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

Ichiro Imai, Tomotaka Shiraishi (Kyoto Univ.), Kiyohito Nagai, Shingo Hiroishi, Shigeru Itakura, Yasunori Watanabe, Akira Ishikawa and Yasuwo Fukuyo
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 μm 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 μm)

Blue light excitation

Blocking of chlorophyll autofluorescence

*H. circularisquama*  
*H. triquetra*
### Reactivity of the monoclonal antibody

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

**Alexandrium catenella**

- Blue light excitation
- Blocking of chlorophyll autofluorescence

Scale Bar, 20 μm
Sampling period: April 2001 ~ March 2003

Once a week in summer and twice a month in other seasons
Pretreatment of seawater sample

- **Filtration** (1 mL~1000 mL)
- **Seawater sample**
- **Prefiltration** (Pore size 30 μm)
- **Fixation** (Final con. 0.37 %)
- **Formaldehyde**
- **Nuclepore filter** (pore size 3.0 μm)
- **Dyeing with** Sudan black B

Filtration process:
1. Prefiltration with a Nuclepore filter (pore size 3.0 μm)
2. Formaldehyde fixation (final concentration 0.37 %)
3. Dyeing with Sudan black B
Indirect fluorescent antibody technique

The primary antibody (monoclonal antibody)
Incubation for 10 minutes
Washing with PBS (Phosphate-buffered saline)

The secondary antibody (FITC conjugated)
Incubation for 10 minutes
Washing with PBS
Collection on the black filter

Mounting of the filter with non-fluorescence immersion oil
Observation with epifluorescence microscopy
Akasaki

April 2001 ~
March 2002

0 m

5 m

B-1 m

Sampling date

2001

2002

Antibody technique

Common counting

cells/L

<1

10^2

10^4

10^6
Akasaki
April 2002 ~ March 2003

Sampling date

0 m

5 m

B-1 m

cells/L

<1

10^6

10^4

10^2

10^6

10^4

10^2

<1

2002

2003

Sampling date
Seasonal changes of water temperature at Akasaki station in Ago Bay

Water temperature (℃)

Sampling date

April 2001 ~ March 2002

April 2002 ~ March 2003
Takonobori 5 m  (Mouth of Ago Bay)

April 2001 ~ March 2002

April 2002 ~ March 2003

Sampling date

Antibody technique

Common counting

<10^2 cells/L

<10^4 cells/L
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

- **Temperature (°C)**
  - R = 0.117, n = 588
  - (P < 0.01)

- **Salinity (PSU)**
  - R = 0.006, n = 588
  - Unrelated (P > 0.05)

- **DO (mL/L)**
  - R = 0.141, n = 573
  - (P < 0.01)

- **in vivo Chl. a (μg/L)**
  - R = 0.357, n = 572
  - (P < 0.01)

*At the Mouth of the Bay*
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 $\geq 12 ^\circ C$) in winter.