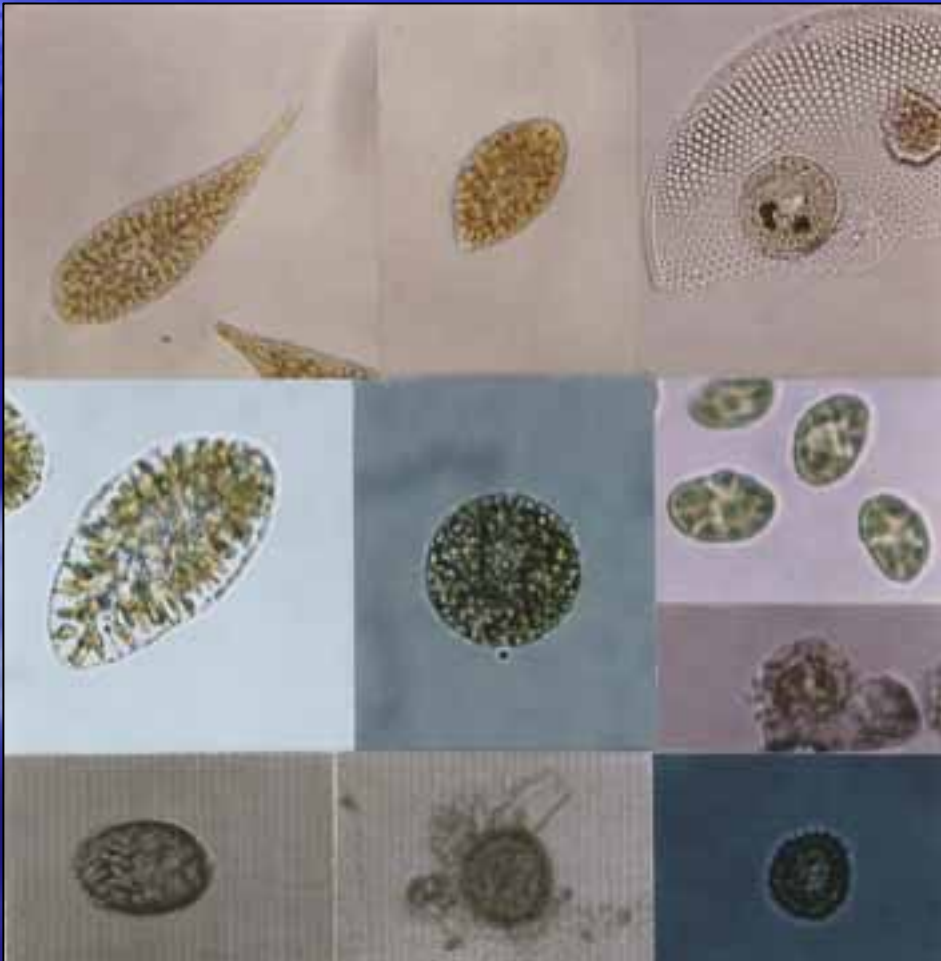
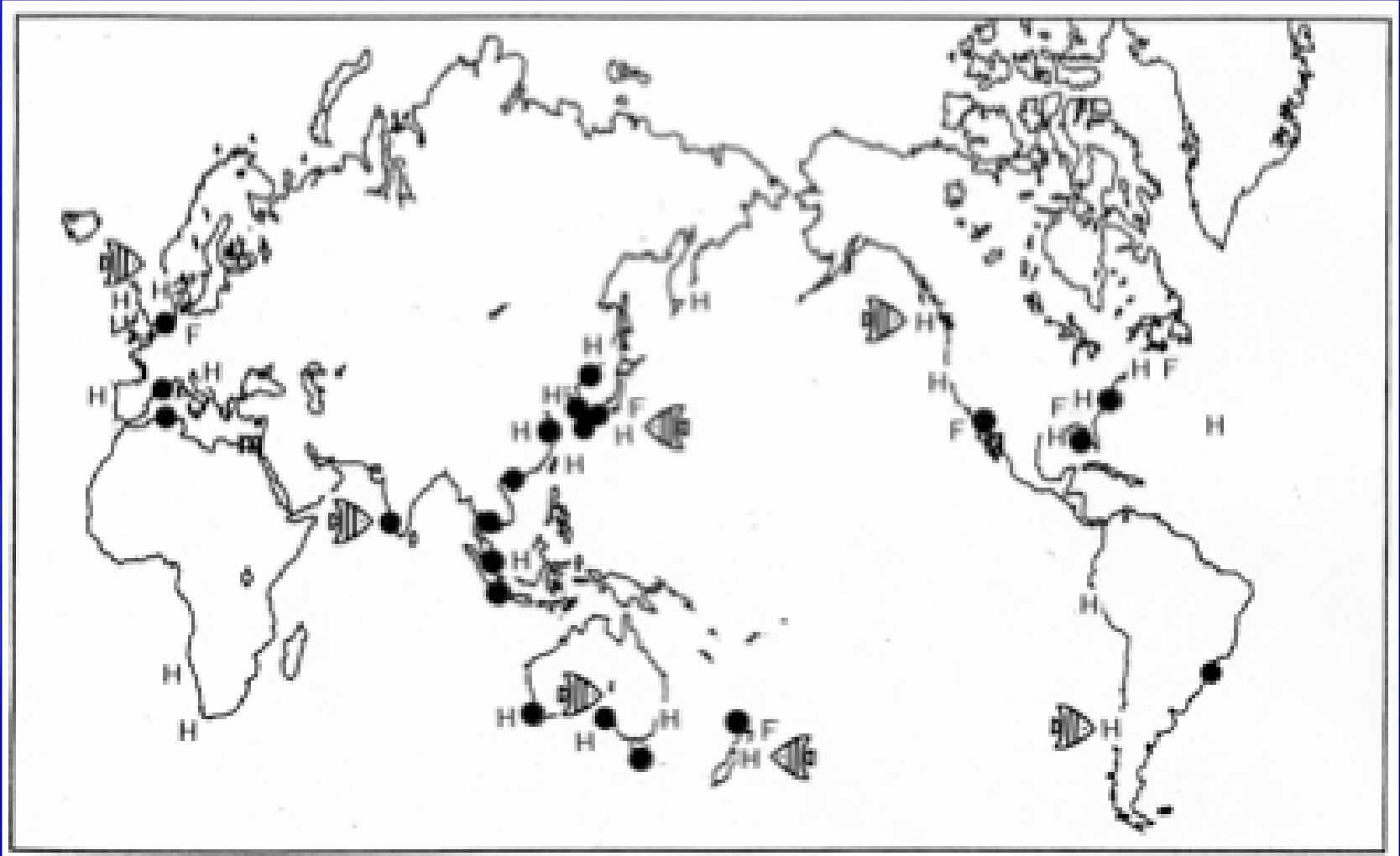


Life cycle strategies and occurrences  
of red tides of *Heterosigma akashiwo*  
and *Chattonella* spp. in temperate  
coastal sea



**Ichiro Imai**  
(Kyoto University),  
Shigeru Itakura,  
Mineo Yamaguchi

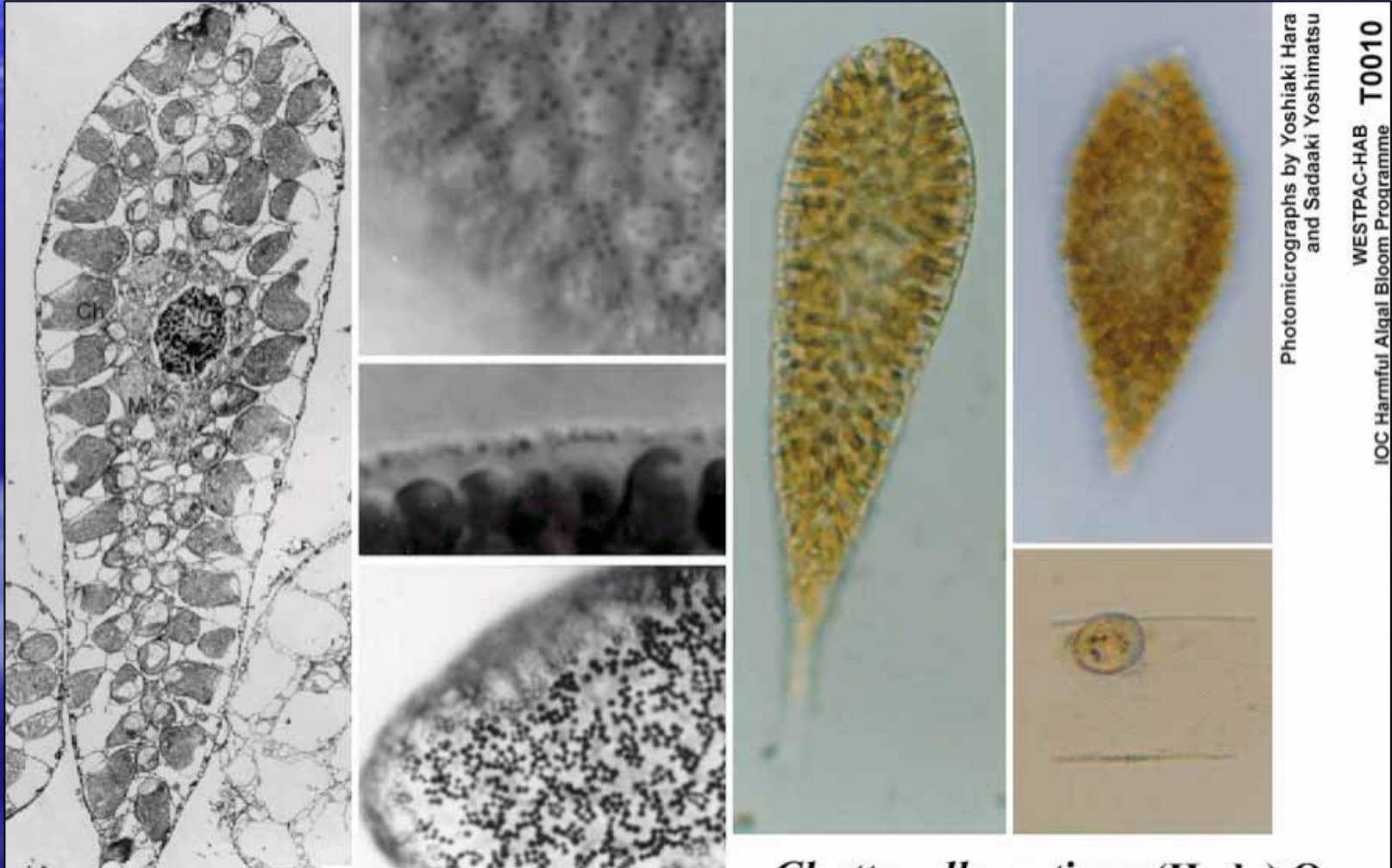
# Global distribution of raphidophytes and fish-kill (Edvardsen and Imai 2006)



# Contents

1. Life cycle strategy of *Chattonella antiqua* and *C. marina*
2. Life cycle strategy of *Heterosigma akashiwo*
3. How do harmful raphidophyte blooms occur predominantly over diatoms in coastal sea?

# Life cycle strategy of *Chattonella* spp.

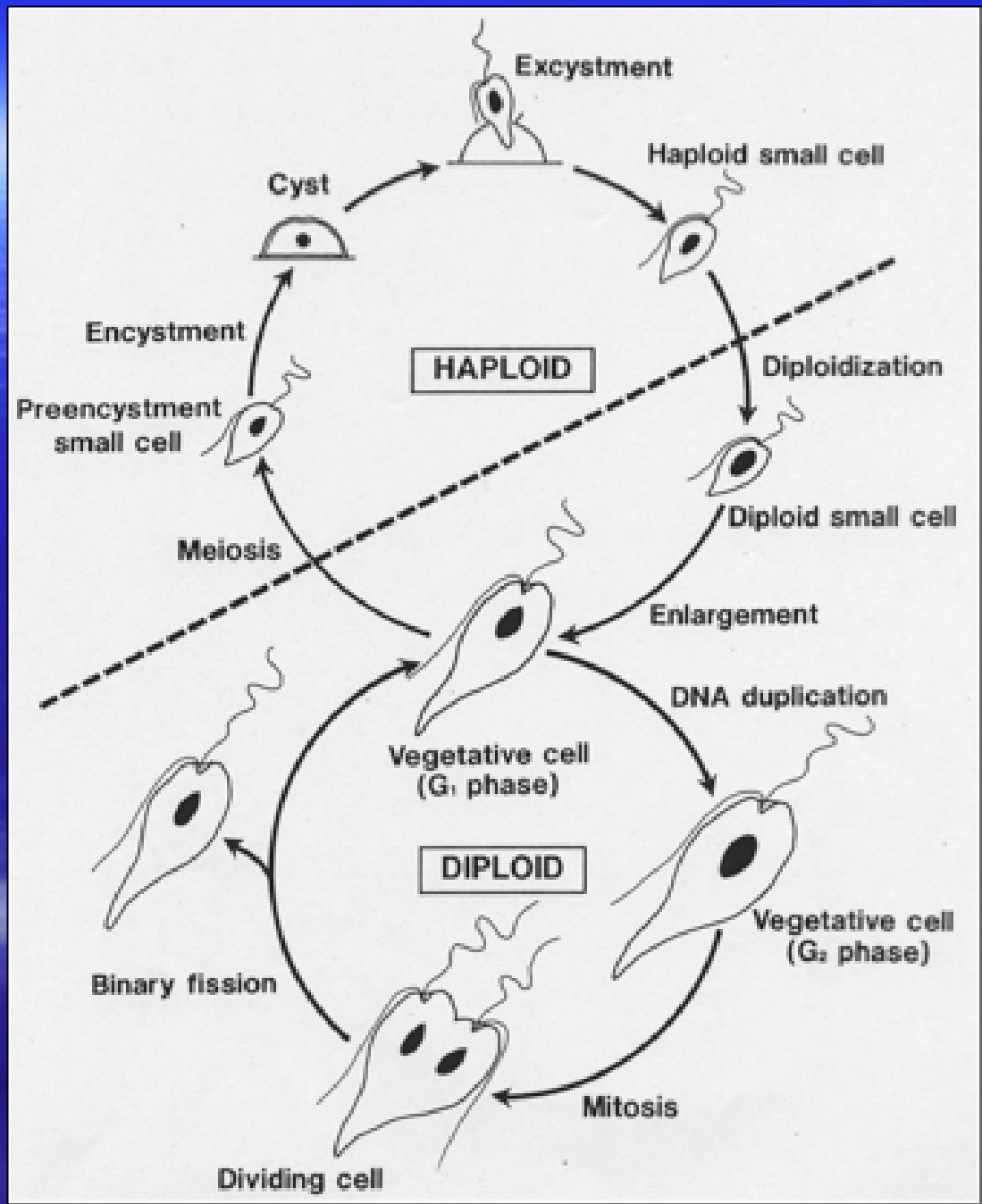


# Growth characteristics

1. *Chattonella antiqua* and *C. marina* are the summer red tide flagellates.
  2. The optimum combination of temperature and salinity
    - C. antiqua*: 25°C and 25psu
    - C. marina* : 25°C and 20psu
  3. Survival range of temperature
    - C. antiqua*: 11 ~ 31°C
    - C. marina* : 13 ~ 31°C
- ∴ *C. antiqua* and *C. marina* can not overwinter as vegetative cells in the Seto Inland Sea, and form cysts for overwintering.

Life cycle of *Chattonella antiqua* and *C. marina* based on DNA microfluorometry (Yamaguchi and Imai 1994)

*Chattonella antiqua* and *C. marina* are the diploid organisms.

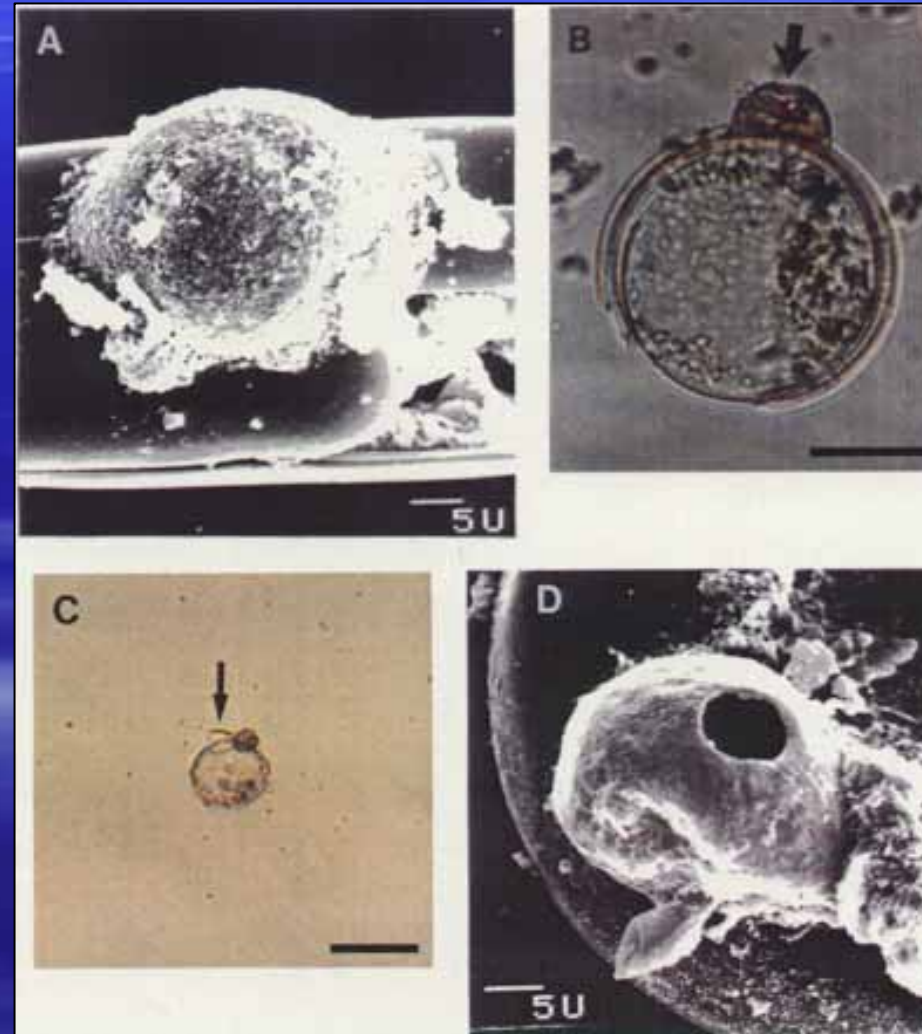
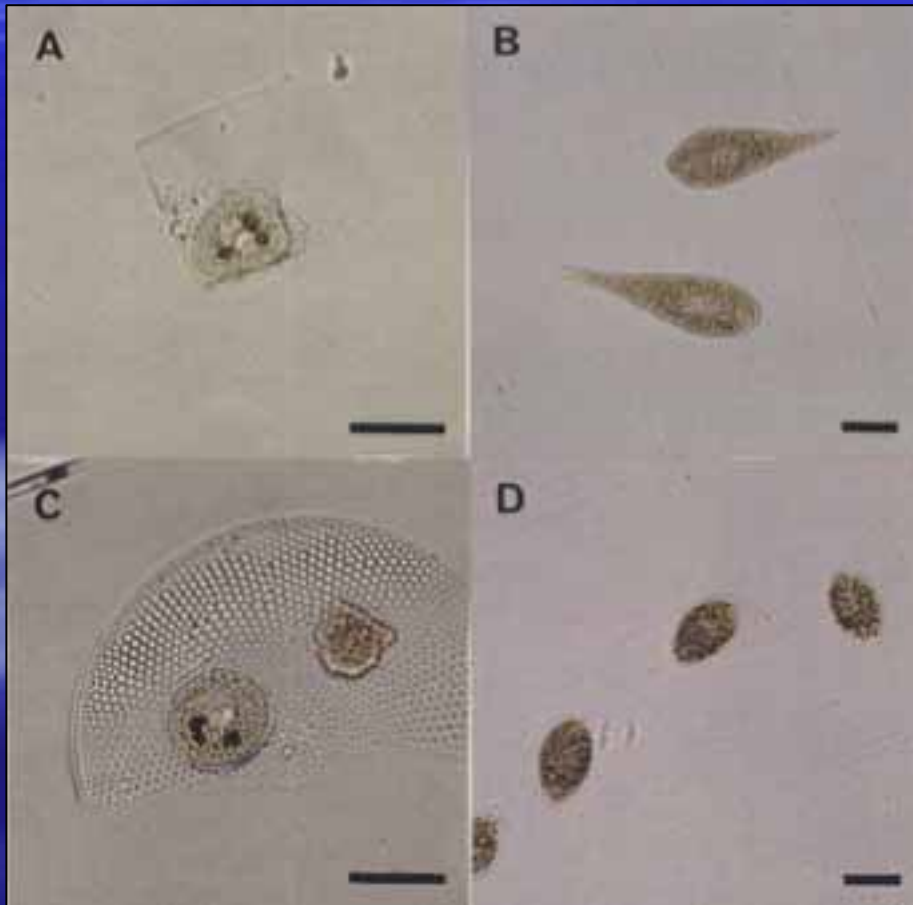


# Cysts of *Chattonella antiqua* and *C. marina*

(Imai and Itoh 1988)

A & B: *C. antiqua*

C & D: *C. marina*



MPN method for enumeration of *Chattonella* cysts having germination ability in sediments (Imai et al. 1984).

Feasible for the cysts without information on morphology

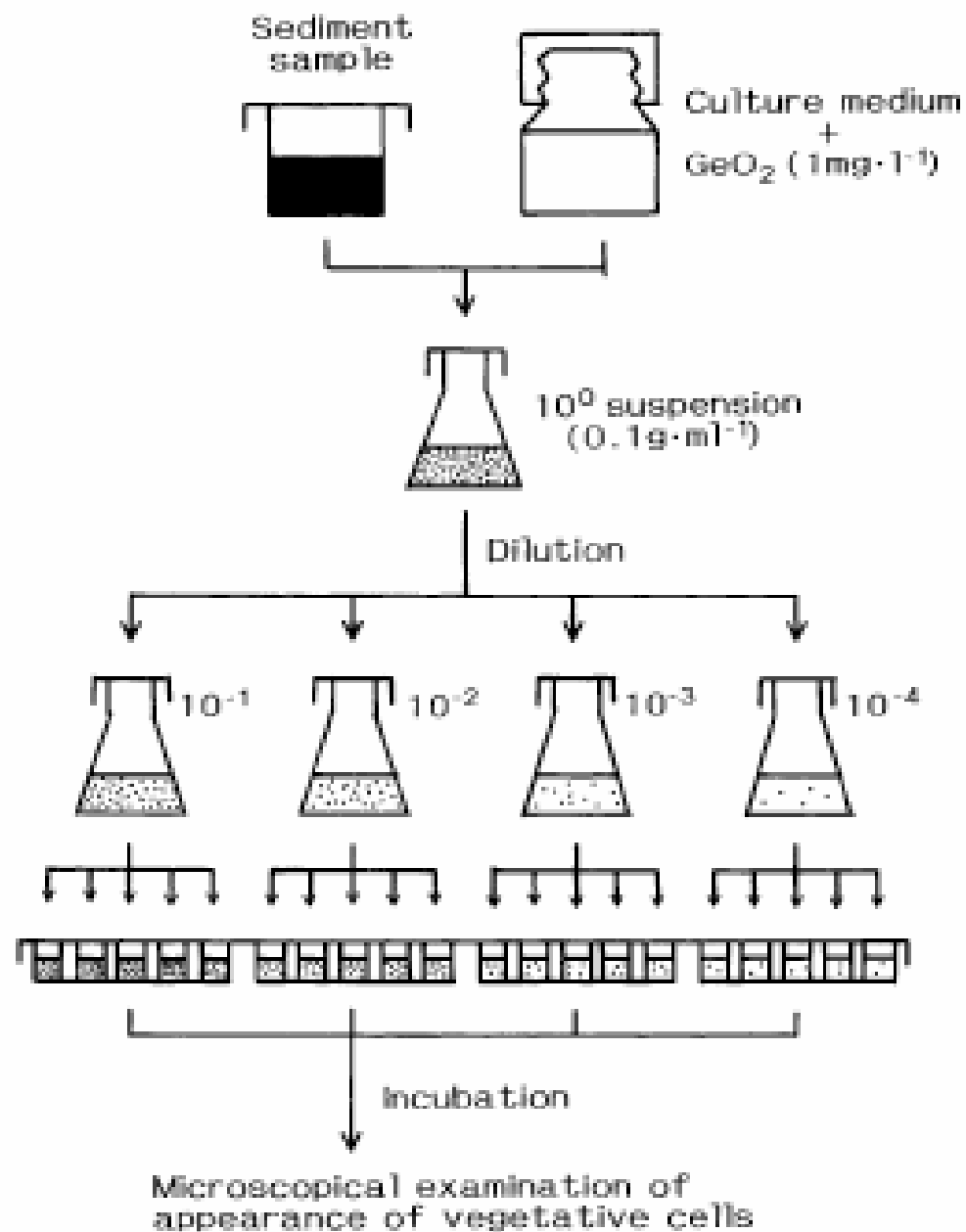
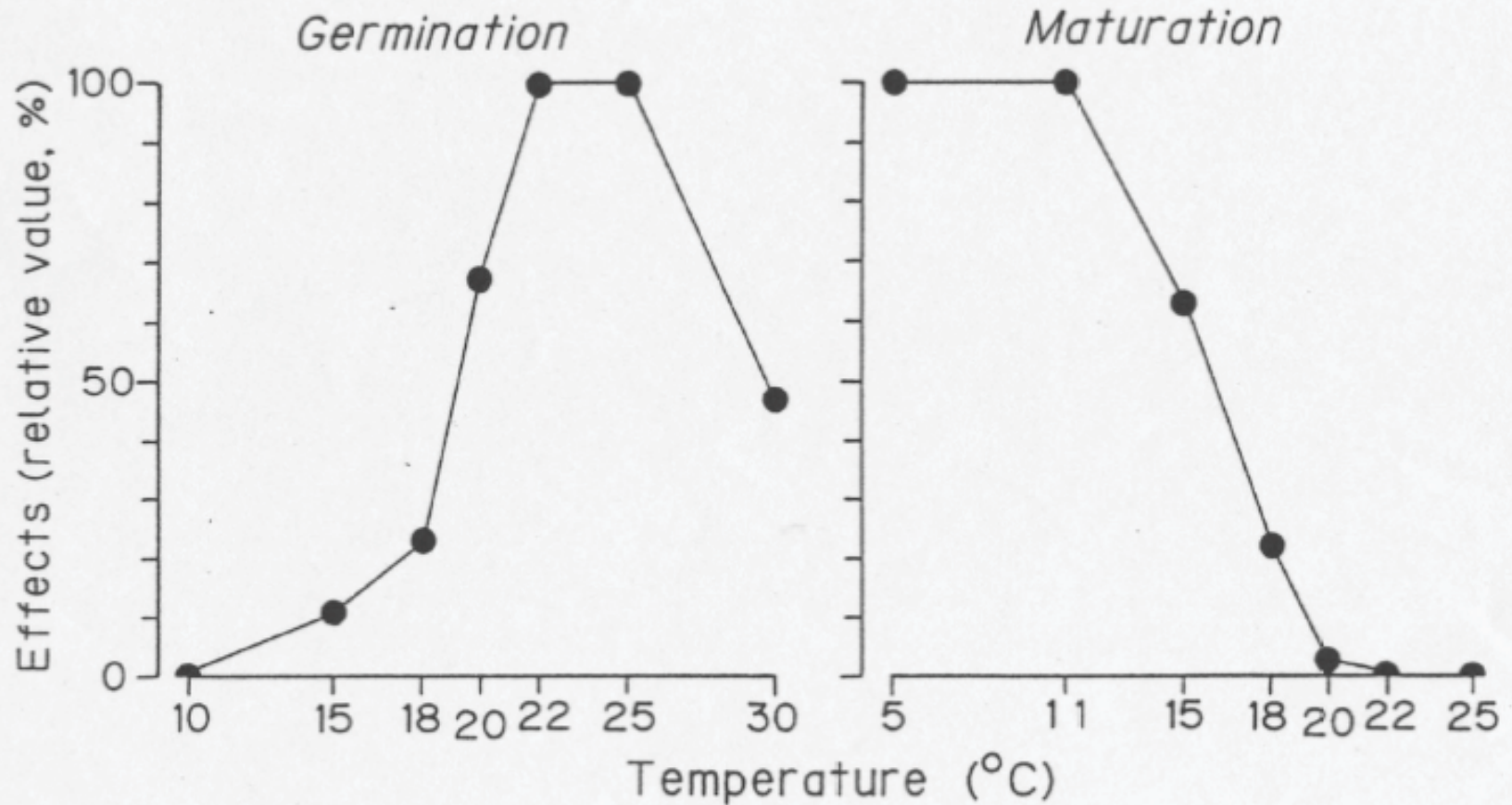


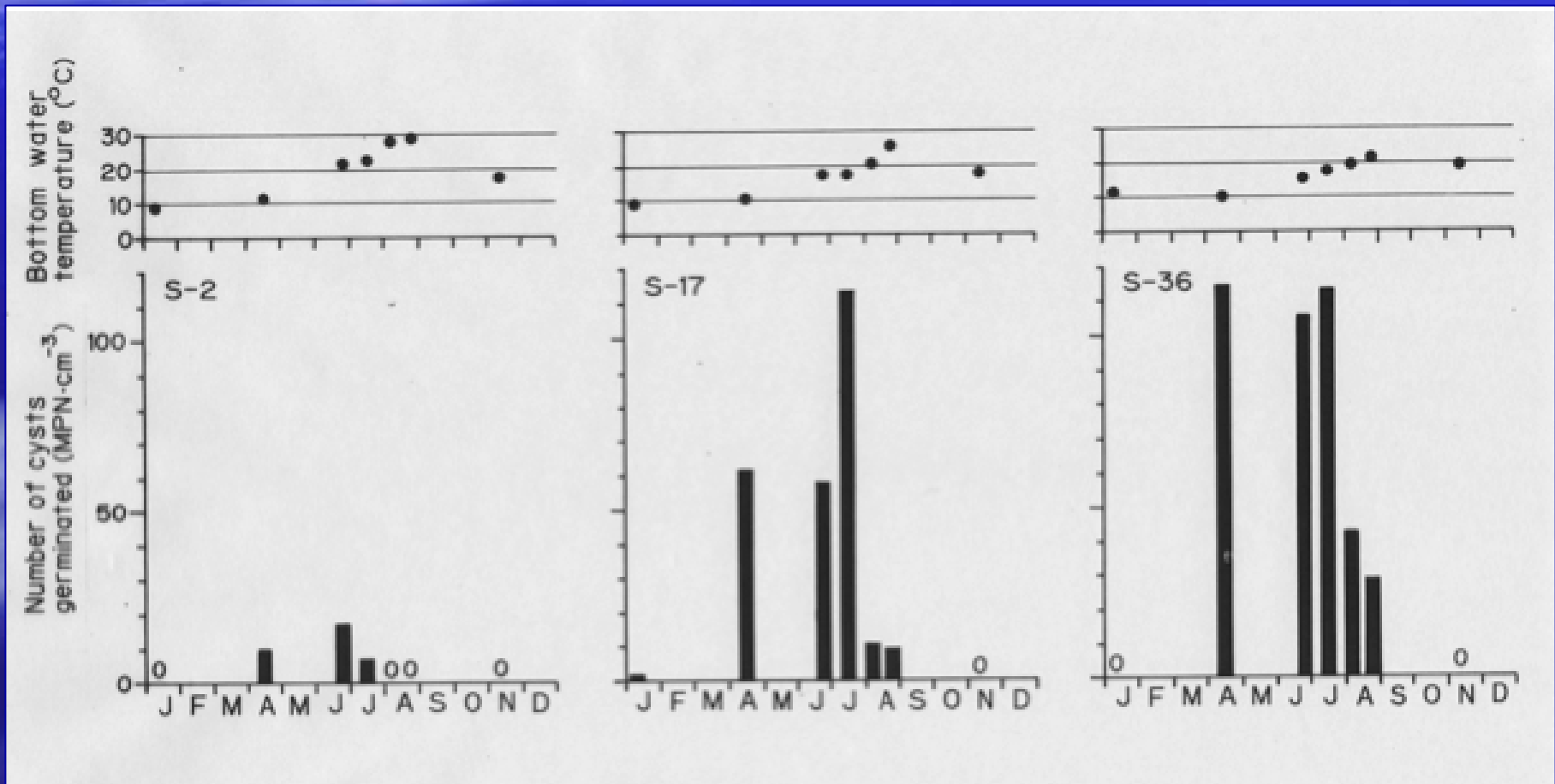
Fig. 2 *Heterosigma akashiwo*. Procedure of the extinction dilution method for enumeration of germinable cysts in sediment samples



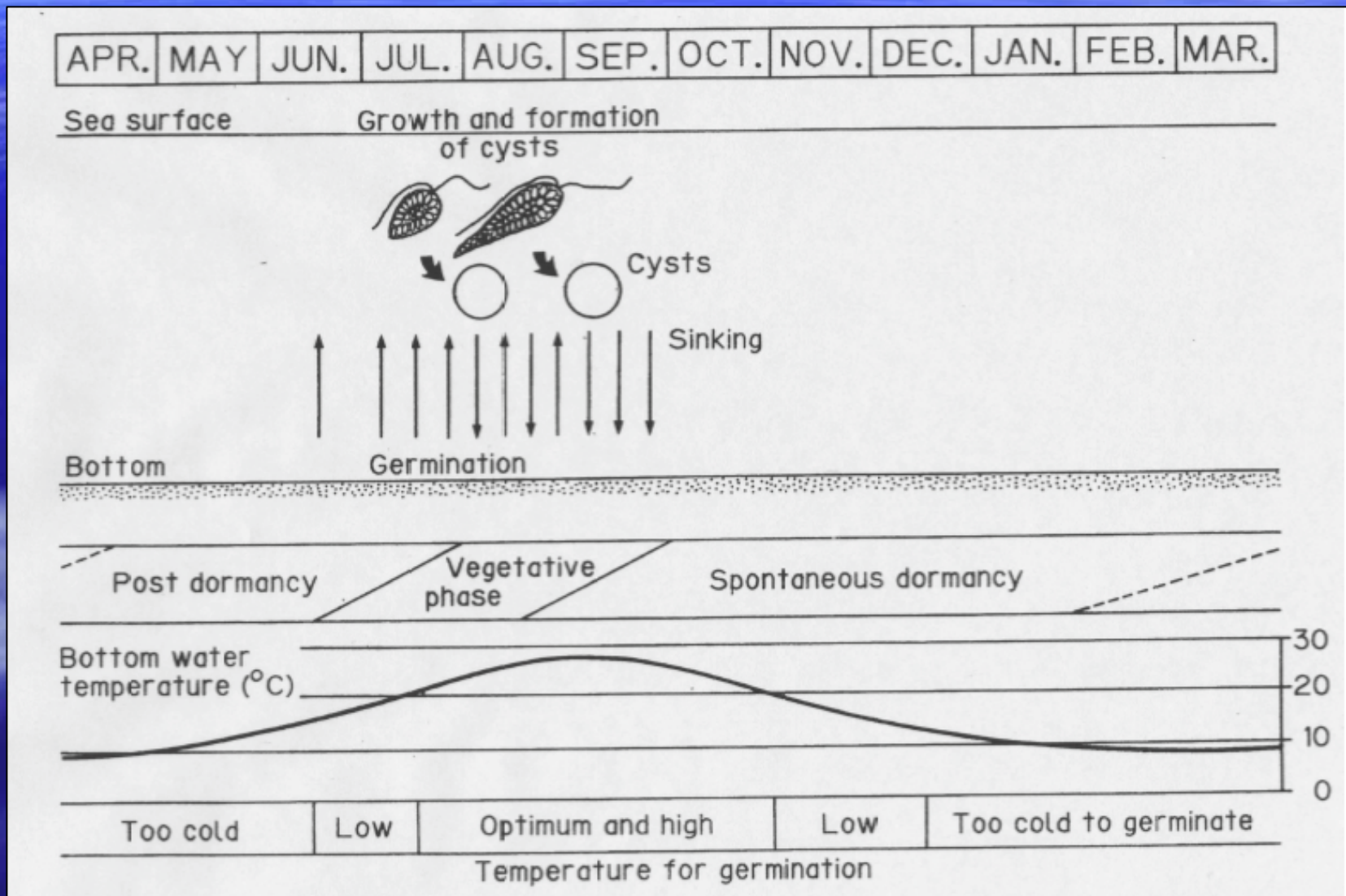
# Comparison of temperature characteristics of germination and maturation in *Chattonella* cysts.



# Seasonal changes in germinability of *Chattonella* cysts in sediments of collected at 3 points in Suo-Nada, the Seto Inland Sea (Imai and Itoh 1987).



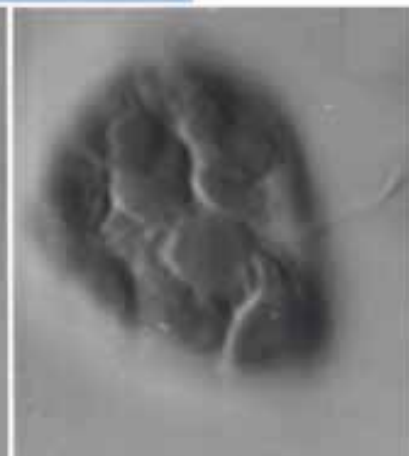
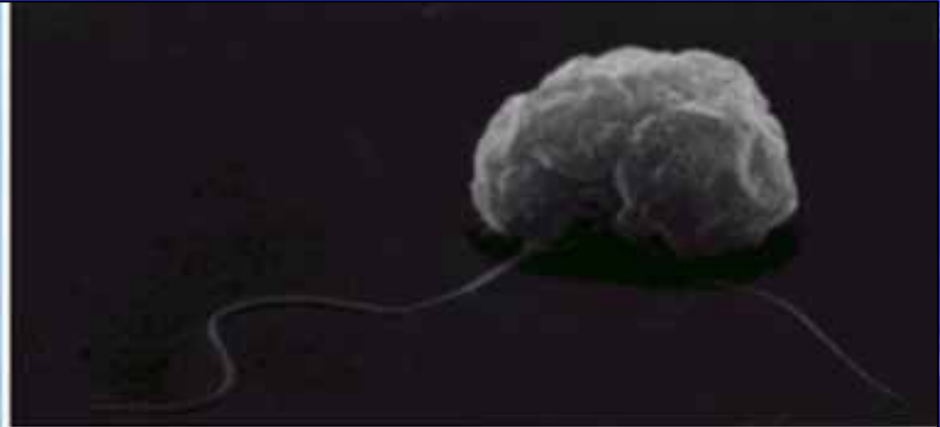
# Annual life cycle of *Chattonella* in the Seto Inland Sea, including vegetative cells and cyst phase. (Imai and Itoh 1987)



# Summary for *Chattonella*

- 1. *Chattonella* spp. are diploid organisms (2n).
- 2. *Chattonella* has cyst stage for overwintering.
- 3. Cyst formation was induced by nutrient depletion such as nitrogen under low light conditions.
- 4. Cysts needs winter for maturation.
- 5. Cysts effectively germinate at 20°C or higher.
- 6. *in situ* germination of cysts show great seasonality.
- 7. Summer red tides are seeded by the germination of cysts in sea bottom.
- 8. Life cycle startegy of *Chattonella* is well adapted to temperate shallow coastal areas; changes between cysts and vegetative cells are easy.

# Life cycle strategy of *Heterosigma akashiwo*



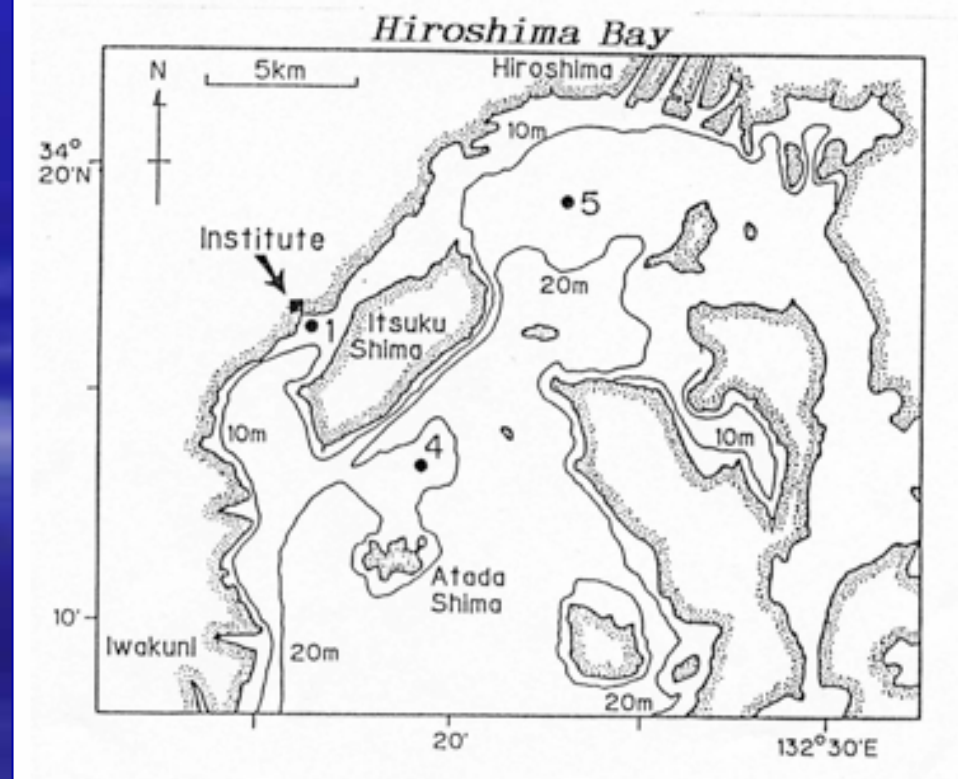
*Heterosigma akashiwo*  
(Hada) Hada

Photomicrographs  
by Yoshiaki Hara

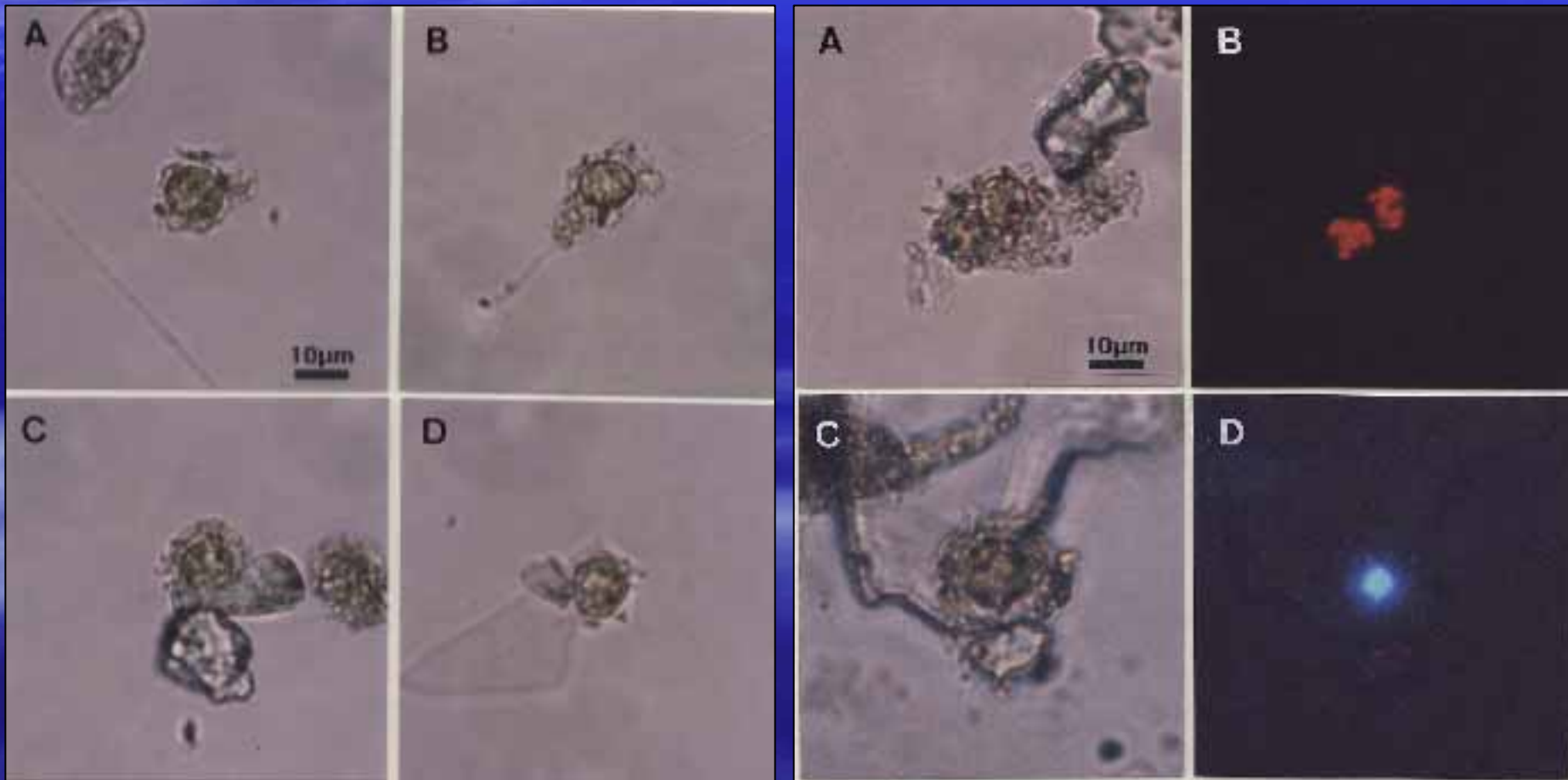
WESTPAC-HAB  
IOC Harmful Algal Bloom Programme

T0007

Sampling stations  
for *H. akashiwo*  
study in Hiroshima  
Bay, the Seto  
Inland Sea.



Cysts of *Heterosigma akashiwo* discovered from the sediments of the Seto Inland Sea (Imai et al. 1993)



MPN method for enumeration of *H. akashiwo* cysts having germination ability in sediments (Imai and Itakura 1991).

Feasible for the cysts without information on morphology

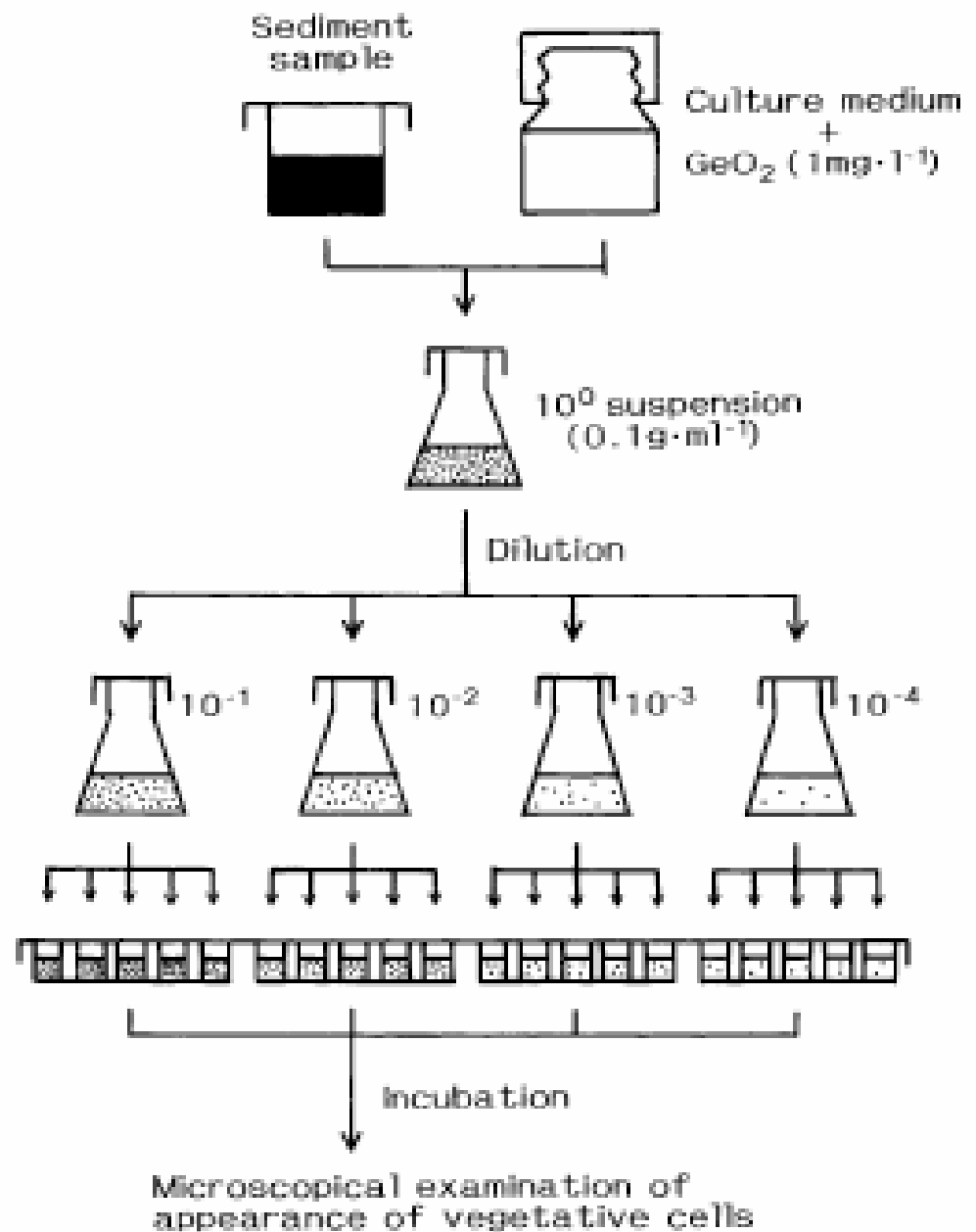


Fig. 2 *Heterosigma akashiwo*. Procedure of the extinction dilution method for enumeration of germinable cysts in sediment samples



Distribution of cysts of *Heterosigma akashiwo* in bottom sediments of Hiroshima Bay, the Seto Inland Sea (Imai and Itakura 1991).

Cysts were abundant in the coast.

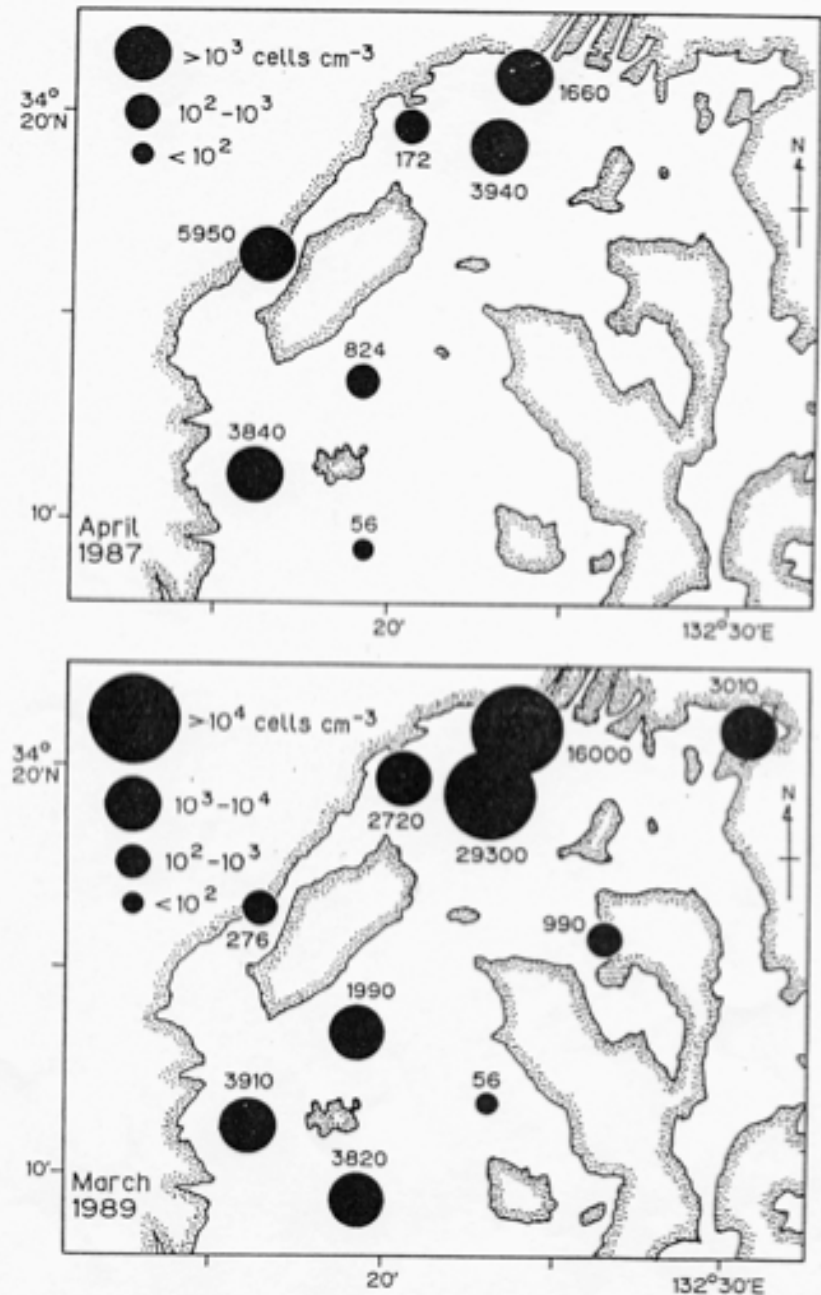
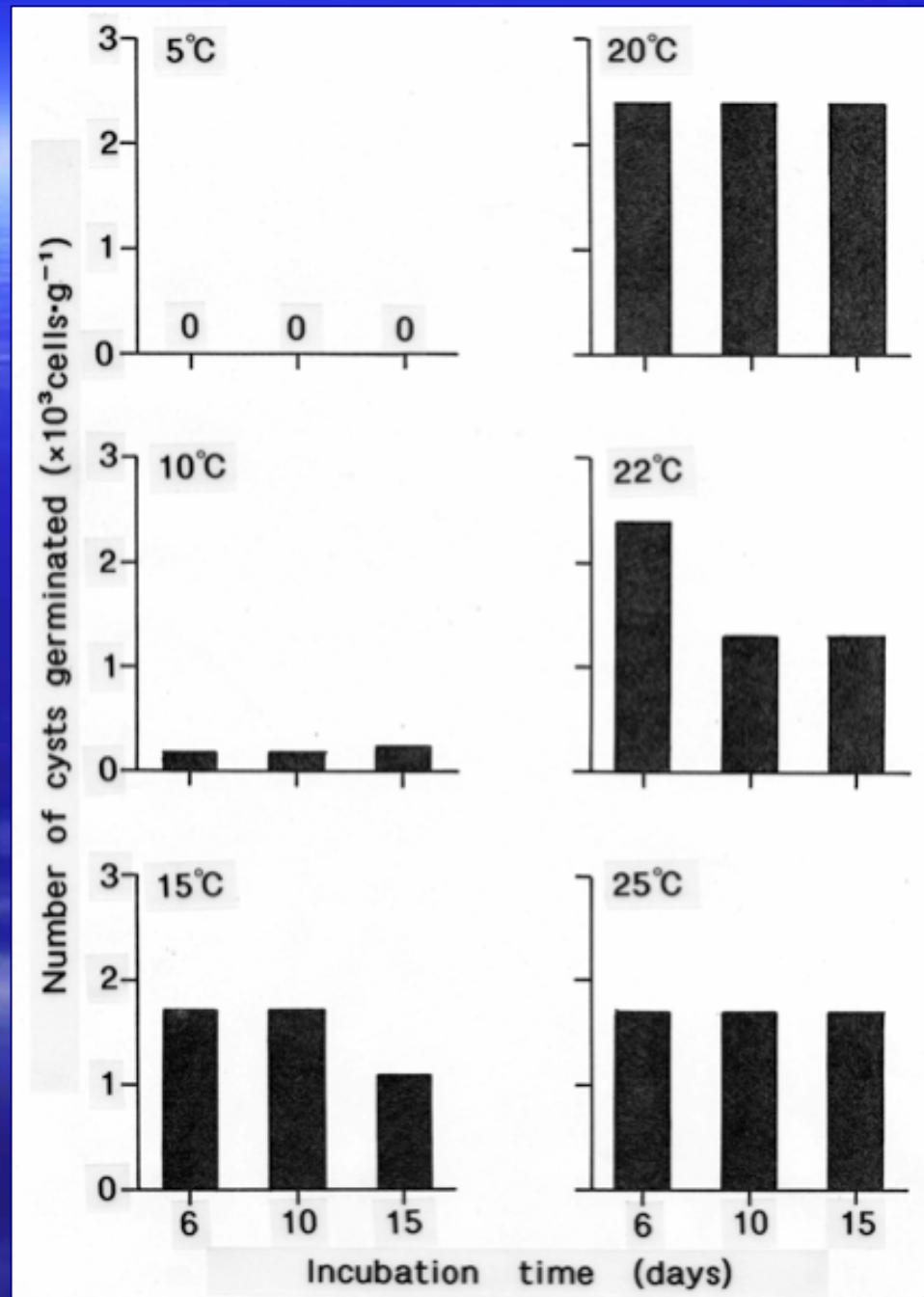


Fig. 3. Densities of dormant cells of *H. akashiwo* in bottom sediments of northern Hiroshima Bay, enumerated by the extinction dilution method. Numerals indicate the number of the dormant cells per cubic centimeter wet sediment.

Effects of temperature on the germination of *Heterosigma akashiwo* cysts in sediments determined by the MPN method (Imai and Itakura 1999).

Vigorous germination at 15°C or higher temperature



Seasonal fluctuations of the highest cell densities of *H. akashiwo* cells in water columns at 3 stations in Hiroshima Bay (Imai and Itakura 1999).

Blooms in May ~ June  
with great seasonality

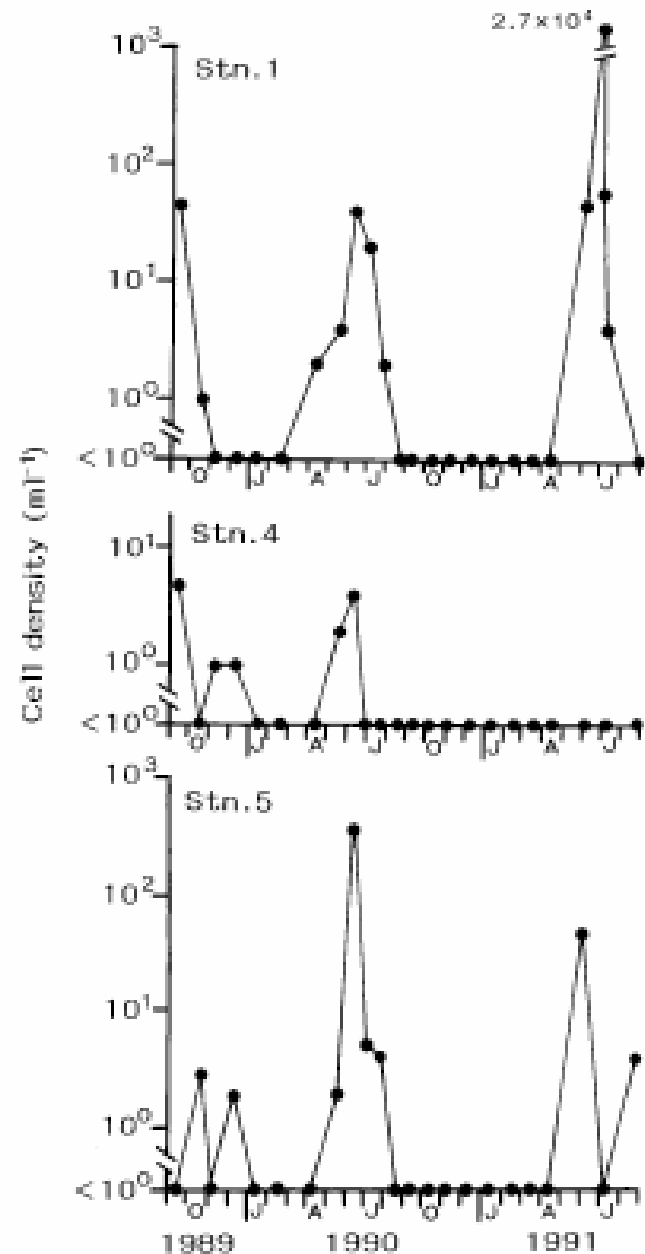


Fig. 3 *Heterosigma akashiwo*. Seasonal fluctuations in the highest densities of vegetative cells in the water columns at three stations in northern Hiroshima Bay during the research period

Seasonal fluctuations of germinable cysts (MPN) in the surface sediments (0 - 1cm) of Hiroshima Bay (Imai and Itakura 1999).

Many cysts are always physiologically germinable.

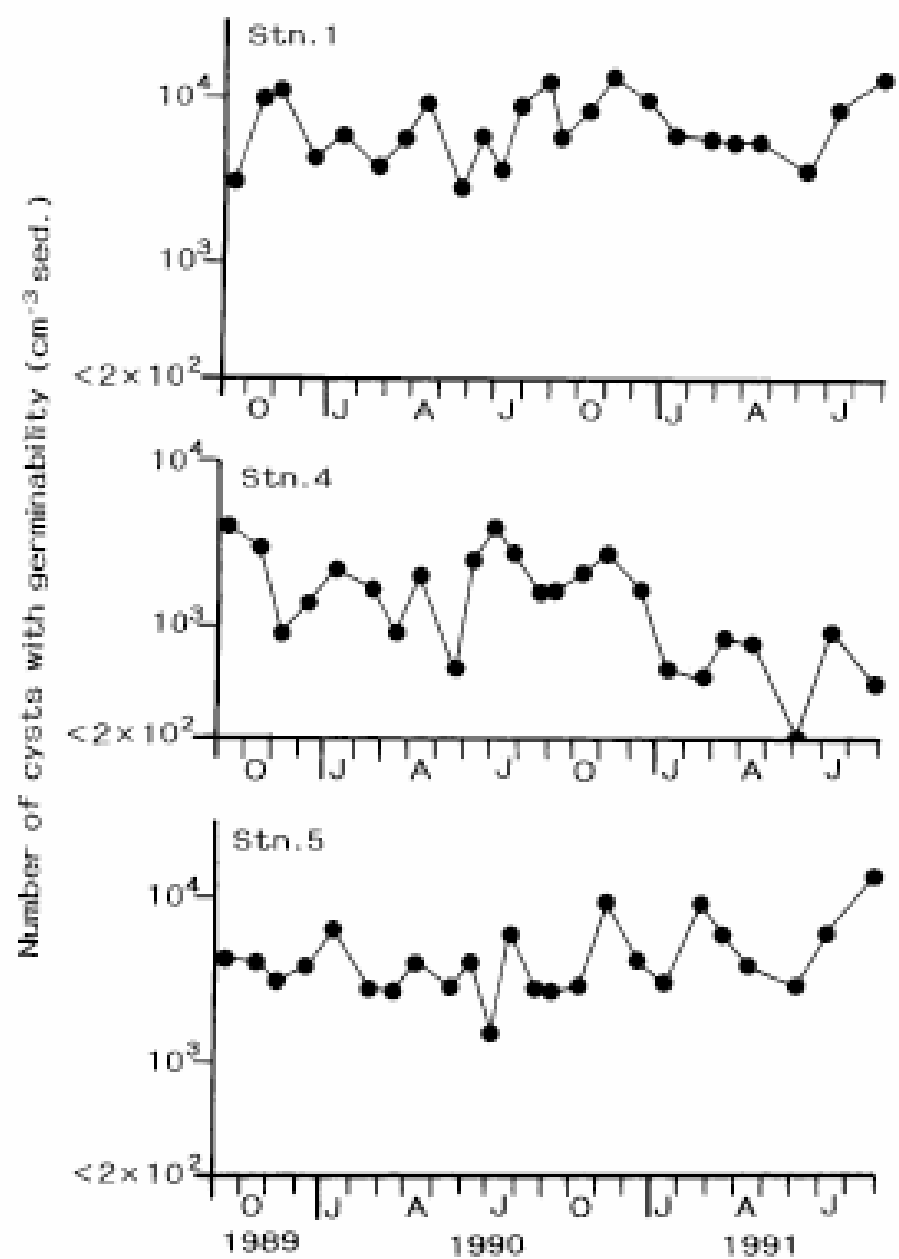
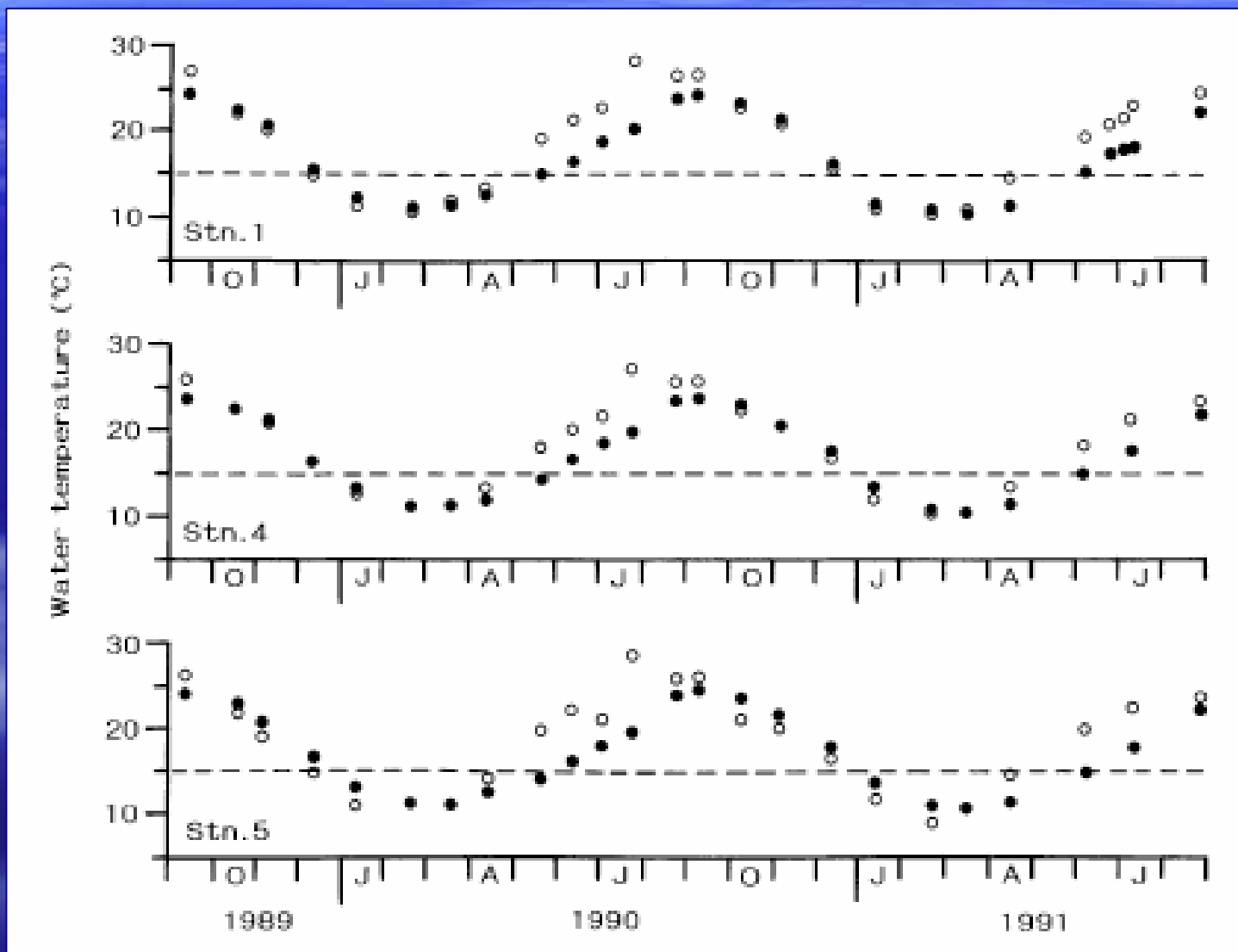


Fig. 5 *Heterosigma akashiwo*. Seasonal fluctuations in the number of germinable *H. akashiwo* cysts in surface sediments (top 1-cm layer) collected at three stations in northern Hiroshima Bay. Enumeration by extinction dilution method

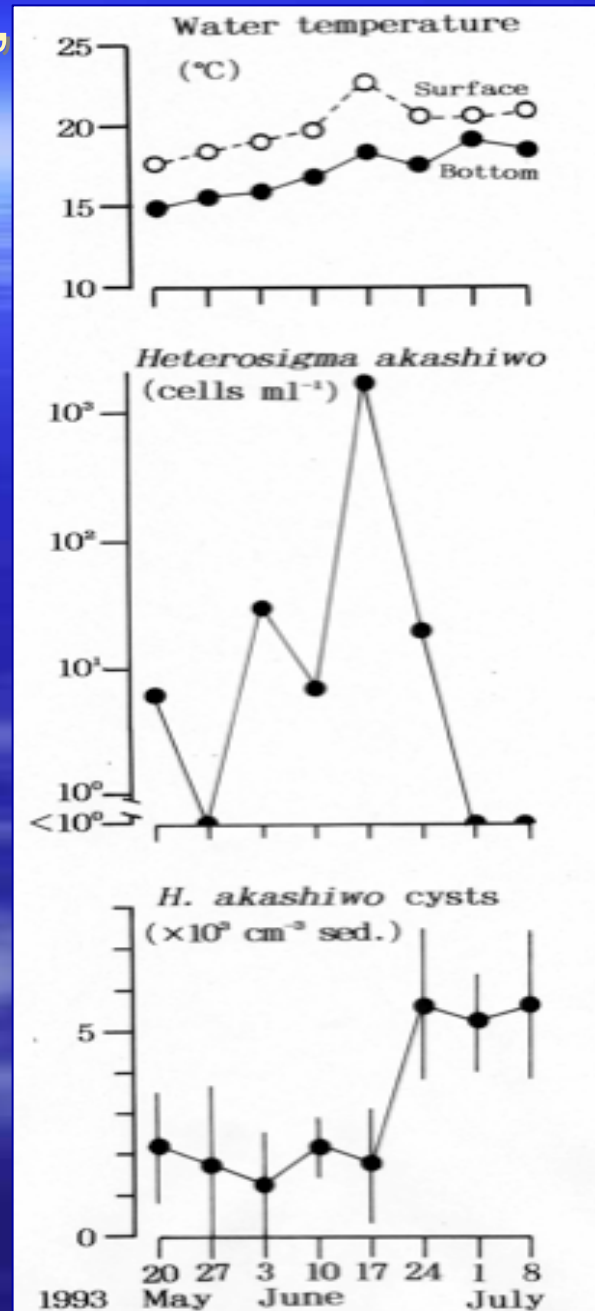
# Seasonal fluctuations of surface (open circle) and bottom (closed circle) water temperatures at 3 stations in Hiroshima Bay.



Weekly changes in water temperature, *H. akashiwo* in surface water, and the total *H. akashiwo* live cysts in surface sediments (0-1cm) at Stn.1 in Hiroshima Bay (Imai and Itakura 1998). Total live cysts increased just after the peak of bloom and needed 1 week for maturation.

Table 1. *Heterosigma akashiwo*. Numbers of the total cysts enumerated by the direct count method and the germinable cysts enumerated by the extinction dilution method in surface (top 1-cm depth) sediments collected at St.1 on 24 June and 1 July, 1993.

Date	June 24	July 1
Total cysts	5676	5240
Germinable cysts (cm <sup>-3</sup> wet sediment)	419	4223



*Heterosigma* red tides usually occur at 20°C or higher with wide range of salinity (Honjo 1993).

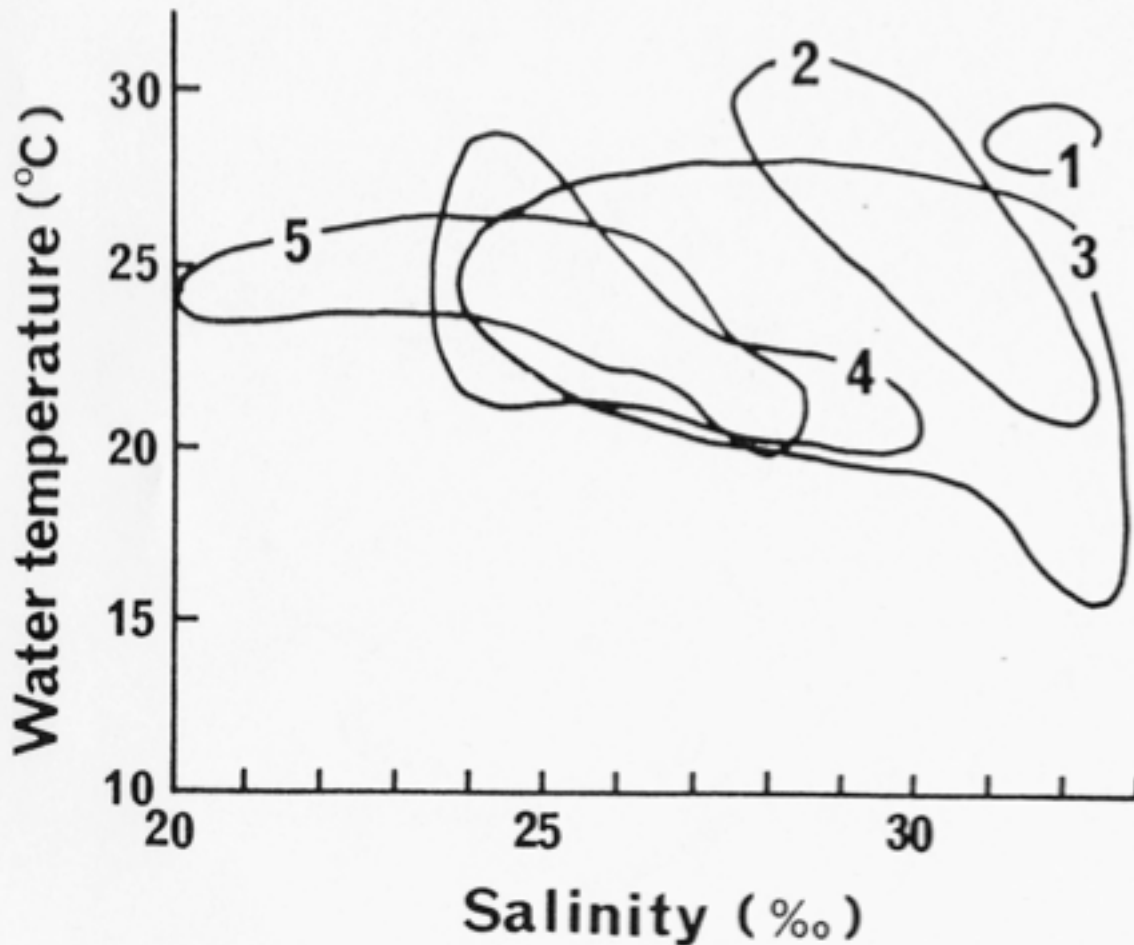


FIG. 2: The ranges of *in situ* water temperature and salinity during *H. akashiwo* red tides: 1. Nagasaki Bay, 2. Hakata Bay, 3. Seto Inland Sea, 4. Mikawa Bay, 5. Narragansett Bay.

Honjo (1993)

# Process and key factors for the occurrence of *Heterosigma akashiwo* red tide (Smayda 1998).

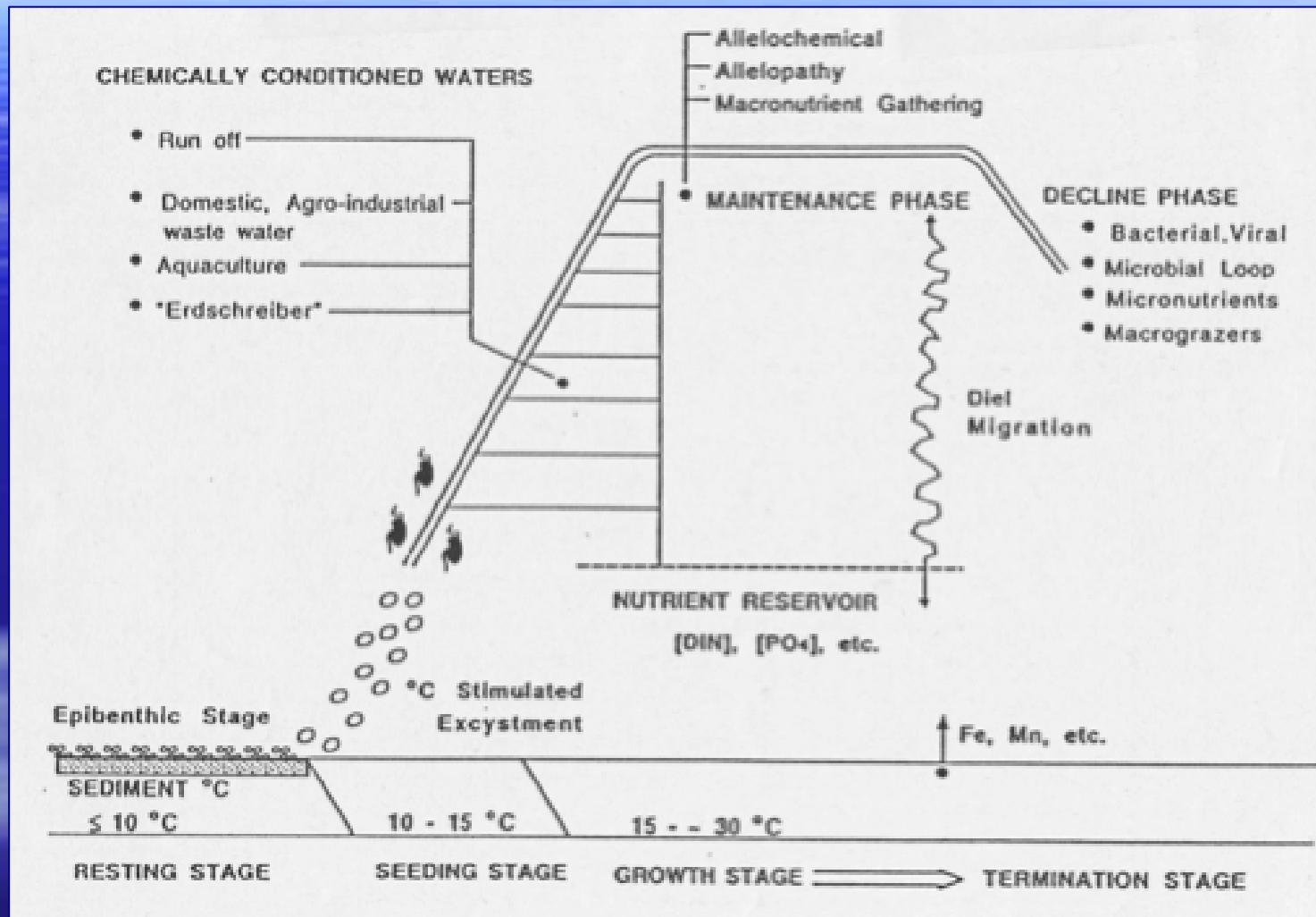


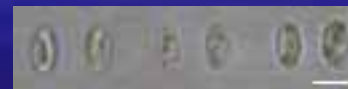
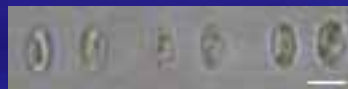
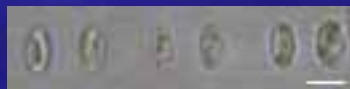
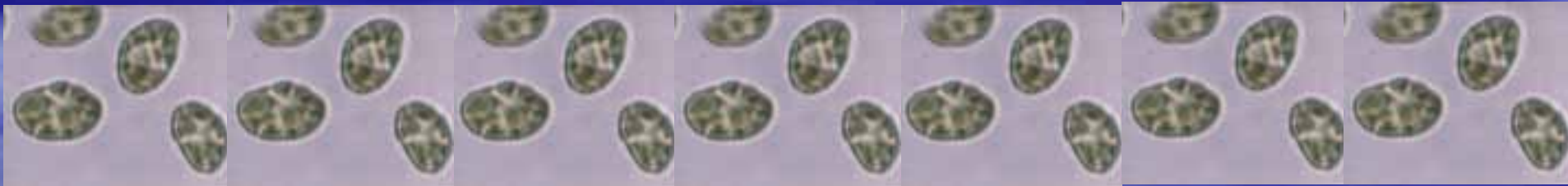
Figure 2. General model of key processes influencing bloom outbreaks of *Heterosigma akashiwo*; see text for details.



# Summary for *Heterosigma akashiwo*

- 1. *Heterosigma akashiwo* has cyst stage for outliving such as overwintering.
- 2. Cyst formation was induced at the end of blooms and completed in the dark (Itakura et al. 1996).
- 3. New cysts need ca. 1 week for maturation.
- 4. Cysts effectively germinate at 15°C or higher.
- 5. *in situ* germination of cysts can always occur.
- 6. Red tides show great seasonality, and hence seeded by the germination of cysts in sea bottom.
- 7. Life cycle strategy of *H. akashiwo* is also well adapted to temperate shallow coastal areas; changes between cysts and vegetative cells are easy.

How do harmful  
raphidophyte blooms occur  
predominantly over diatoms  
in coastal sea?



# Change of blooms from diatoms to *H. akashiwo* and to diatoms.

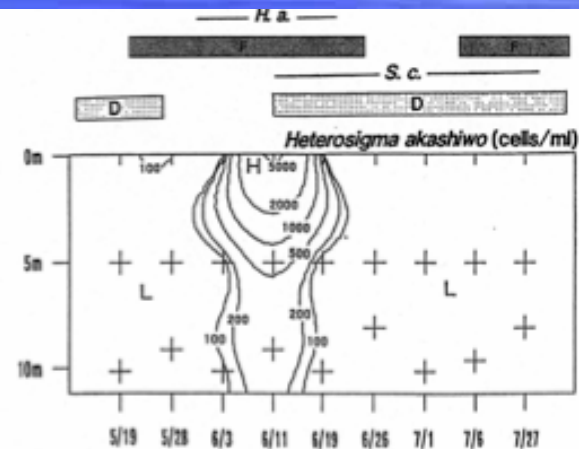
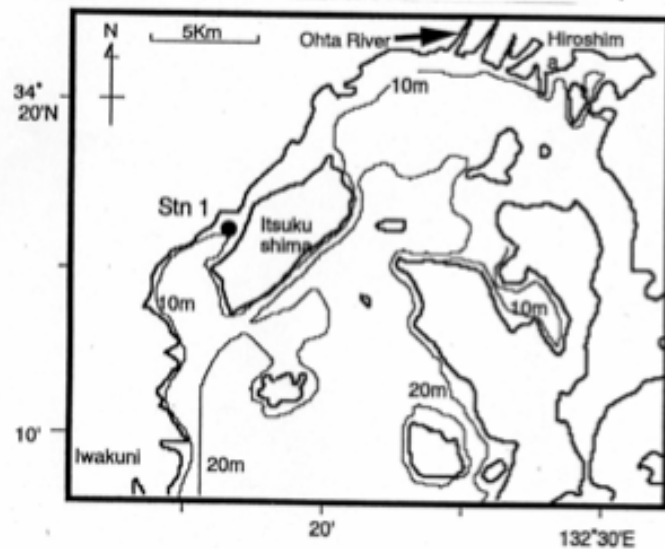


Fig. 1-19. Temporal change of the numbers of *Heterosigma akashiwo* in the water column at Station 1 in 1992.

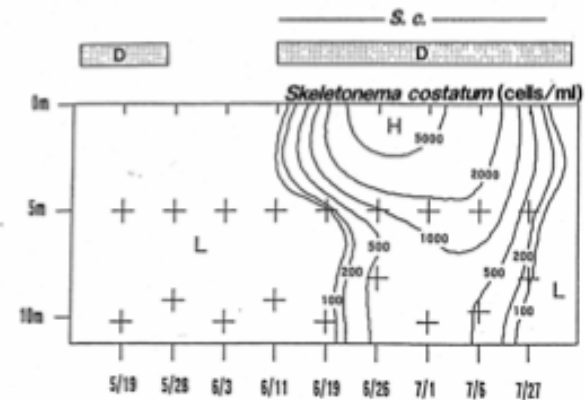


Fig. 1-17. Temporal change of the numbers of *S. costatum* in the water column at Station 1 in 1992.

# Growth parameters of diatoms and raphidophytes

Species	Nutrients	$\mu_{\max}$ (division/day)	Ks ( $\mu\text{M}$ )
<b>Diatoms</b>			
<i>Chaetoceros didymum</i>	NO <sub>3</sub> -N	1.82	1.29
	PO <sub>4</sub> -P	2.16	0.48
<i>Ditylum brightwellii</i>	NO <sub>3</sub> -N	2.02	0.73
	PO <sub>4</sub> -P	2.00	0.16
<i>Thalassiosira rotula</i>	NO <sub>3</sub> -N	1.69	1.28
	PO <sub>4</sub> -P	1.70	0.23
<b>Raphidophytes</b>			
<i>Chattonella antiqua</i>	NO <sub>3</sub> -N	0.74	0.87
	PO <sub>4</sub> -P	0.74	0.29
<i>Heterosigma akashiwo</i>	NO <sub>3</sub> -N	1.7 – 1.9	1.99 - 2.45
	PO <sub>4</sub> -P	1.7 – 1.9	1.00 – 1.98

# Raphidophytes have cysts. Diatoms have resting stage cells.

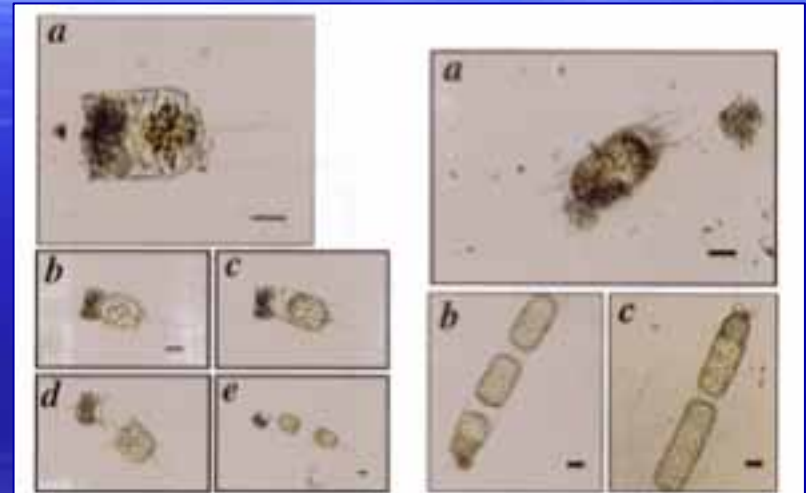
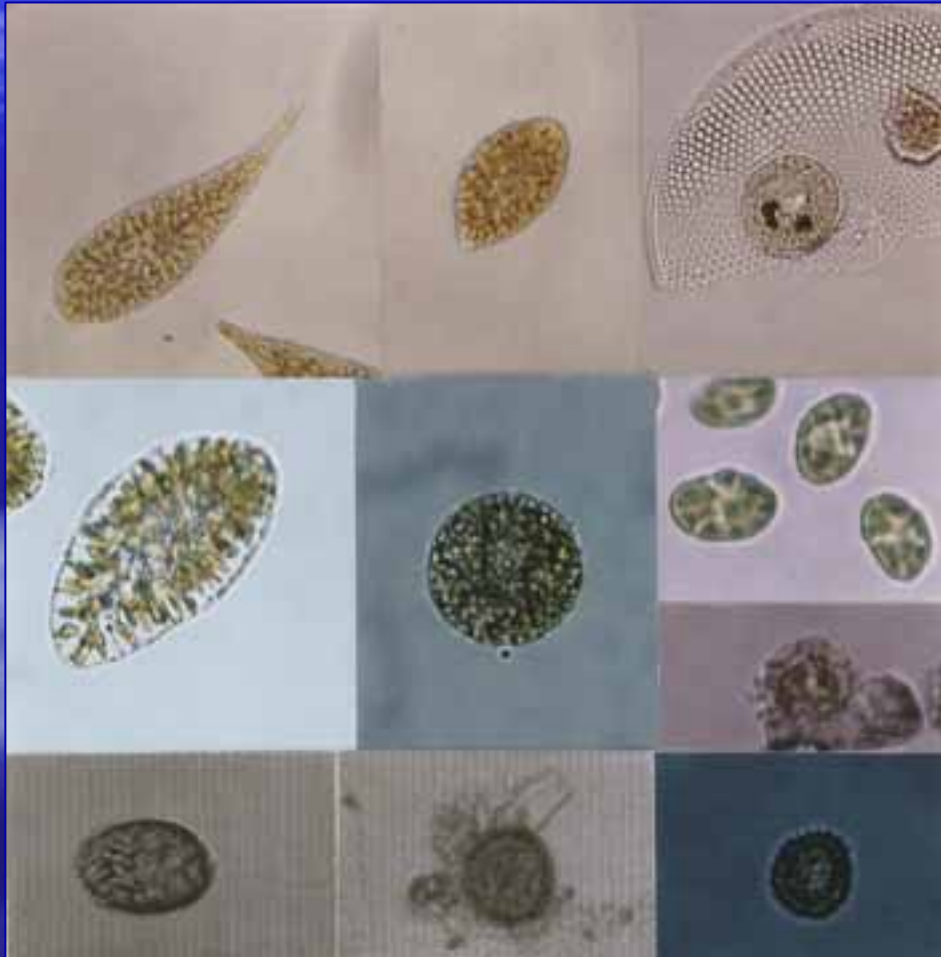


Plate 14. Resting spore (a) and germinating cells (b, c, d, e) of *Dielum frigidum* (Wien) Grunow. Bar = 20  $\mu$ m.

Plate 15. Resting spore (a) and germinating cells (b, c) of *Stephanodiscus* sp. Bar = 20  $\mu$ m.

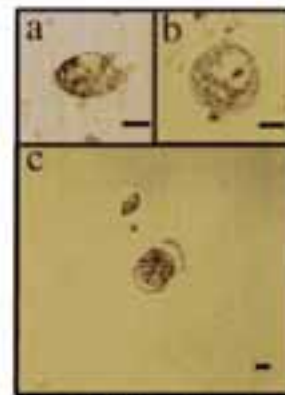


Plate 16. Resting spore (a) and germinating cells (b, c) of *Actinocyclus laticornis* Lohman. Bar = 10  $\mu$ m.

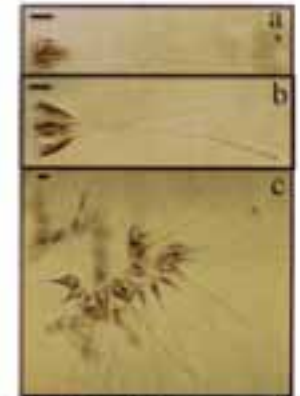


Plate 17. Resting cells (a) and germinating cell (b, c) of *Actinocyclus gracilis* (Carmichael) Round. Bar = 10  $\mu$ m.

Seeds of diatoms and raphidophytes are abundant in sediments of coastal sea.

**Table 1-3.** Abundance of resting stage cells of flagellates (upper column) and diatoms (lower column) in bottom sediments of Hiroshima Bay<sup>a</sup>

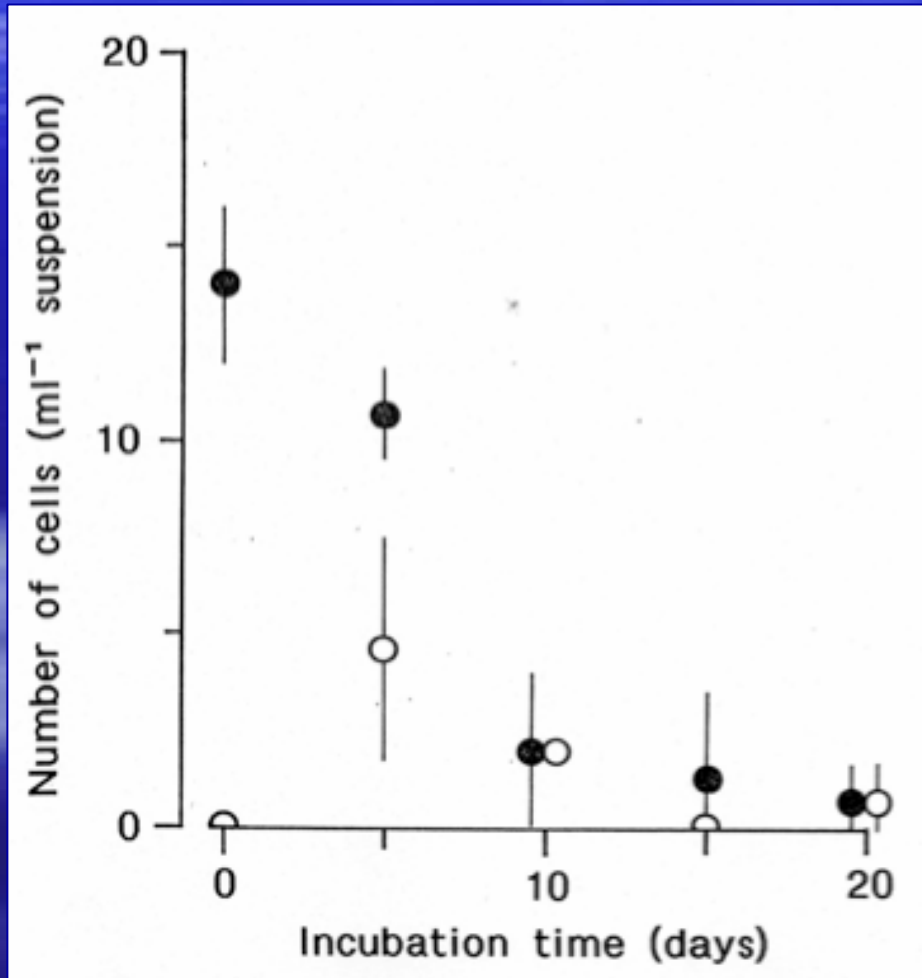
Species	Abundance (cm <sup>-3</sup> · wet sediment)
<b>Raphidophyceae</b>	
<i>Heterosigma akashiwo</i> <sup>*1</sup>	5.6 × 10 <sup>1</sup> ~ 2.9 × 10 <sup>4</sup>
<i>Chattonella</i> spp. <sup>*2</sup>	0 ~ 7.7 × 10 <sup>2</sup>
<b>Dinophyceae</b>	
<i>Alexandrium</i> spp. <sup>*3</sup>	5.0 × 10 <sup>1</sup> ~ 1.3 × 10 <sup>3</sup>
<b>Bacillariophyceae</b>	
<i>S. costatum</i>	8.0 × 10 <sup>3</sup> ~ 2.1 × 10 <sup>6</sup>
<i>Chaetoceros</i> spp.	2.7 × 10 <sup>3</sup> ~ 6.6 × 10 <sup>5</sup>
<i>Thalassiosira</i> spp.	3.7 × 10 <sup>3</sup> ~ 1.5 × 10 <sup>5</sup>

Note: Data of flagellates cysts were compiled from Imai and Itakura (1991),<sup>\*1</sup> Imai et al. (1993),<sup>\*2</sup> Yamaguchi et al. (1995)<sup>\*3</sup>

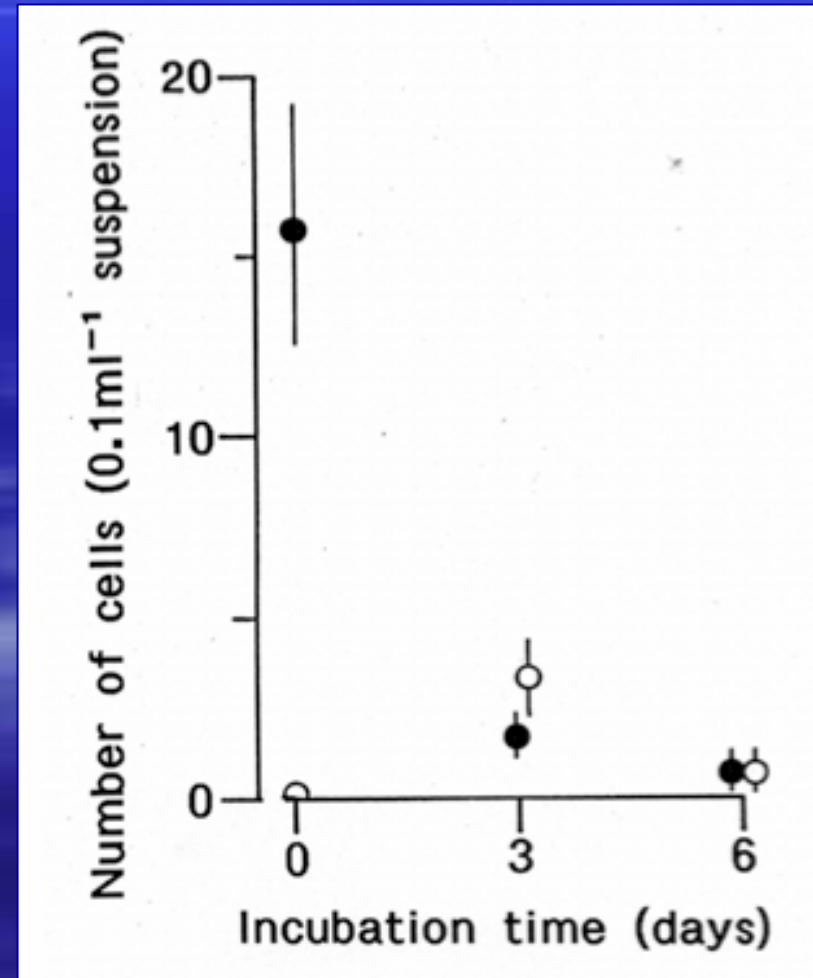
Abundance of diatom resting stage cells (cm<sup>-3</sup> · wet sediment) were calculated from the present data

# Germination of raphidophyte cysts in the dark

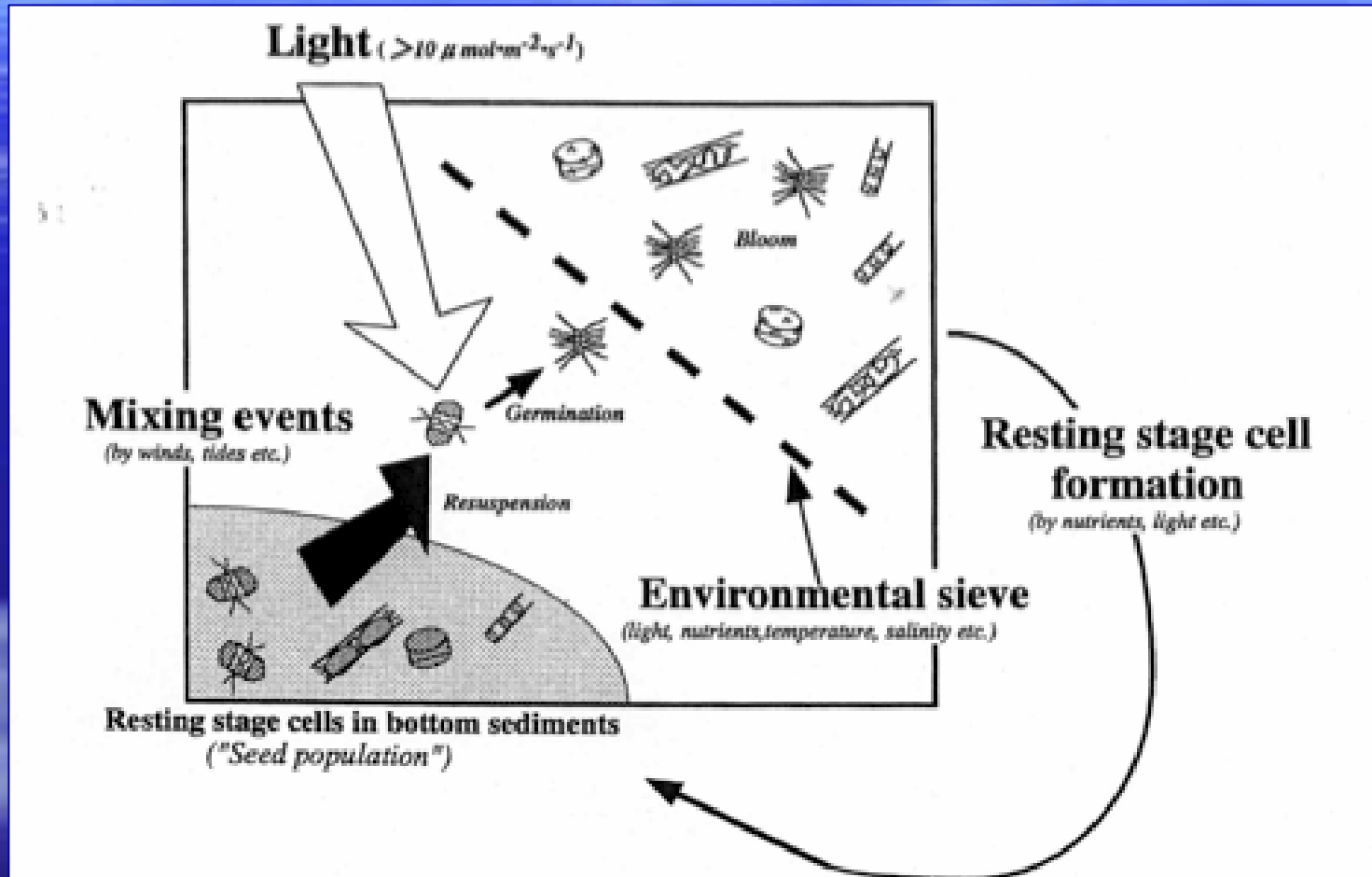
*Chattonella* spp.



*Heterosigma kashiwo*



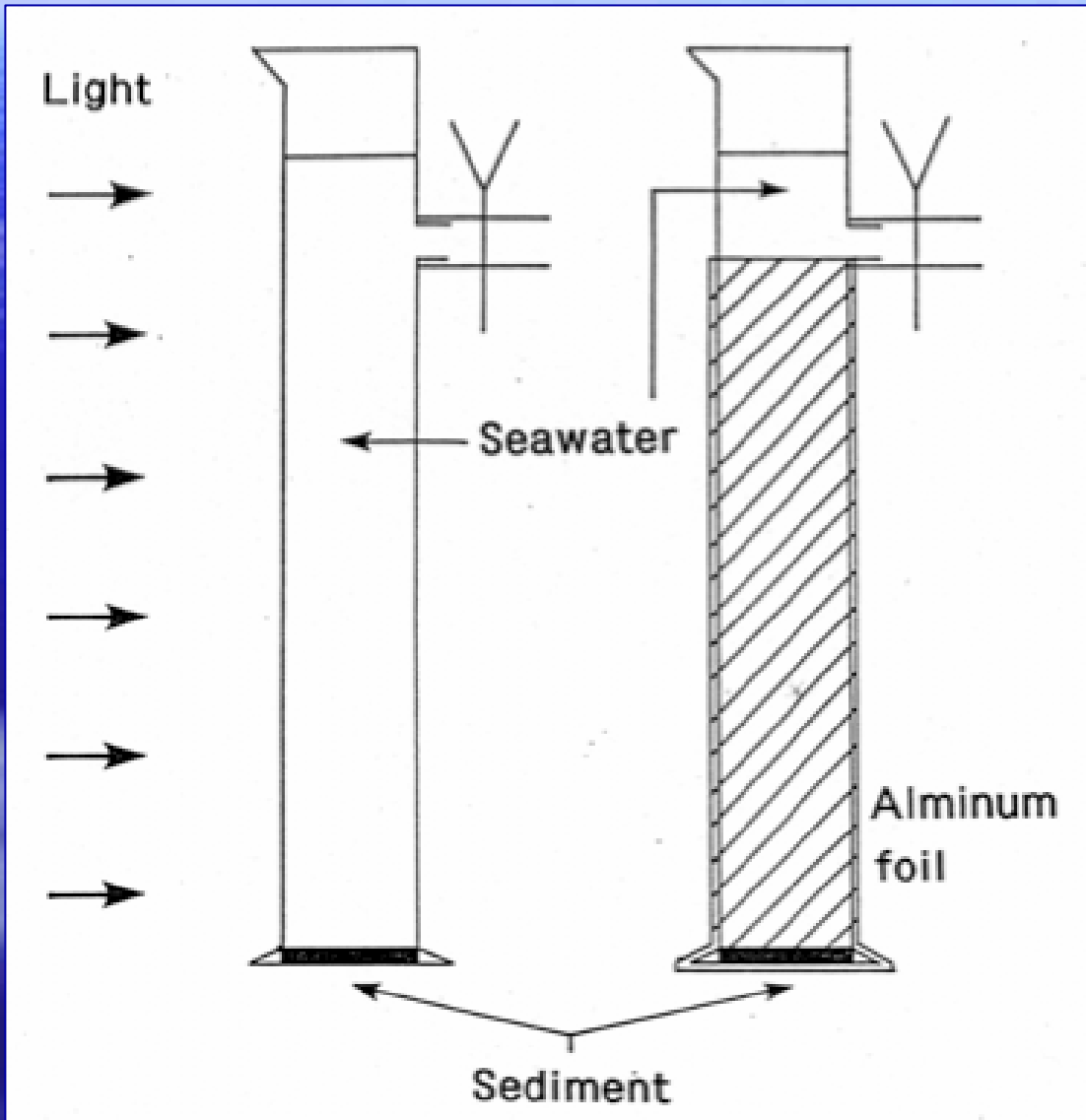
# Diatom resting stage cells needs light for germination and rejuvenation



**Fig. 4-1.** A schematic representation of the role of diatom resting stage cell in the coastal environment.



# Germination experiment



*H. akashiwo* cysts  
330 / g sediment

Diatom resting stage  
cells  
 $1.6 \times 10^5$  / g sed.

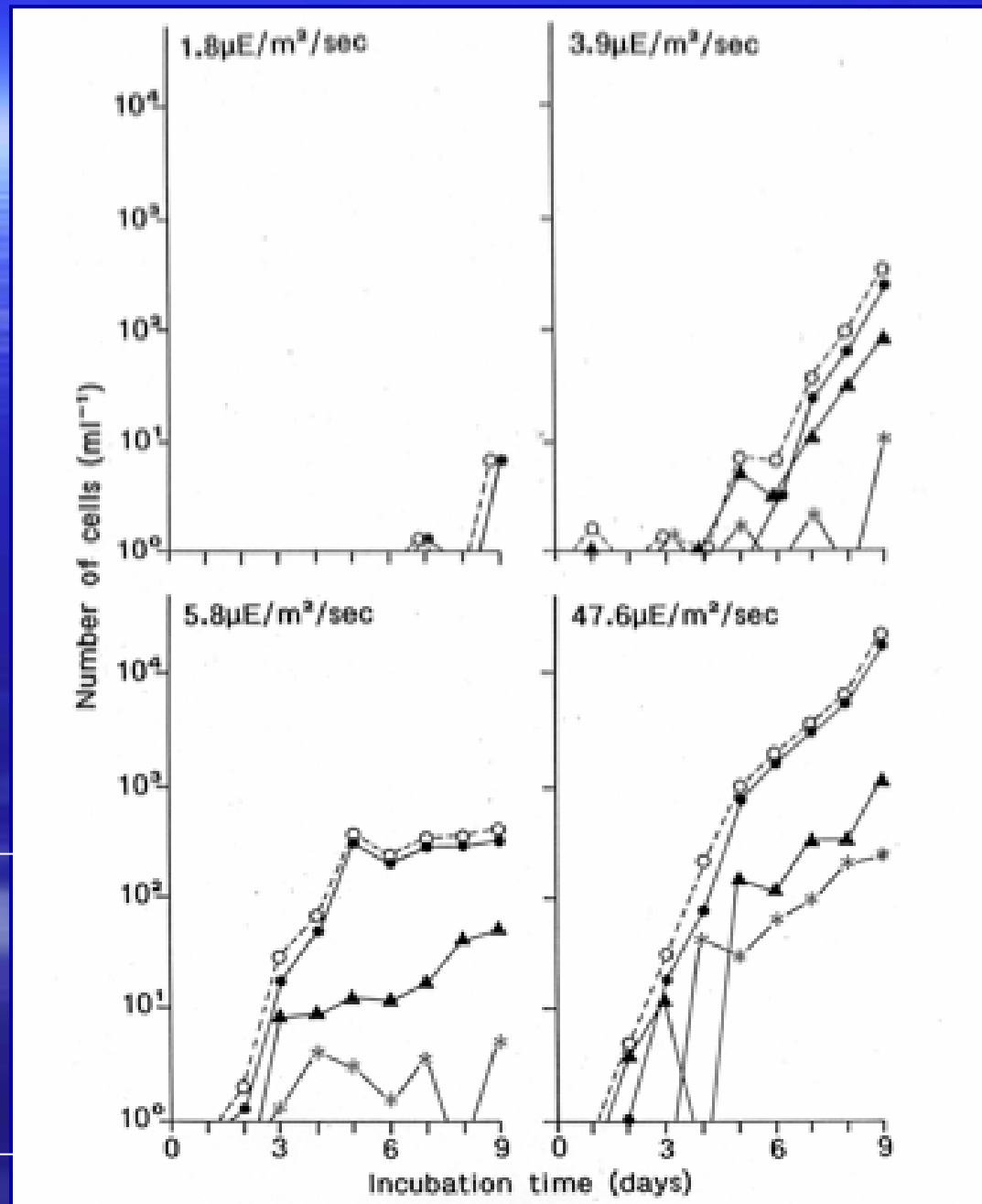
Light:  $110 \mu\text{E} / \text{m}^2 / \text{sec}$   
14hL : 10hD

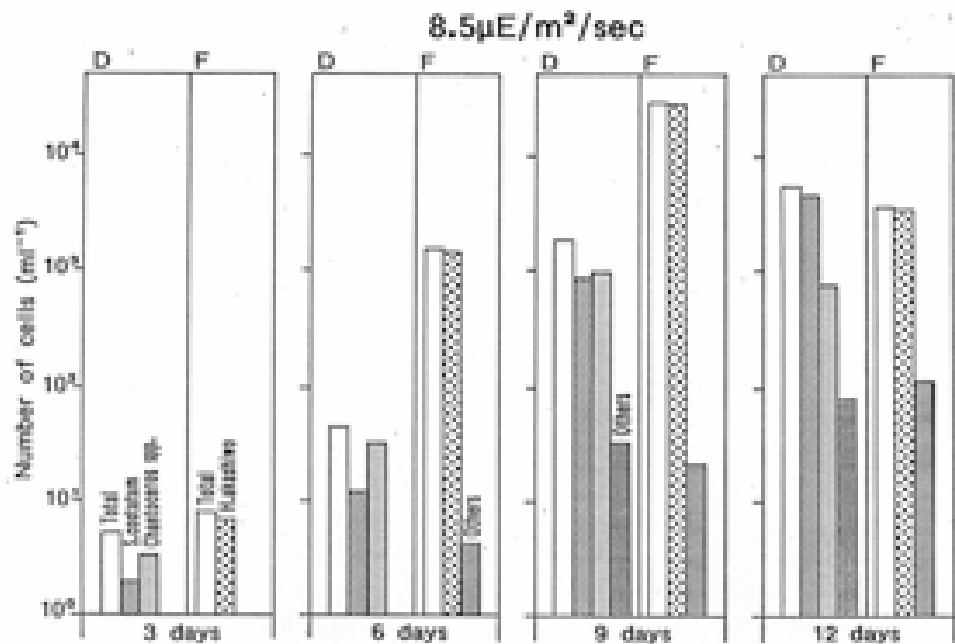
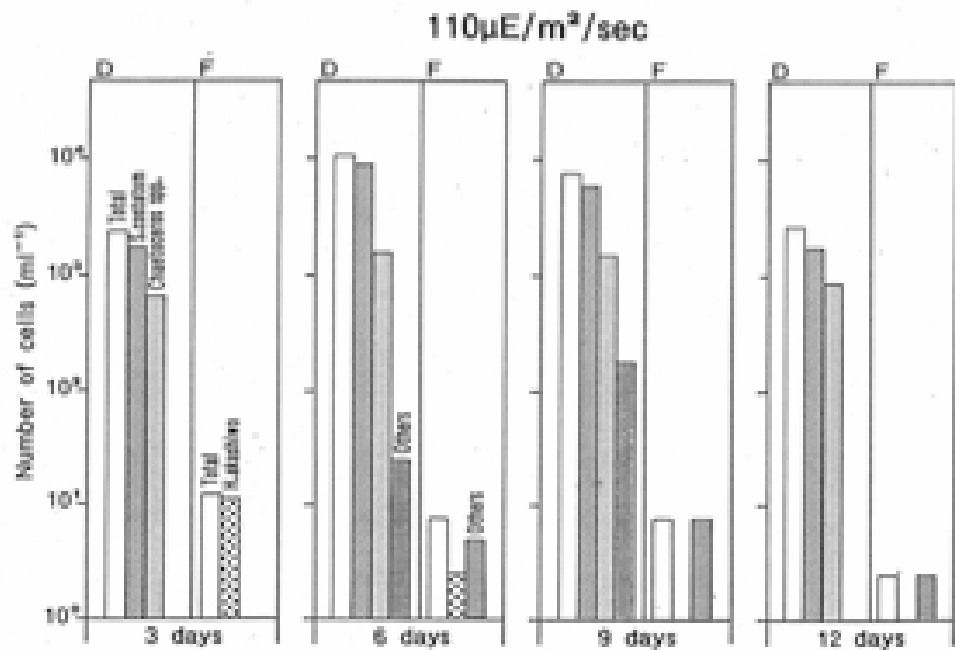
Temperature:  $20^\circ\text{C}$

Effects of light intensity on germination and/or rejuvenation of diatom resting stage cells.

Diatom resting stage cells need light for germination.

Open circle: total diatoms  
Closed circle: *S. costatum*  
Black triangle: *Chaetoceros*  
Star: *Thalassiosira*





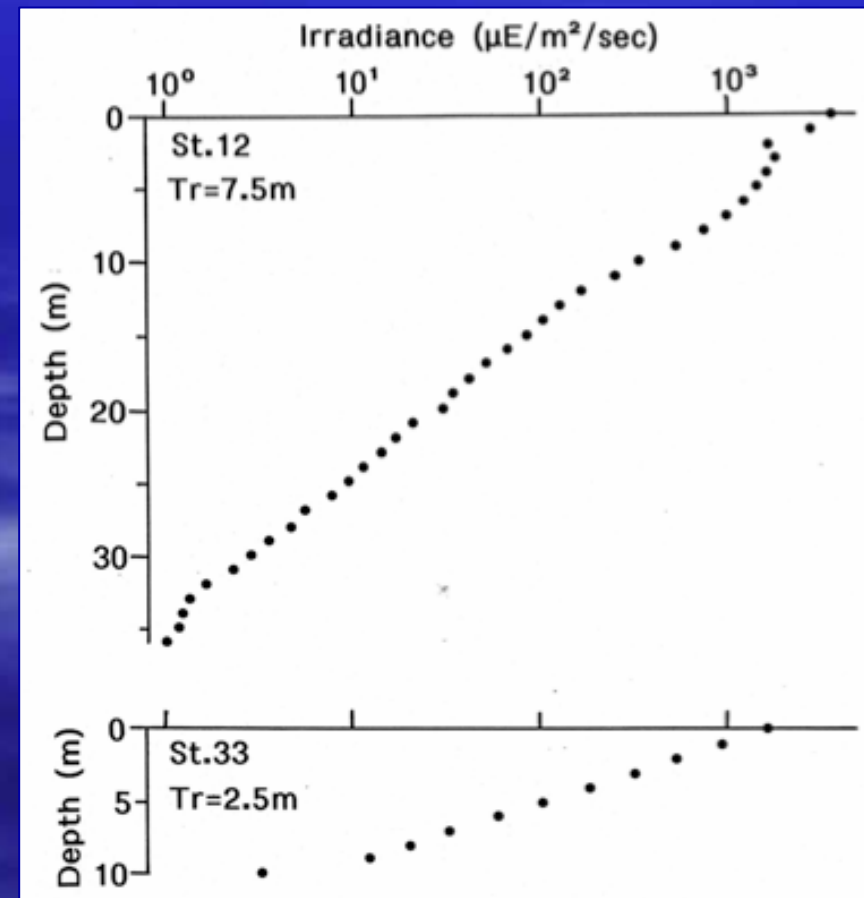
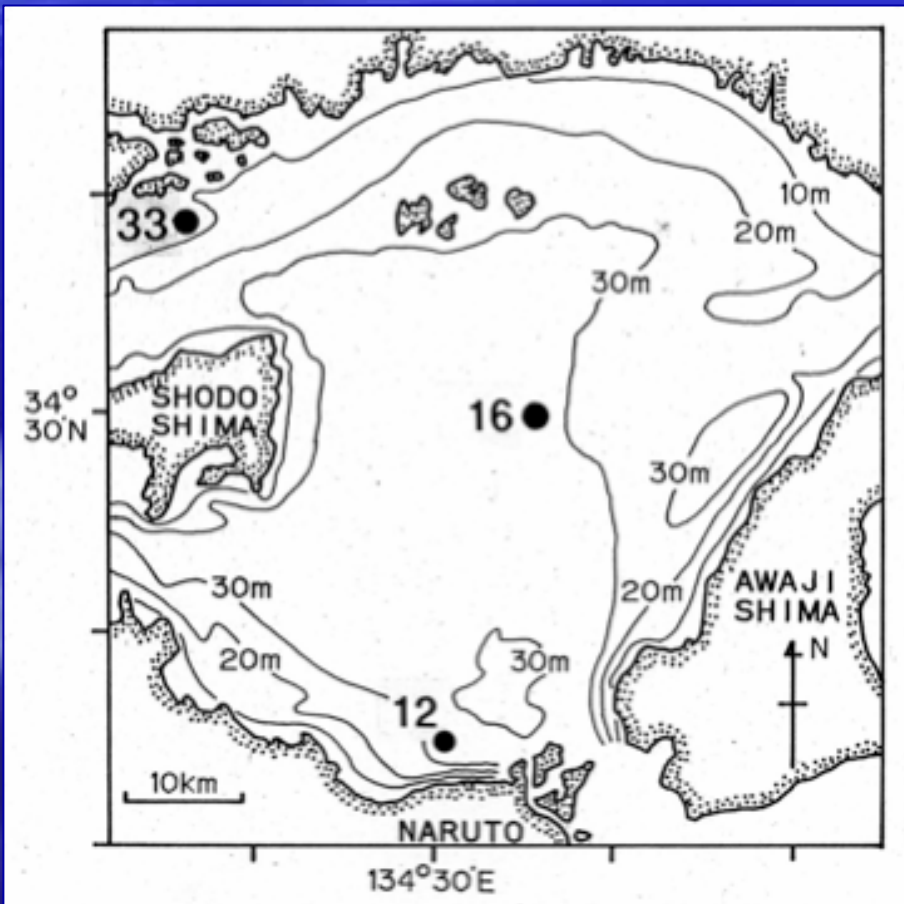
Comparison of the effects of light intensity to cysts and diatom resting stage cells on dominance of *H. akashiwo*.

In surface water:

- 1) Diatoms dominated in higher light.
- 2) *H. akashiwo* dominated in low light

# Vertical profiles of light intensity in coastal sea

Harima-Nada,  
Seto Inland Sea



*Chaetoceros* spp. form  
spores in low nitrogen  
concentration

*Chaetoceros curvusetus*, *C.*  
*distans*, *C. lauderi*

Harima-Nada:  
July 14 - 18, 1991

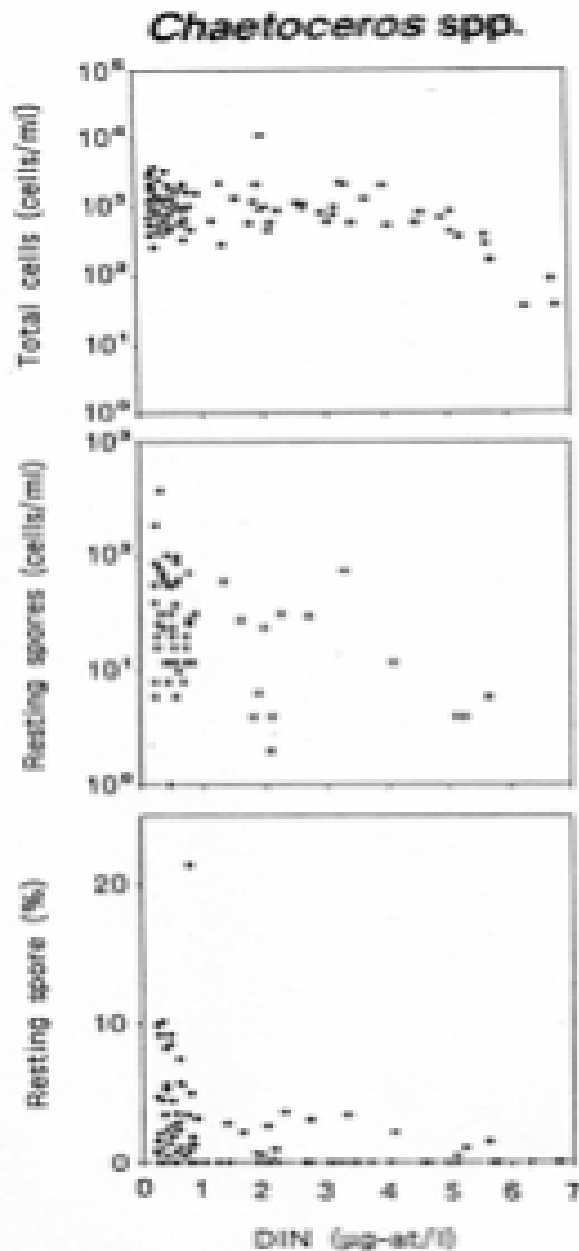
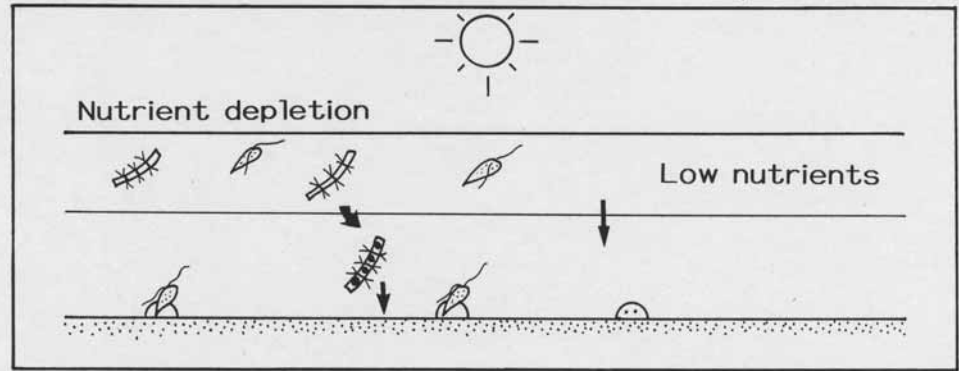


Fig. 3-14. Relationship between ambient DIN concentrations versus total cells (upper), resting spores (middle), and resting spore percentages (bottom) of *Chaetoceros* spp.

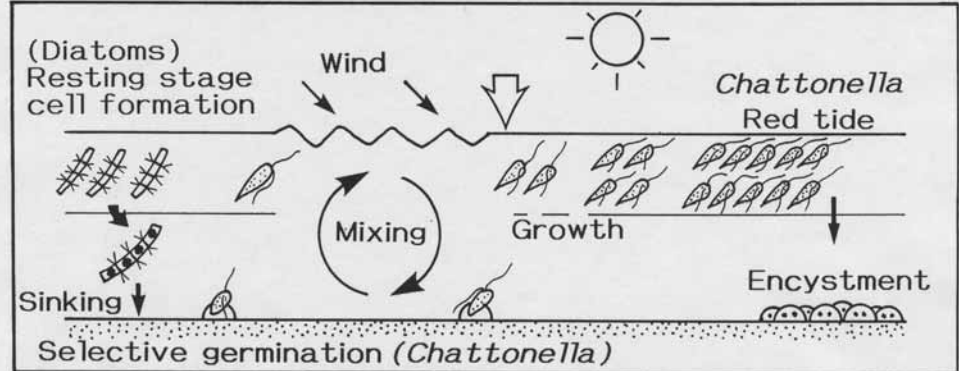
# Diatom resting hypothesis

Nutrient depletion after stratification induce resting stages of diatoms.

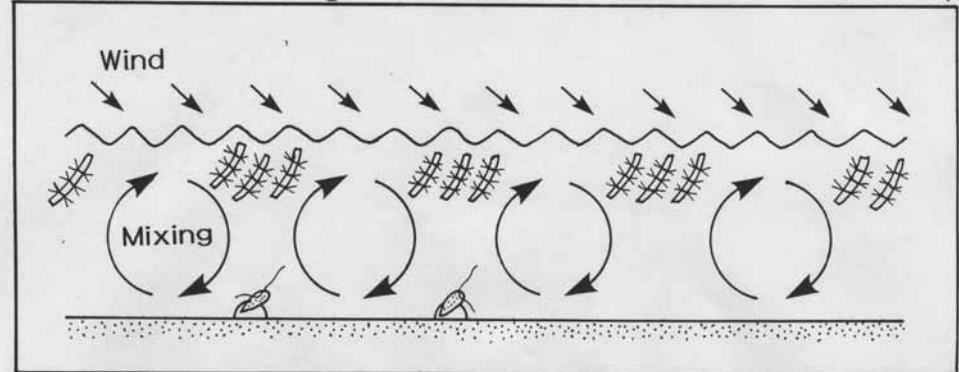
Continuous stratification (No bloom)



Stratification → Mixing → Stratification (Bloom)



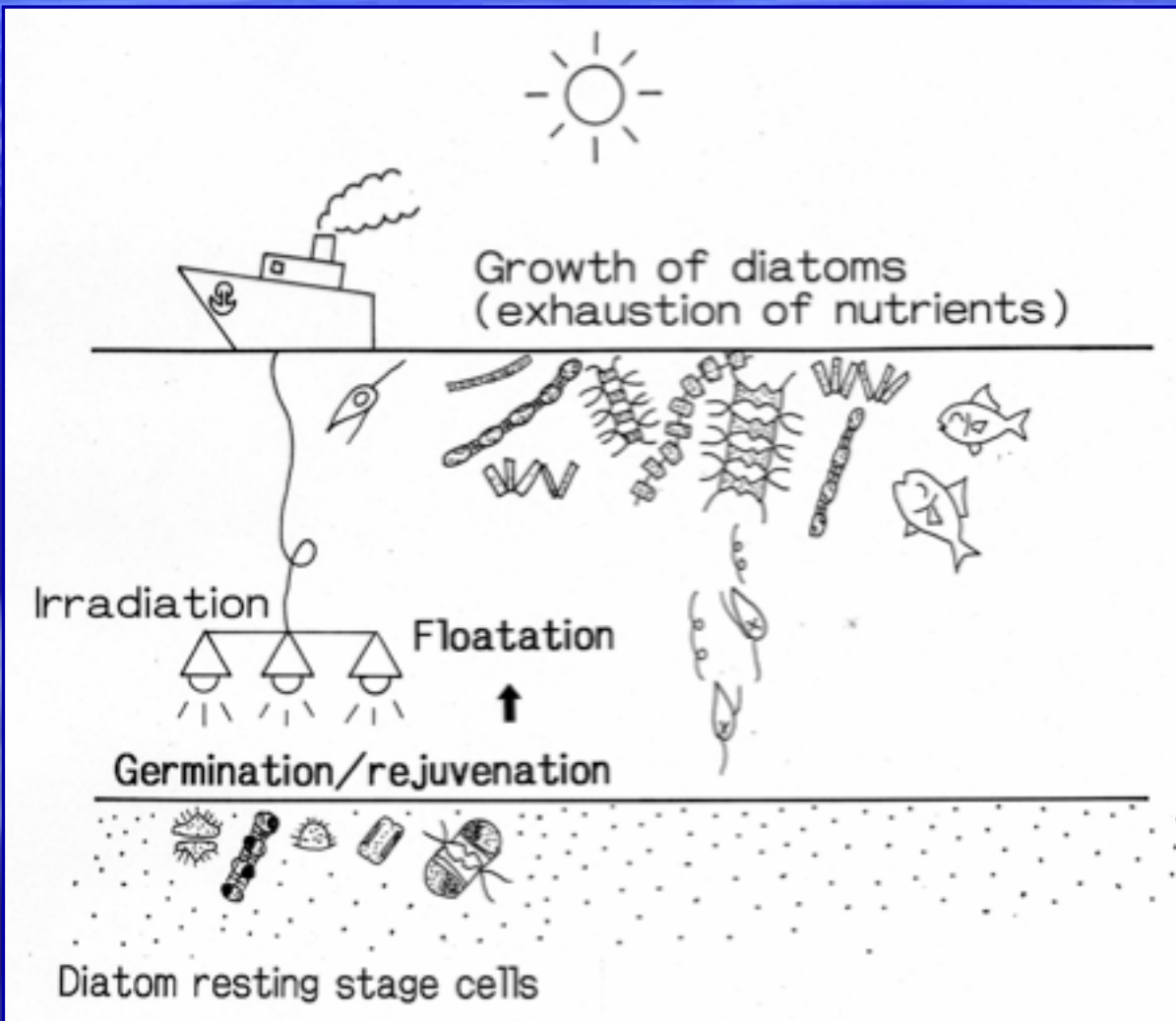
Frequent mixing (Diatom blooms)



# Summary

- 1. Diatoms are stronger than raphidophytes in vegetative growth.
- 2. Raphidophytes have cyst stage and diatoms have resting stage in their life cycles.
- 3. Raphidophyte cysts can germinate in the dark and diatom resting stage cells need light for germination.
- 4. Nutrient depletion induce resting stage in diatoms.
- 5. Low light irradiation to sediments induced predominancy of raphidopyhtes in water columns.
- 6. The diatom resting hypothesis is presented for the mechanism of occurrences of raphidophyte red tides.

# A proposal of idea for prevention of harmful blooms by bottom irradiation



No toxic effects on environments

Problems for feasibility

1. Timing, period, and intensity of irradiation
2. Scale of irradiation