Prospect for the biological control of *Heterocapsa circularisquama* bloom by inoculating frozen bottom sediment with HcRNAV viruses

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Biological control of HABs
Study on application of virus infectious to
*H. circularisquama*

To propose an idea for mitigation of HABs by using natural viruses in the bottom sediments
Phage therapy

Phage therapy or viral phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections (Wikipedia).

The direct human use of phages:
In August 2006, the United States Food and Drug Administration (FDA) approved spraying meat with phages (Wikipedia).
Presentation Outline

【Background and objective】
- *Heterocapsa circularisquama* as HABs in Japan
- Viruses infectious to *H. circularisquama*
- Recent occurrence of the *H. circularisquama* and its virus
- Variability of infectious type of *H. circularisquama* and its virus
- Availability of sediment including viruses

【Biological control Experiment】
- Study on the application of virus to diminish *H. circularisquama* bloom
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Heterocapsa circularisquama (Dinophyceae)

Specifically harmful to shellfish

Oysters killed by Hc-bloom

pearl oyster
## Damage to Japanese fisheries

<table>
<thead>
<tr>
<th>Year</th>
<th>Area</th>
<th>Causative species</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>Harima-Nada</td>
<td><em>Chattonella</em></td>
<td>Y</td>
</tr>
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<td>1977</td>
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<td><em>Chattonella</em></td>
<td>Y</td>
</tr>
<tr>
<td>1984</td>
<td>Kumano-Nada</td>
<td><em>Karenia</em></td>
<td>Yellow</td>
</tr>
<tr>
<td>1991</td>
<td>Aki-Nada etc.</td>
<td><em>Karenia</em></td>
<td>Red sea</td>
</tr>
<tr>
<td>1992</td>
<td>Ago Bay</td>
<td><em>Heterocapsa</em></td>
<td>Red sea</td>
</tr>
<tr>
<td>1995</td>
<td>Kagoshima Bay</td>
<td><em>Heterosigma</em></td>
<td>Yellowtail etc.</td>
</tr>
<tr>
<td>1998</td>
<td>Hiroshima Bay</td>
<td><em>Heterocapsa</em></td>
<td>4 billion JPY</td>
</tr>
<tr>
<td>2000</td>
<td>Yatsushiro Bay</td>
<td><em>Cochlodinium</em></td>
<td>Yellowtail etc.</td>
</tr>
<tr>
<td>2000-2001</td>
<td>Ariake Bay</td>
<td><em>Diatoms</em></td>
<td>Porphyra</td>
</tr>
<tr>
<td>2009-2010</td>
<td>Yatsusiro Bay</td>
<td><em>Chattonella</em></td>
<td>Yellowtail etc.</td>
</tr>
<tr>
<td>2012</td>
<td>Bungo-Suido</td>
<td><em>Karenia</em></td>
<td>Yellowtail etc.</td>
</tr>
</tbody>
</table>

**390 billion US$**
Lytic infection to dinoflagellate

Two virus species infecting *Heterocapsa circularisquama*

- **HcV** (DNA virus)
  - Large
- **HcRNAV** (RNA virus)
  - Small

100μm
H. circularisquama and its virus closely correlate to each other.

Virus in the sediment (g⁻¹)

Mie

Virus in the water-column

► H. circularisquama and its virus (HcRNAV) showed a similar pattern of fluctuation in natural environments.

► There are large amounts of HcRNAV in sediment.
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Biological Control: Advantages of using virus

- Artificially promoting events that naturally occur in nature

**Environmental friendly**

- Virus controls the population dynamics of *H. circularisquama*

- Highly host-specificity
  - Killing only the target

- Virus increases as it destroys the host cells.
  - Effect on the wide range in a small amount of virus
But, it is difficult to spray directly viruses.

I. The word 'Virus' makes negative impression.
II-① Variability of infectious type of HcRNAV

At least three groups of infectious type of HcRNAV

(Tomaru et. al 2004, Nakayama et al. 2013)
II.-② In natural sea water and sediment...  
① before bloom
② bloom: Increasing variability of *H. circurarisquama*
increase of variable viruses attacking the host
accumulation of valuable viruses in sediment
5 sampling of the sediment, cryopreservation
Advantages of using frozen sediment

- Large amount of HcRNAVs in sediment
- Frozen sediment keeps high-titer of HcRNAV
- Harmful cysts die by sediment freezing
- A variety of virus (differing in host range) is included.

spraying the virus-containing sediment means:

Making the environment more disadvantageous to *H. circularisquama*
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Field Experiment (Lake Kamo)

Blackish lake: **Hc bloom occurred in the summer of 2009-2014.**

- Enclosed environment
- No cultured fish
- Bottle experiment is performed to verify its effect to natural Hc population.
Bottle experiment in 2011

Objective:
verification of effect sediment on natural HC population

No sediment
Autoclaved sediment
+ Sediment

Red tide
natural sea water

natural sea water + sediment

0.5m
Result I

- *H. circularisquama* showed a remarkable decrease in cell density (from ca. 7,500 to ca. 75 cells/mL)
Result II

- HcRNAV significantly increased within the 6 days. (from 40 to 72,000 infectious units/mL)
Future Subject

1. Effect of the virus in the sediment
2. How much volume does it need?
3. Safety
Field test

【Simulated experiment by large-scaled test】

【Lake Kamo is only field that verification test was allowed by public administration.】
*Summary*

★ *H. circularisquama* and its virus (HcRNAV) showed a similar pattern of fluctuation in natural environments.

★ There are large amounts of HcRNAV in sediment.

★ Inoculating frozen sediment is available to decrease *H. circularisquama* in cell density.

We will develop the application of preventing HABs.
Thank you all for your attention