A stylized world map in shades of blue, serving as a background for the text.

Recent progress on
Cochlodinium polykrikoides
(Dinophyceae)

Kazumi Matsuoka
Institute for East China Sea Research
(ECSER)
Nagasaki University

Red-tides of *Cochlodinium polykrikoides*

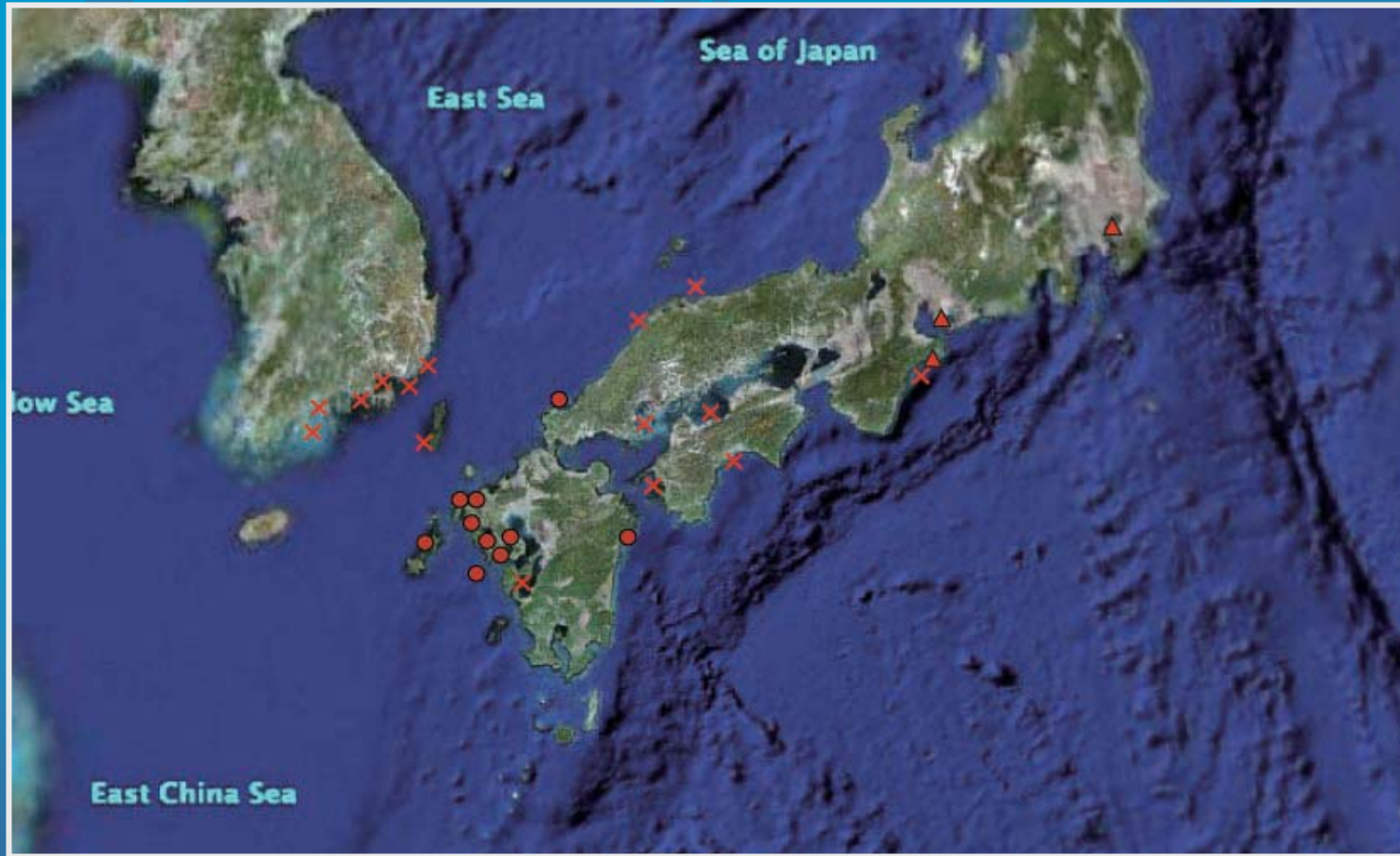


Busan, Korea in Aug. 2002



Guatemala in 1995

Location of *Cochlodinium polykrikoides* (●, X) and *C. catenatum* (▲) around Japan and Korea



Fish mass mortality by *C. polykrikoides* in Asia



Mass mortality of flat-fish by a red tide of *Cochlodinium polykrikoides*

Flat-fish culture farms



Photo
by Mr. San Geun Lee
of National Fisheries
Research & Development
Agency of Korea

WESTPAC-HAB
R0005

Korea

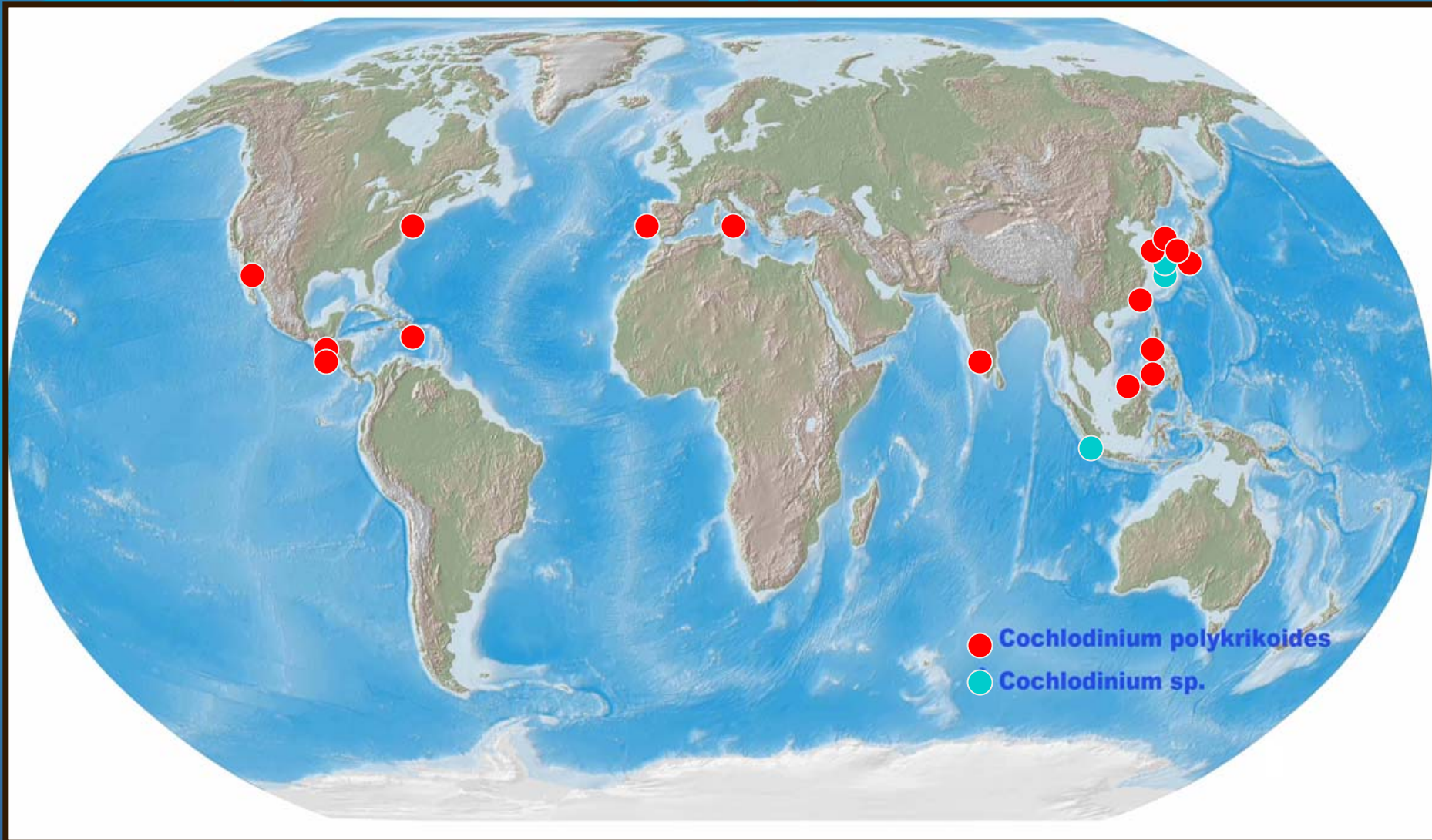


Japan



Saba

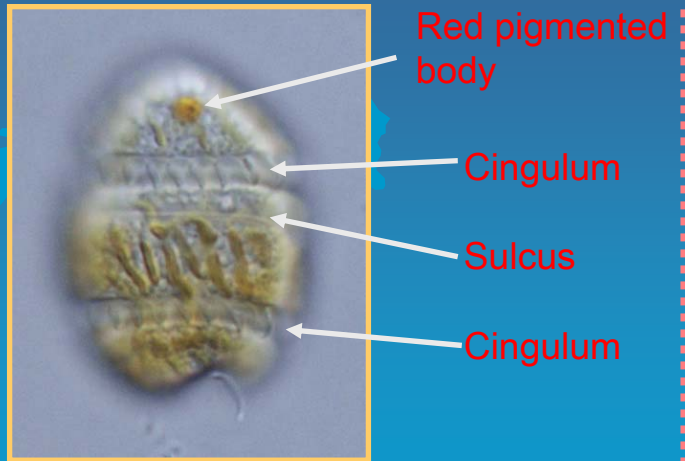
Geographical distribution of *Cochlodinium polykrikoides* and *Cochlodinium* sp.



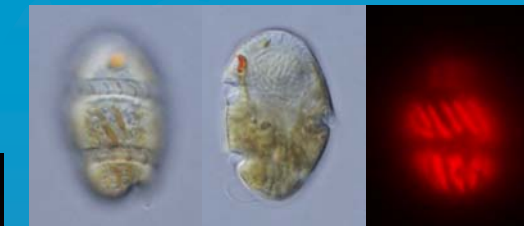
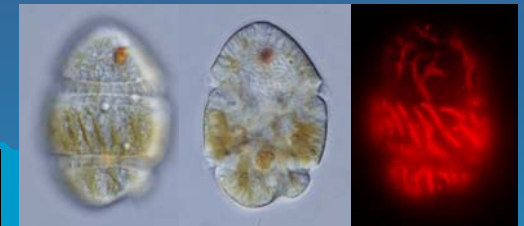
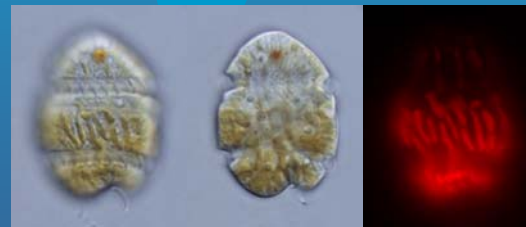
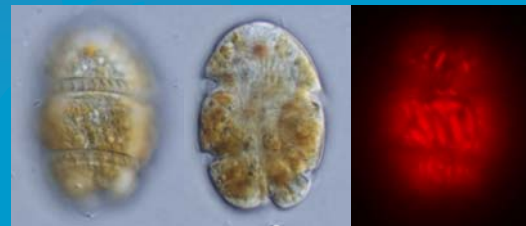
Contents

1. Morphology and taxonomy of *C. polykrikoides* and related taxa
2. Life cycle of *C. polykrikoides* (Resting cyst or over-wintering cell)
3. Reproduction eco-physiology and environmental requirements
4. Toxicology of *C. polykrikoides*

Cochlodinium polykrikoides Margalef 1961



Single cell



Chain-forming cell



Two cells chain



August 2002, Busan, Korea

The genus *Cochlodinium*

The genus *Cochlodinium* has been established by Schütt (1894), circumscribing unarmored dinoflagellates possessing the cingulum encircling the cell >1.5 times (Kofoid & Swezy 1921).

Unarmored dinoflagellates have been classified based mainly on the position and torsion of the cingulum, however, this classification was incongruent with phylogenetic relationship based on recent molecular analyses.

Cochlodinium is still classified based on characteristics of the cingulum, because their phylogenetic relationships have not been examined.



C. catenatum



C. citron



C. lebourae

Kofoid & Swezy 1921

Cochlodinium species described

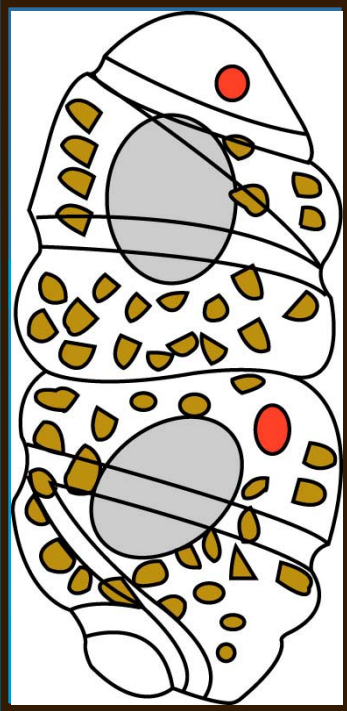
- C. achromaticum* Lebour 1925
- C. adriaticum* Schiller 1933
- C. angustatum* Kofoid et Swezy 1921
- C. archimedes* (Pouchet) Lemmermann 1899
- C. atromaculatum* Kofoid et Swezy 1921
- C. augustum* Kofoid et Swezy 1921
- C. brandtii* Wulff 1916
- C. catenatum* Okamura 1916*
- C. cavatum* Kofoid et Swezy 1921
- C. cereum* Kofoid et Swezy 1921
- C. cnidophorum* Biecheler 1939
- C. citron* Kofoid et Swezy 1921
- C. clarissimum* Kofoid et Swezy 1921
- C. conspiratum* Kofoid et Swezy 1921
- C. constrictum* (Schütt) Lemmermann 1899
- C. convolutum* Kofoid et Swezy 1921
- C. distortum* Kofoid et Swezy 1921
- C. elongatum* Kofoid et Swezy 1921
- C. faurei* Kofoid et Swezy 1921
- C. flavum* Kofoid et Swezy 1921
- C. geminatum* (Schütt) Schütt 1896
- C. helikoides* Lebour 1925
- C. helix* (Pouchet) Lemmarmann 1899
- C. heterolobatum* Silva 1967 *
- C. lebourae* Kofoid et Swezy 1921
- C. longum* Lohmann 1908
- C. miniatum* Kofoid et Swezy 1921
- C. moniliforme* Margalef 1961
- C. pellucidum* Lohmann 1908
- C. pirum* (Schütt) Lemmermann 1899
- C. polykrikoides* Margalef 1961*
- C. pulchellum* Lebour 1917
- C. pupa* Lebour 1925
- C. radiatum* Kofoid et Swezy 1921
- C. rosaceum* Kofoid et Swezy 1921
- C. schuettii* Kofoid et Swezy 1921
- C. scintillans* Kofoid et Swezy 1921
- C. strangulatum* (Schütt) Schütt 1896 (Type)
- C. turbineum* Kofoid et Swezy 1921
- C. vinctum* Kofoid et Swezy 1921
- C. virescens* Kofoid et Swezy 1921
- C. volutum* Kofoid et Swezy 1921

Morphology and Taxonomy of *C. polykrikoides* and its related taxa

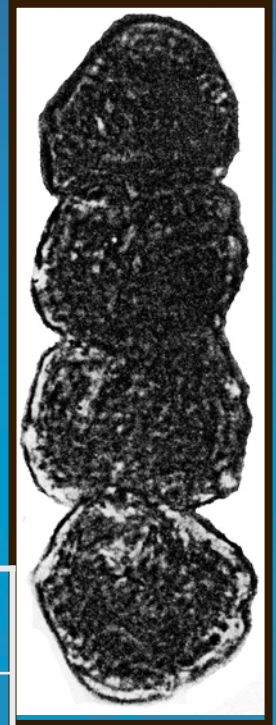
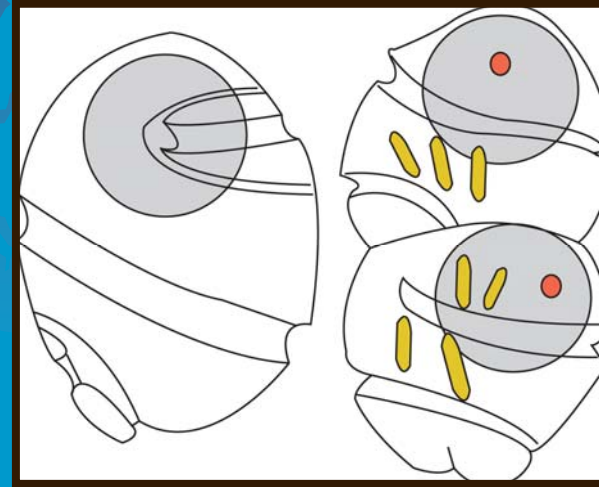
- Morphology of *C. polykrikoides*, *C. heterolobatum*, and *C. catenatum*
- Taxonomic relationships between *C. polykrikoides* and its related taxa
 - Morphological view point
 - Molecular phylogentic view point

Morphological comparison

Cochlodinium heterolobatum Silva 1967



Cochlodinium polykrikoides
Margalef 1961



Red pigmented body	Dorsal, epicone
Cingulum	ca. twice
Sulcus	Shallow, immediately below the cingulum
Nucleus	Spherical, anterior
Chloroplast	Rod-like, aligned longitudinally

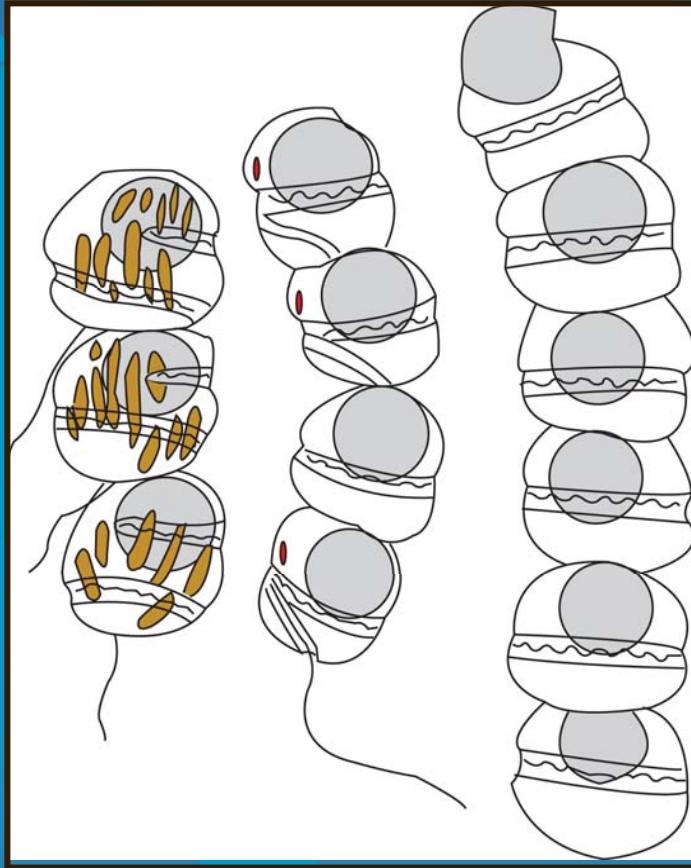
Taxonomic conclusion

Cochlodinium polykrikoides Margalef 1961
(Puerto Rico)

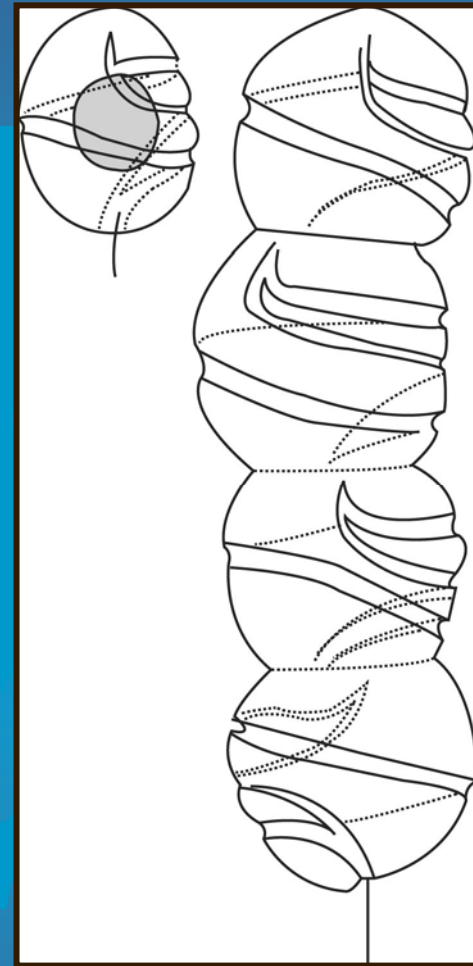
is the senior synonym of

Cochlodinium heterolobatum Silva 1967
(New Jersey, USA)

Cochlodinium catenatum Okamura 1916



Okamura 1916
Loc.:Tokyo Bay



Kofoid & Swezy 1921
Loc.:Off California

A faint world map is visible in the background of the slide, showing the continents in a light blue color against the dark blue background.

Cochlodinium catenatum Okamura

is different form

Cochlodinium catenatum Okamura sensu Kofoid and Sweezy

in Nucleus is located in the center of the cell.

Chloroplasts are lacking.

Taxonomic subject

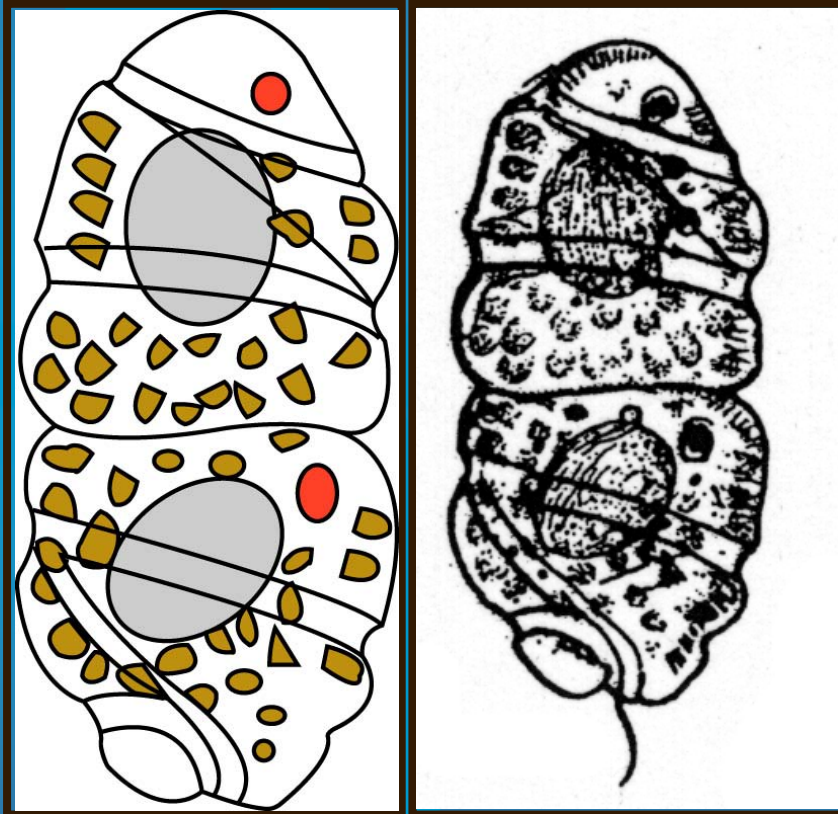
Cochlodinium polykrikoides Margalef 1961
(Type locality; Puerto Rico)

VS

Cochlodinium catenatum Okamura 1916
(Type locality; Tokyo Bay, Japan)

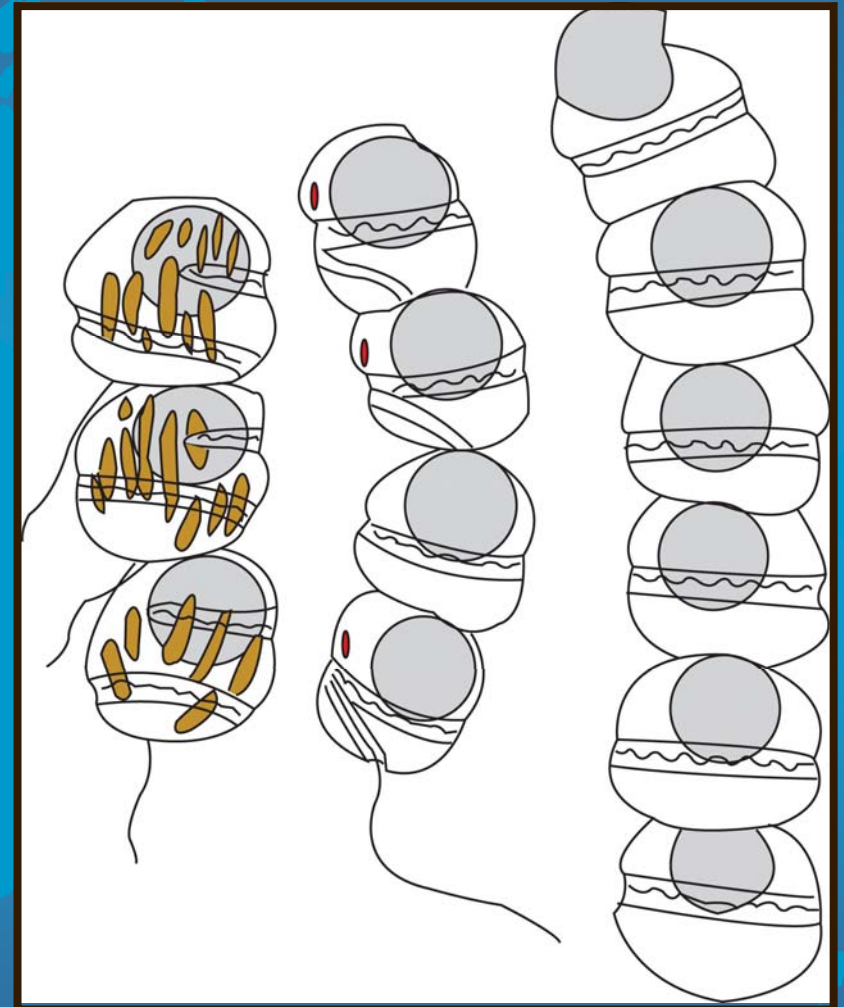
C. polykrikoides vs *C. catenatum*

Cochlodinium catenatum Okamura



Cochlodinium polykrikoides Margalef

Holotype



Holotype

Morphological change



Cochlodinium polykrikoides

ellipsoidal

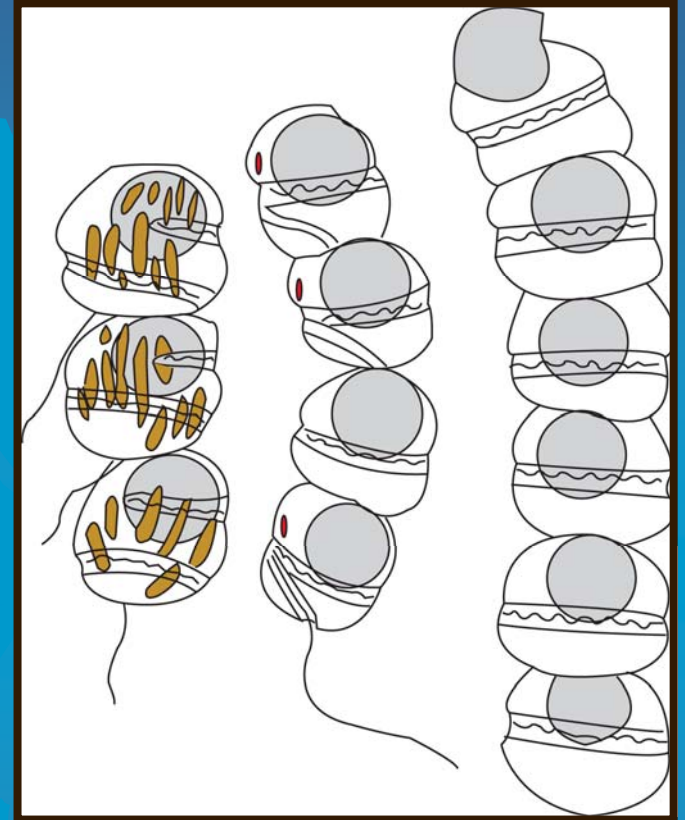
subspherical

Chloroplast

Rod-like

unclear

Red tide
in Korea Aug, 2001

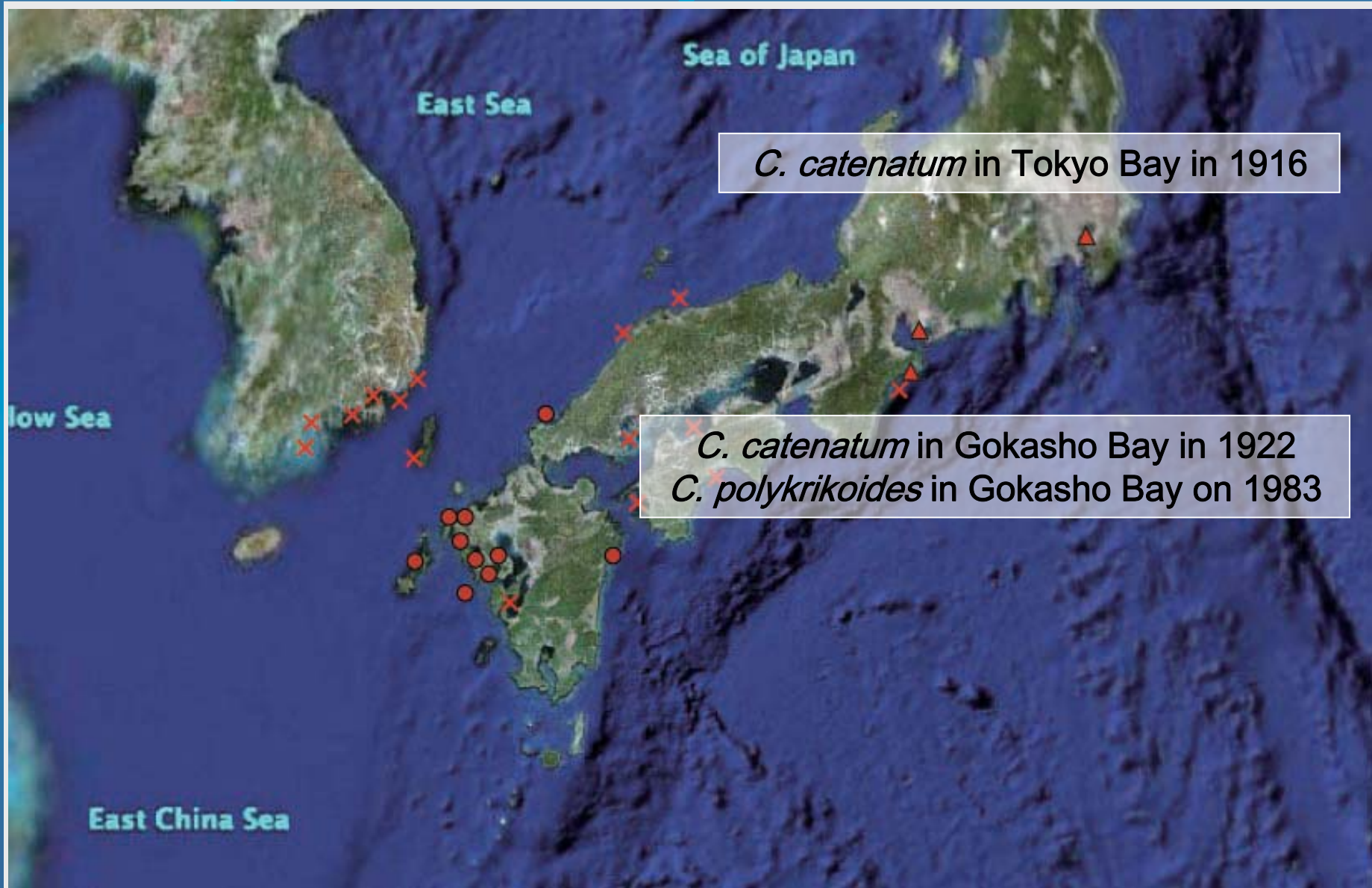


Cochlodinium catenatum

Holotype by Okamura

Transparent membrane

Location of *Cochlodinium catenatum* and *C. polykrikoides* around Japan



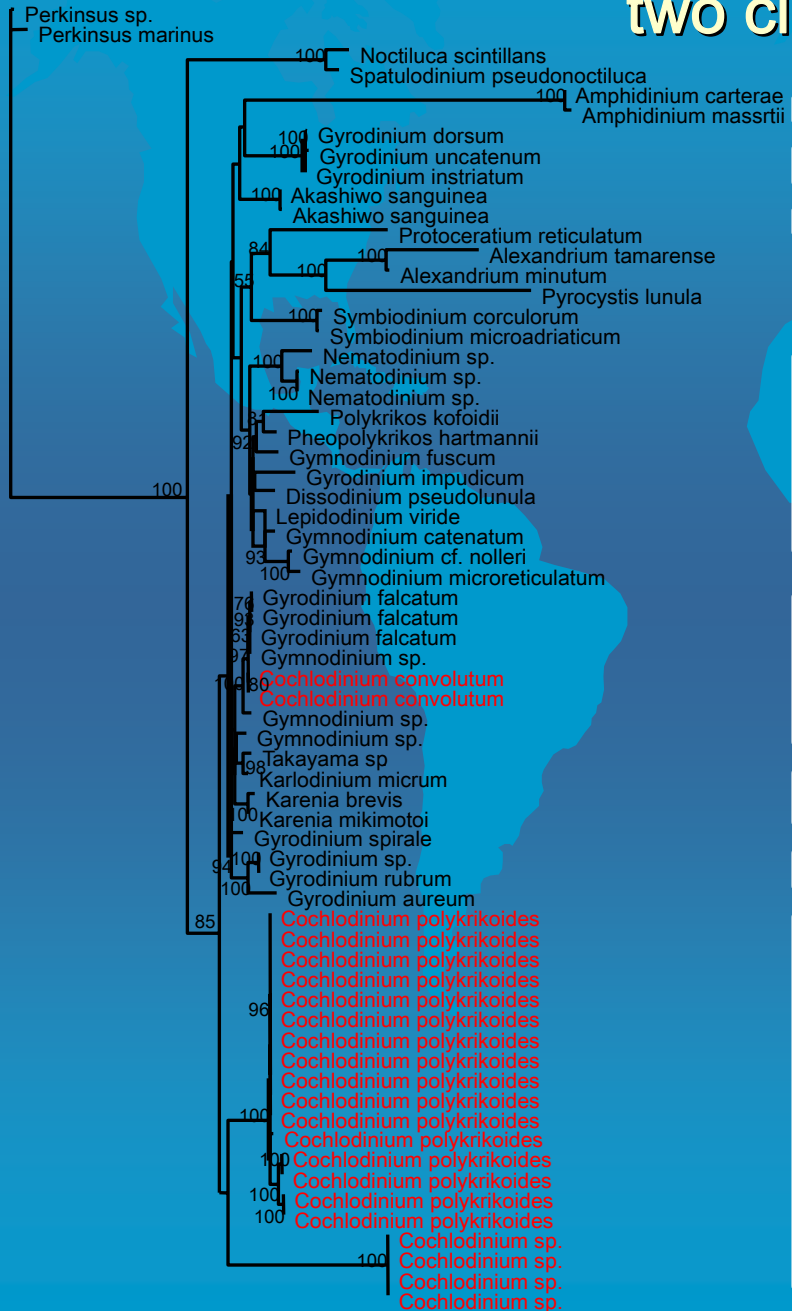
Summary

Taxonomy of *Cochlodinium polykrikoides* and related taxa

Cochlodinium heterolobatum Silva 1967 are a junior synonym of *Cochlodinium polykrikoides* Margalef 1961.

Cochlodinium polykrikoides Margalef 1961 and *Cochlodinium heterolobatum* Silva 1967 are probably a junior synonym of *Cochlodinium catenatum* Okamura 1916.

The genus *Cochlodinium* is separated into two clusters (SSU rDNA)



- Amphidinium*
- Gyrodinium instriatum*
- Akashiwo sanguinea*
- Gymnodinium sensu stricto*
- Gymnodinium*
- Gyrodinium*
- Cochlodinium convolutum*
- Karenia + Karlodinium*
- Gyrodinium sensu stricto*
- Cochlodinium polykrikoides*
- Cochlodinium sp.*

Gamma weighted NJ tree,
 Substitution model:
 Tamura & Nei (1993),
 Proportion of invariable
 site: 0.3834, Shape
 parameter: 0.5580



Summary

Phylogenetic position of the genus *Cochlodinium*

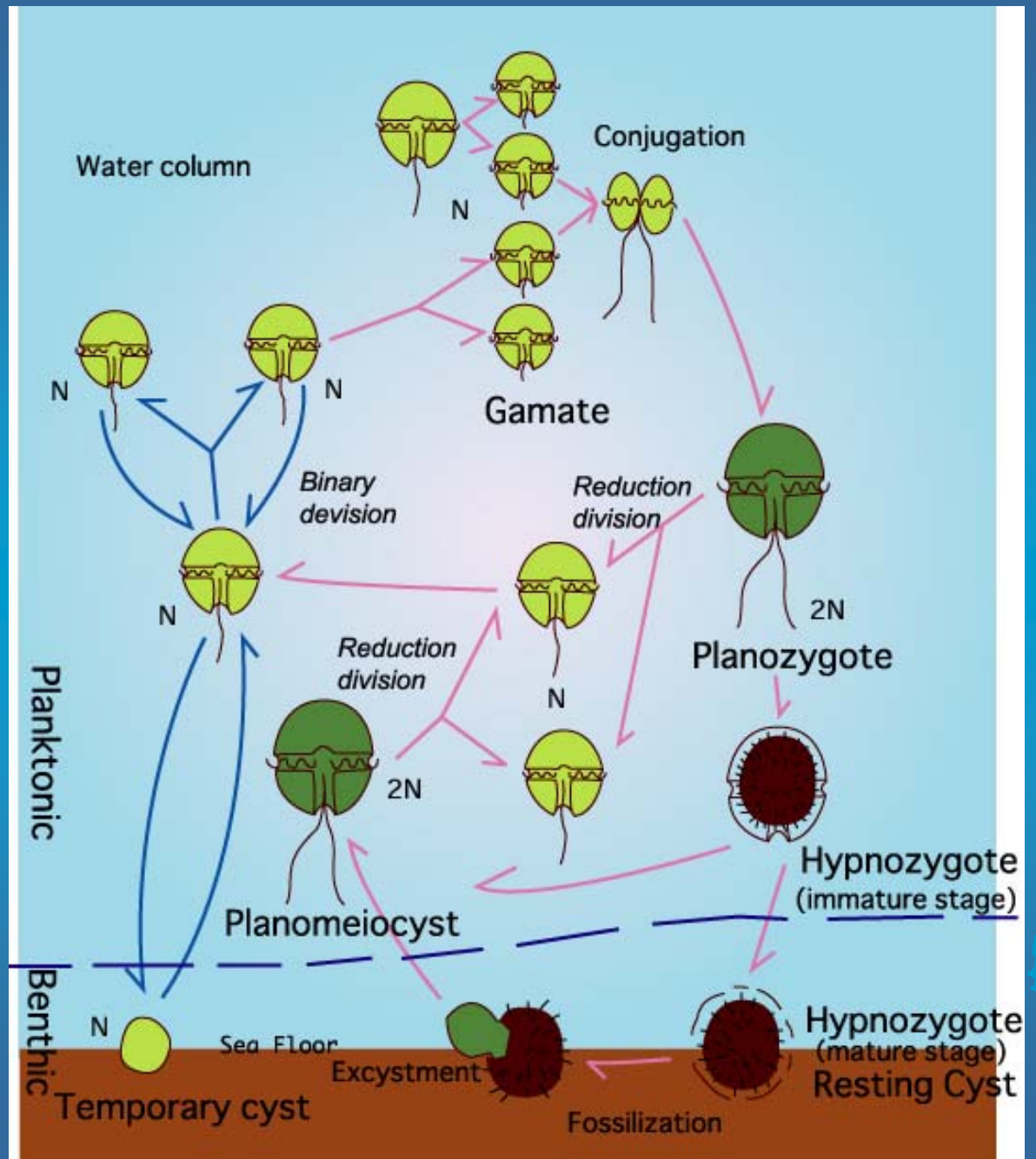
The genus *Cochlodinium* is probably subdivided into two different taxonomic groups.

A faint, light blue world map is visible in the background of the slide, showing the outlines of continents and oceans.

2. Life cycle of *Cochlodinium polykrikoides*

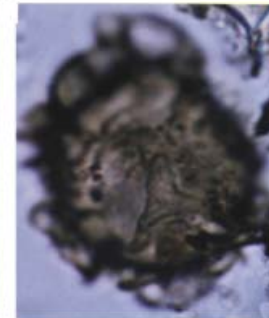
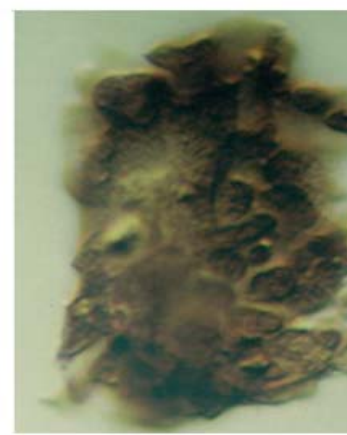
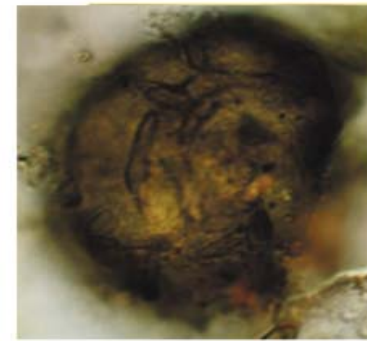
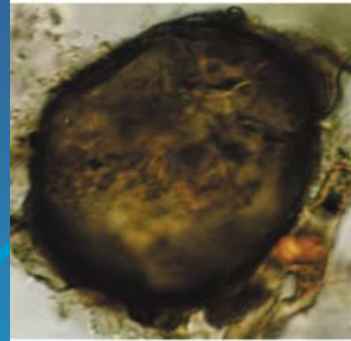
(Resting cyst and/or over-wintering cell)

General Life cycle of dinoflagellates

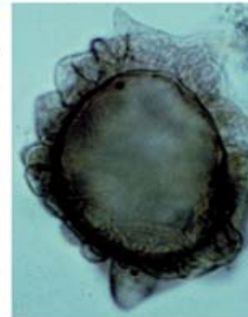
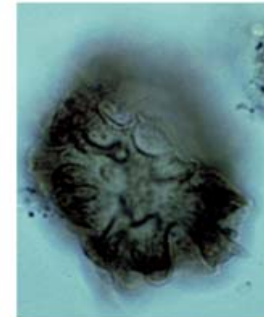


Cysts of *Cochlodinium* “*polykrikoides*”

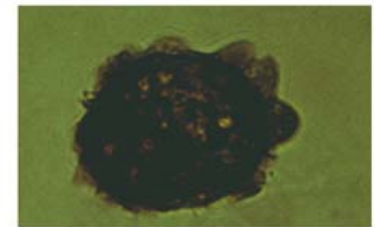
- Brown, subspherical to ellipsoidal, covered with fin-like (*Cochlodinium* sp. 1) or
- Rod-like ornaments (*Cochlodinium* sp. 2), chasmic archeopyle .



cyst form



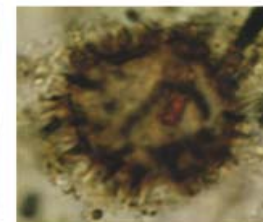
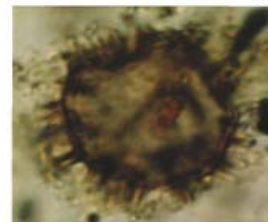
motile form



Cochlodinium sp. 1

(= *C. cf. polykrikoides* Margalef)

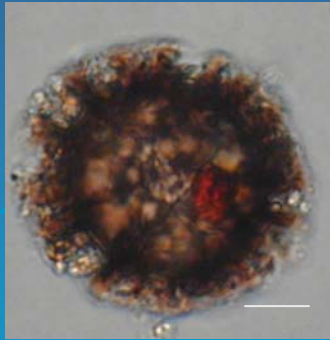
cyst form



Cochlodinium sp. 2

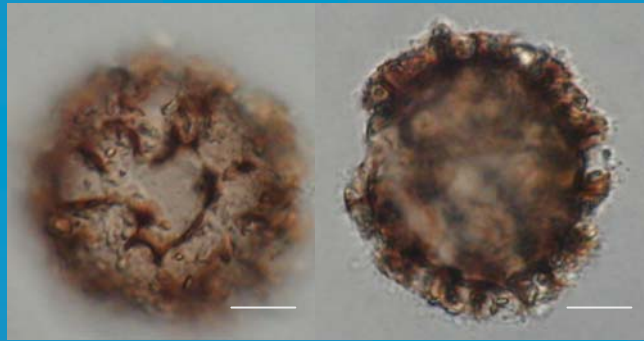
microphotographs by K. Matsuoka & Y. Fukuyo

Cyst-motile form relationship of *Cochlodinium* sp 2.

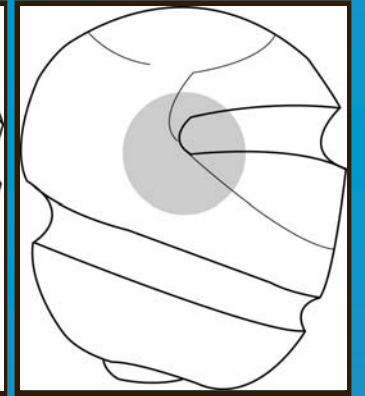
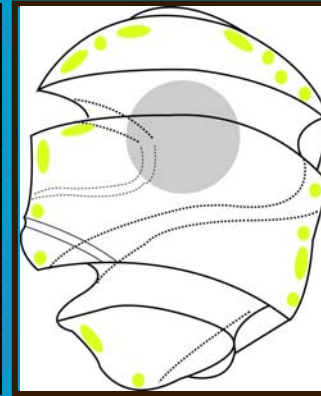
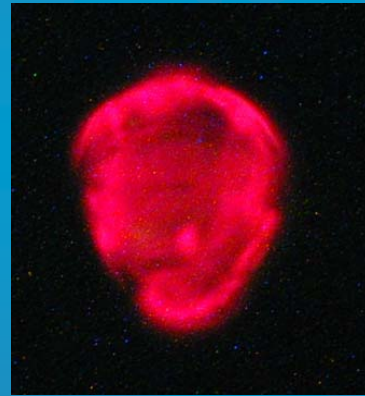


Living cyst

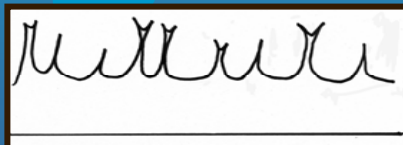
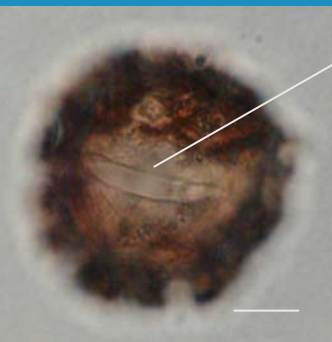
Motile cell
germinated



Empty cyst



Archeopyle



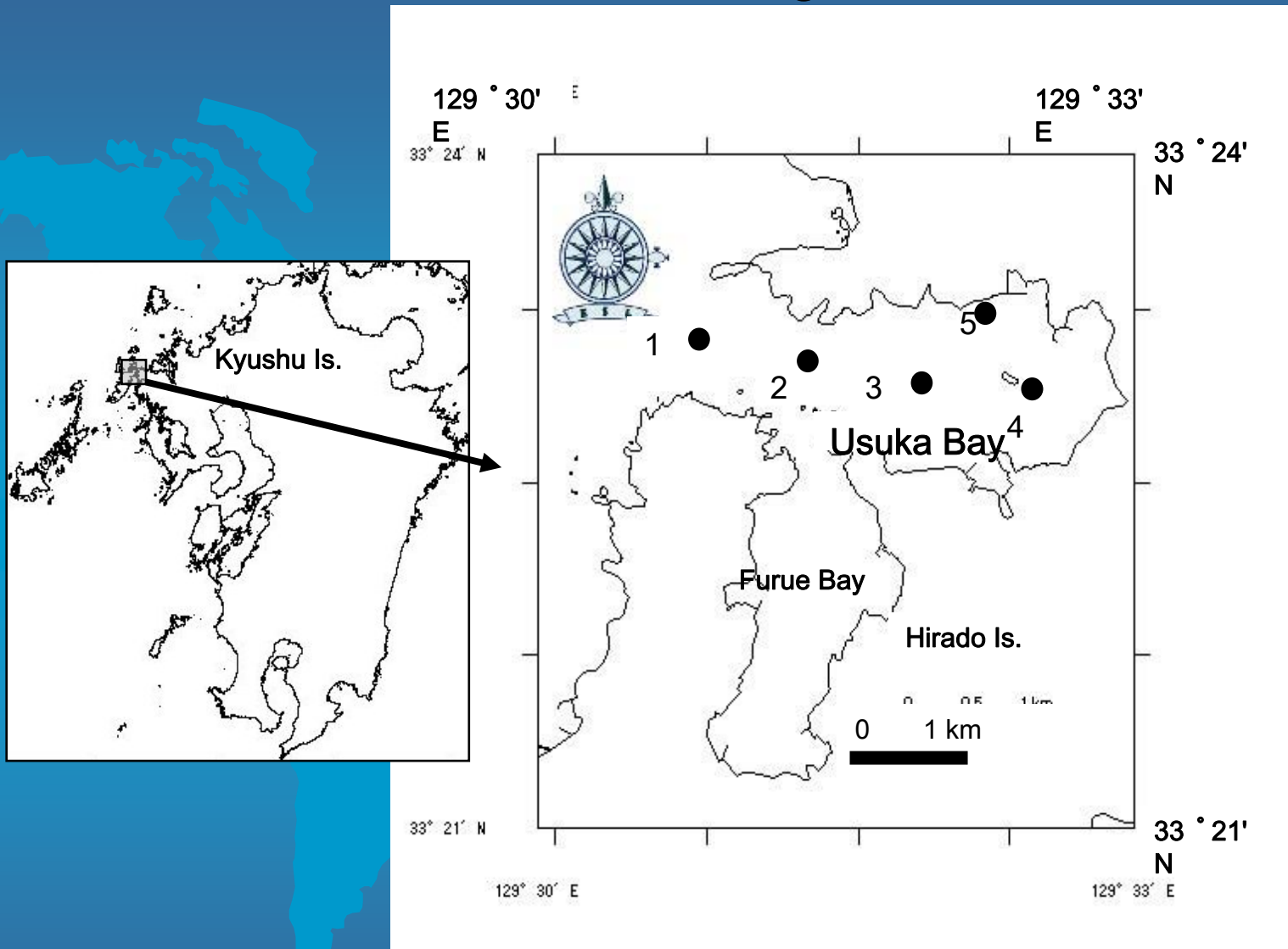
Processes hystriate



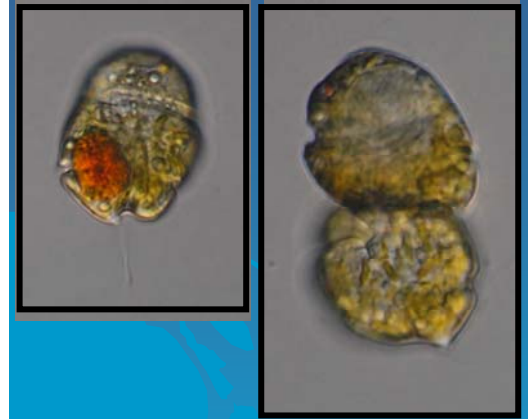
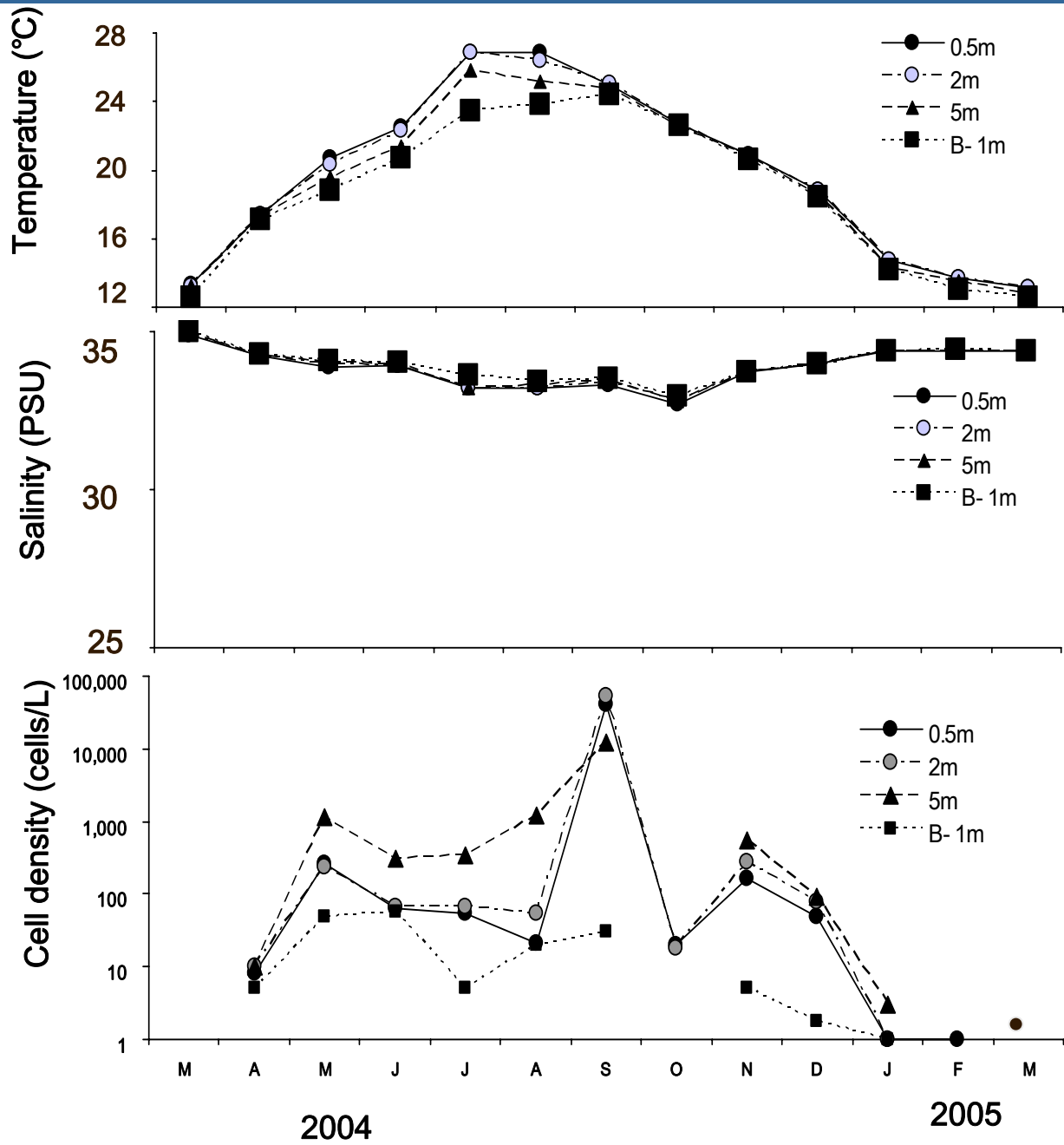
Chain
composed of
two cells

Scale bar 10 μ

Over-wintering cells



Stn.1-5; observation once per month from April of 2004 to March of 2005



April of 2004-
March of 2005

Cell density:
2-320,000 cells L⁻¹

Water temperature:
13.5 - 27.6°C

PSU: 32.83 - 34.42

Summary

Cyst of *C. polykrikoides* has been not yet confirmed on the basis of germination or cultural experiment.

C. polykrikoides can survive over winter as a vegetative cell in more than 13°C water temperature based on field observation.

A world map is visible in the background, rendered in a light blue color against a darker blue gradient. The map shows the outlines of the continents.

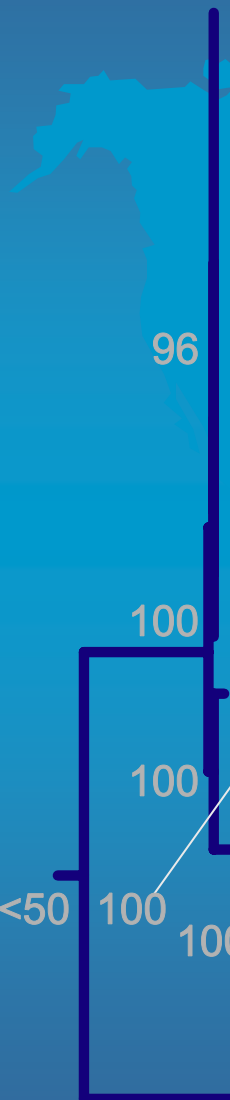
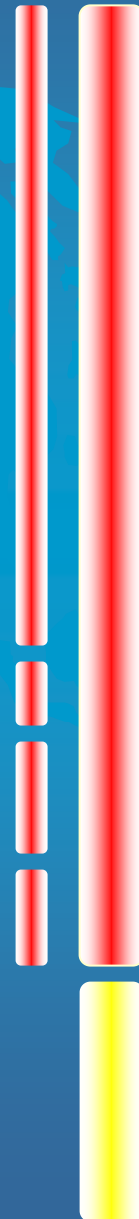
3. Reproductive physiology and environmental requirements

C. polykrikoides and *Cochlodinium* sp. in Asia

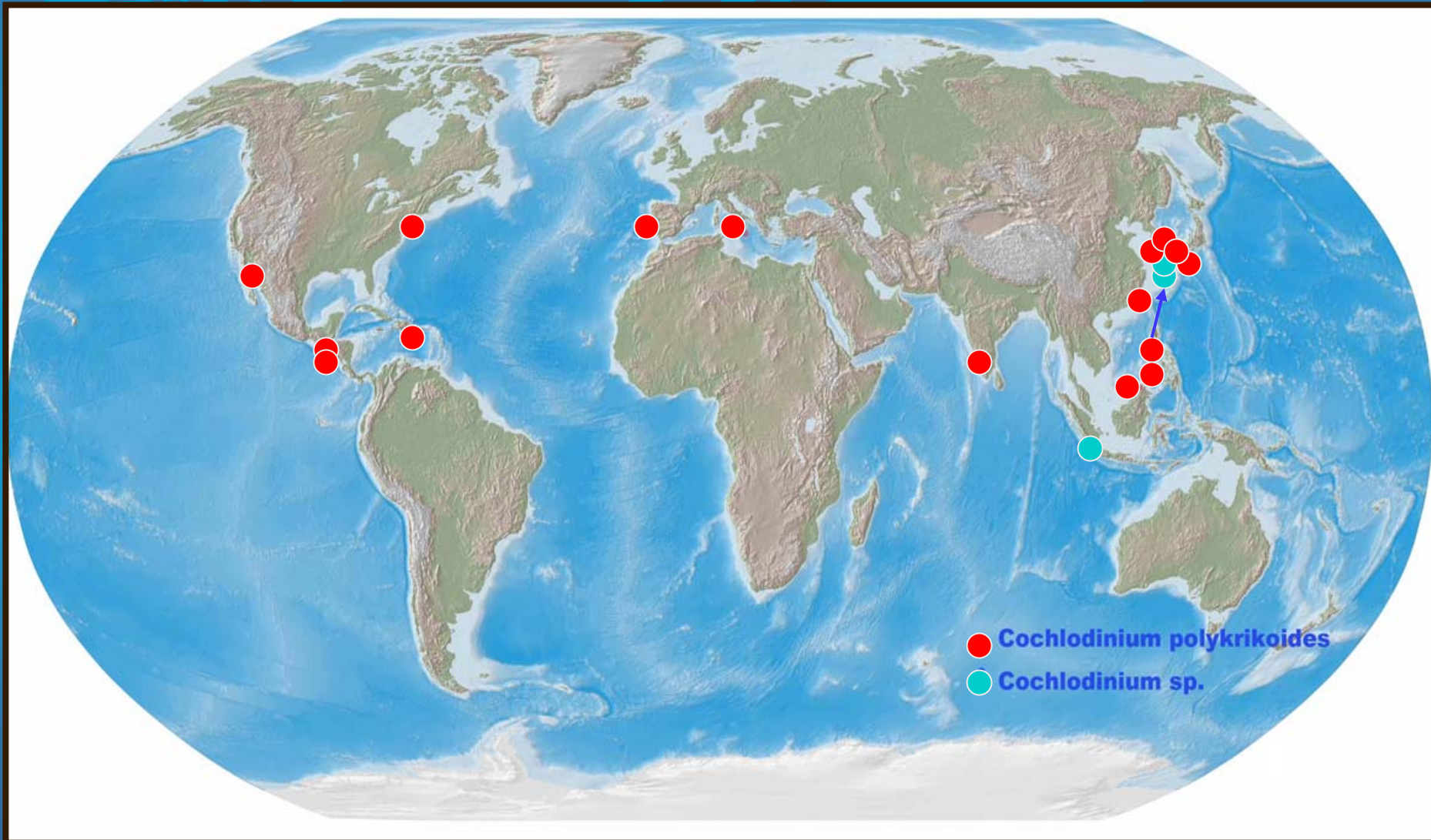


Populations in *C. polykrikoides* in and around the East China Sea based on SSU rDNA

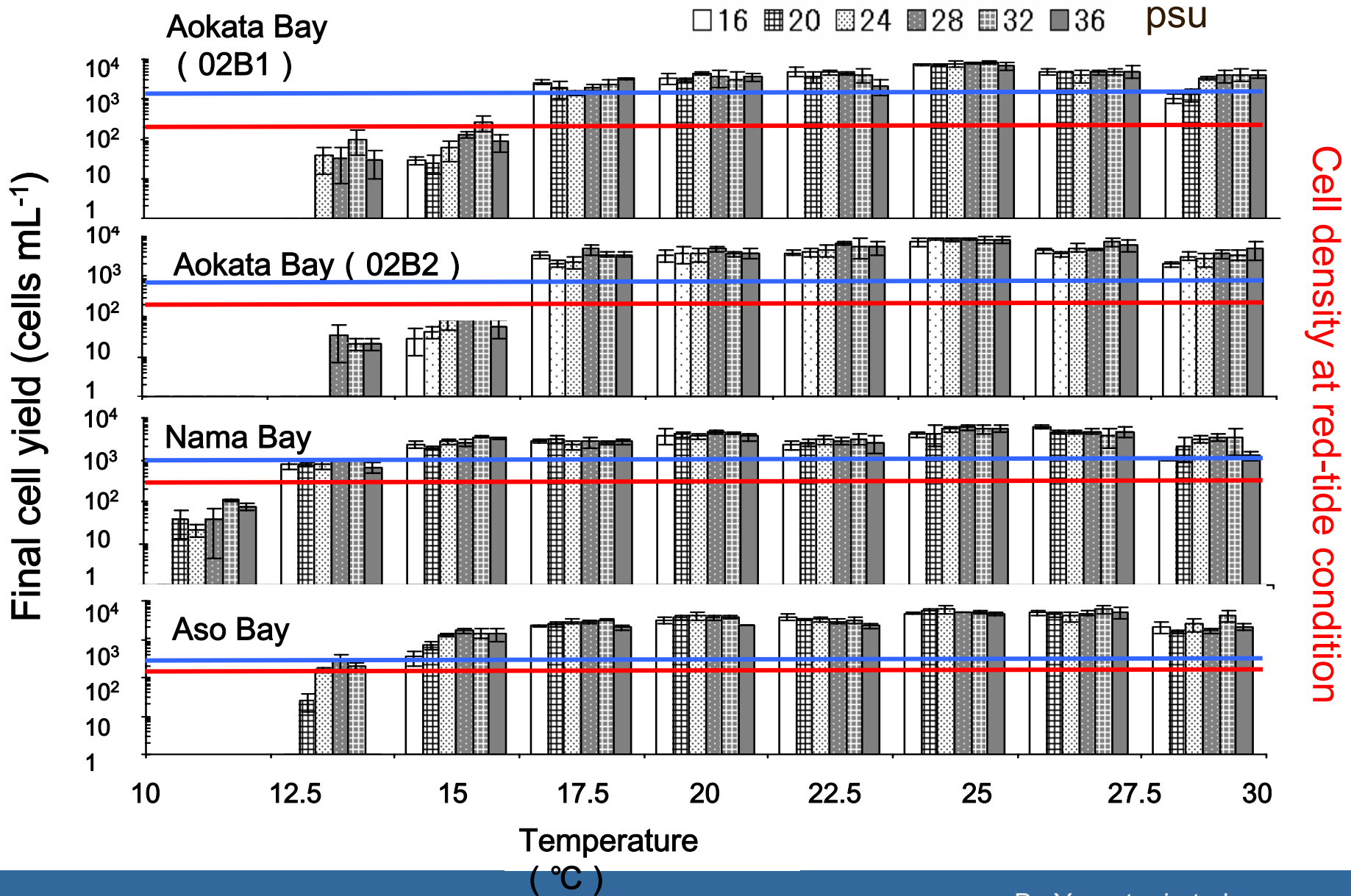
Cochlodinium polykrikoides Geoje, Korea
Cochlodinium polykrikoides Saryang-do, Korea
Cochlodinium polykrikoides Busan, Korea
Cochlodinium polykrikoides Yoesu, Korea
Cochlodinium polykrikoides Yoesu, Korea
96 *Cochlodinium polykrikoides* Kamigoto, Japan
Cochlodinium polykrikoides Hirado, Japan
Cochlodinium polykrikoides Mishima, Japan
Cochlodinium polykrikoides Inokushi Bay, Japan
100 *Cochlodinium polykrikoides* Isahaya Bay, Japan
Cochlodinium polykrikoides Tachibana Bay, Japan
Cochlodinium polykrikoides Imari Bay, Japan
100 *Cochlodinium polykrikoides* Omura Bay, Japan
100 *Cochlodinium polykrikoides* Manila Bay, Philippines
<50 100 *Cochlodinium polykrikoides* Sabah, Malaysia
100 *Cochlodinium polykrikoides* Sabah, Malaysia
100 *Cochlodinium* sp. East China Sea, Japan
Cochlodinium sp. Lampung Bay, Indonesia
Cochlodinium sp. Lampung Bay, Indonesia
Cochlodinium sp. Lampung Bay, Indonesia



Geographical distribution of *Cochlodinium polykrikoides* and *Cochlodinium* sp.

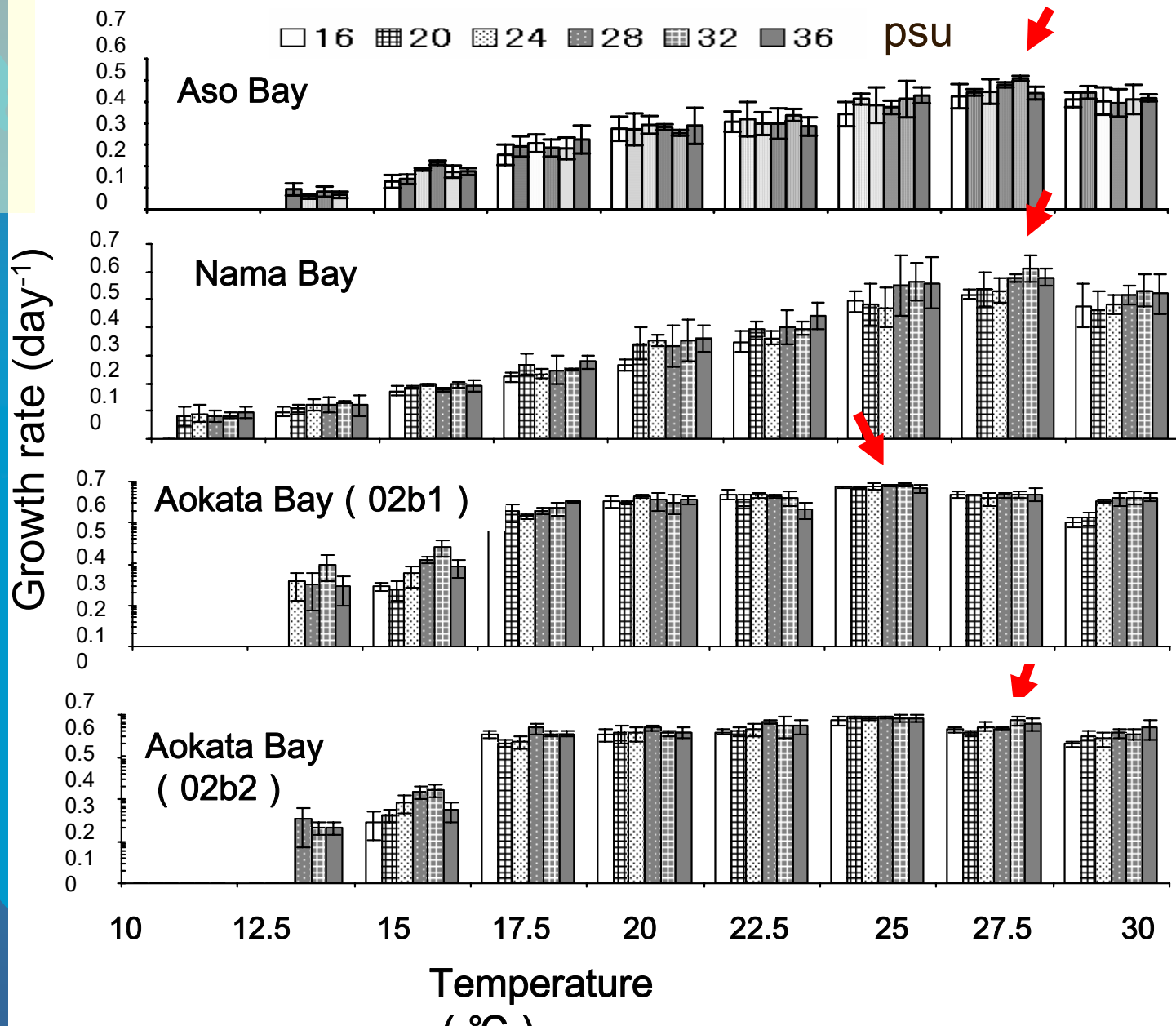


Final Cell Yield of *C. polykrikoides*

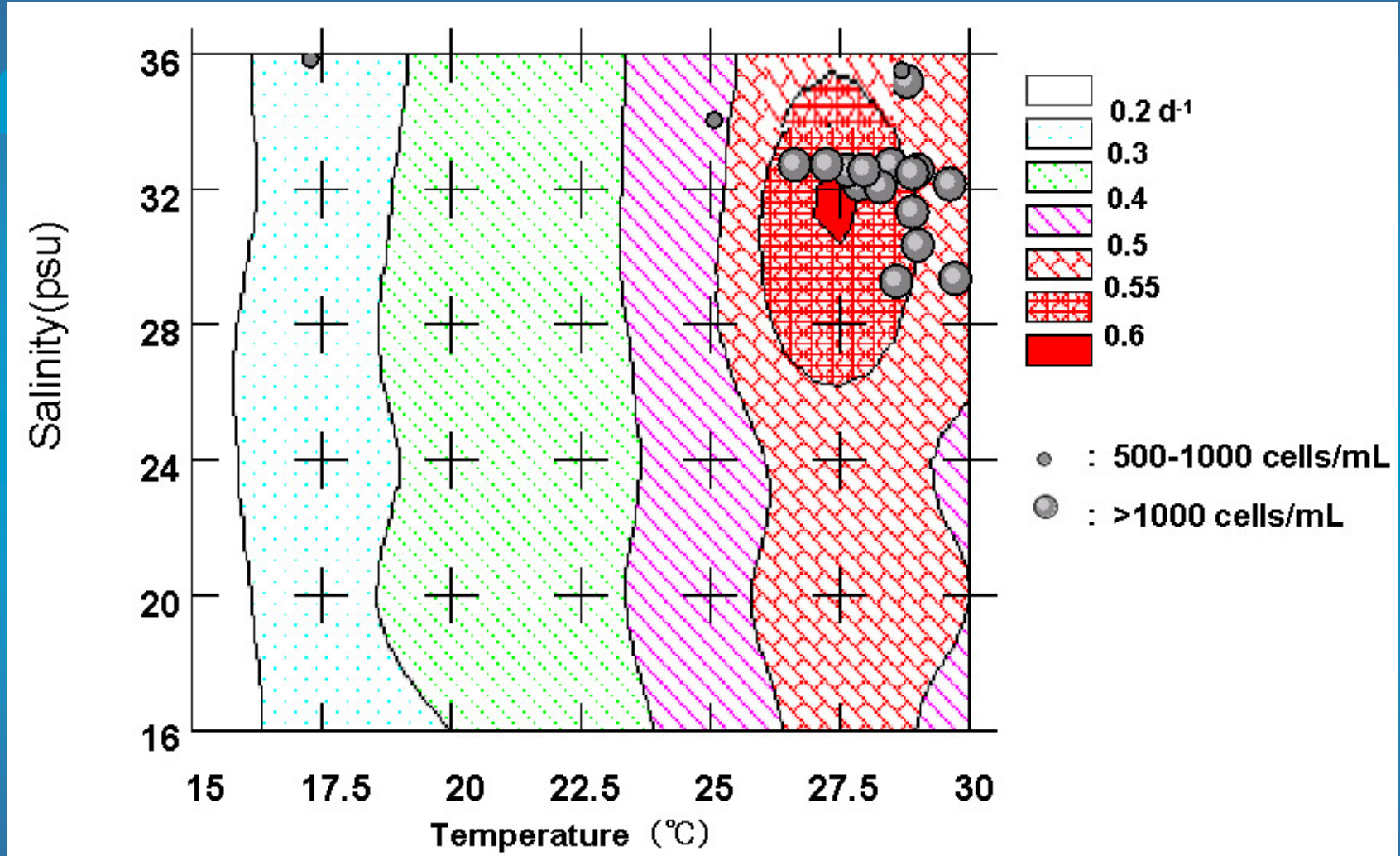


Growth rate in different conditions of *C. polykrikoides*

Maximum growth rate; 0.56 ~ 0.64 day⁻¹ under 27.5°C and 28 ~ 32PSU



Growth rate of *C. polykrikoides* and its relationship to water temperature and salinity



Environmental condition
For more than 1000 cells/L
26-30.4°C, 29.36- 35.21PSU

Summary

1. Maximum growth rate ($0.56 \sim 0.64 \text{ day}^{-1}$) of *C. polykrikoides* is under 27.5°C and $28 \sim 32\text{PSU}$.
2. This environmental condition is concordant with that of red-tide of *C. polykrikoides* in the field.
3. The physiological requirements for the growth of *C. polykrikoides* suggest that *C. polykrikoides* is originally subtropical to tropical species (High temperature and wide salinity range) .



4. Toxicology of *Cochlodinium polykrikoides*

Organisms killed by *C. polykrikoides* bloom

Coral; *Pocillopora* spp.
Porites lobata
Pavona gigantea etc.

by Guzmán et al. (1990)

Shellfish; *Batillus cornutus*
Haliotis discus
Chlorostoma lischkei etc.

by Miyahara et al. (2005)

Fish; *Trachinocephalus maypos*
Sebastiscus inermis
Dasyatis akajei
Pagrus major
Oplegnathus fasciatus

by Kim et al. (2000), Miyahara et al. (2005)

Others; *Octopus vulgaris*
Anthocidaris crassispina

by Miyahara et al. (2005)

Ichthyotoxicity of *C. polykrikoides*

Three toxic fractions (Onoue et al., 1985)

Neurotoxic, hemolytic, hemagglutinating

Paralytic shellfish poisoning (Onoue & Nozawa 1989)

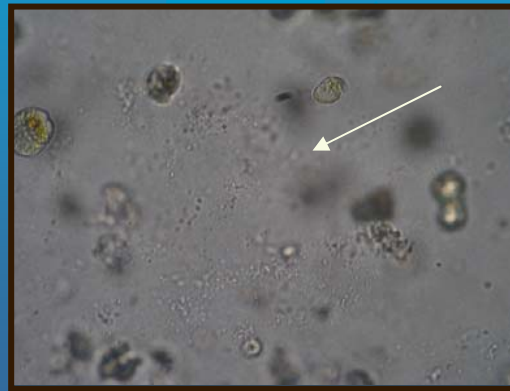
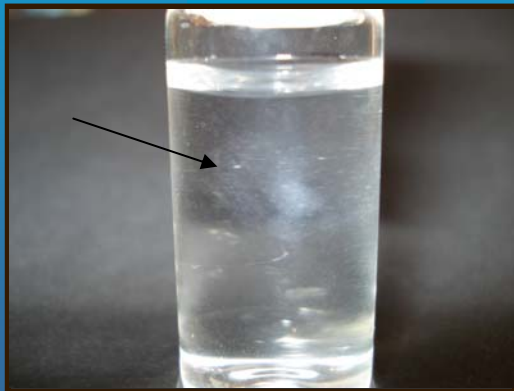
Reactive oxygen species (Kim, C-S et al, 1999)

Superoxide anion (O_2^-)

Hydroxide preoxide (H_2O_2)

Hydroxyl radical (OH)

Mucus-like substance (Hallegraeff 1992, Kim D-K et al, 2002)



Mucus substance
produced by
C. polykrikoides
culture

Hyaline cyst of *C. polykrikoides* during culture

All cells consisting of chain covered with a hyaline membrane on the bottom .

Kim et al. (2002)

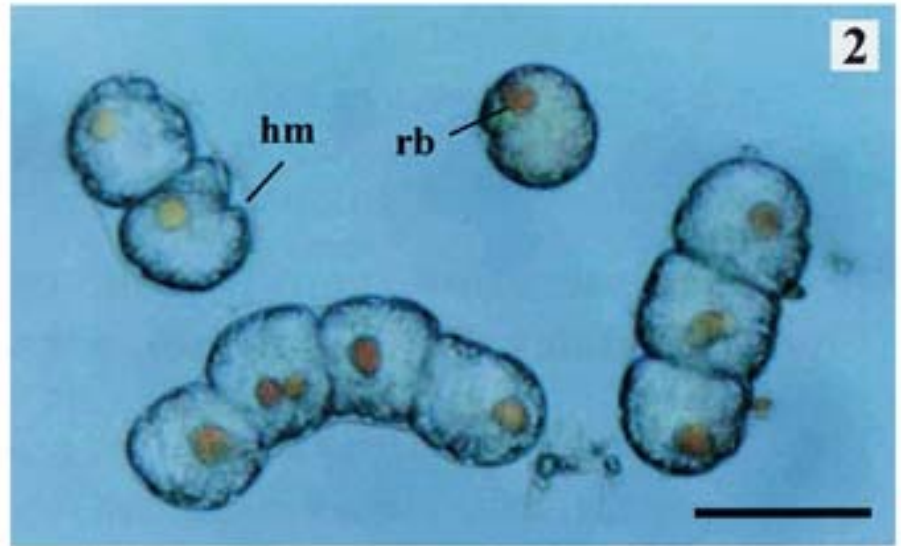
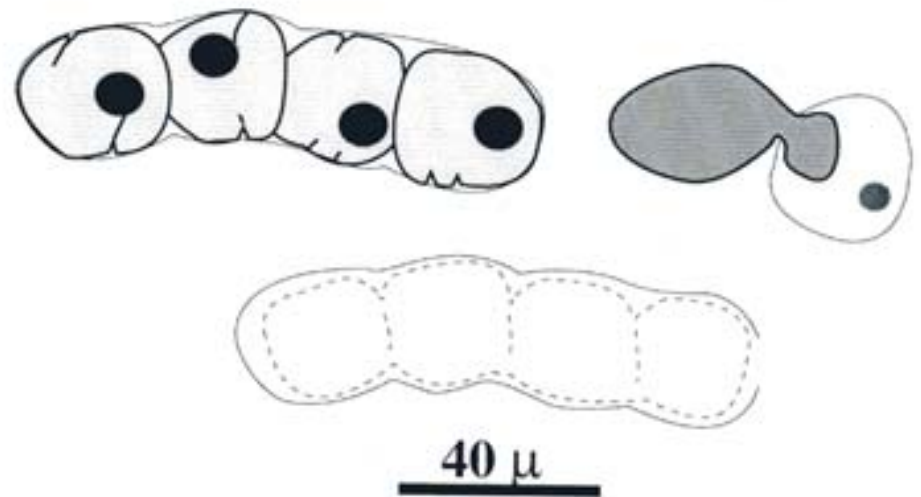


Fig. 2. Chains of hyaline cysts of *C. polykrikoides*; rb = red accumulation body, hm = thin hyaline membrane. Scale bar = 40 μ m.



Hyaline cyst of *Cochlodinium polykrikoides*

Possible causative substance for toxicity of *C. polykrikoides*

- No significant increase of O_2^- generated by *C. polykrikoides*
- Gradual accumulation of polysaccharides during the culture
- Many benthic organisms were damaged after the bloom of *C. polykrikoides*

Summary

Mainly mucus substance and additionally reactive oxygen species (ROS)

Future studies

- Nutrient strategies ; macro- and limited nutrients oligotrophic, mixotrophic
- Mechanism for recruitment ; over-wintering cells, cysts, and/or transportation
- Toxic substance ; reactive oxygen species or mucus
- Phylogenetic position of the genus *Cochlodinium*

Acknowledgements

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The data were provided by following researches;

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Mr. Kazuyoshi Miyamura (Oita Institute of Marine and Fisheries Science, Japan)

Dr. Haruyoshi Takayama

Mr. Koichiro Mizushima (Nagasaki University, Japan)

Ms Ryoko Kida (Nagasaki University)

Ms Akane Mizuno (Nagasaki University)

Mr. Juan Relox Jr. (National Fisheries Research and Development Institute, Philippines)

Dr. Ann Anton (University of Malaysia Sabah, Malaysia)

Dr. Keun-Yong Kim (Pukyong University, Korea)

Thank you!!