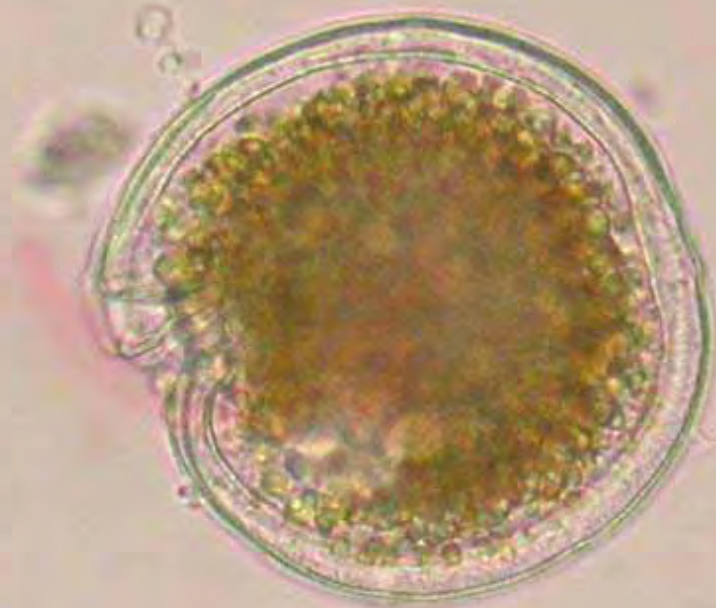


**Year-round occurrence of the benthic  
dinoflagellate *Gambierdiscus* sp. in  
temperate coastal waters of Japan**



Akira Ishikawa, Yumi Takeichi  
(Mie University)

Setsuko Sakamoto, Mineo Yamaguchi

(National Research Institute of Fisheries and Environment of Inland Sea)

# Background

*Gambierdiscus* spp. is the benthic and epiphytic dinoflagellate, commonly found on the macroalgae in coral reefs.

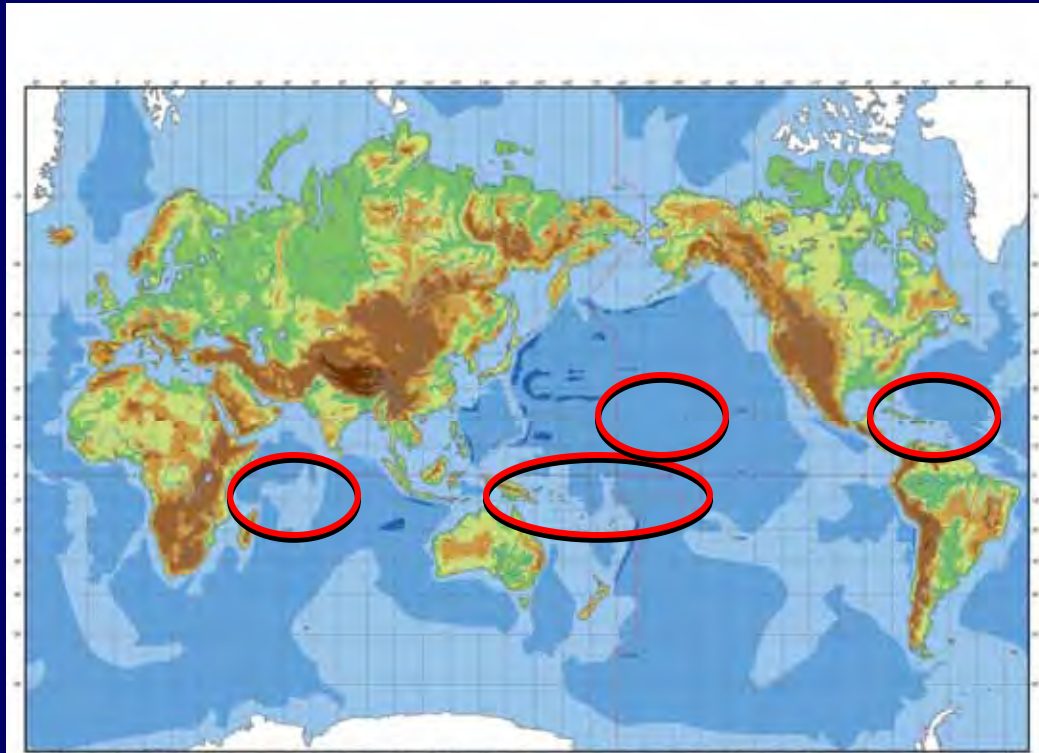
Some of the *Gambierdiscus* spp. have been confirmed to produce maitotoxins and ciguatoxins, being the primary causative organism of ciguatera fish poisoning (CFP).



# Background

They are distributed widely in tropical and subtropical regions, where CFP mainly occurs.

It has been reported that > 50,000 people suffer from CFP annually.

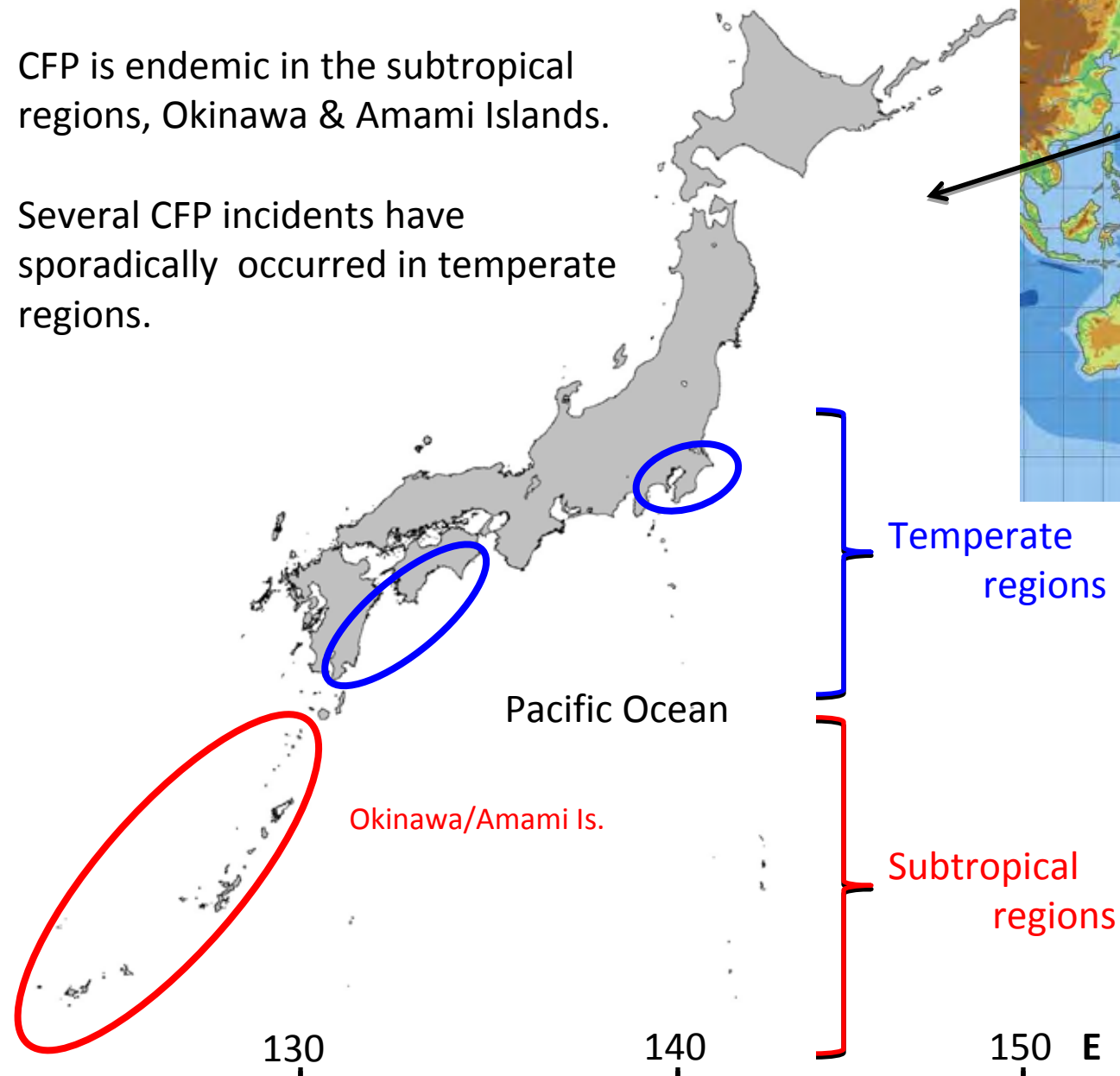


# In Japan

CFP is endemic in the subtropical regions, Okinawa & Amami Islands.

Several CFP incidents have sporadically occurred in temperate regions.

45 N  
40  
35  
30  
25



Temperate regions

Subtropical regions

150 E

# In Japan

45 N

CFP is endemic in the subtropical region, Okinawa & Amami Islands.

40

Several CFP incidents have sporadically occurred in temperate region.

Pacific Ocean

35

Temperate regions

30

In Kii peninsula, CFP occurred in 2007, for the first time, and subsequently in 2008.

Okinawa/Amami Is.

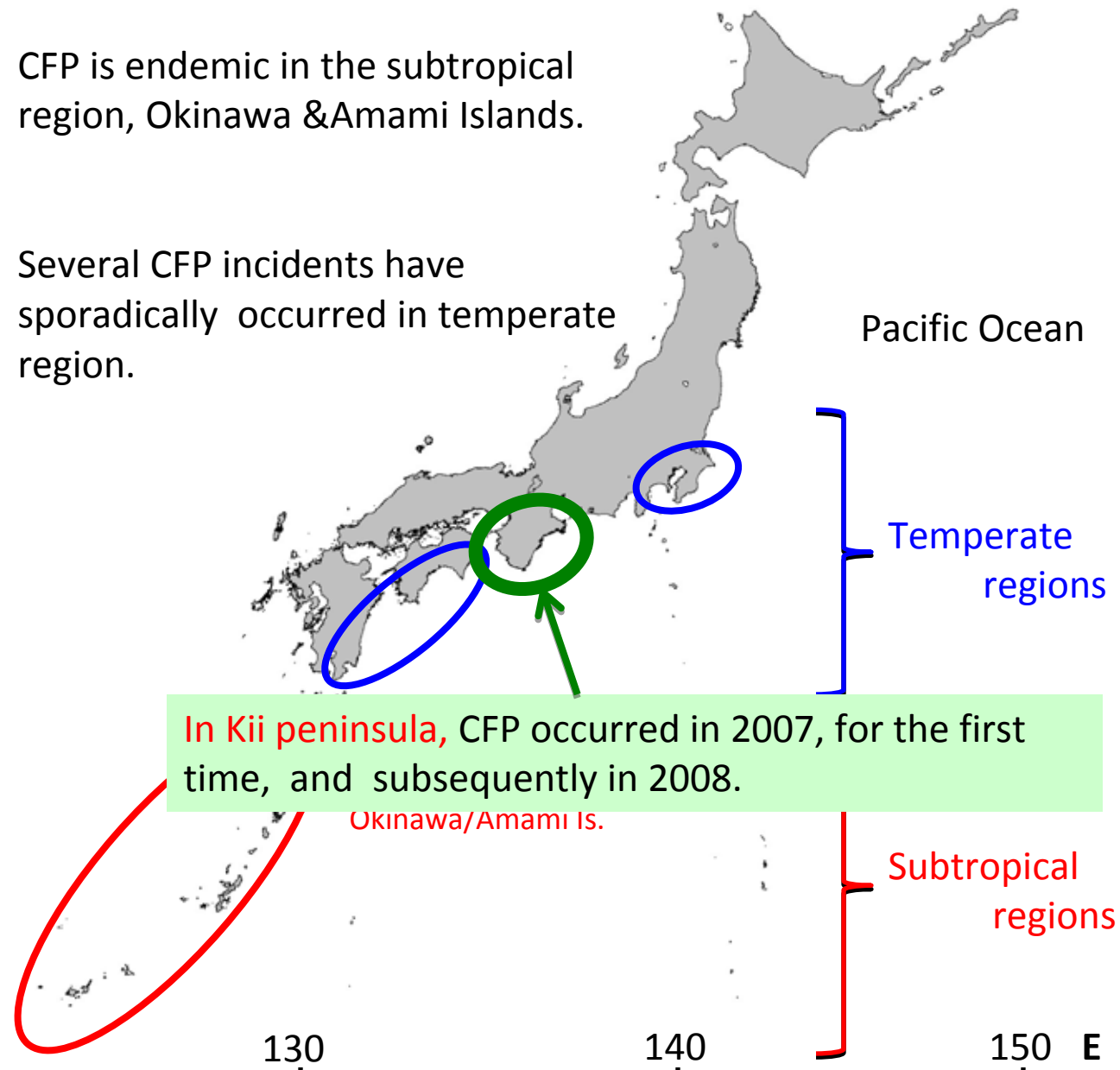
Subtropical regions

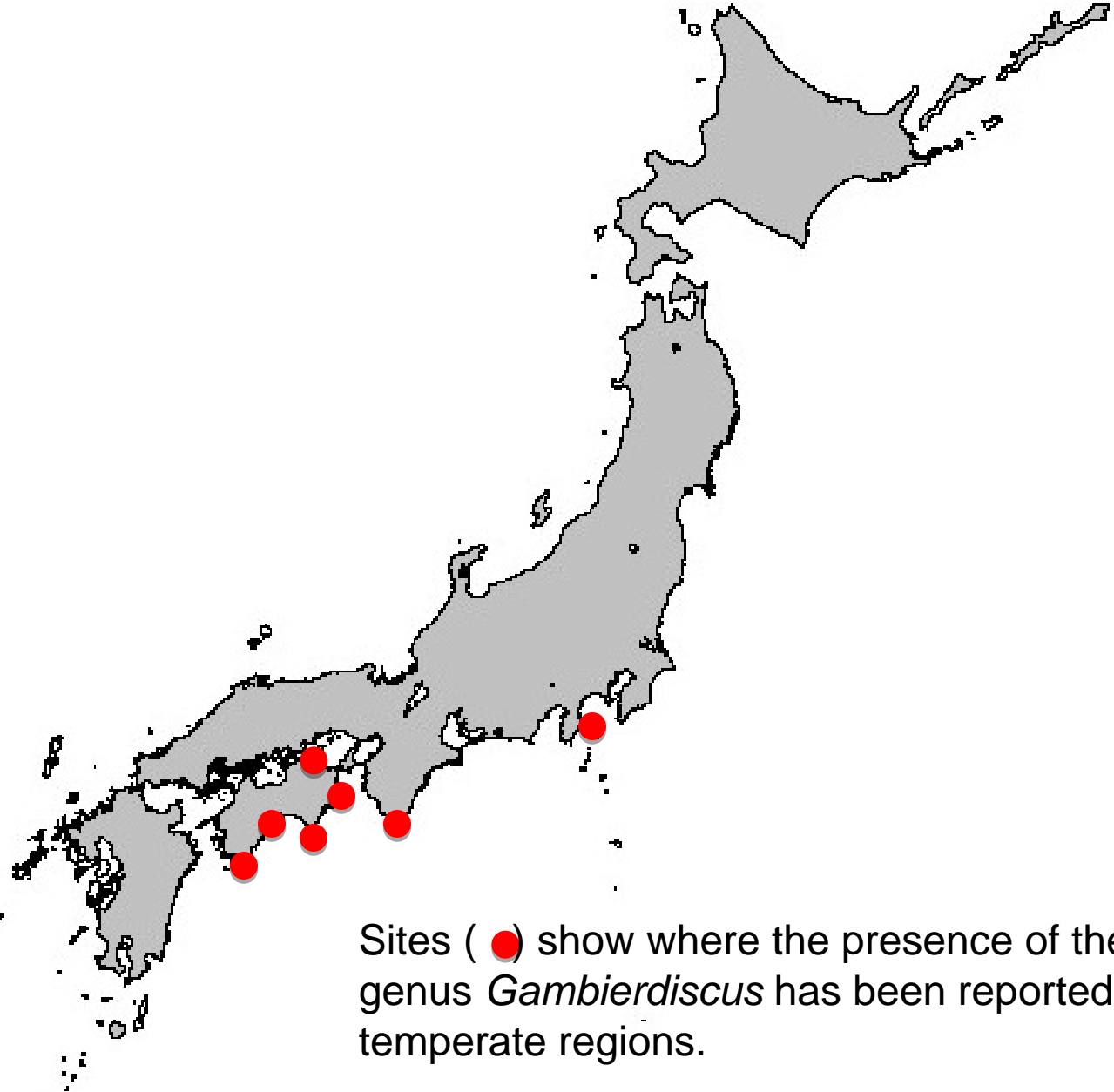
25

130

140

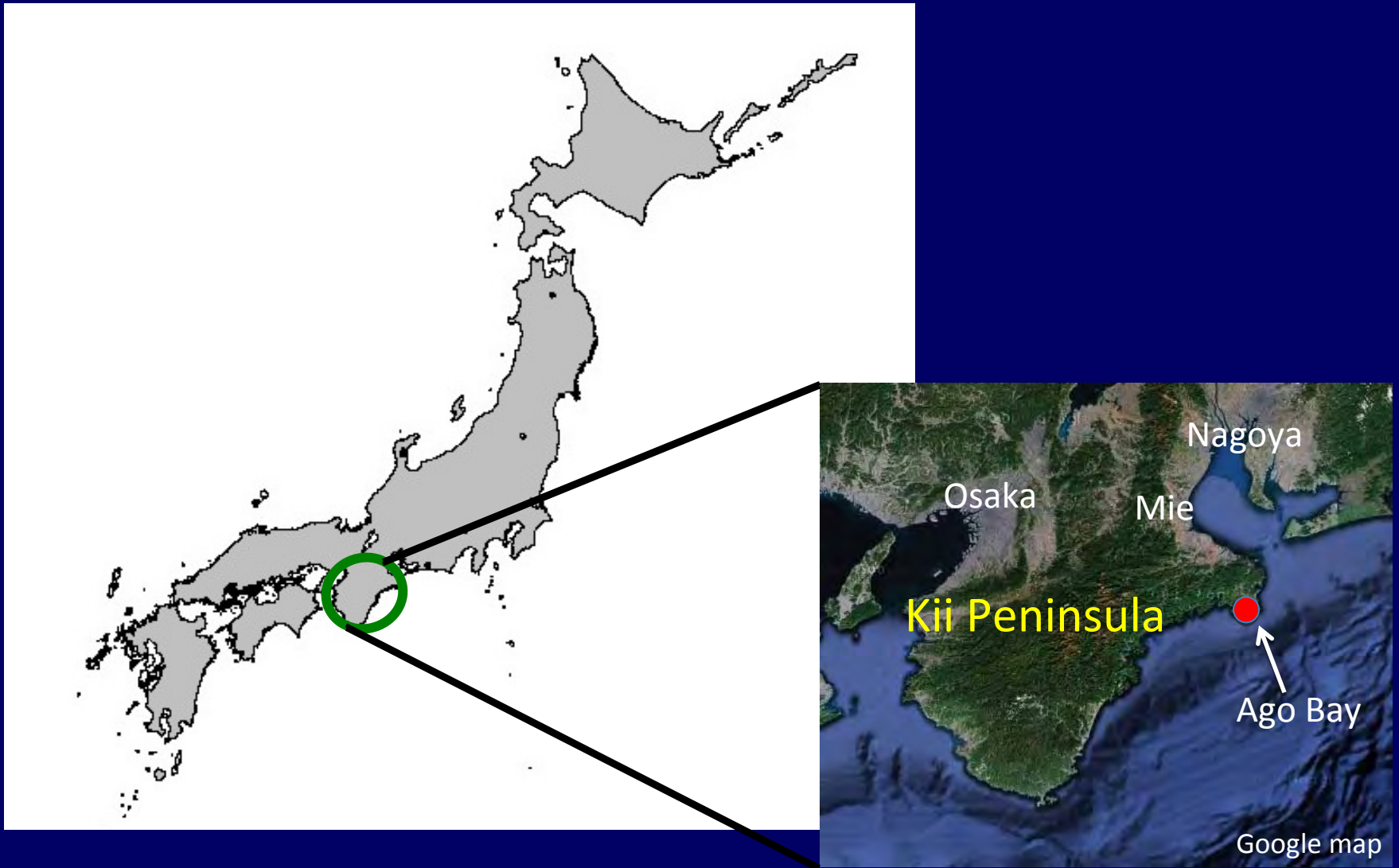
150 E





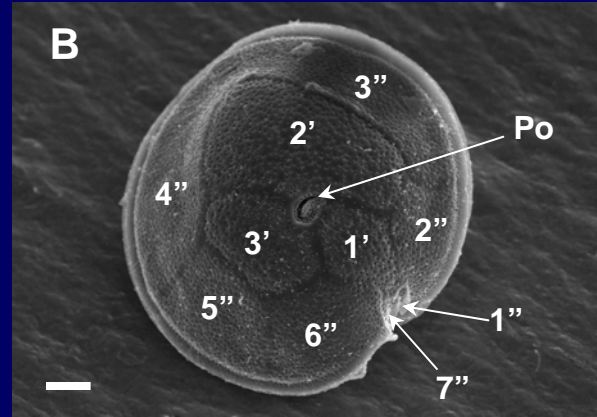
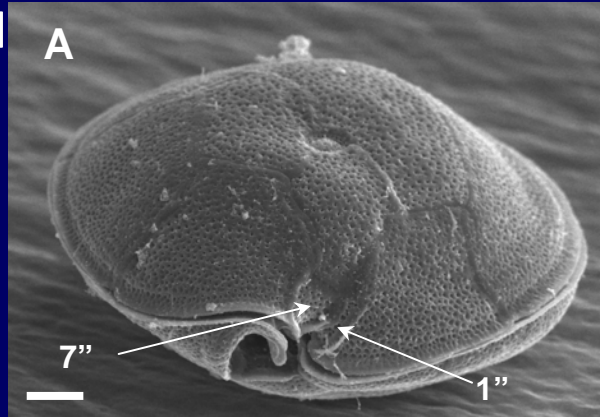
Sites ( ● ) show where the presence of the genus *Gambierdiscus* has been reported in temperate regions.

Having recent CFP incidents in Kii Peninsula,  
We examined for the presence of the genus *Gambierdiscus* in Ago Bay.



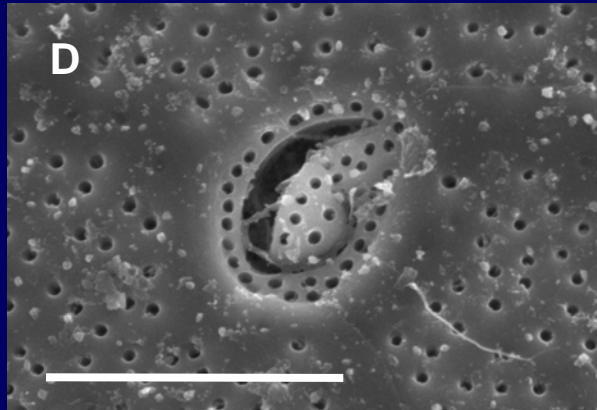
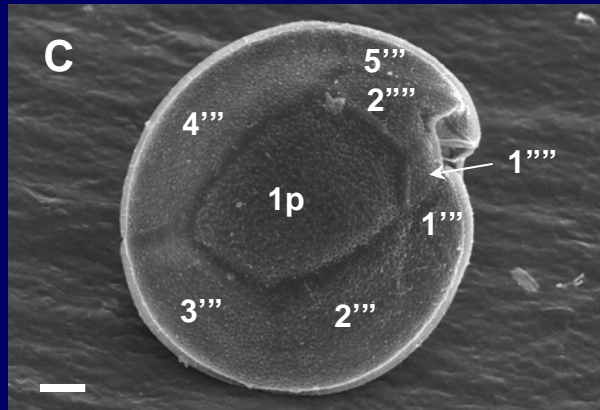
# Gambierdiscus found in Ago Bay

Oblique ventral view



Epitheca

Hypotheca



Apical pore Plate (Po)

Scal bars: 10  $\mu$ m

Main plate formula : Po, 3', 7'', 5''', 1p, 2''''

(Ishikawa & Kurashima 2010: Bull Jpn Soc Fish Oceanogr)



# Flowchart to distinguish the various *Gambierdiscus* species

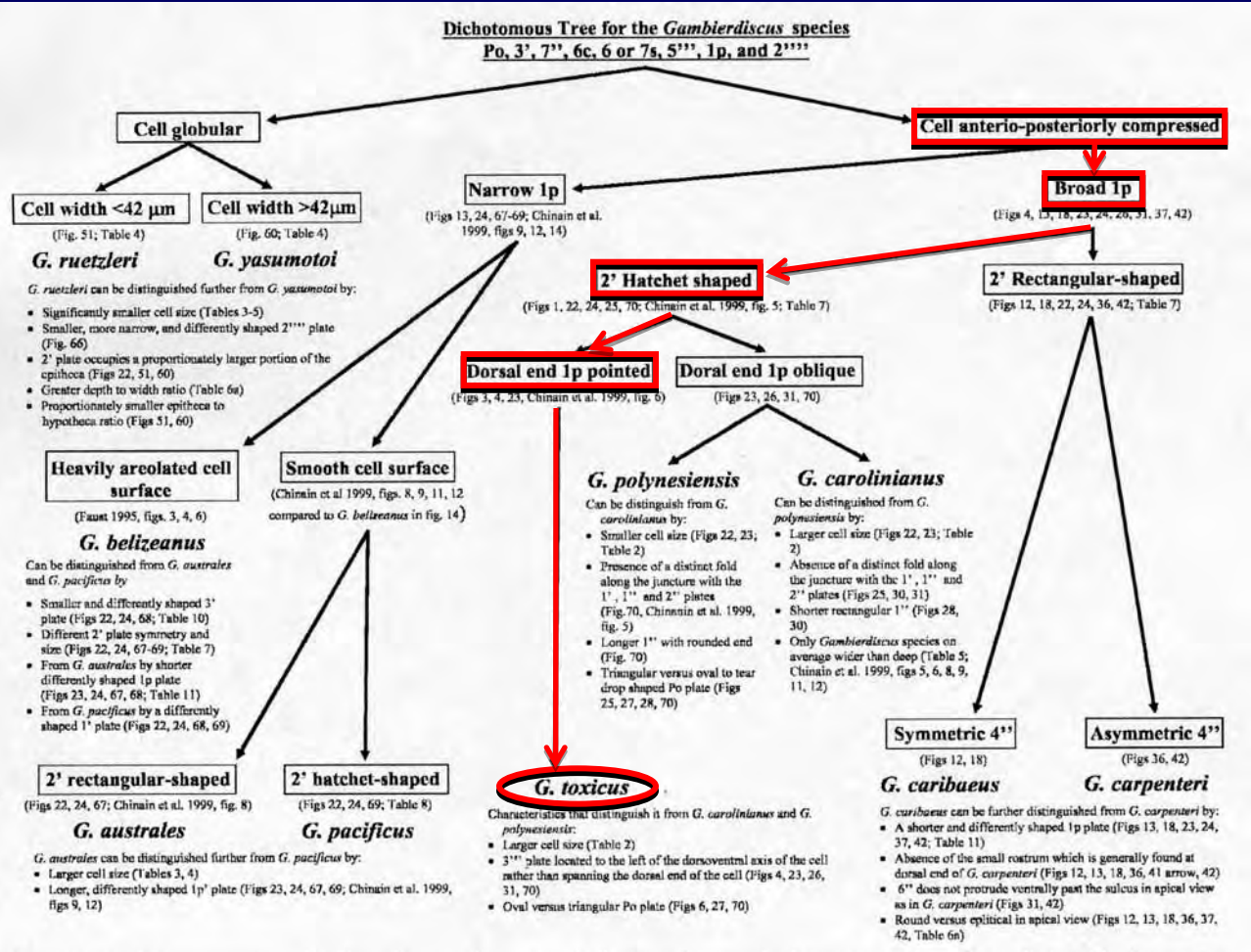
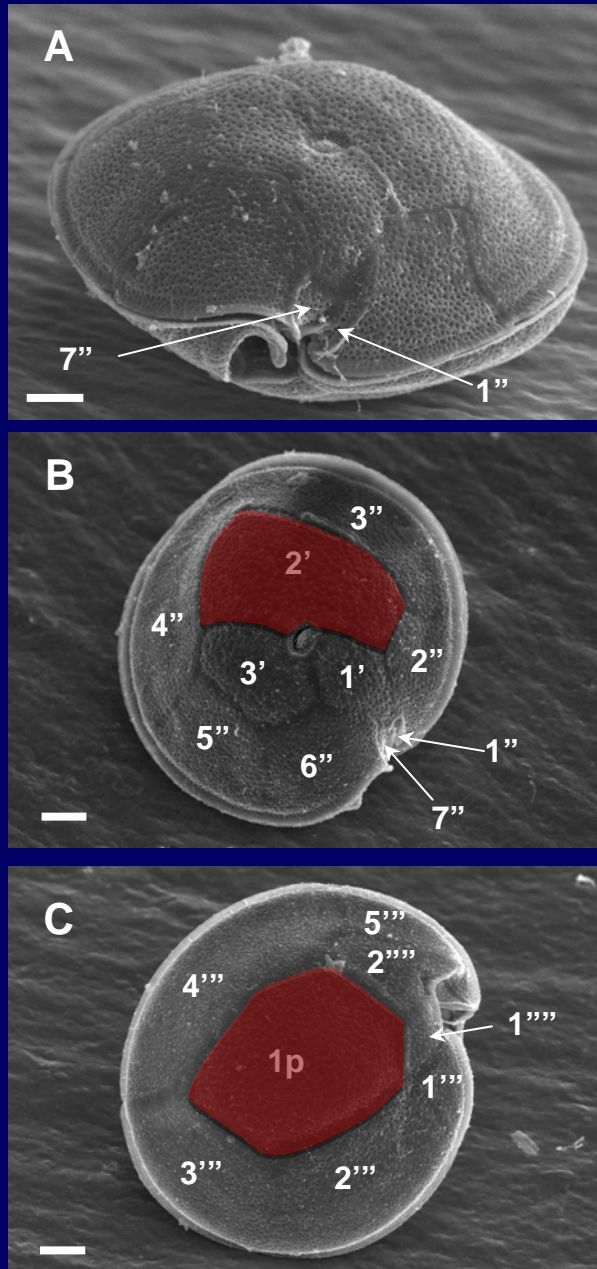


Fig. 10. A dichotomous tree detailing the morphometrics (cell size, shape and plate structure) used to distinguish the various *Gambierdiscus* species.

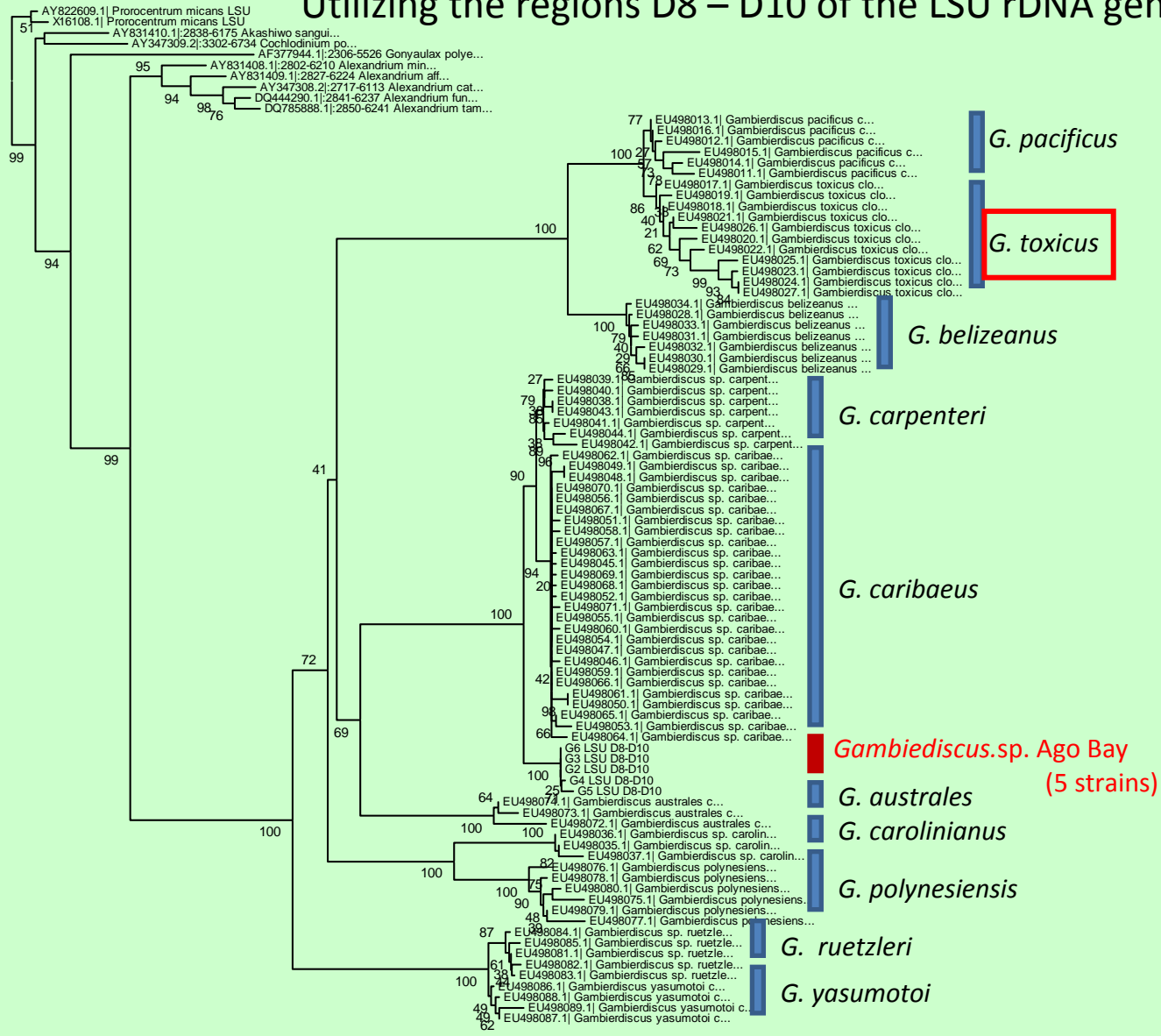
Fig. 10 in Litaiker et al. (2009): *Phycologia*



Scale bars: 10 μm

# Result of phylogenetic analysis (NJ)

Utilizing the regions D8 – D10 of the LSU rDNA gene



# Aim of this study

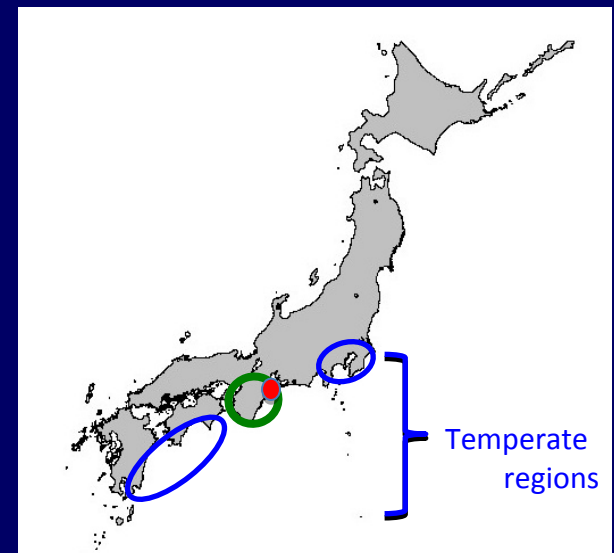
To evaluate future risk of CFP in temperate regions, it is necessary to understand the population dynamics of *Gambierdiscus* sp.



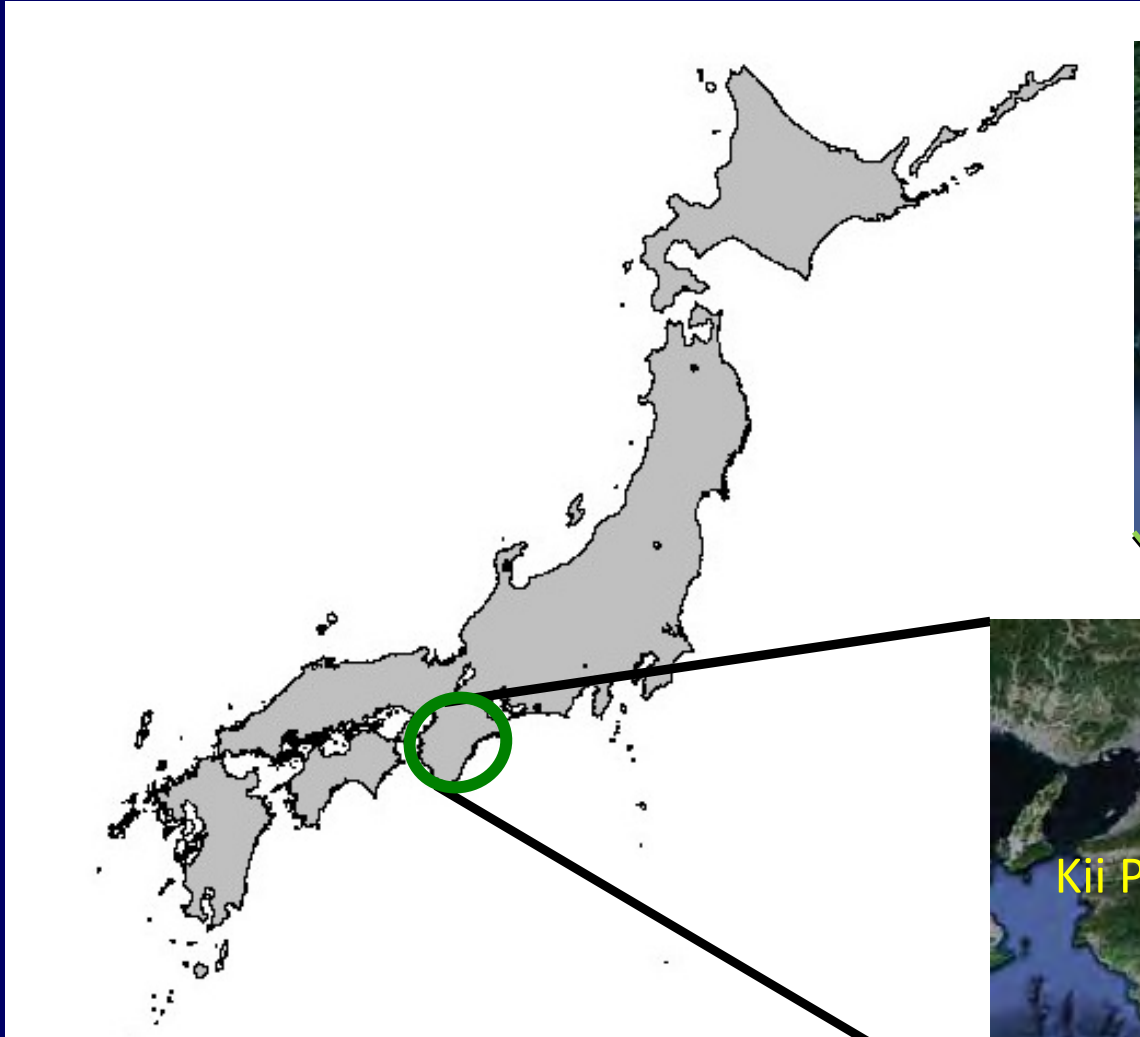
But no ecological studies on *Gambierdiscus* have yet been done in temperate regions.....

This study aimed to reveal the seasonal change in abundance of *Gambierdiscus* sp. in Ago Bay.

In addition, the effect of temperature on the growth of *Gambierdiscus* sp. isolated from the bay was also investigated in this study.



# Study site



# Seaweed bed



# Field works



Depth: 1 - 3 m

Sampling period: November 2008 - March 2011  
(once a month or more in warmer seasons)

Various macroalgal species were collected by snorkeling and sealed within plastic bags while underwater.

# Examples of macroalgae collected

## Chlorophyceae



*Codium fragile*

## Phaeophyceae



*Padinaarborescens*



*Sargassumthunbergii*

## Phodophyceae

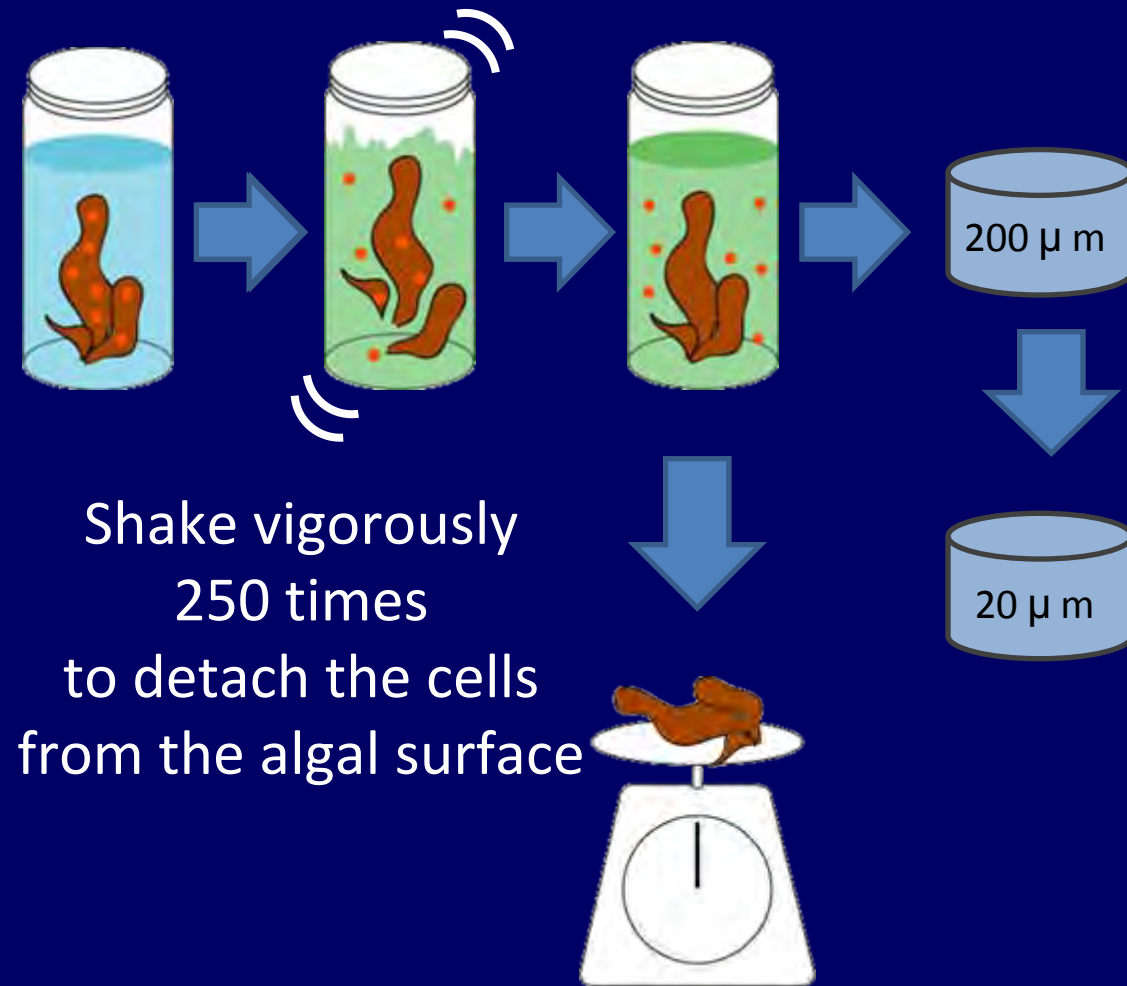


*Amphiroazonata*



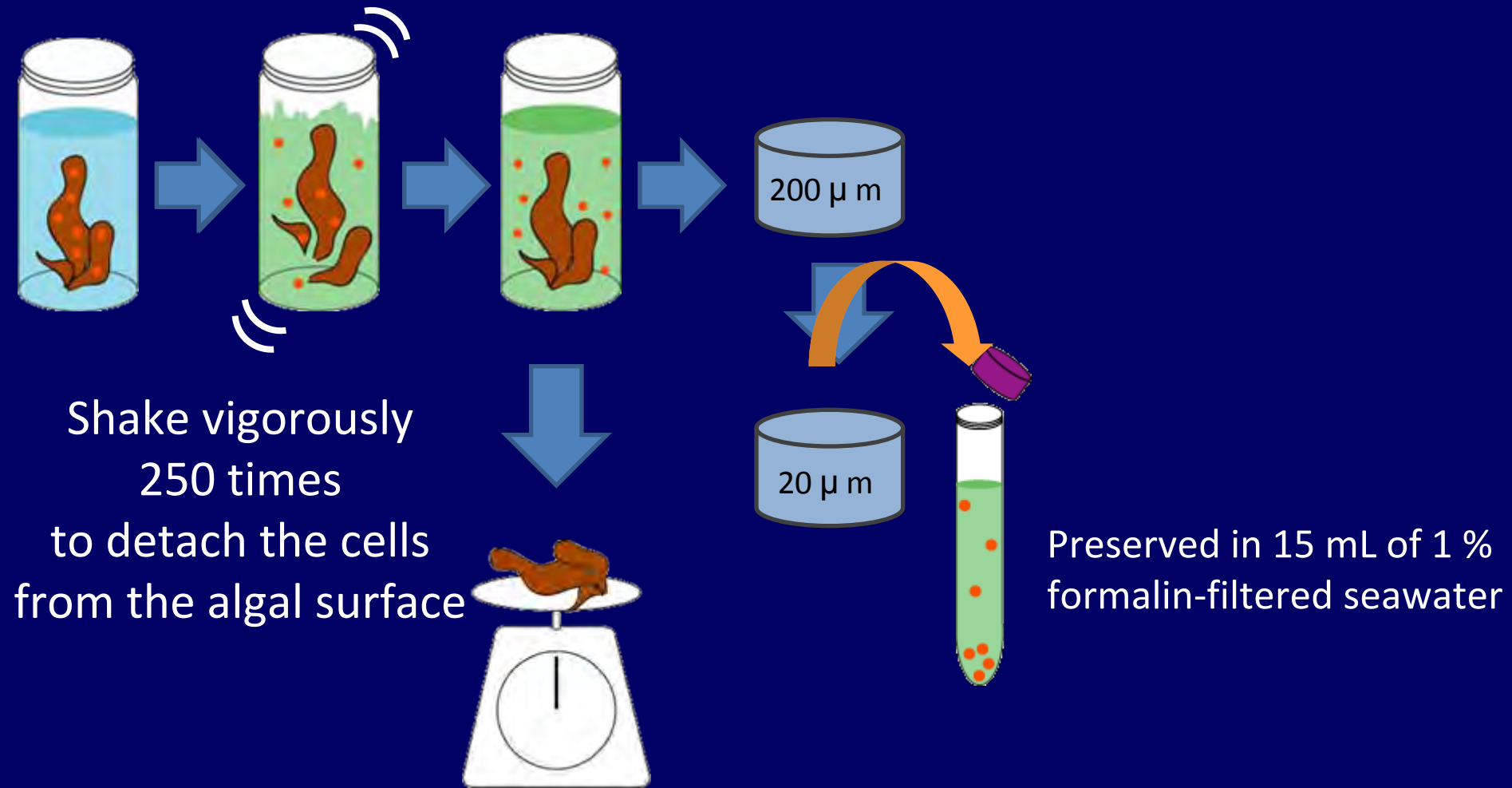
*Gelidiumelegans*

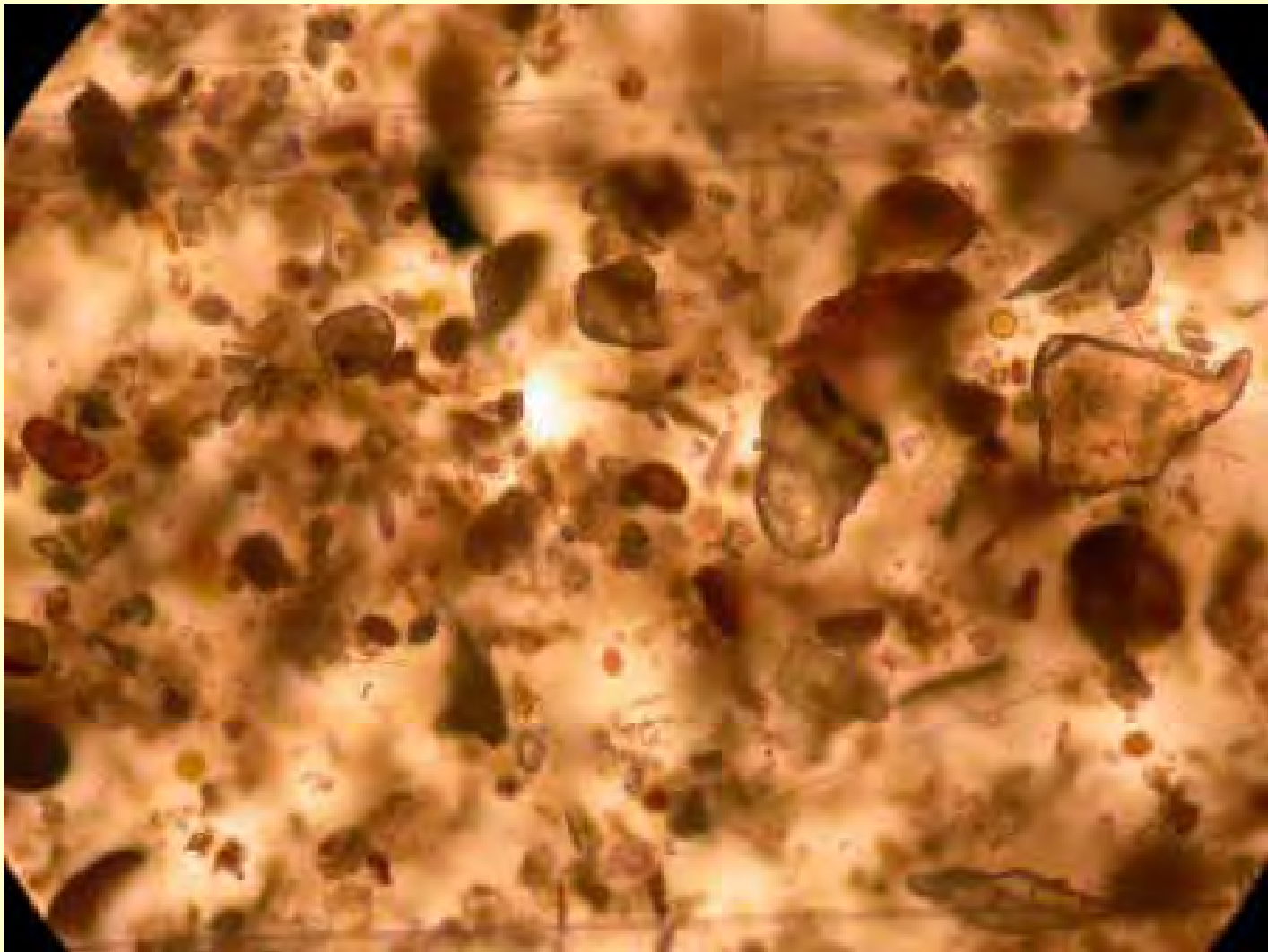
# Procedure to quantify *Gambierdiscus* sp.



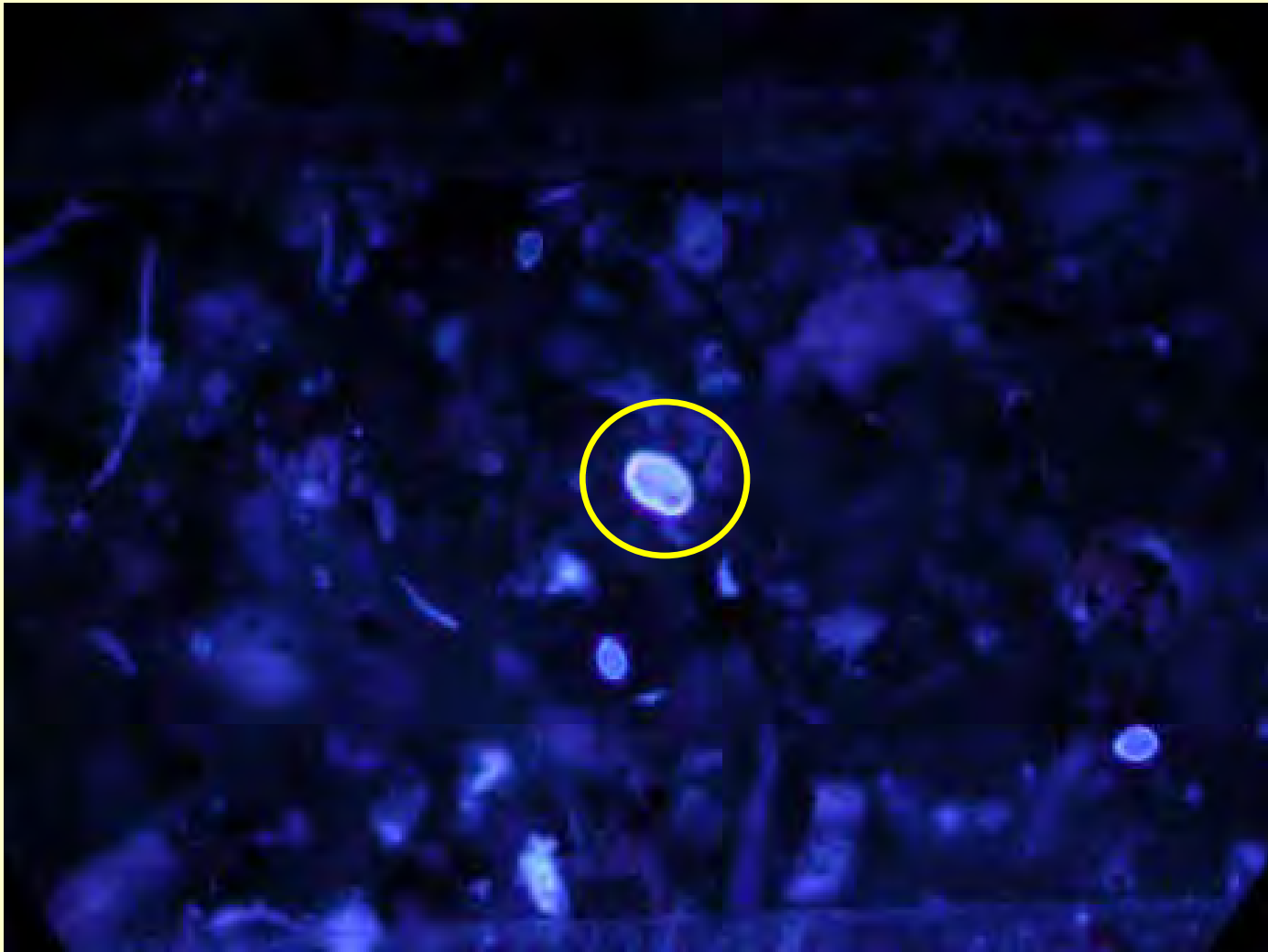


# Procedure to quantify *Gambierdiscus* sp.



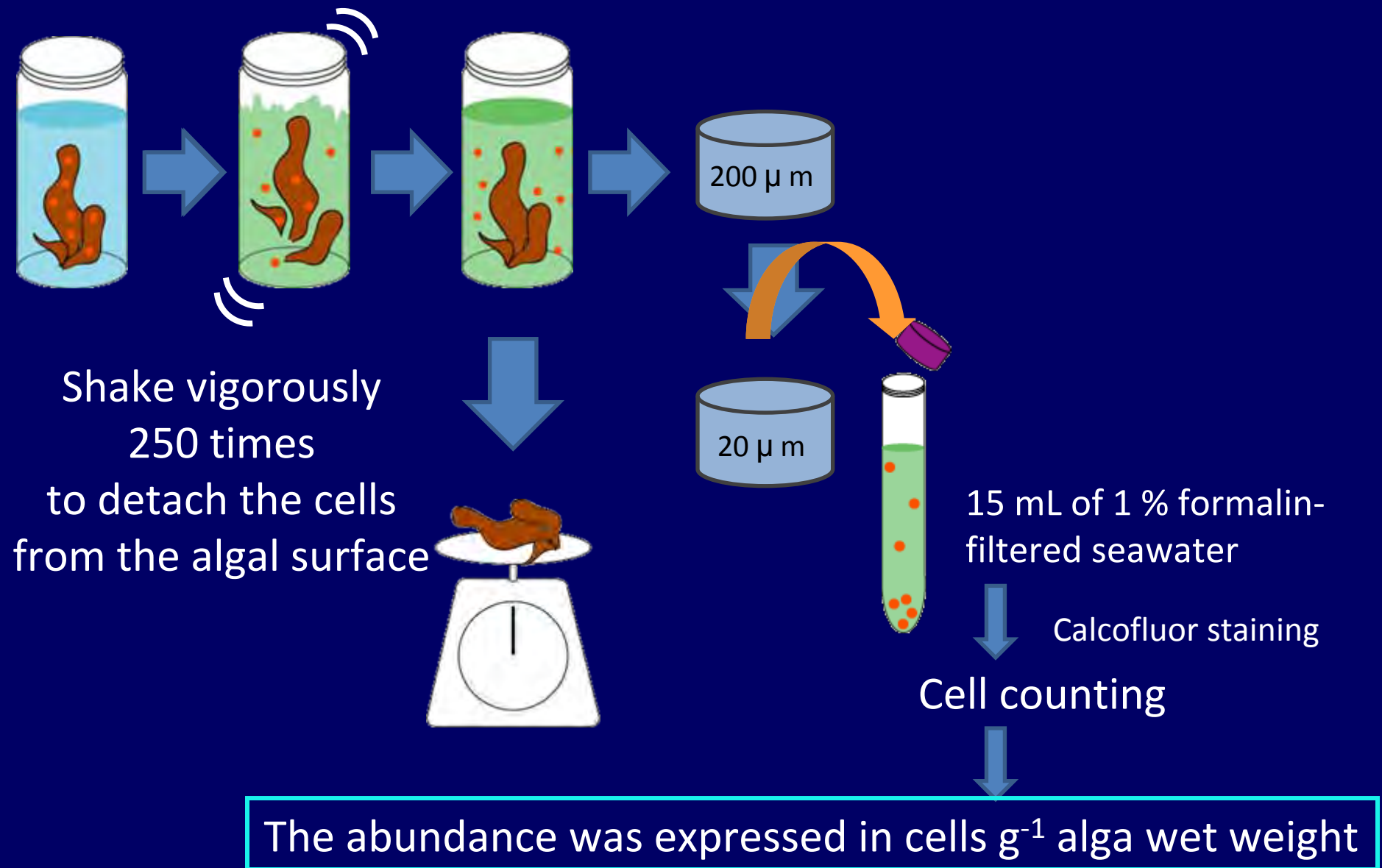


Under the light microscope



Under the epifluorescent microscope  
The cells were stained with calcofluor.

# Procedure to quantify *Gambierdiscus* sp.



# Growth experiments

## Effects of temperature on the growth

### Experimental conditions

Temperature : 10, 15, 20, 25, 30 , 32, 35

Medium : f/2

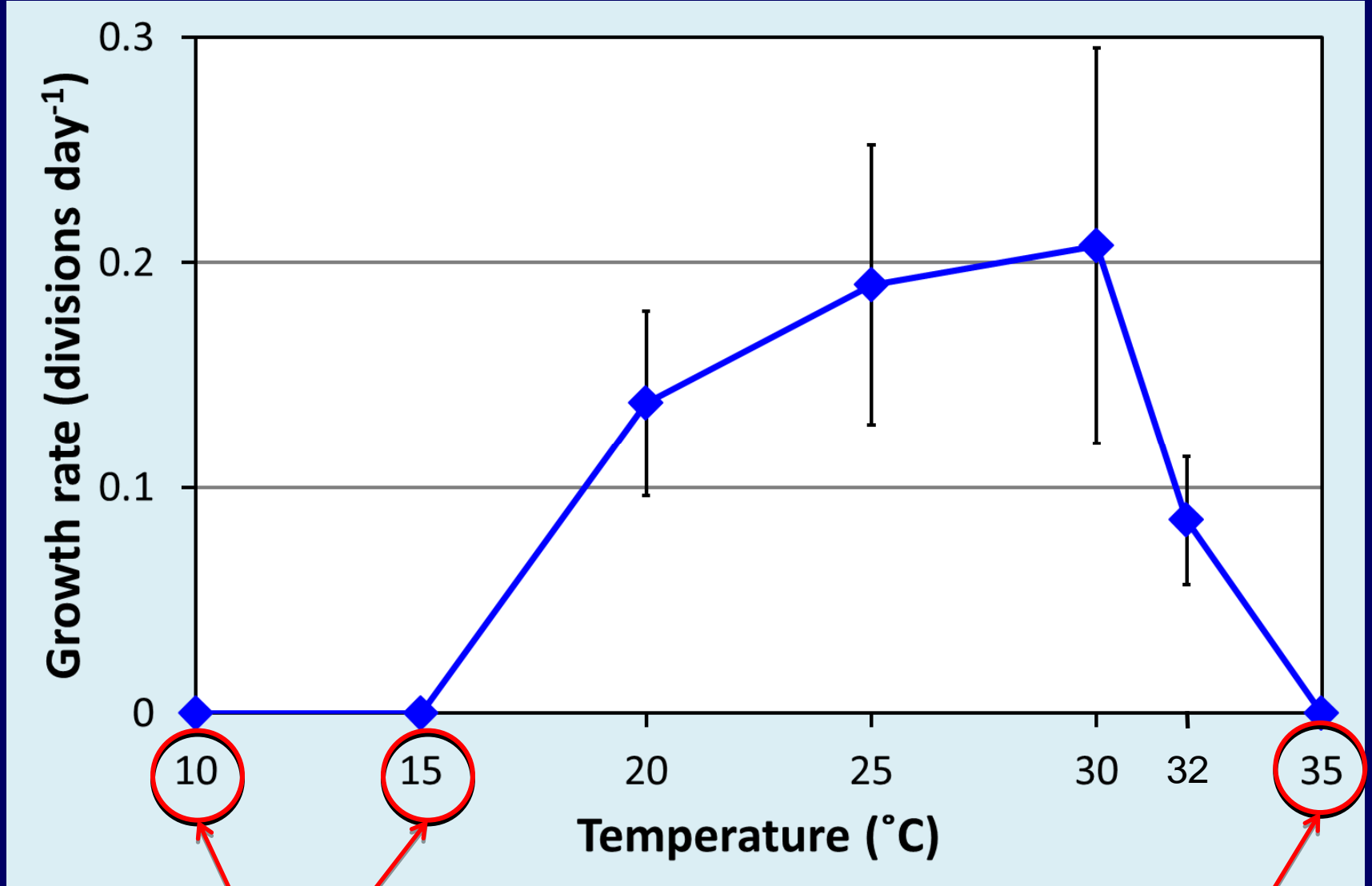
Light intensity :  $150 \mu \text{ mol m}^{-2} \text{ s}^{-1}$

Photo cycle : 12h:12h



Growth rate (divisions  $\text{day}^{-1}$ ) was calculated.

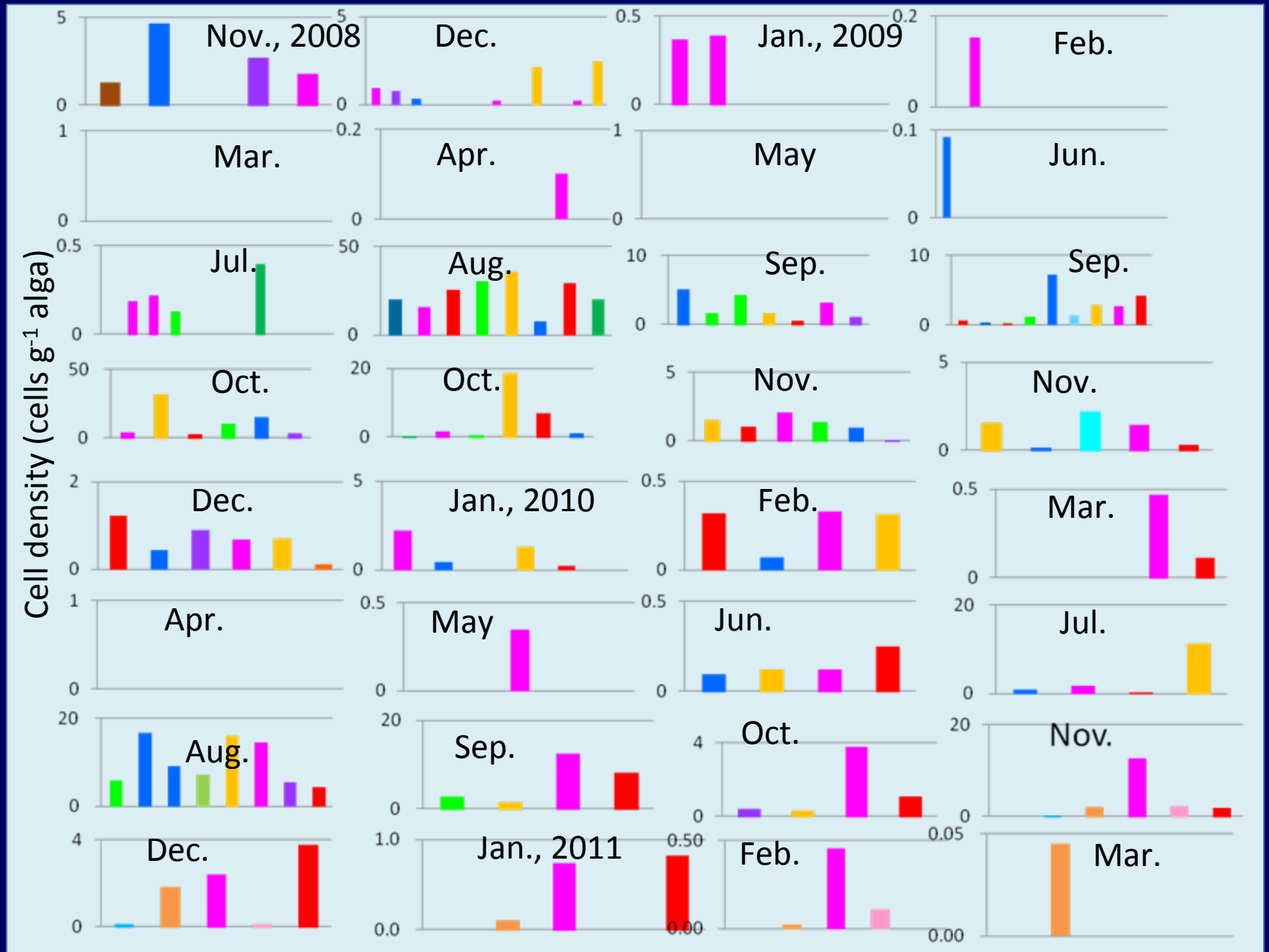
# Effect of temperature on the growth



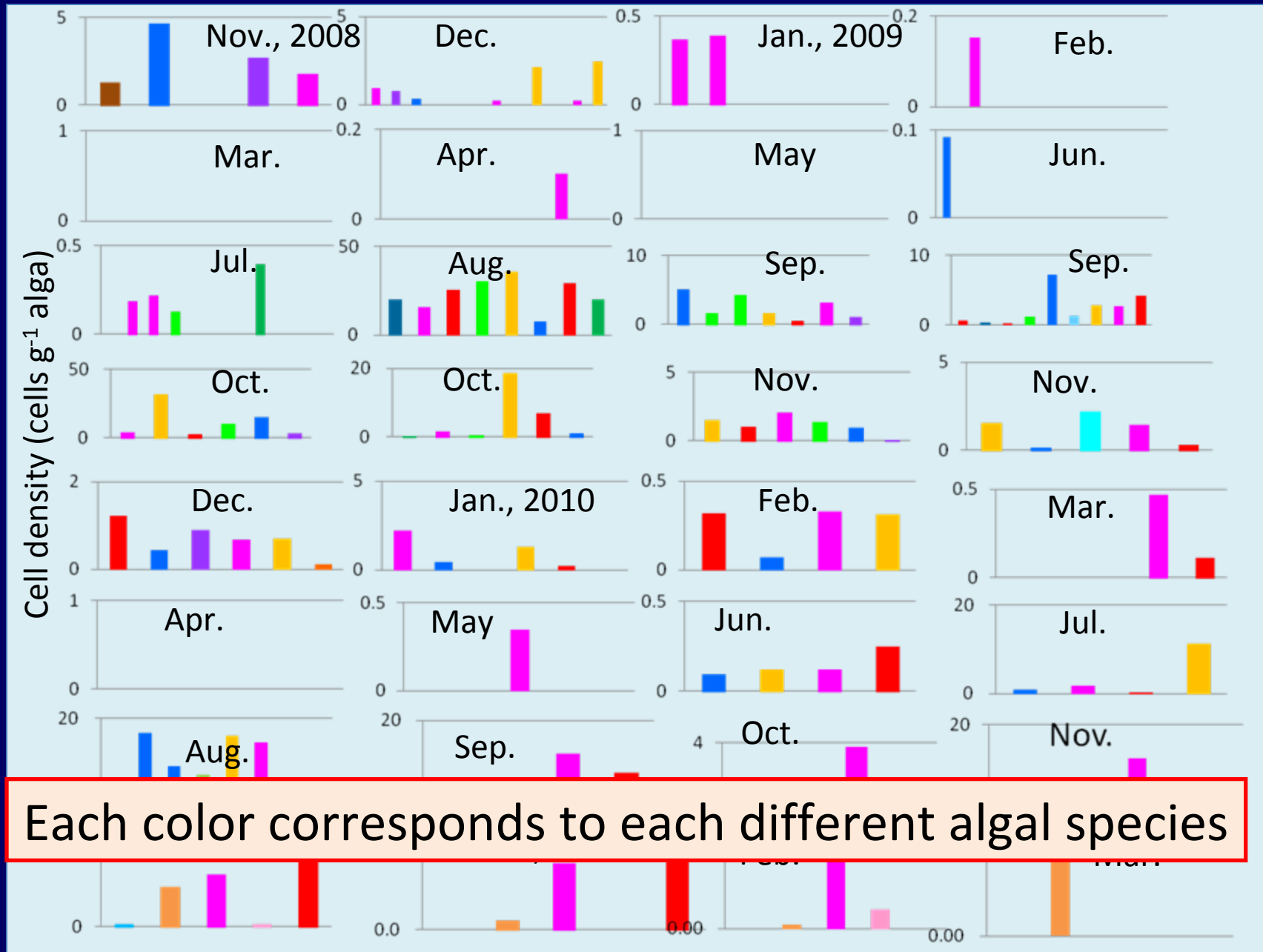
No growth but

No growth & dead

# Cell densities of *Gambierdiscus* sp. attached to each different macroalga

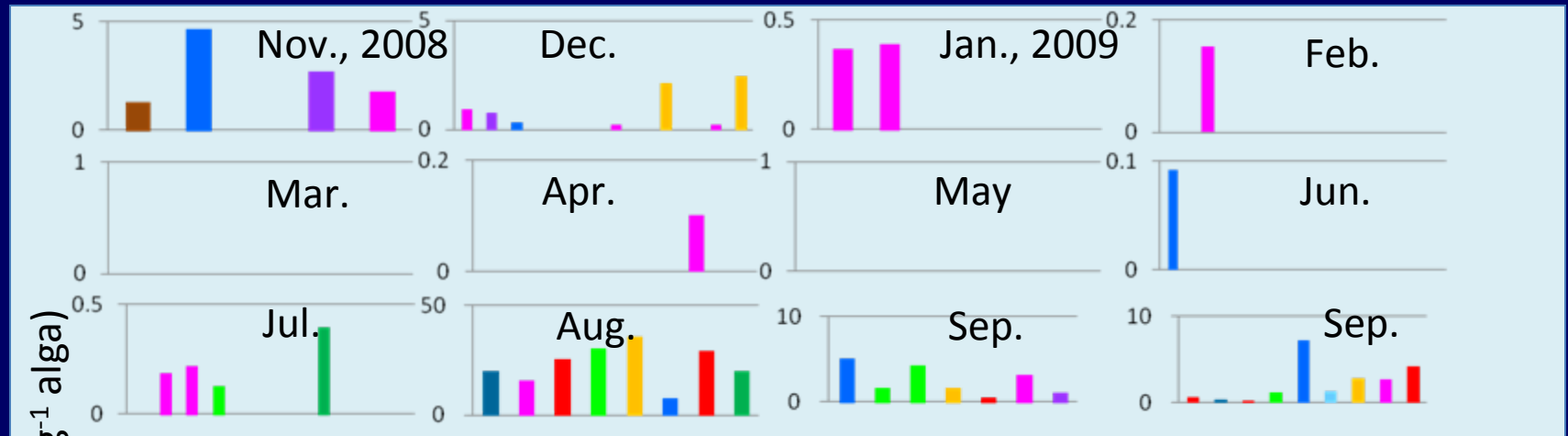


# Cell densities of *Gambierdiscus* sp. attached to each different macroalga

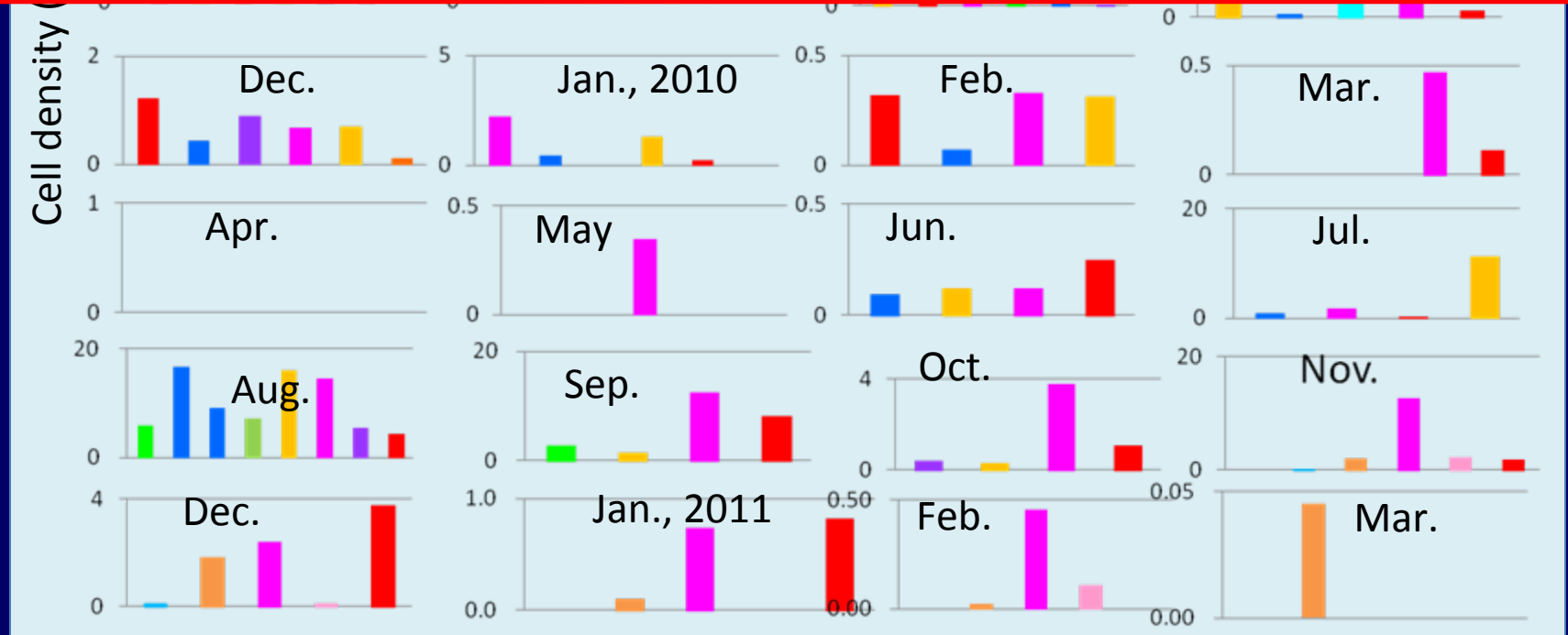




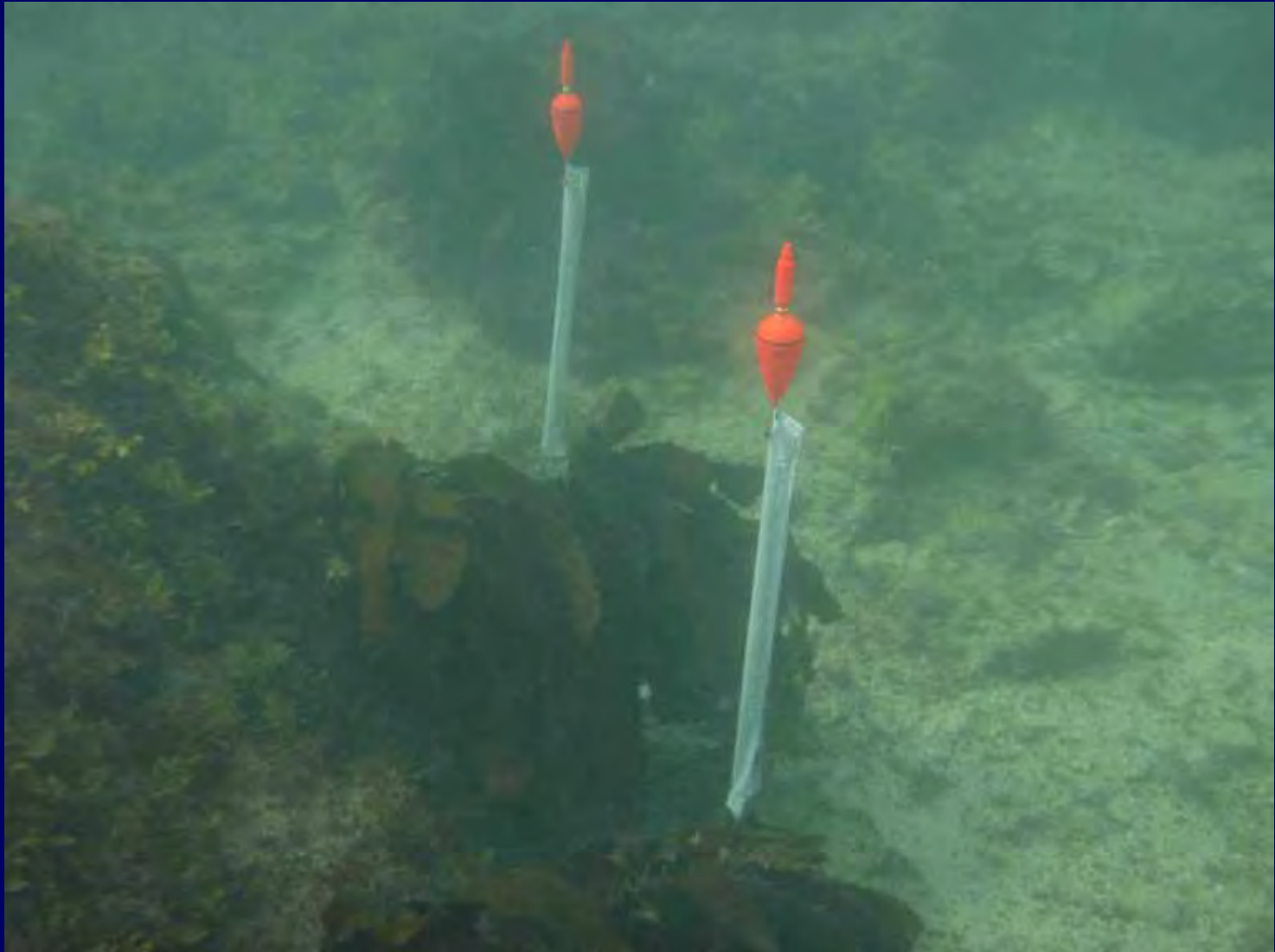
# Cell densities of *Gambierdiscus* sp. attached to each different macroalga



No preference of *Gambierdiscus* sp. to attach to macroalga

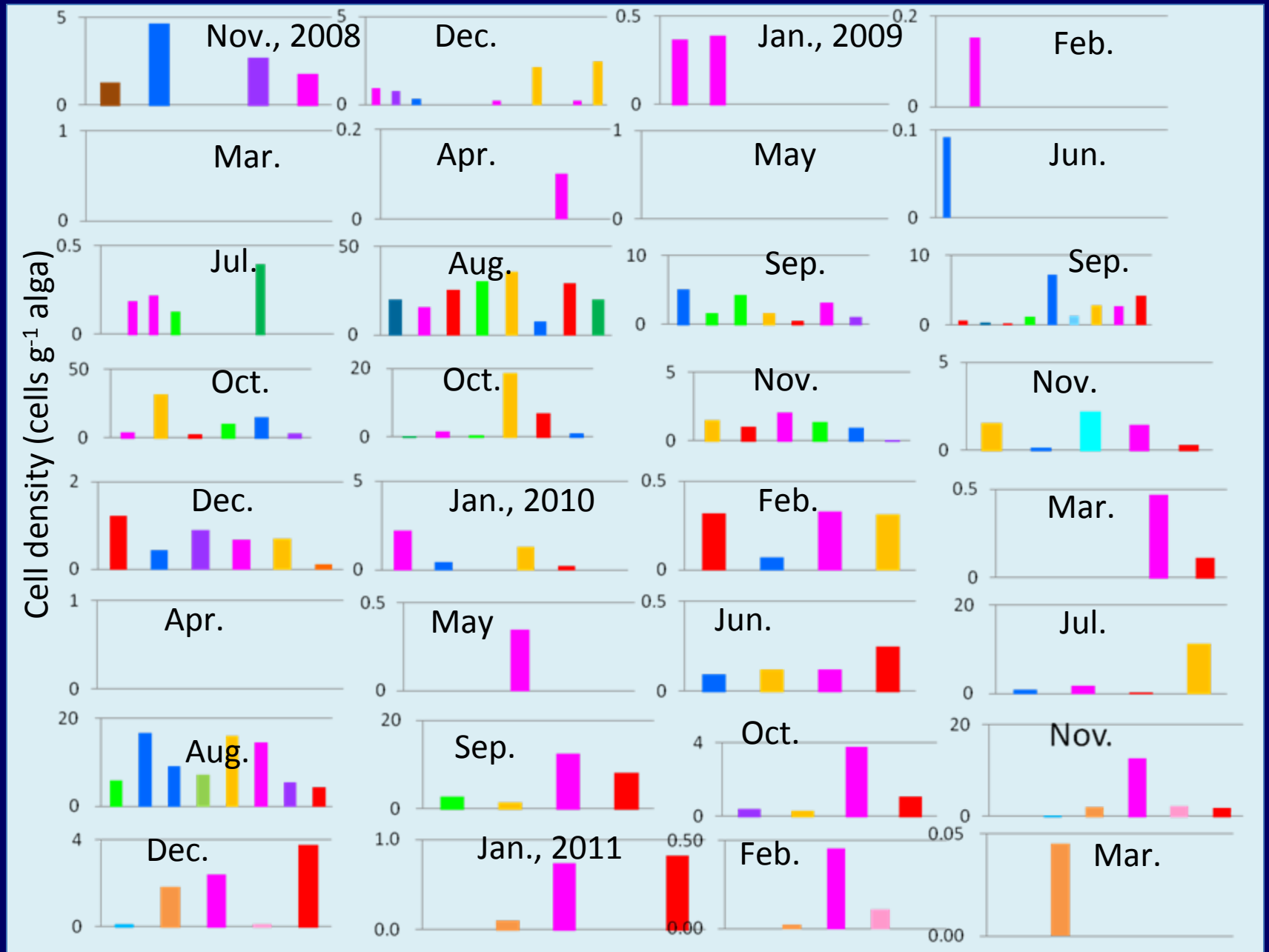


# Artificial seaweed experiment

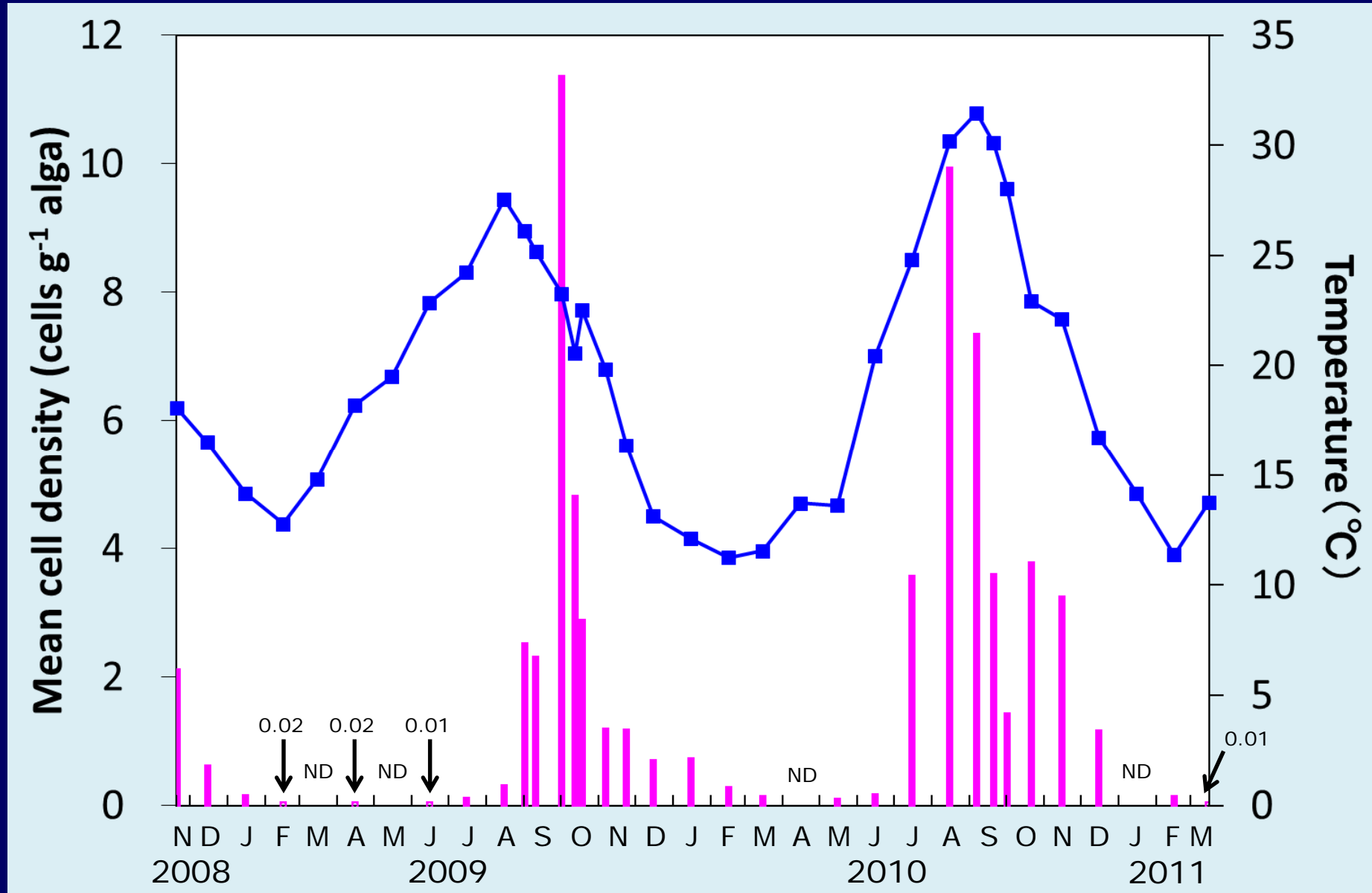


Cloth (Cotton: 65% , Chemical fiber: 35%)

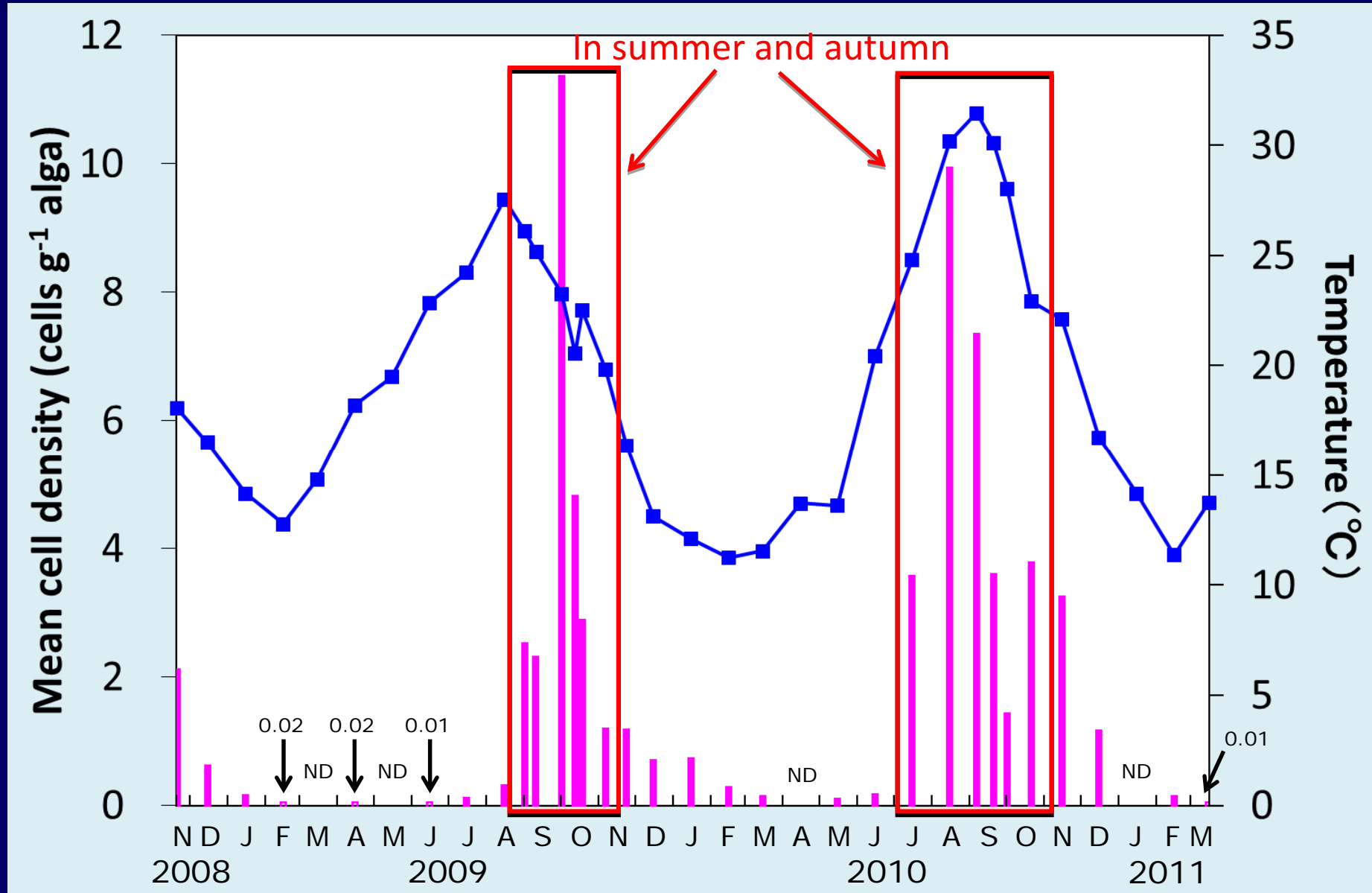
# Cell densities of *Gambierdiscus* sp. attached to each different macroalga



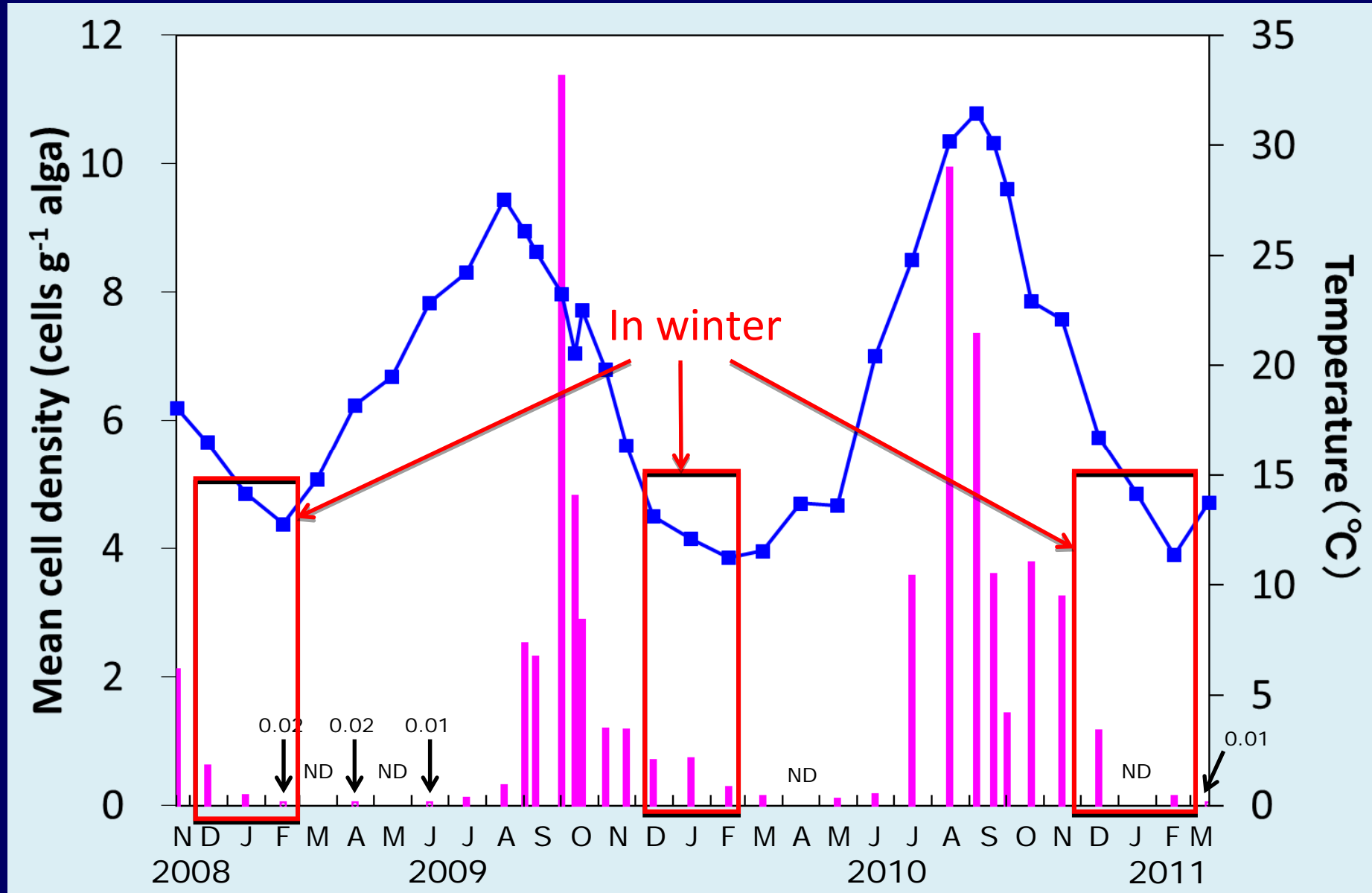
# Seasonal change in mean abundance of *Gambierdiscus* sp. attached to the macroalgae



# Seasonal change in mean abundance of *Gambierdiscus* sp. attached to the macroalgae

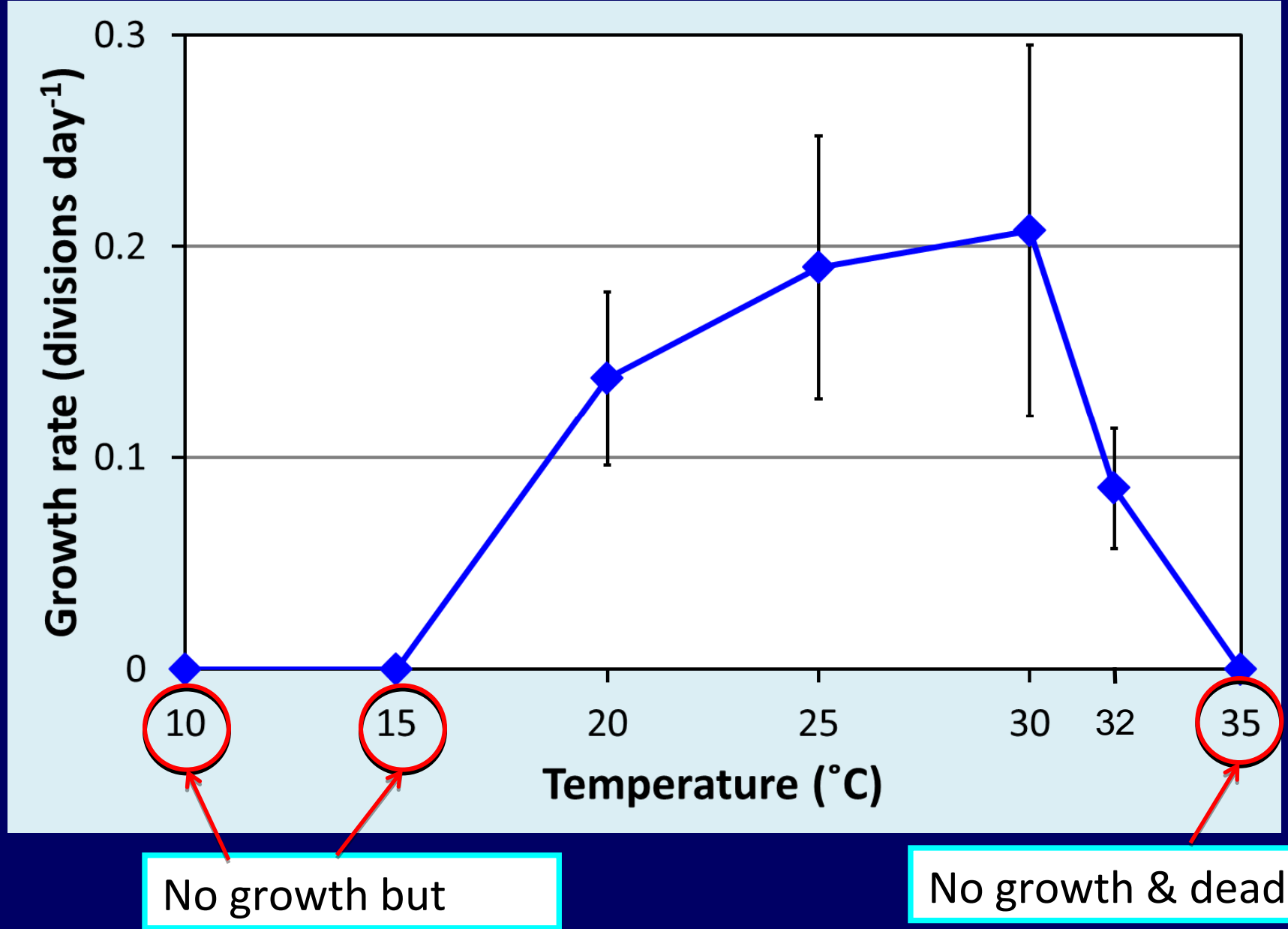


# Seasonal change in mean abundance of *Gambierdiscus* sp. attached to the macroalgae

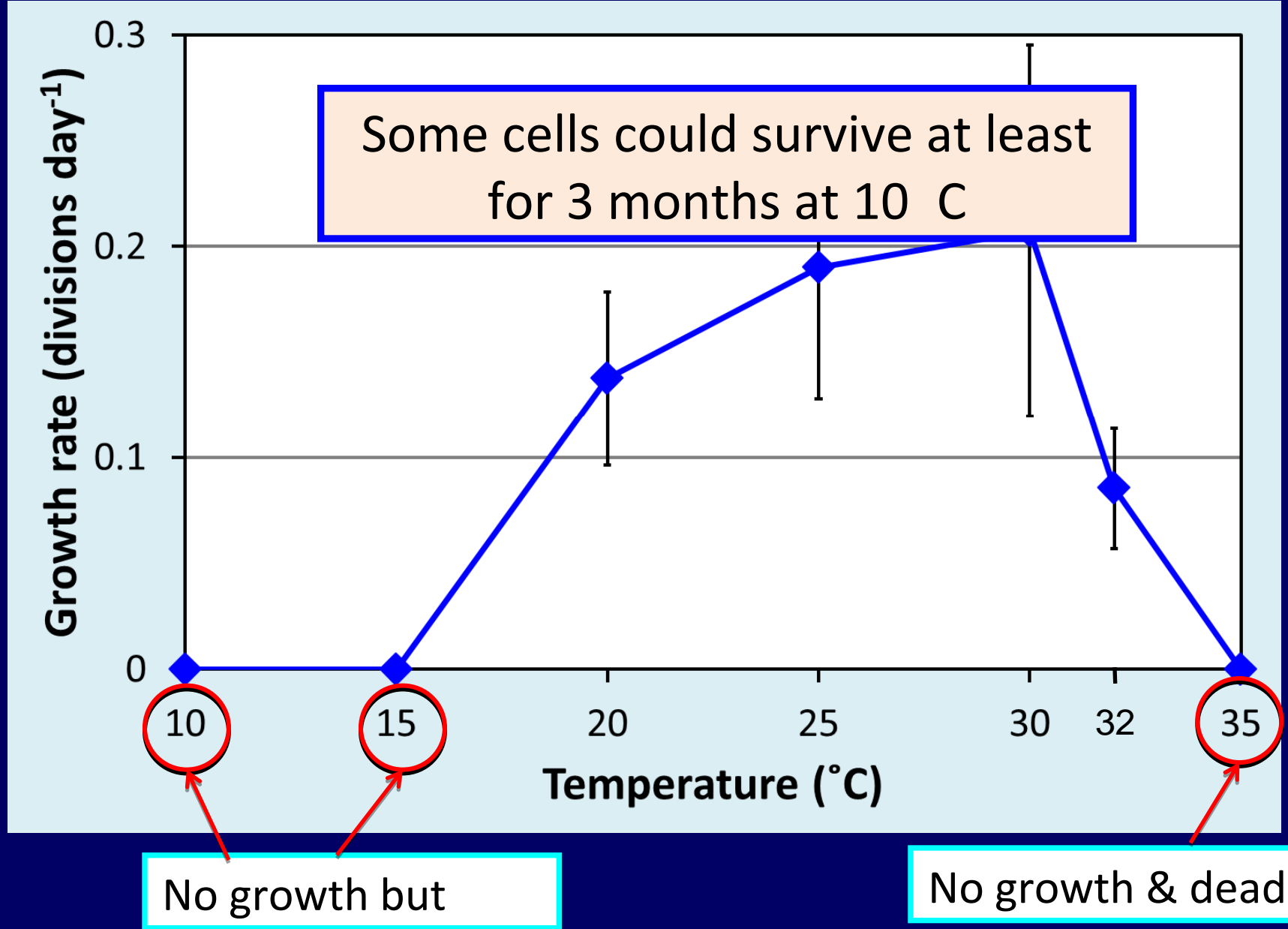


ND: Not detected

# Effect of temperature on the growth

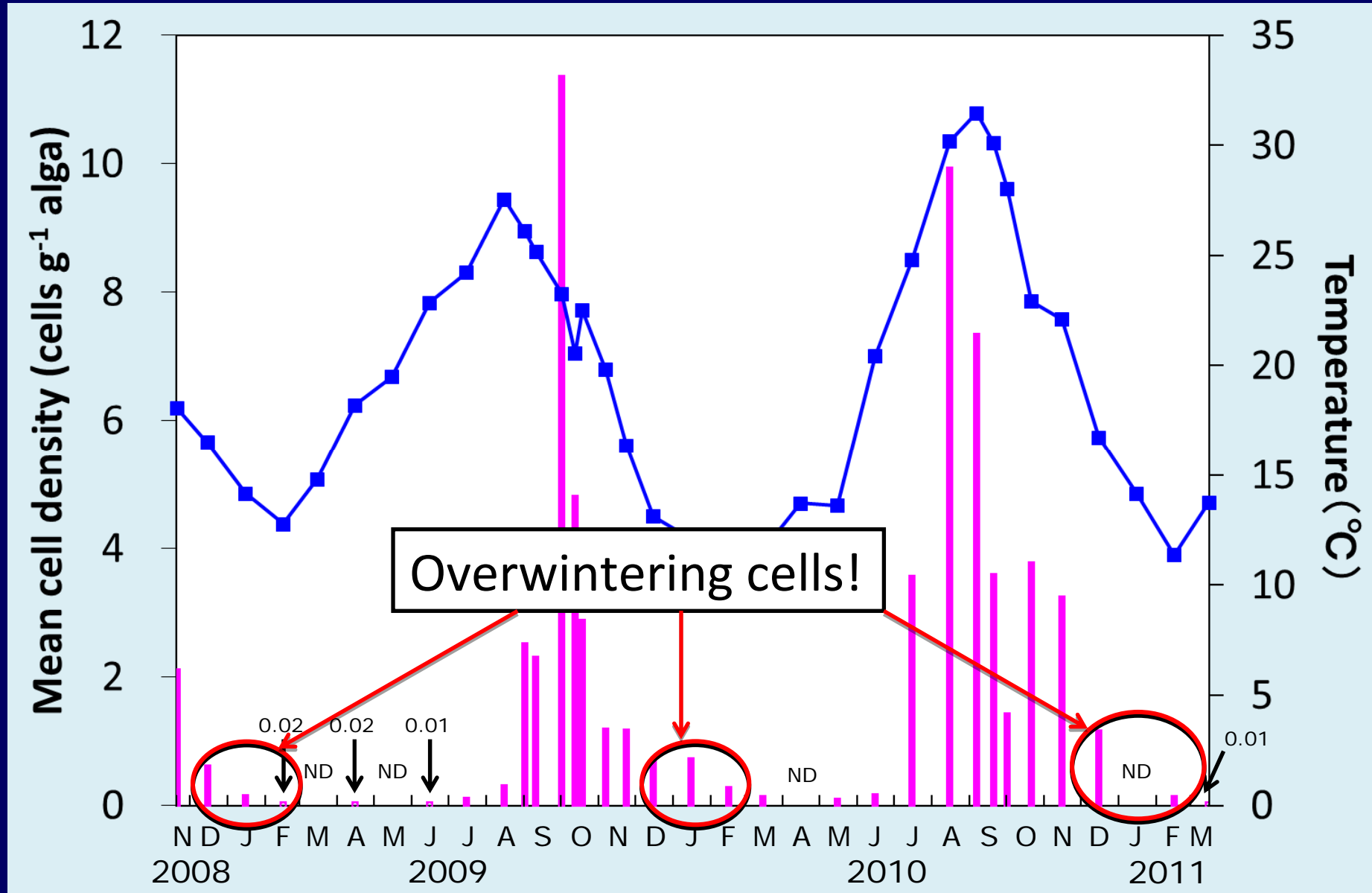


# Effect of temperature on the growth

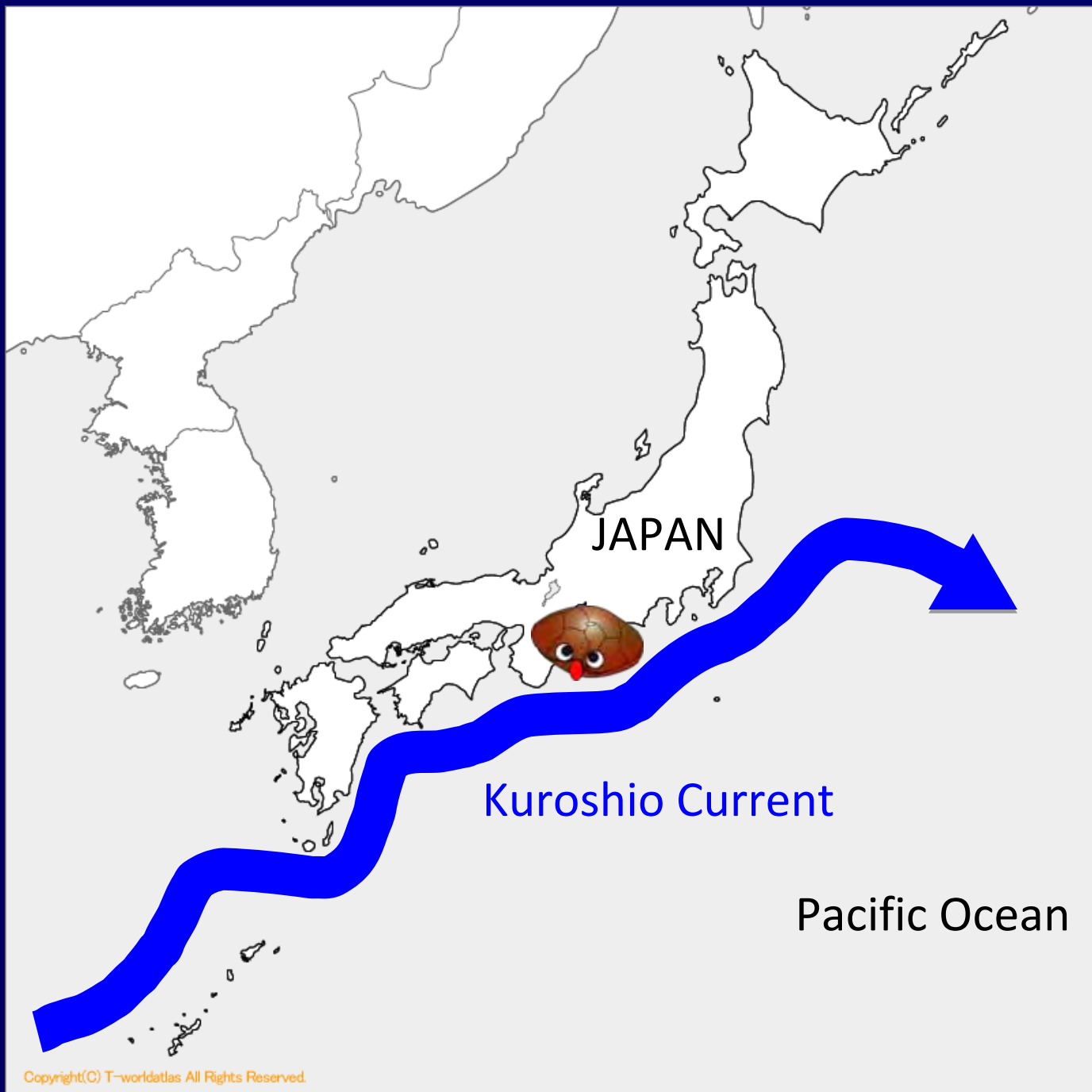




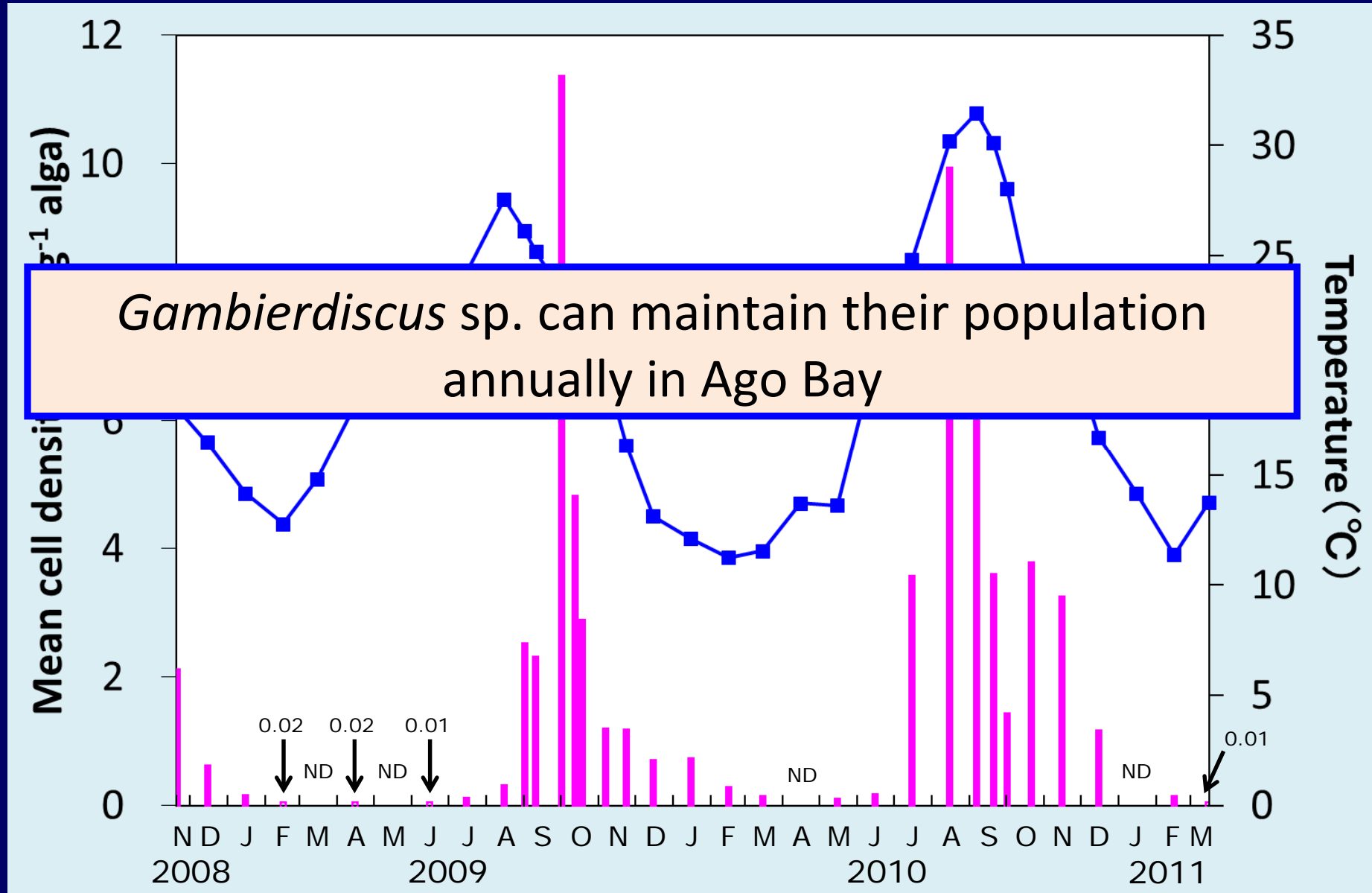
# Seasonal change in mean abundance of *Gambierdiscus* sp. attached to the macroalgae



ND: Not detected



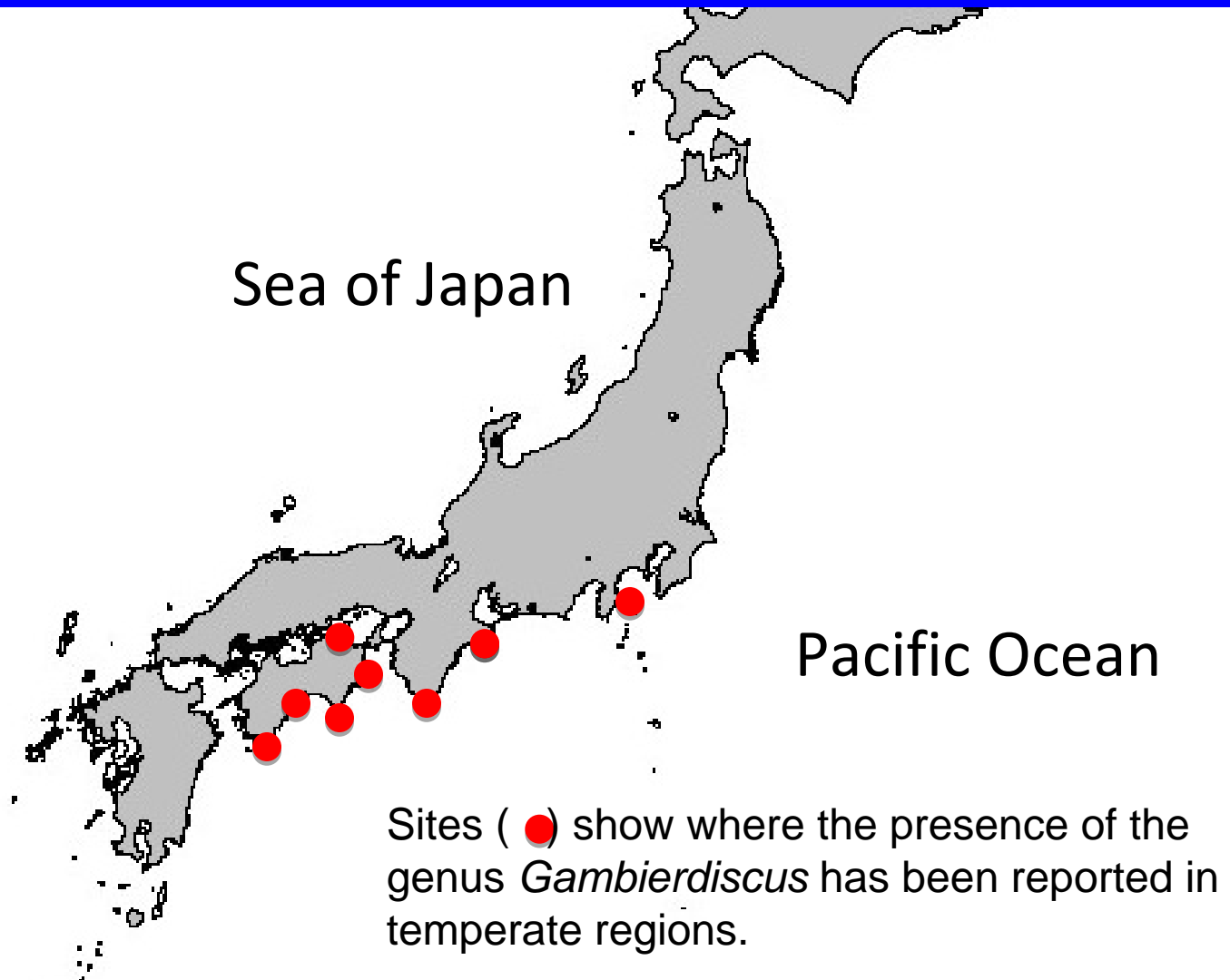
# Seasonal change in mean abundance of *Gambierdiscus* sp. attached to the macroalgae

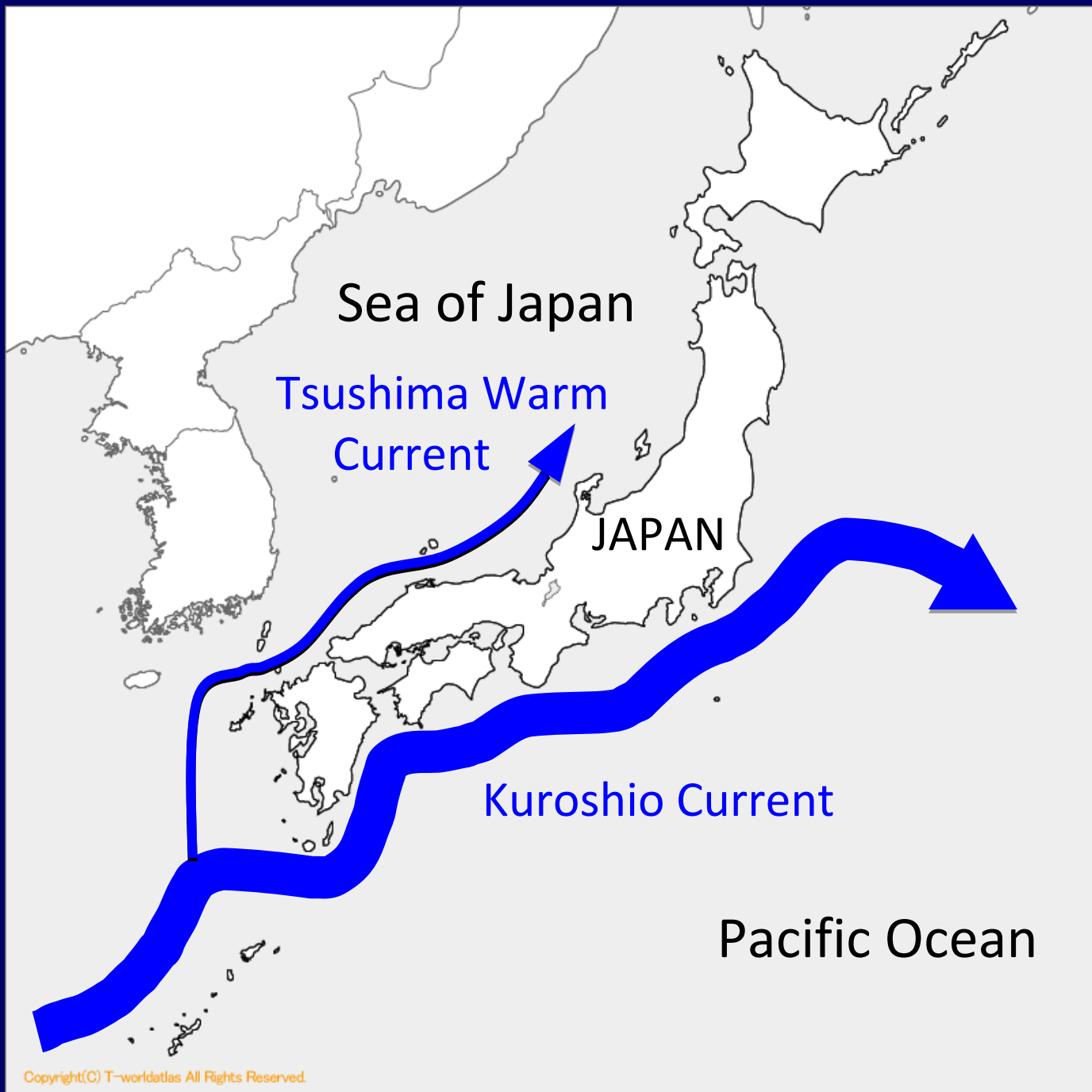


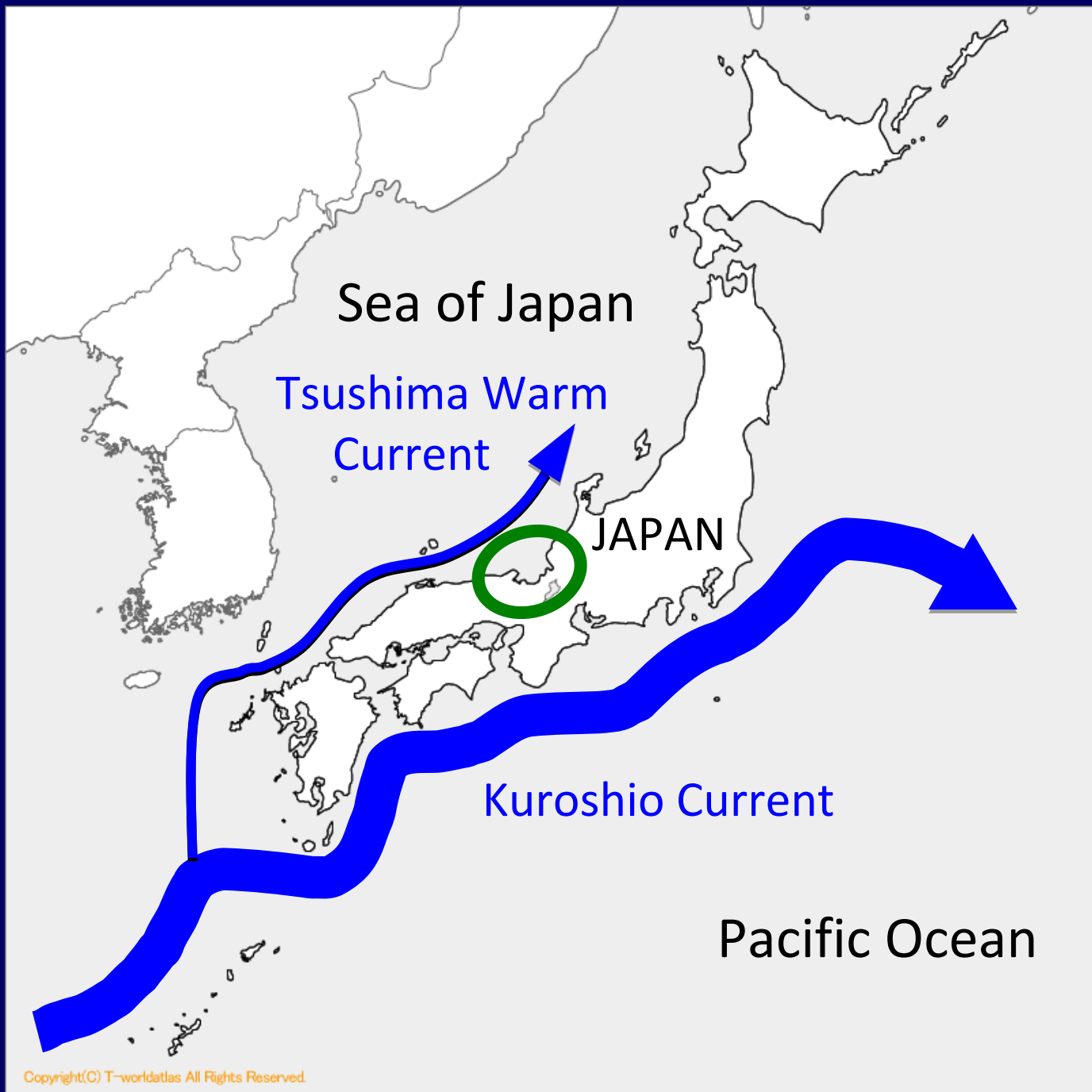
ND: Not detected

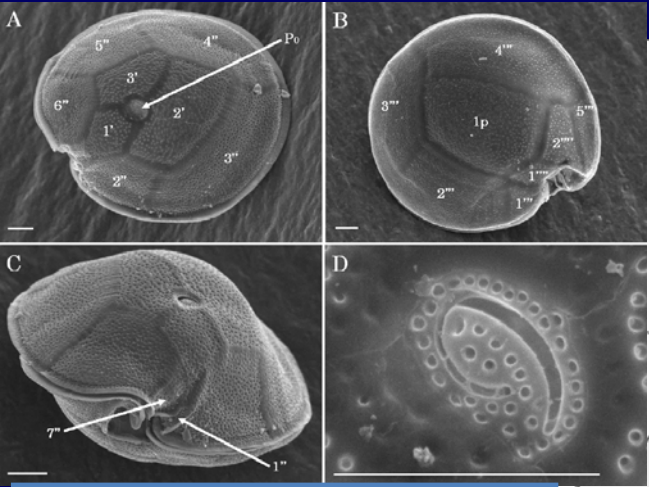


No reports of the presence of *Gambierdiscus*  
from the coast in Sea of Japan

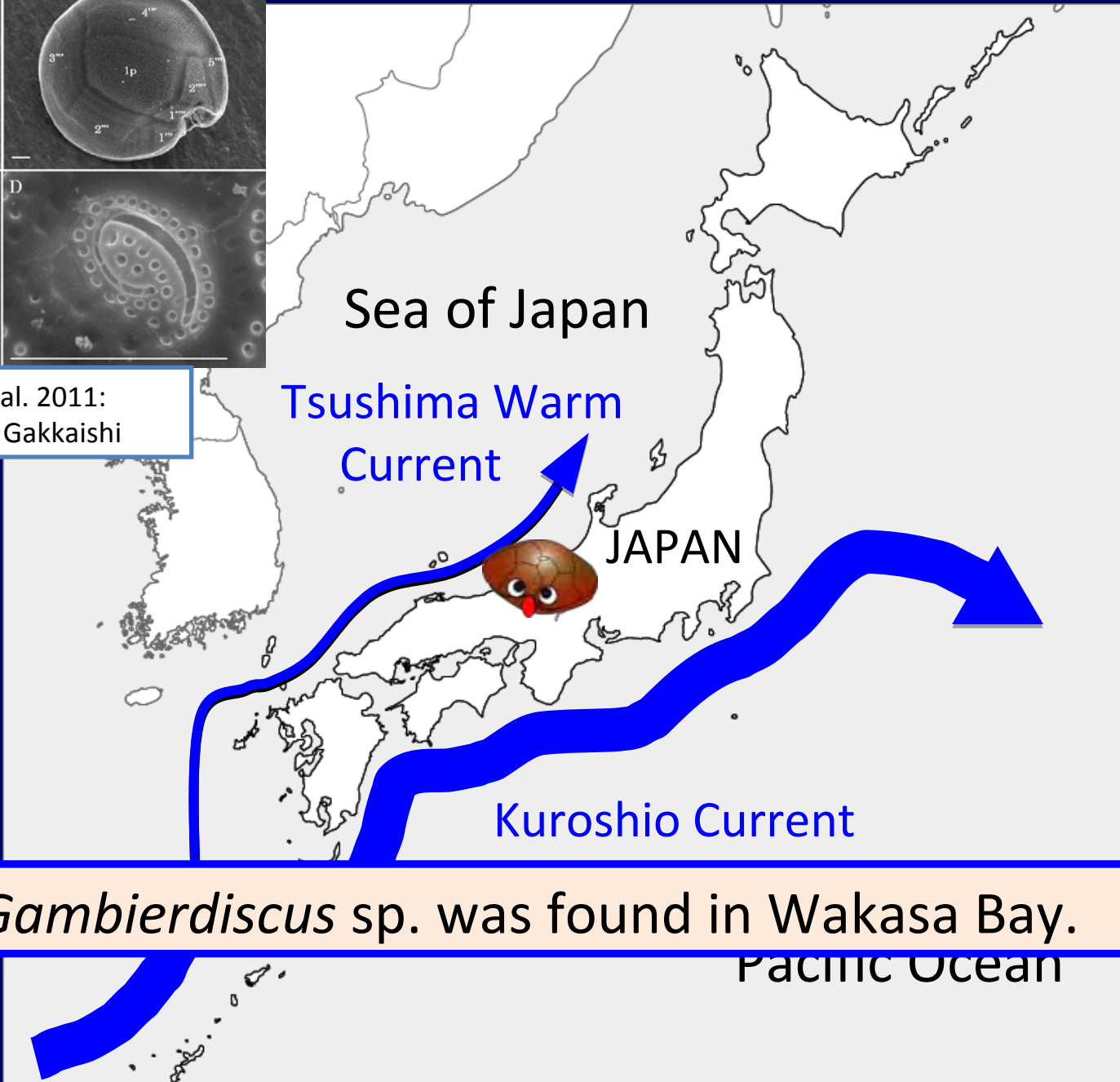






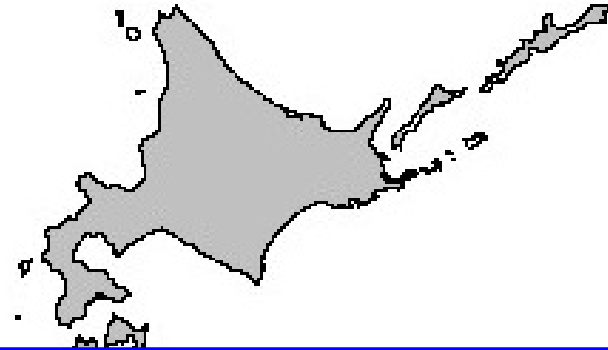


Hatayama et al. 2011:  
Nippon Suisan Gakkaishi

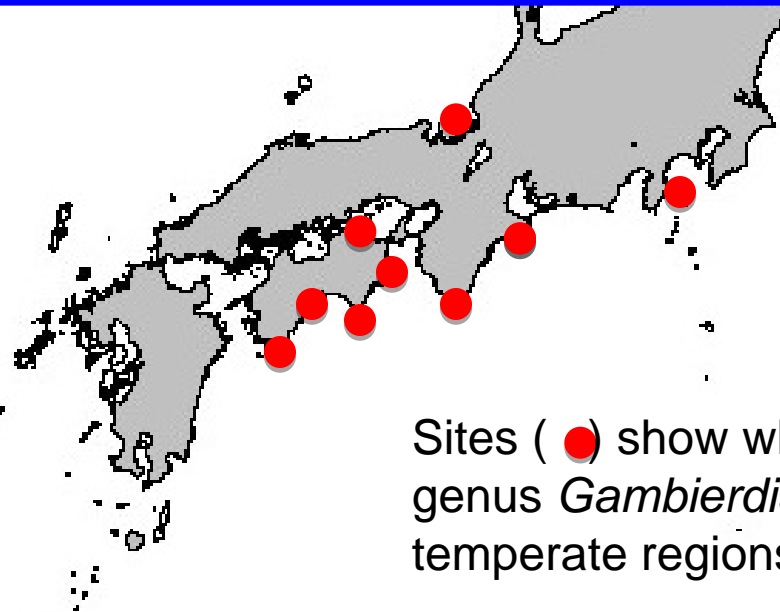


*Gambierdiscus* sp. was found in Wakasa Bay.

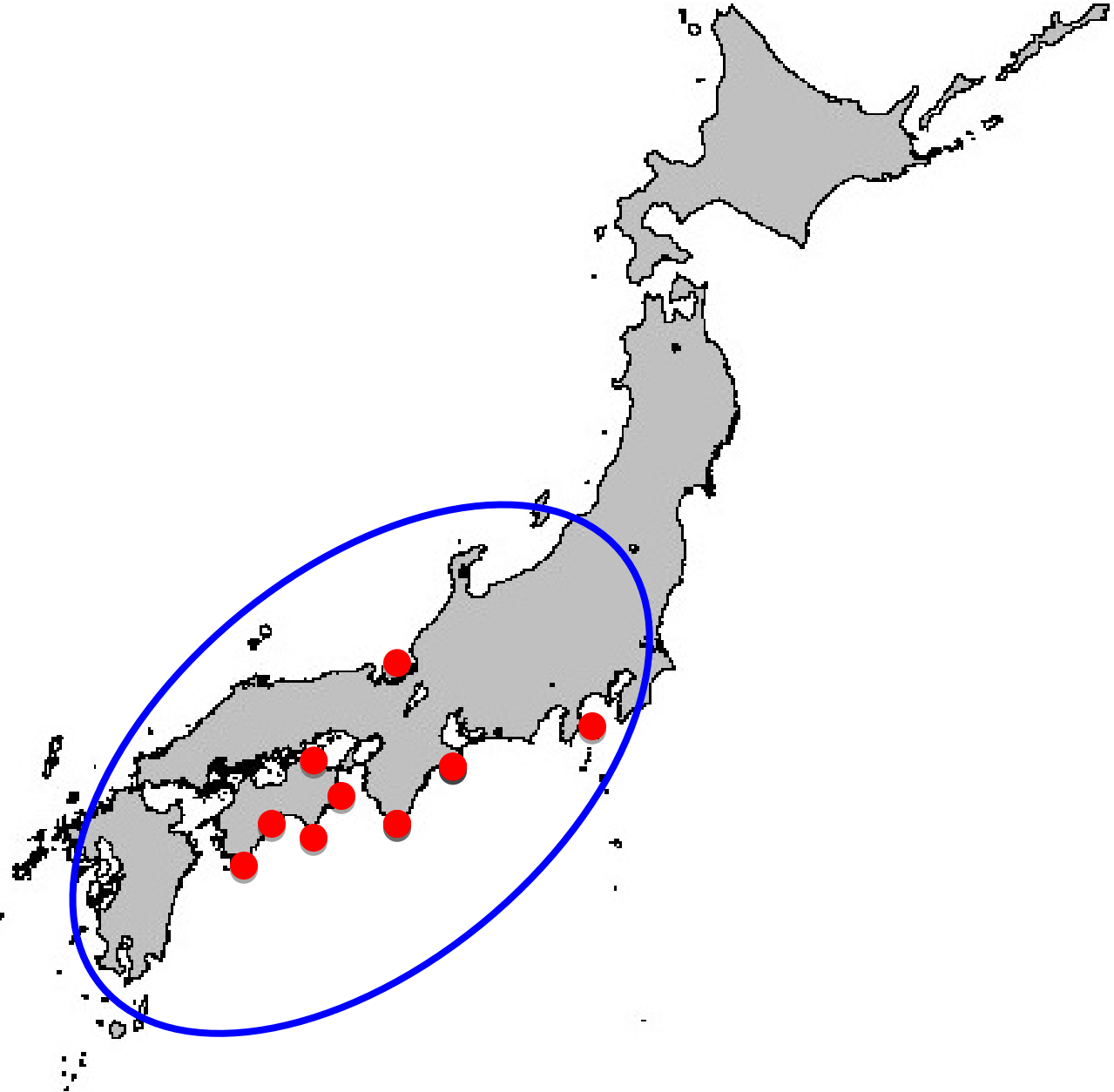




*Gambierdiscus* sp. (spp.) may build autochthonous populations widely along the Japanese coast in temperate regions

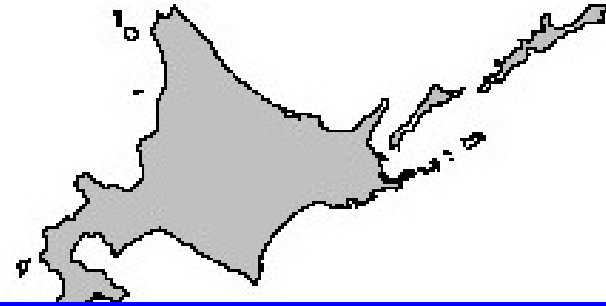


Sites ( ● ) show where the presence of the genus *Gambierdiscus* has been reported in temperate regions.

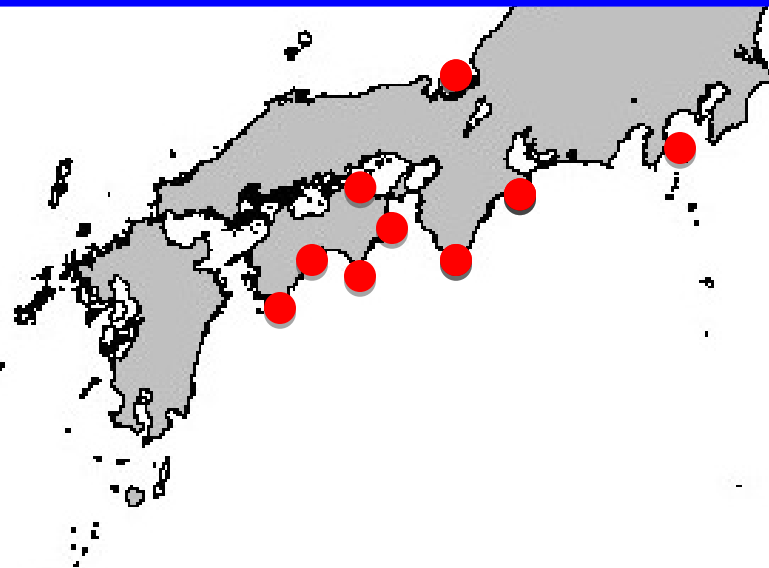




First report of occurrence  
from Shimoda  
(Hara & Horiguchi 1982)



Increase of water temperature would induce *Gambierdiscus* sp. (spp.) to build larger populations in temperate regions and also spread their distribution toward north



# Maximum cell density of *Gambiediscus* sp. in Ago Bay



*Sargassum thunbergii* (Phaeophyceae)

October 1<sup>st</sup>, 2009

32 cells g<sup>-1</sup> alga

## *Gambierdiscus* spp. abundance in other regions

Regions	Maximum cell density (cells g <sup>-1</sup> )	References
Hawai (USA)	823	Shimizu et al. 1982
Florida (USA)	8,191	Bomber et al. 1989
Virgin Islands (Puerto Rico)	75,793	Carlson and Tindall 1985
Mayotte (Mozambique channel)	60,463	Turquett et. al. 2001
Queensland (Australia)	2,180	Gillespie et al. 1985
Great Barrier Reef (Australia)	250	Heil et al. 1998
Tahiti (French Polynesia)	10,880	Chinain et al. 1999
<b>JAPAN</b> <b>Ago Bay, Mie</b>	<b>32</b>	<b>This study</b>

## *Gambierdiscus* spp. abundance in other regions

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Queensland (Australia)	2,180	Gillespie et al. 1985

The maximum cell density in Ago Bay is comparable to those recorded in Okinawa Islands located in subtropical part of Japan, where CFP have often been reported.

### JAPAN

**Ago Bay, Mie**

**32**

**This study**

Kerama Islands, Okinawa

51

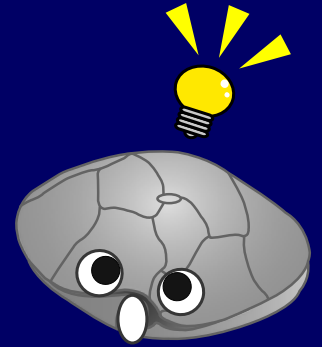
Koike et al. 1991

Nago, Okinawa

10

Ishikawa, unpublished.

NOW



Further studies on ecology and physiology, including toxicology, of *Gambierdiscus* sp. are certainly needed to evaluate future risk of CFP, even in temperate waters of Japan.