

MONITORING OF THE SHELLFISH-KILLING
DINOFLAGELLATE
HETEROCAPSA CIRCULARISQUAMA IN JAPANESE
COASTAL SEA BY INDIRECT FLUORESCENT ANTIBODY
TECHNIQUE

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What is *Heterocapsa circularisquama* ?

Class DINOPHYCEAE
Order Peridiniales
Family Peridineaceae



- Its red tides cause mass mortalities of bivalves.
- This species grows well at high temperatures (optimum 30°C) .
- No growth at 10°C or below.
- Temporary cyst formation under bad conditions.

Mass mortality of short-necked-clam in an intertidal flat



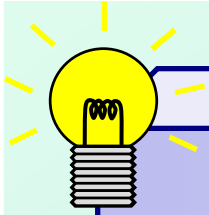
Excerpted from the web site of National Research
Institute of Fisheries and Environment of Inland
Sea ,Fisheries Research Agency

Problem

The population dynamics has not yet
been clarified
(difficulty in monitoring)

Reason

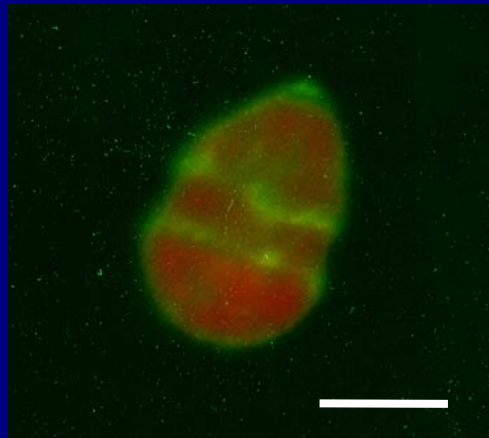
- Small size (ca. $20 \mu\text{m}$ length)
- Presence of morphologically similar species



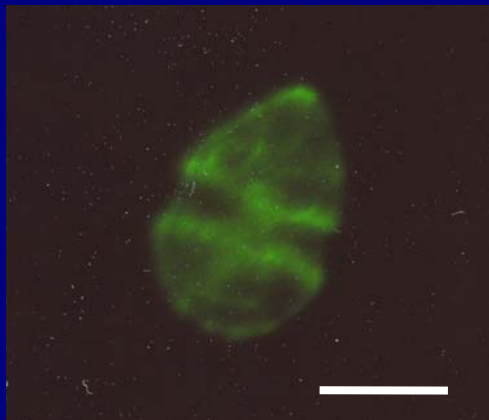
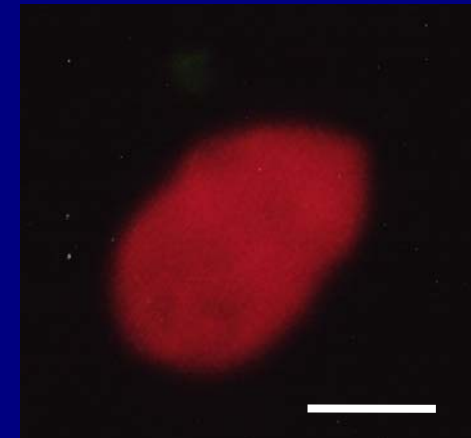
Monitoring by the indirect fluorescent antibody technique using monoclonal antibody

Monitoring of the population dynamics throughout the year in Ago Bay, the red tide area, Mie Prefecture, Japan.

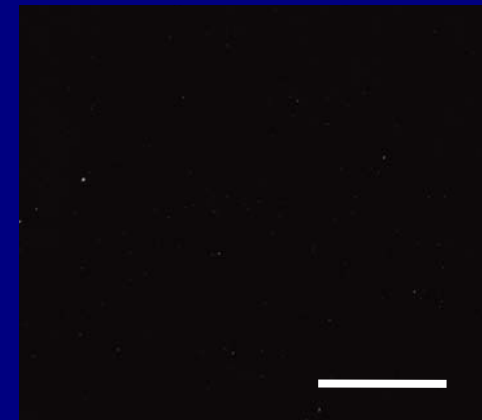
Epifluorescence photomicrographs of *H. circularisquama* and *H. triquetra* treated with the indirect fluorescent antibody technique (Scale Bar, 10 μ m)



Blue light
excitation



Blocking of chlorophyll
autofluorescence



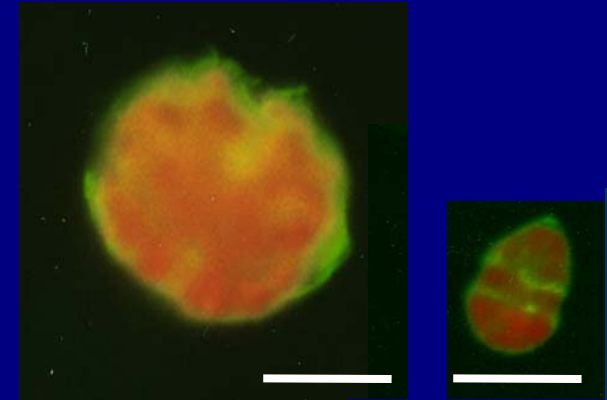
H. circularisquama

H. triquetra

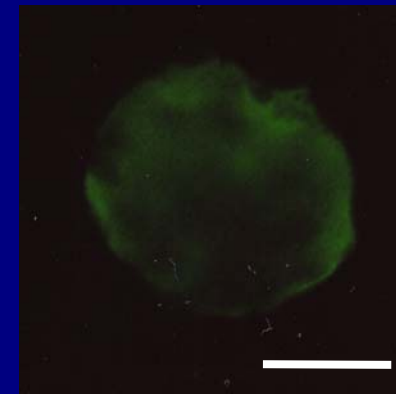
Reactivity of the monoclonal antibody

Species	Reactivity
<i>H. circularisquama</i> HU9433	+
<i>H. circularisquama</i> HU9436	+
<i>H. circularisquama</i> HA92-1	+
<i>H. circularisquama</i> HI9428	+
<i>H. circularisquama</i> HY9423	+
<i>H. triquetra</i>	-
<i>Scrippsiella trochoidea</i>	-
<i>Alexandrium catenella</i> TN-11	+
<i>Alexandrium catenella</i> OF-072	+
<i>Karenia mikimotoi</i>	-
<i>Chattonella antiqua</i>	-
<i>C. marina</i>	-
<i>Heterosigma akashiwo</i>	-
<i>Fibrocapsa japonica</i>	-
<i>Ditylum brightwellii</i>	-
<i>Oltmannsiellopsis viridis</i>	-
<i>Eutreptiella gymnastica</i>	-

Alexandrium catenella



Blue light excitation



Blocking of chlorophyll
autofluorescence

Scale Bar, 20 μ m

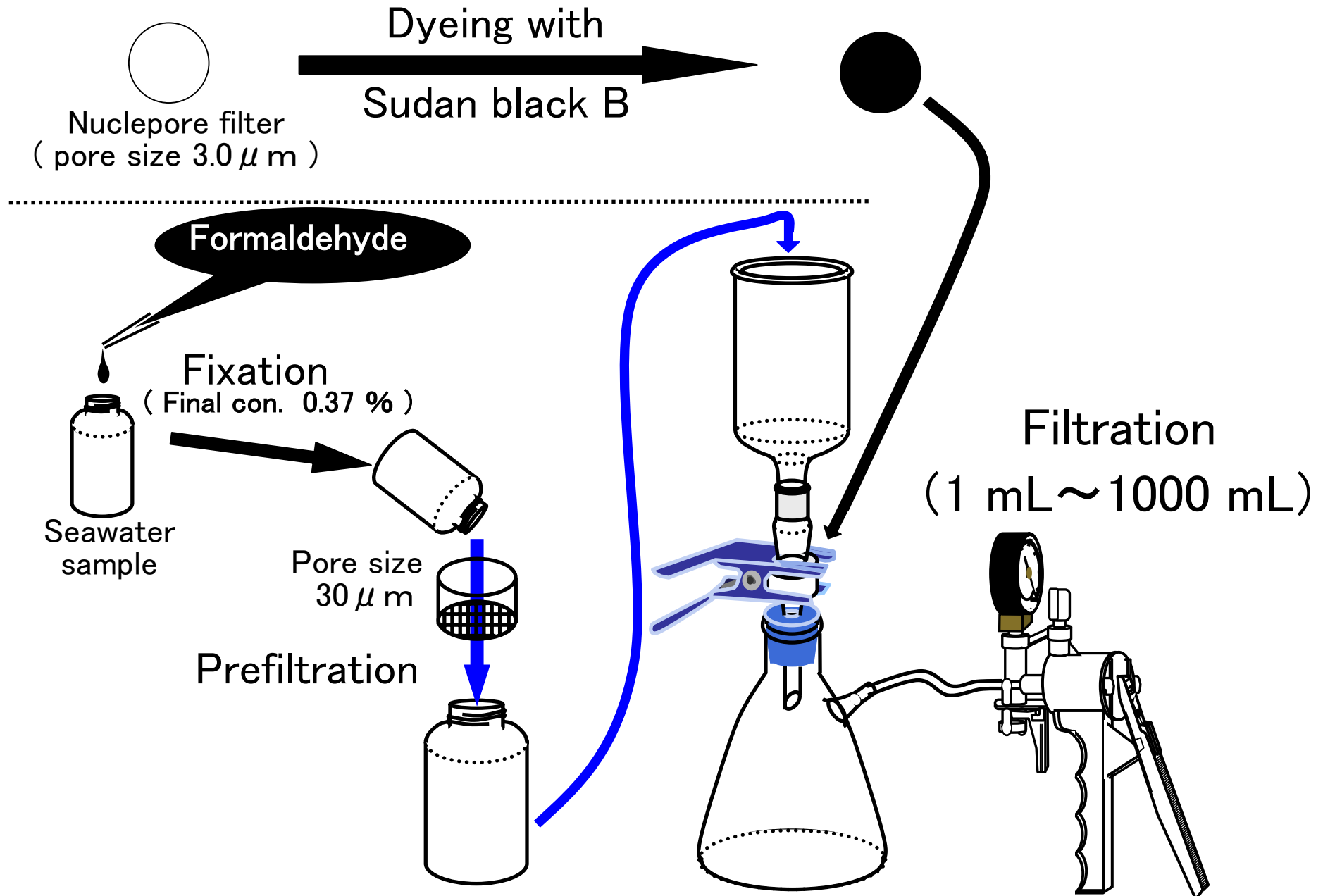
Location of the sampling stations



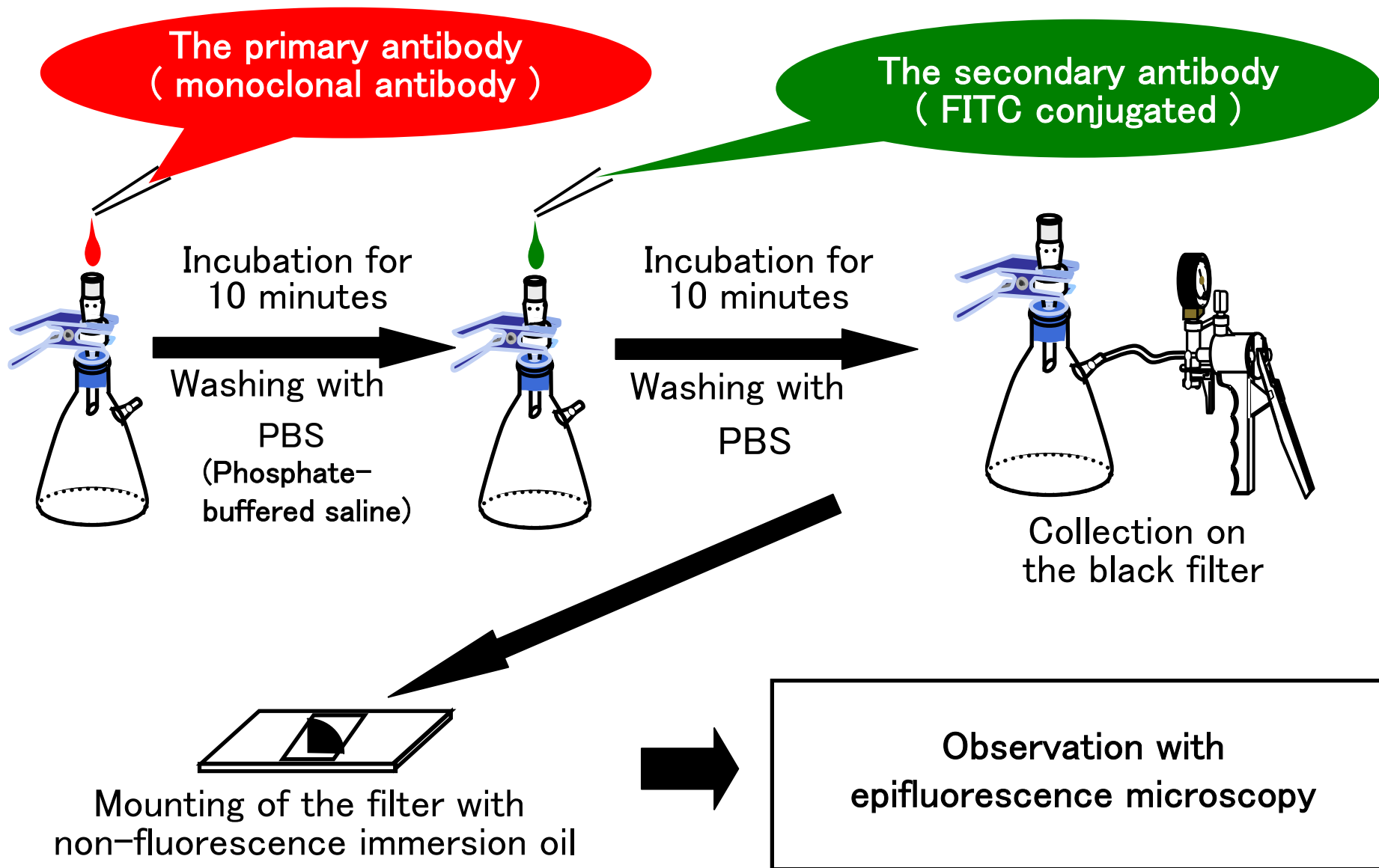
Sampling period: April 2001 ~ March 2003

Once a week (in summer and
twice a month in other seasons)

Pretreatment of seawater sample

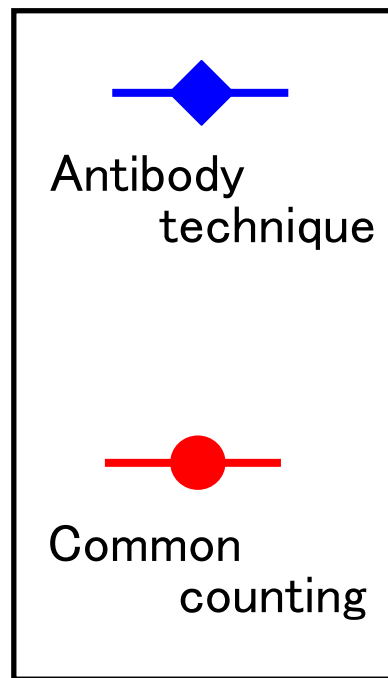
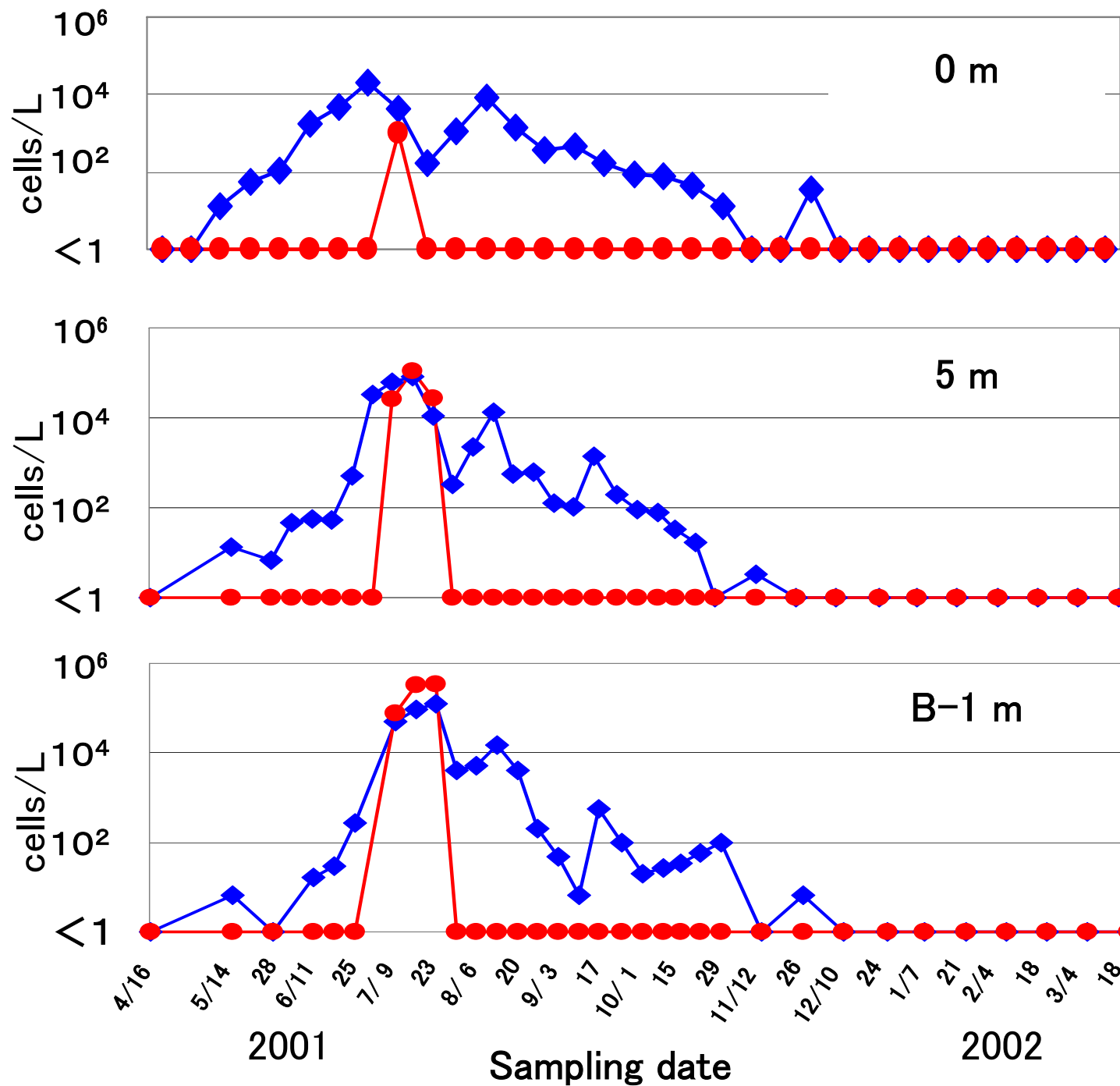


Indirect fluorescent antibody technique



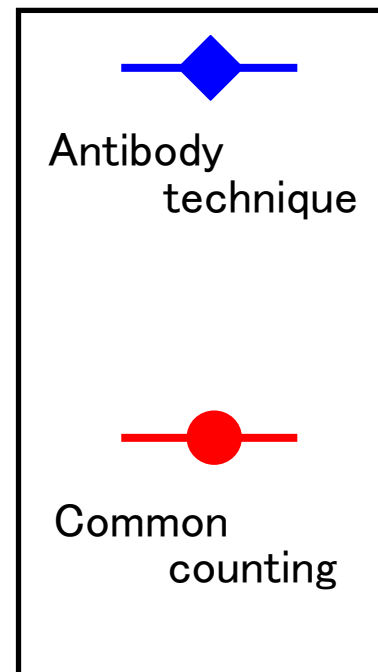
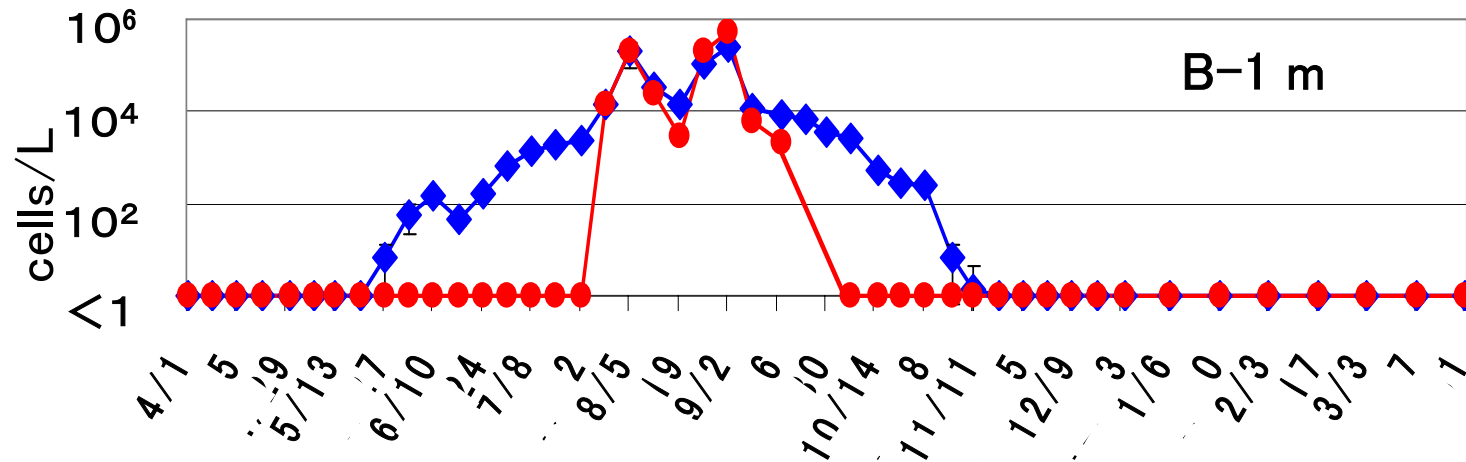
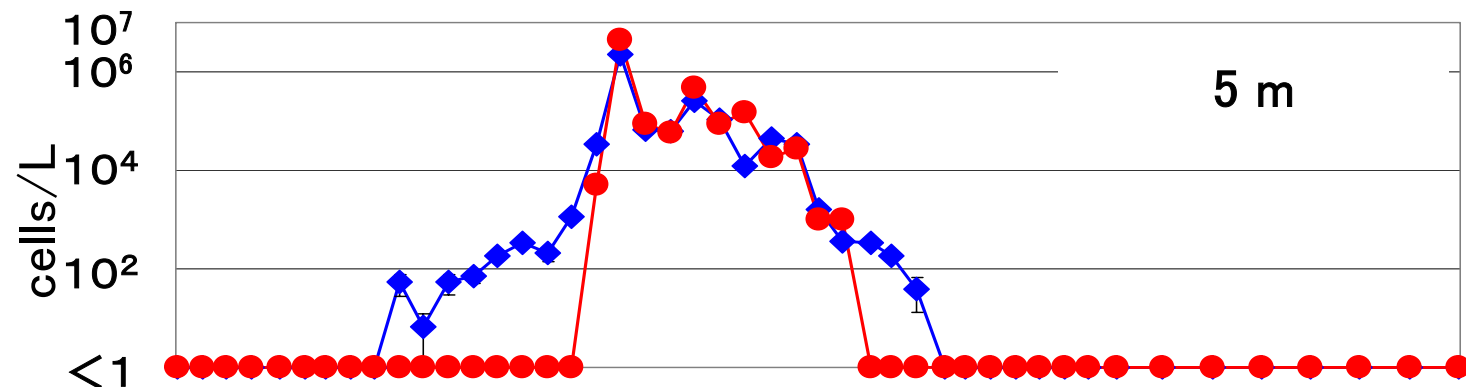
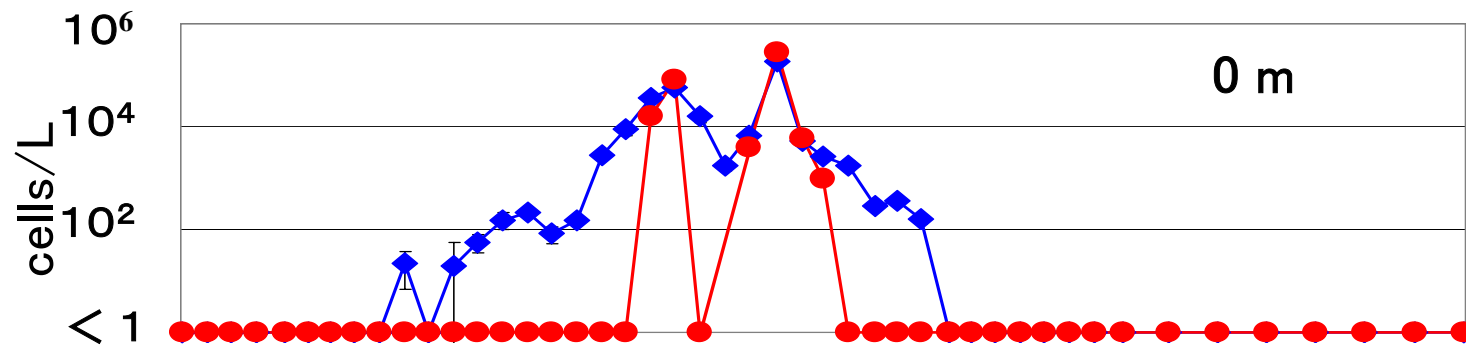
Akasaki

April 2001 ~
March 2002



Akasaki

April 2002 ~
March 2003



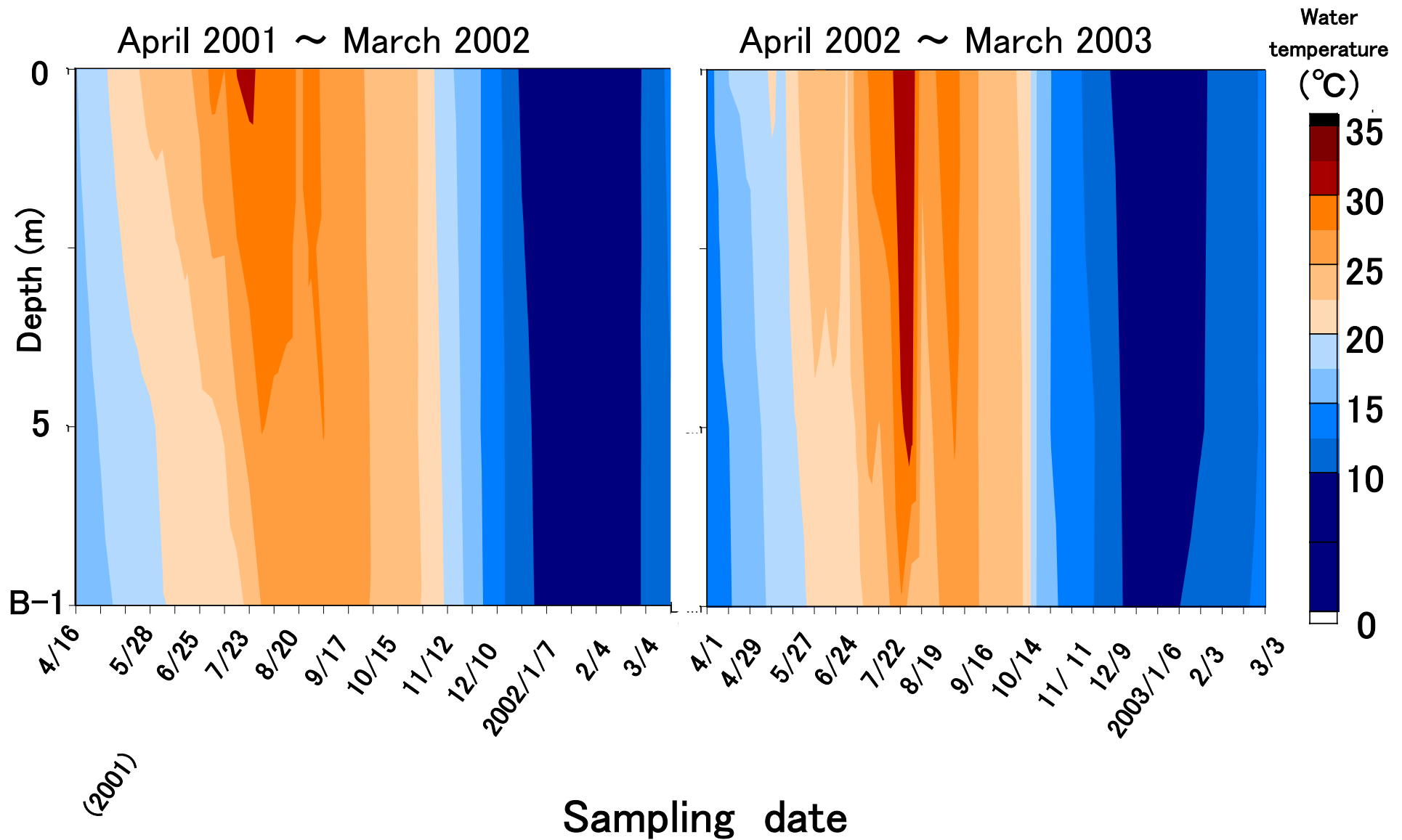
2002

2003

Sampling date

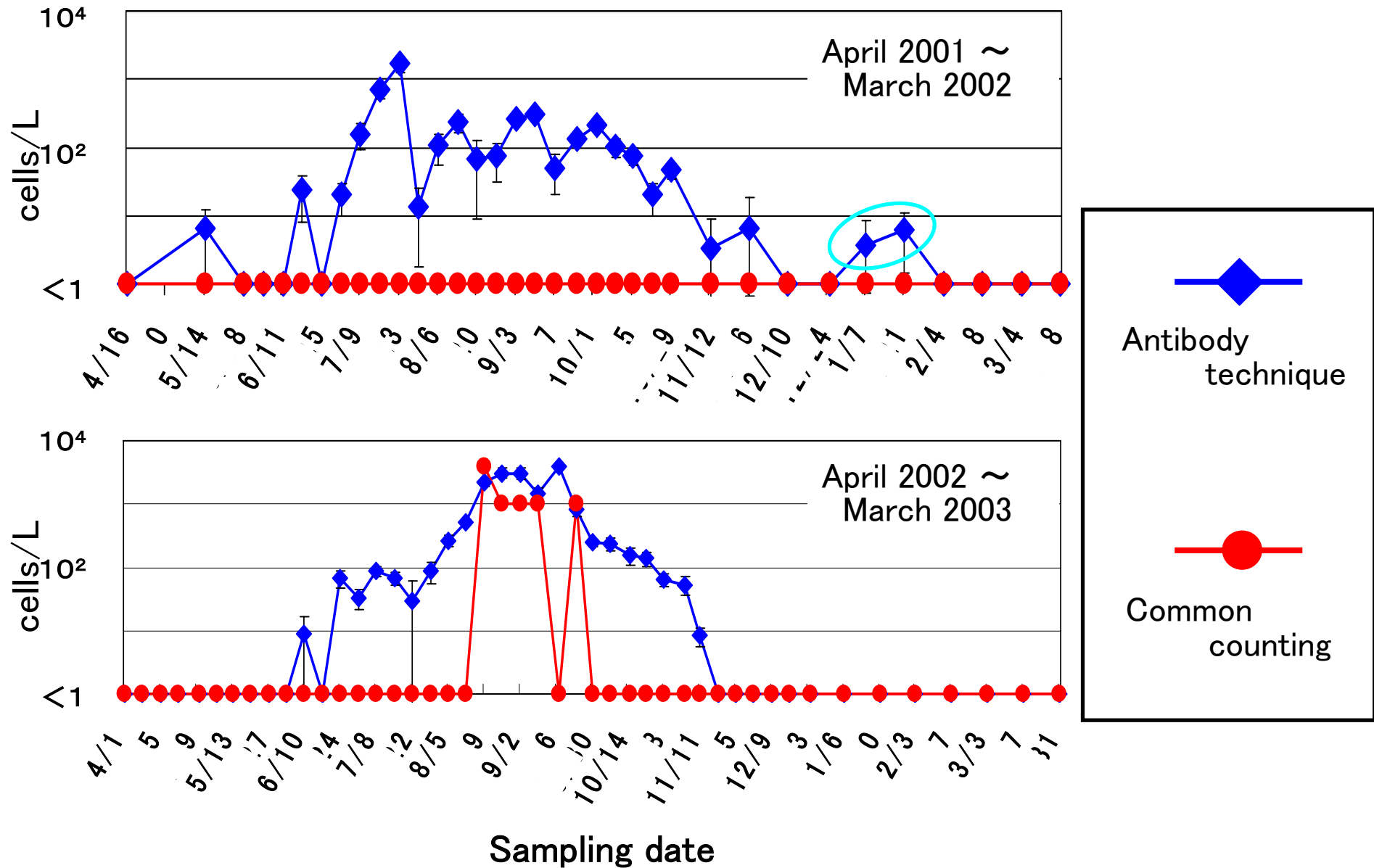
4/1 5 5/19 5/13 6/7 6/10 7/14 7/8 8/2 8/5 8/19 9/2 9/6 10/10 10/14 11/8 11/11 12/5 12/9 1/3 1/6 2/0 2/3 3/17 3/3 7 1

Seasonal changes of water temperature at Akasaki station in Ago Bay



Takonobori 5 m

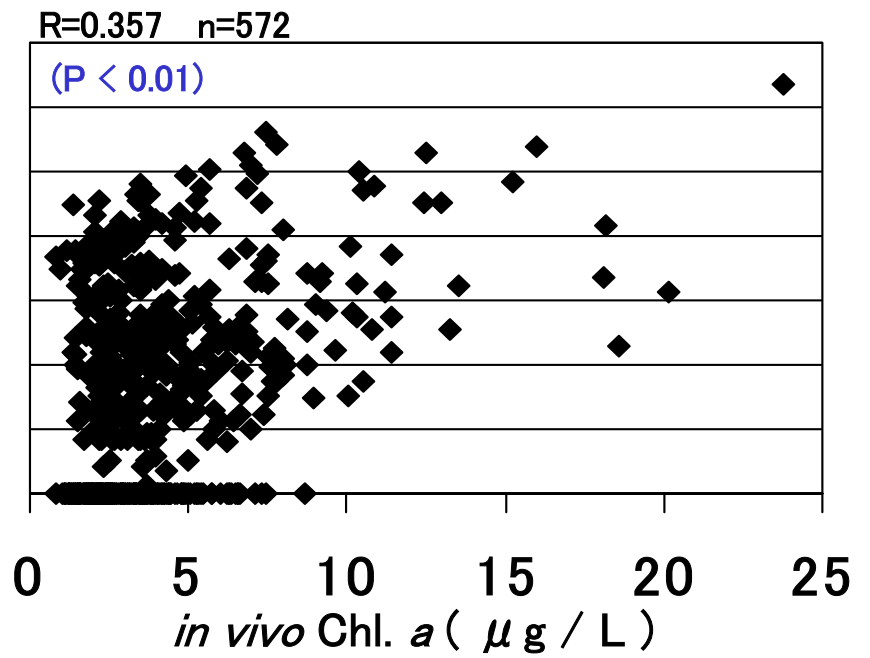
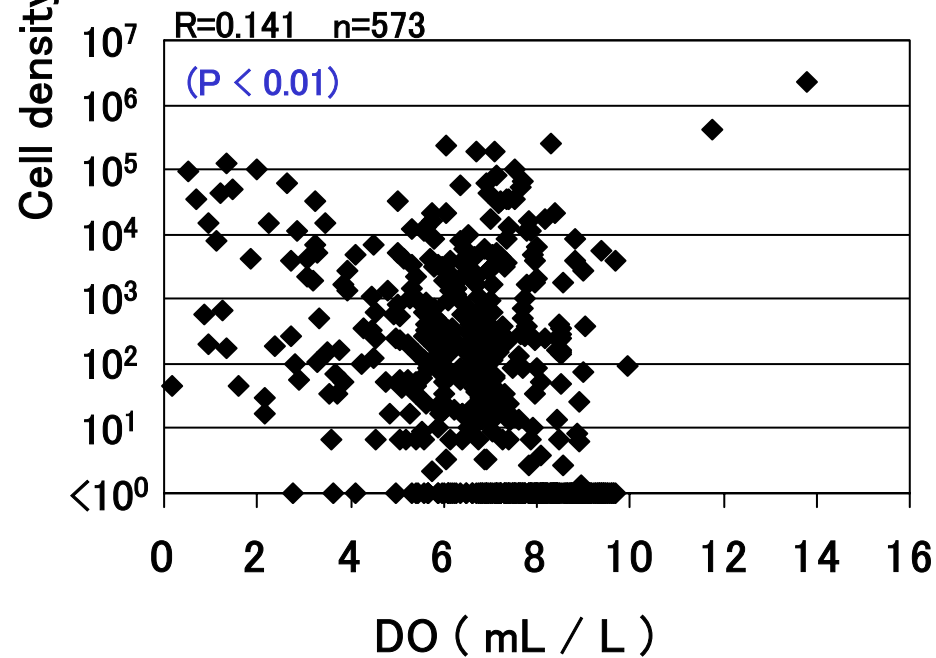
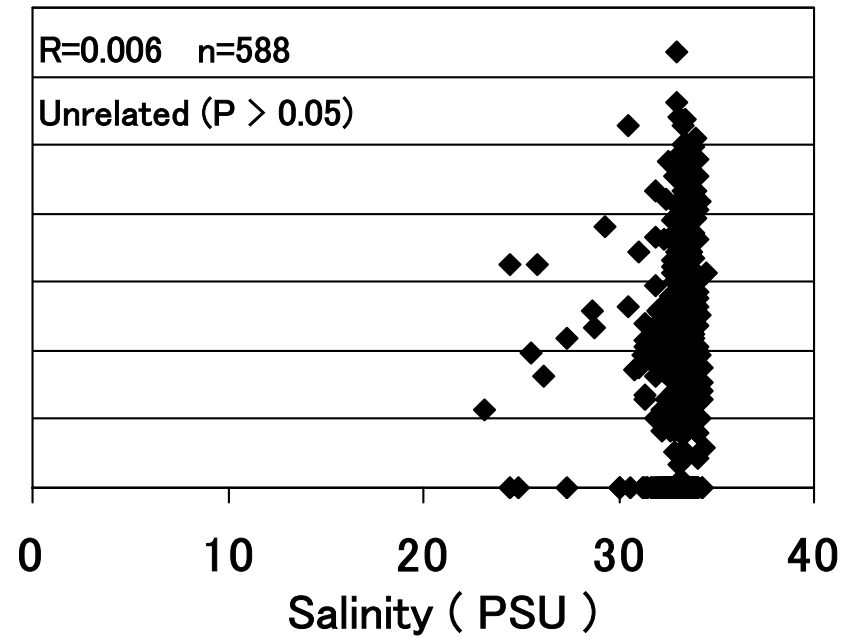
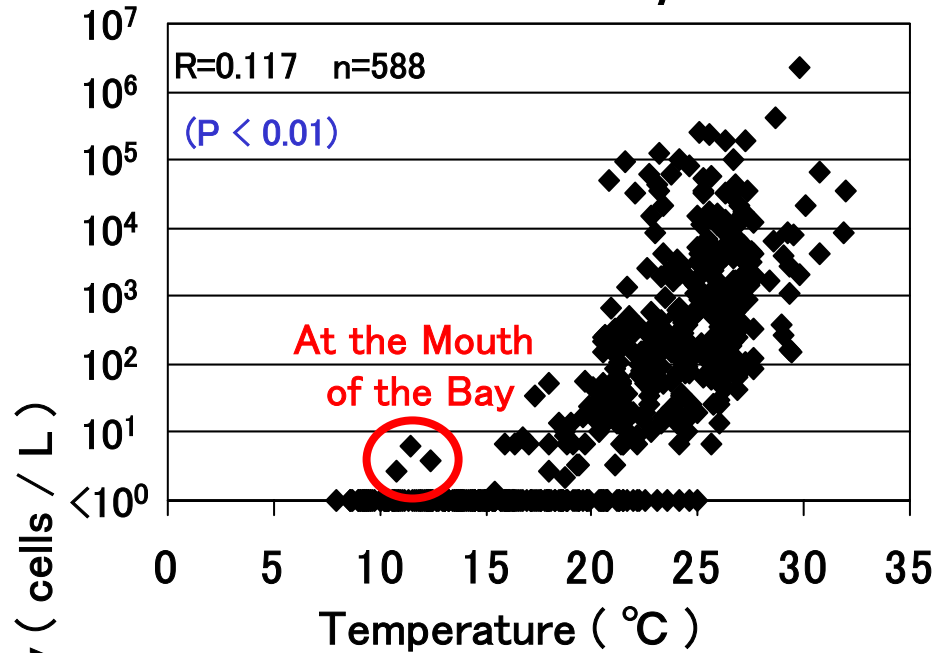
(Mouth of Ago Bay)



Characteristics of population dynamics of *H. circularisquama* in Ago Bay

- *H. circularisquama* cells were detected from July to October by the common microscopic counting.
- *H. circularisquama* cells were detected from May to November and in January by the indirect fluorescent antibody technique.
- The cell densities of *H. circularisquama* were high in summer.
- Decline of the cell density of *H. circularisquama* delayed at the mouth of Ago Bay, and the cells were detected even in January.

H. circularisquama and environmental factors



Summary

- Common microscopic counting detect *H. circularisquama* only at cell density of 1000 cells / L or higher.
- The present antibody technique can detect *H. circularisquama* at cell density of 10 cells / L or lower.



We could follow the population dynamics of *H. circularisquama* throughout the year by the indirect fluorescent antibody technique.

- *H. circularisquama* always exists at high temperature (>25°C) in Ago Bay.

Future study

Overwintering mechanisms are unknown in *Heterocapsa circularisquama*.



- Detection of *H. circularisquama* cells in winter season is essential with this method using large volume sea water in Ago Bay.
- Detection of *H. circularisquama* in warmer bays (winter temperature $> 12\text{ }^{\circ}\text{C}$) in winter.