

3 Brownish cyst wall with long acuminate processes ----- *Protoperidinium minutum*

----- *Protoperidinium amphidosum*

---- *Diplopelta parva*

3 Brownish cyst wall with acuminate processes striated proximally ----- *Pheopolykrikos hartmanı*

4 Long spines developed on each corner of peridinioid shape ----- *Protoperidinium compressum*

(Stelladinium)

4 Parasutural and intratabular short spines ----- *Protoperidinium pentagonum*

(Trinovantedinium)

4 A single apical and two antapical horns well developed ----- *Protoperidinium leonis*

----- *Protoperidinium latissinum*

(Lejeunecysta)

5 Parasutural furcate processes	----- *Gonyaulax spinifera* complex
	----- *Gonyaulax scrippsae*
	----- *Gonyaulax* sp.
	(Spiniferites)
5 Parasutural furcate processes connected with ectophragm	----- *Gonyaulax spinifera* complex
	(Nematosphaeridinium)

0: name for cyst based taxa.

Table 6 Key to modern dinoflagellate cysts based on shape and archeopyle type

1 Saphopylic archeopyle ------ **2**
1 Theropylic archeopyle ------- **11**
1 Cryptopylic archeopyle ------ **14**

2 Epicystal archeopyle ---------***Polysphaeridium***
2 Hypocystal archeopyle ------ ***Tuberculodinium***
2 Intercalary archeopyle ------ **3**
2 Precingular archeopyle ------- **7**
2 Combination archeopyle ----- **10**

3 Cordate in dorso-ventral view ------ ***Votadinium***
3 Spehrical and brownish autophragm without ornament ------ ***Brigantedinium***
3 Peridinioid with dome-like epicyst and two antapical horn ---- **6**
3 Brownish wall with several spines ----- **4**
3 Peridinioid with a single apical and two antapical horns ----- **5**

4 Single apical, two antapical and a few cingular spines ----- ***Stelladinium***
4 Roundly hexagonal, antero-posteriorly compressed ----- ***Selenopemphix***
4 Subcircular to ellptical in dorso-ventral view with hollow spines closed distally ------***Xandarodinium***

5 Transparent wall with intratabular and parasutural spines ------ ***Trinovantedinium***
5 Epicyst triangular in dorso-ventral view with distinct apical and antapical horns -------***Lejeunecysta***

6 Two small antapical horns ------ ***Leipokatium***
6 A single broad antapical boss ---- ***Selenopemphix***

7 Various ornaments and processes ----- **8**
7 No ornament ----- **9**

8 Parasutural furcate processes and coasely reticulate ectophrag ----- ***Nematosphaeropsis***
8 Furcate gonal and / or processes ------ ***Spiniferites***

8 Slender capitate processes ------ ***Operculodinium***

9 Subspherical with thick spongy cyst wall ----- ***Tectatodinium***

9 Spherical to ovoidal with distinct paratabulation indicated by parasutural septa ***—Impagidinium***

9 Subspherical endophragm covered with membranous periphragm -----***Ataxiodinium***

10 Spherical, without ornament and with operculumcorresponding two precingular paraplates -----***Bitectatodinium***

10 Spherical with bullbous processes and opercula corresponding four precingular and/or anterior intercalary and apical paraplates -----***Lingulodinium***

11 Spherical with brownish wall and acuminate processes, and apical archeopyle***—Diplopeltaparva***

11 Spherical with brownish wall and apical archeopyle ------- ***Diplopsalis lenticula***

11 Spherical with brownish wall and a simple intercalary archeopyel ------- ***Diplopsalis lebourae***

11 Subspherical to lenticular with epicystal archeopyle ------- **12**

11 Combination archopyle ------- **13**

12 Brounish wall with distinct paracingulum -----***Dubridinium (Zygabikodinium lenticulatum)***

12 Thin and smooth transparent wall ----- ***Helgolandinium sunglobosum***

------ ***Fragilidium heterolobum(?)***

13 Subspherical with opercula comprising apical and anterior intercalary paraplates ----- ***Diplolsalopsis orbicularis***

13 Subspherical to lenticular, with opercula consisting of two anterior intercalary parap ----- ***Gotoius abei***

13 Ovoidal with calcareous wall and ornaments ----- ***Scrippsiella trochoidea***

13 Transapical archoepyle ------ ***Peridinium limbatum***

14 Tremic archeopyle (hole type) -----***Polykrikos schwartzii***

----- ***Gyrodinium instriatum***

14 Chasmic archeopyle (slit type)-----***Pheopolikrikos hartmannii***

----- ***Gymnodinium catenatum***

----- ***Gymnodinium pseudopalustre***

Table 7 Equipment for dinoflagellate cyst studies in this technical guide book

For field sampling

TFO corer
Acrylic resin transparent inner tube (1.1mm in diameter, 32cm in length)
Steel ball stopper for corer
Rubber stopper for inner tube
Roup
Tin foil (or plastic sheet) for rapping

For laboratory work

Stainless sieve (mesh-sizes with 250µm, 125µm and 20µm)
Ultrasonic probe or bath
Plastic bottles (10*ml*, 50*ml*)
Petri-dish
Watch glass
Polyethylene centrifuge tube (15*ml*)*
Water bath*
Rubber or vinyl gloves*
Gas musk*
Needle (glass rod with insect pin)
Pasteur pipette with capillary tip (50-100µm in hole diameter)
Pipette(5*ml*)
Silicone tube (60cm in length) for Pasteur pipette
Counting chamber slides
Slides glass
Cover slip (18x24mm)
Beaker (100*ml*, 500*ml*)

Chemicals

Hydrochloric acid (HCl)*

Hydrofluoric acid (HF)*
Glacial acetic acid (CH3COOH)*
Acetic anhydride [(CH3CO)2O]*
Sulfuric acid (H2SO4)*

Bioplastic**
Polyvinyl alcohol**
Glycerin jelly**

Neutralized formalin(3%)***
Glutaraldehyde (10%)***

*:Palynological processing, **:permanent slide, ***: sample preservation