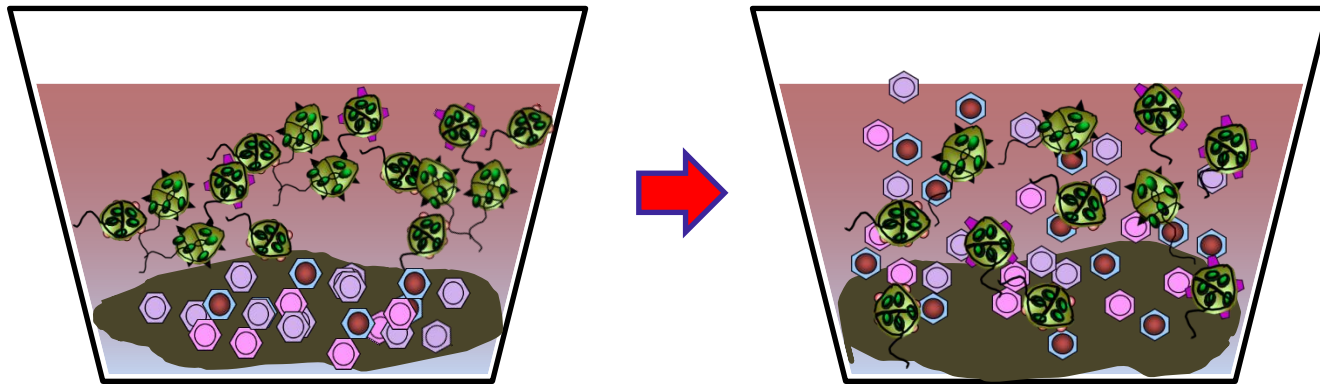


Prospect for the biological control of *Heterocapsa circularisquama* bloom by inoculating frozen bottom sediment with HcRNAV viruses



Natsuko Nakayama¹, Shinichi Kondo³, Naotsugu Hata², Yuji Tomaru¹,
Masami Hamaguchi¹, Keizo Nagasaki¹ and Shigeru Itakura¹

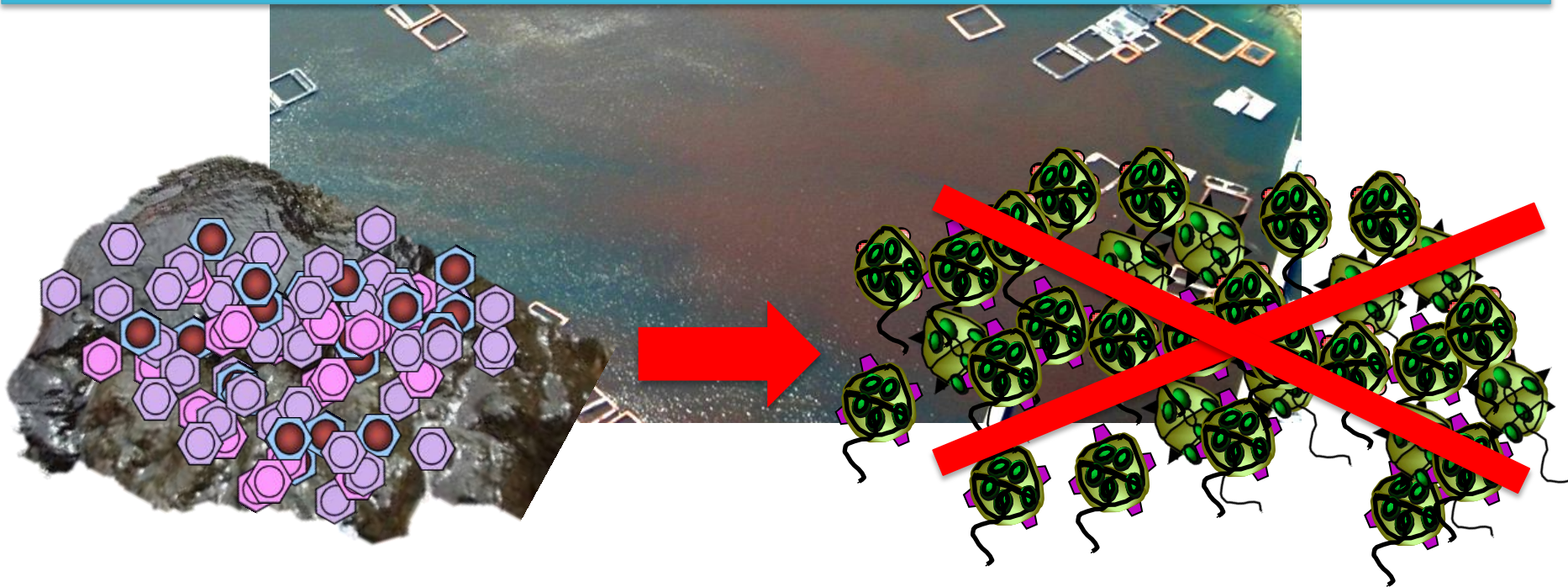
¹Fisheries Research Agency, ²Mie pref. Fisheries Research Institute,
³Niigata pref. Fisheries Research Institute

Biological control of HABs

Study on application of virus infectious to
H. circularisquama



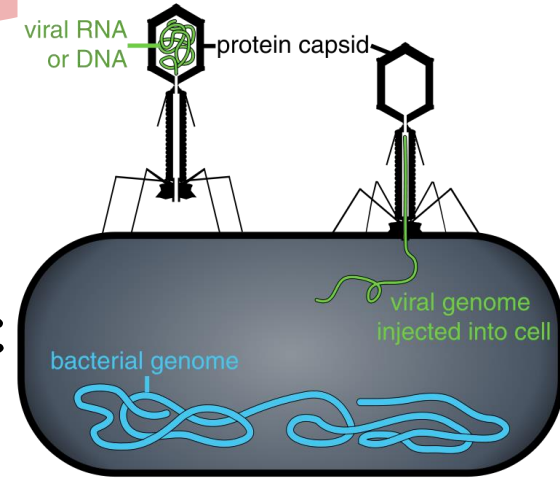
To propose an idea for mitigation of HABs
by using natural viruses in the bottom sediments



ref.

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

Presentation Outline

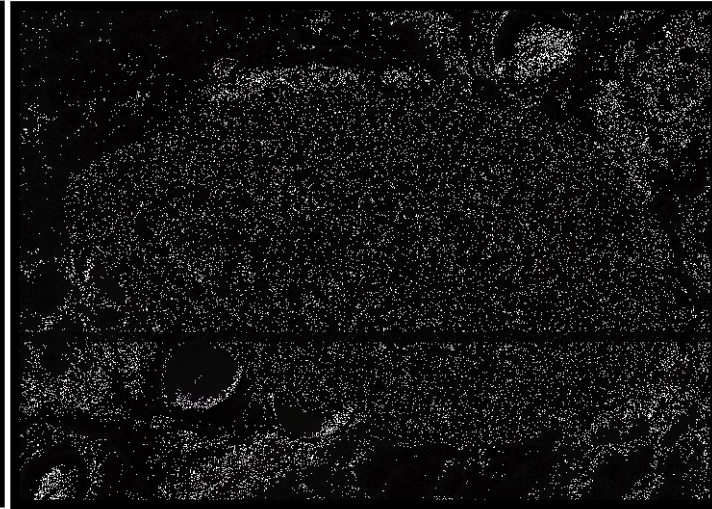
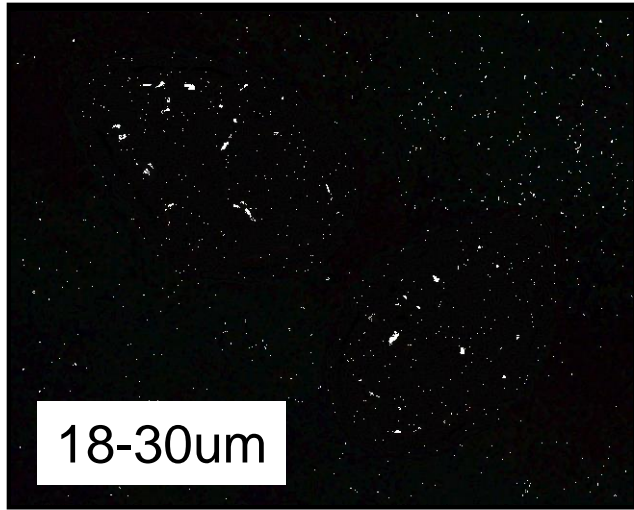
【Background and objective】

- *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

Heterocapsa circularisquama (Dinophyceae)



Specifically harmful to shellfish

Oysters killed
by Hc-bloom

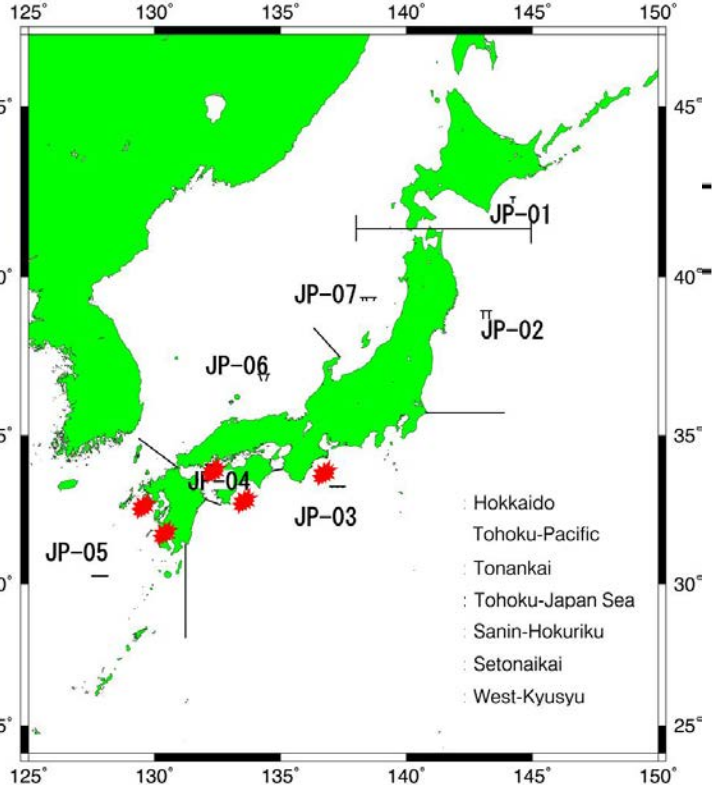


pearl oyster



Damage to Japanese fisheries

Year	Area	Causative species	Damage	Value
1972	Harima-Nada	<i>Chattonella</i>	Yellowtail etc.	4 billion JPY
1977	Harima-Nada	<i>Chattonella</i>	Yellowtail etc.	>1 billion JPY
1978	Harima-Nada	<i>Chattonella</i>	Yellowtail etc.	8.7 billion JPY
1984	Kumano-Nada	<i>Karenia</i>	Yellowtail etc.	1.3 billion JPY
1991	Aki-Nada etc.	<i>Karenia</i>	Yellowtail etc.	
1992	Ago Bay	<i>Heterocapsa</i>	Yellowtail etc.	
1995	Kagoshima Bay	<i>Heterosigma</i>	Yellowtail etc.	
1998	Hiroshima Bay	<i>Heterocapsa</i>	Yellowtail etc.	
2000	Yatsushiro Bay	<i>Cochlodinium</i>	Yellowtail etc.	4 billion JPY
2000-2001	Ariake Bay	Diatoms	Porphyra	>1 billion JPY
2009-2010	Yatsusiro Bay	<i>Chattonella</i>	Yellowtail etc.	8.7 billion JPY
2012	Bungo-Suido	<i>Karenia</i>	Yellowtail etc.	1.3 billion JPY



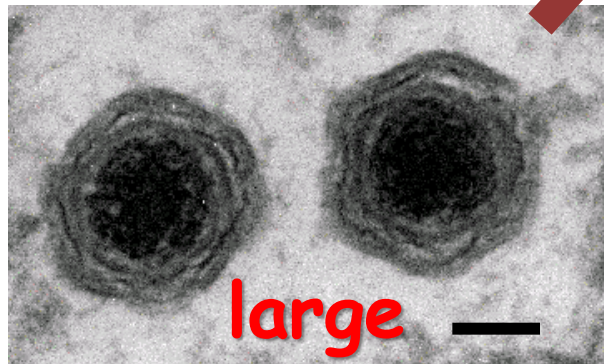
390 billion US\$

Lytic infection to dinoflagellate

Two virus species infecting *Heterocapsa circularisquama*

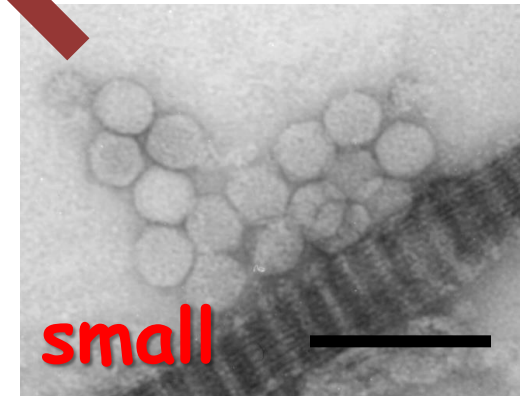


HcV
(DNA virus)



large

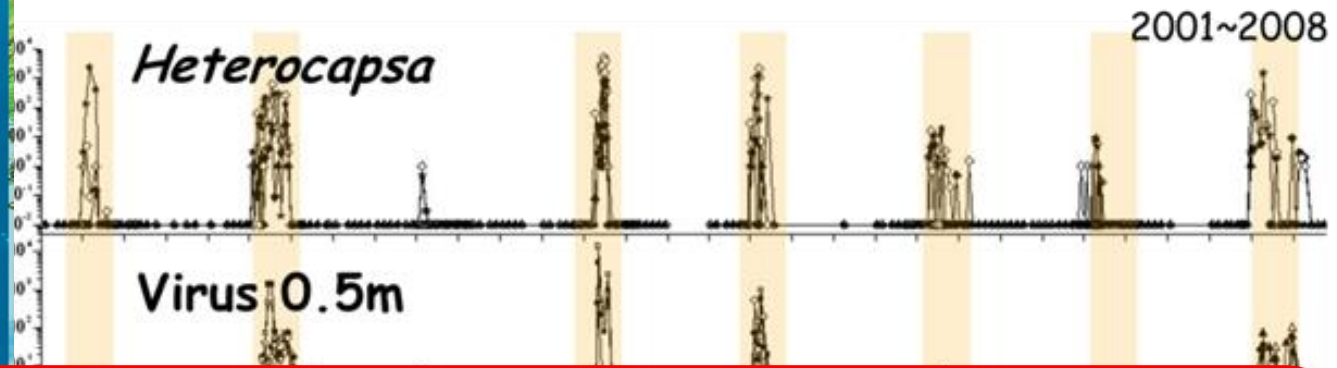
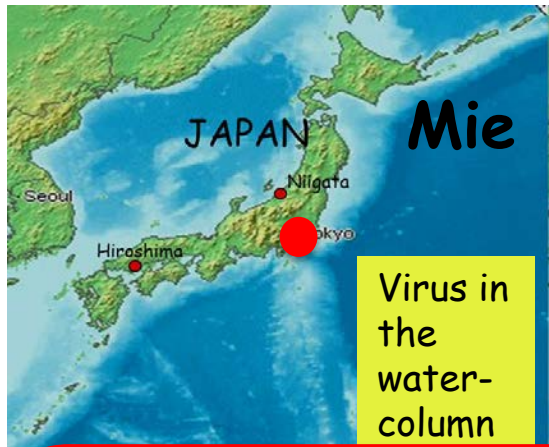
HcRNAV
(RNA virus)



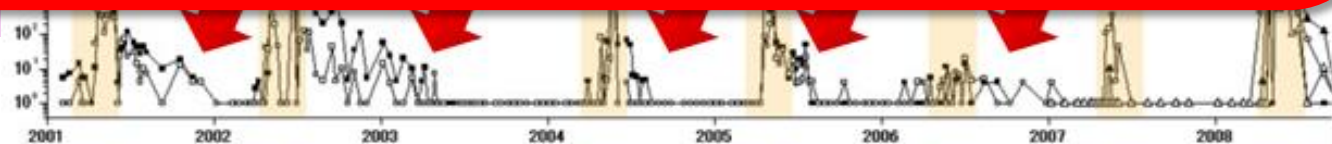
small

100μm

Occurrence in recent years of the *H.circularisquama* and its virus



- ▶ *H. circularisquama* and its virus (HcRNAV) showed a similar pattern of fluctuation in natural environments.
- ▶ There are large amount of HcRNAV in sediment.



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】

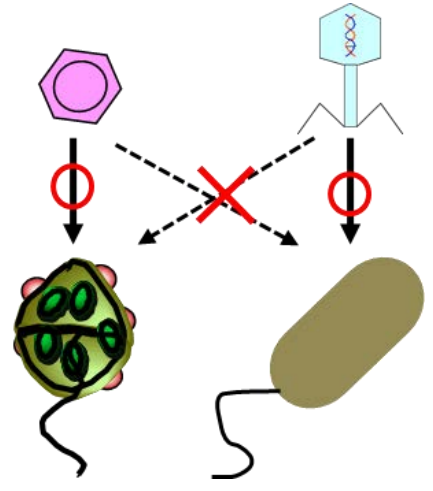
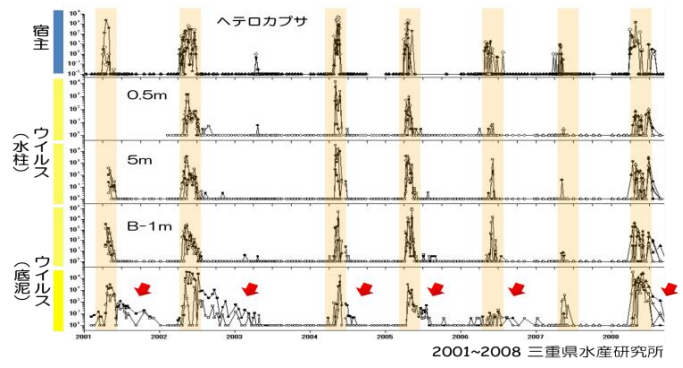
- Study on the application of virus to diminish *H. circularisquama* bloom

Biological Control : Advantages of using virus

~Artificially promoting events that naturally occur in nature~

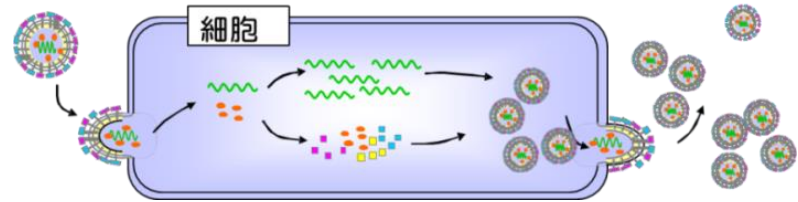
Environmental friendly

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

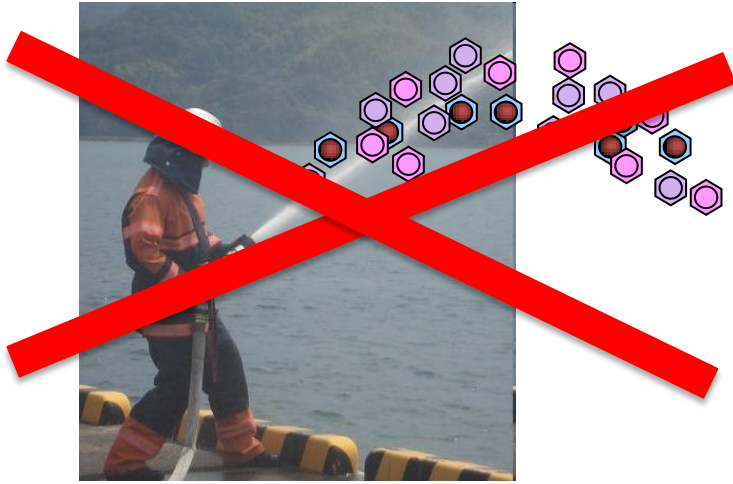


◆ Highly host-specificity
→ Killing only the target

◆ 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.

tobacco mosaic virus

Virus!?

dangerous!

Virus!?

emergency!

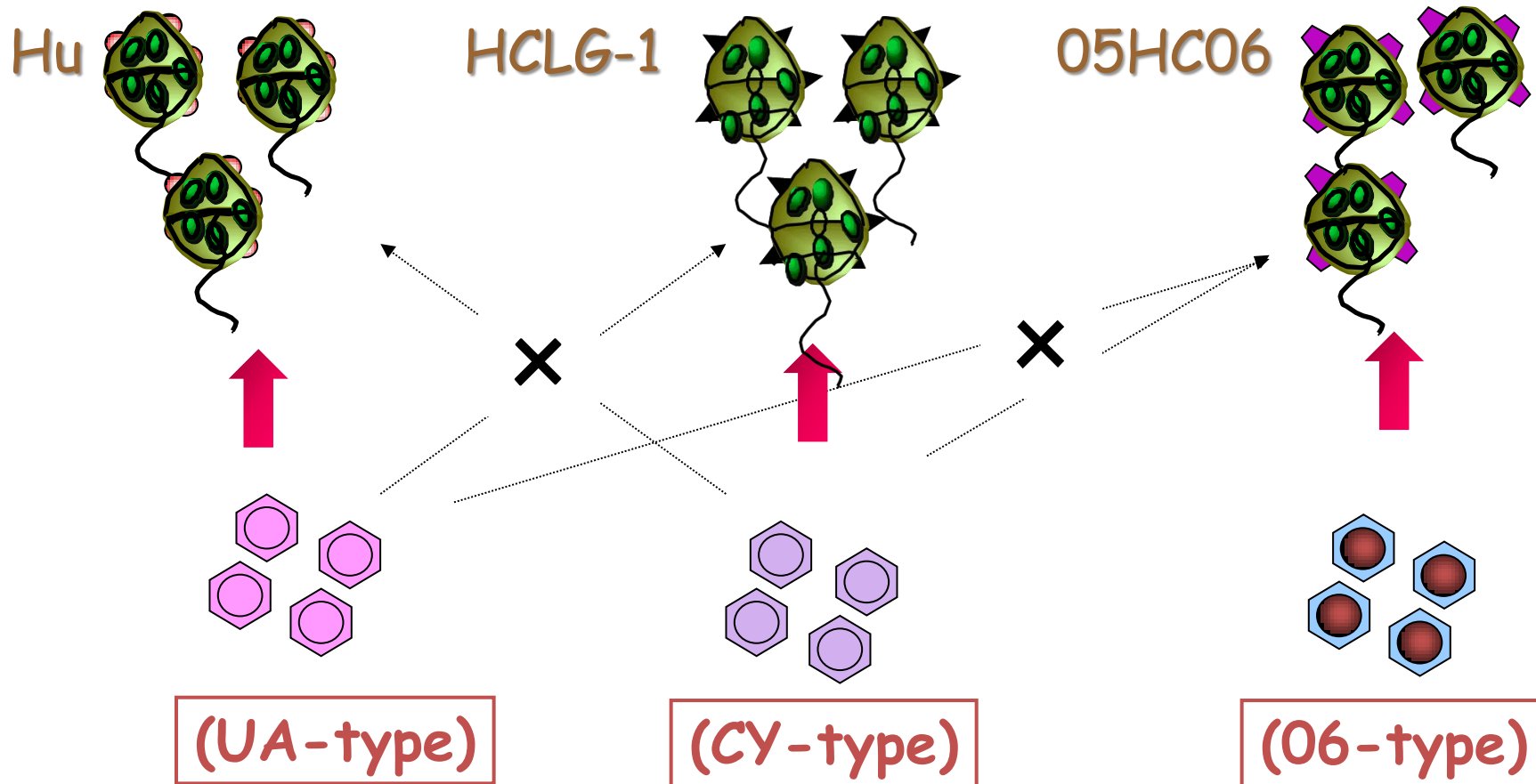
Virus!?

influenza virus

SARS coronavirus

II. ① Variability of infectious type of HcRNAV

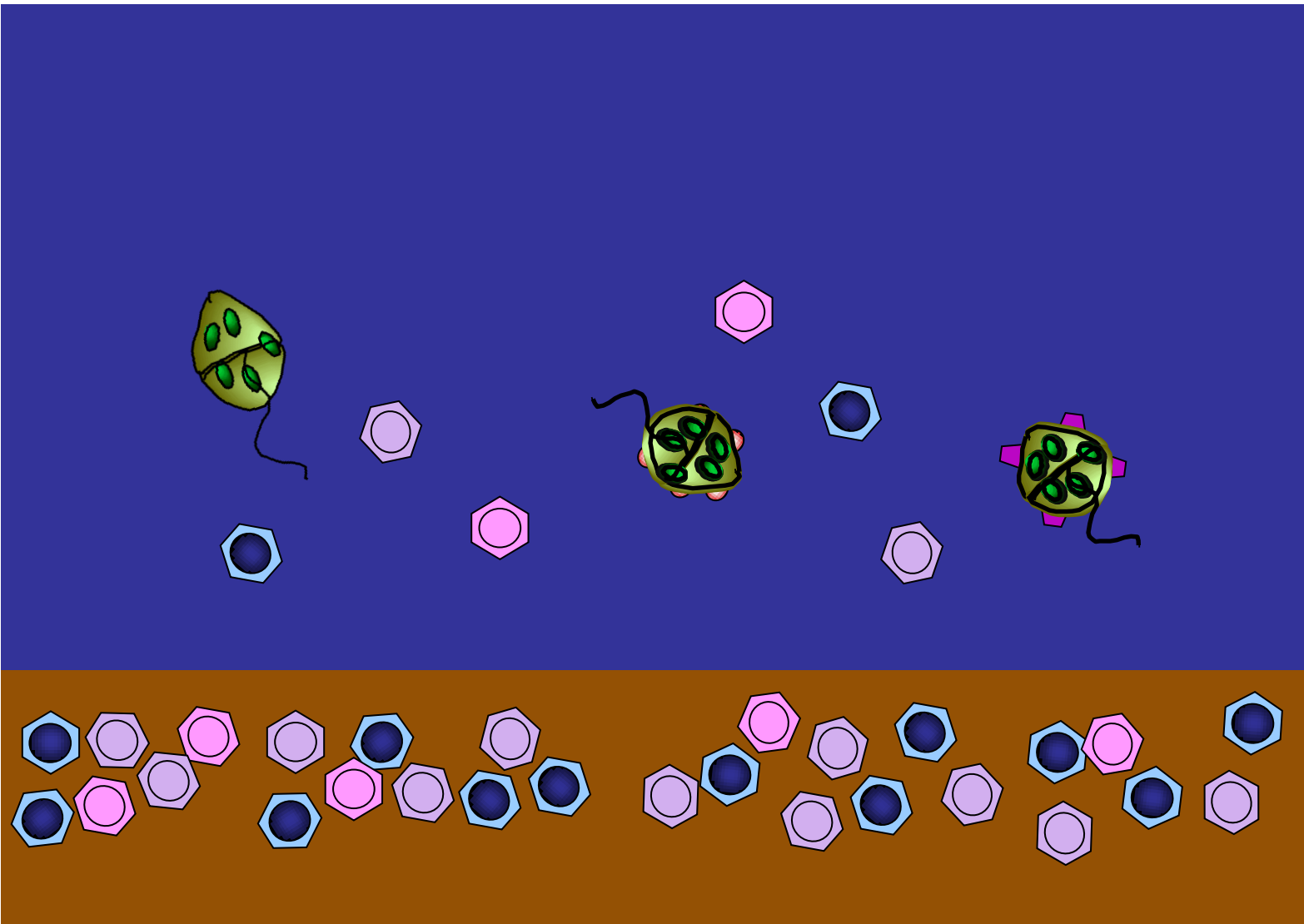
At least **three groups** of infectious type of HcRNAV



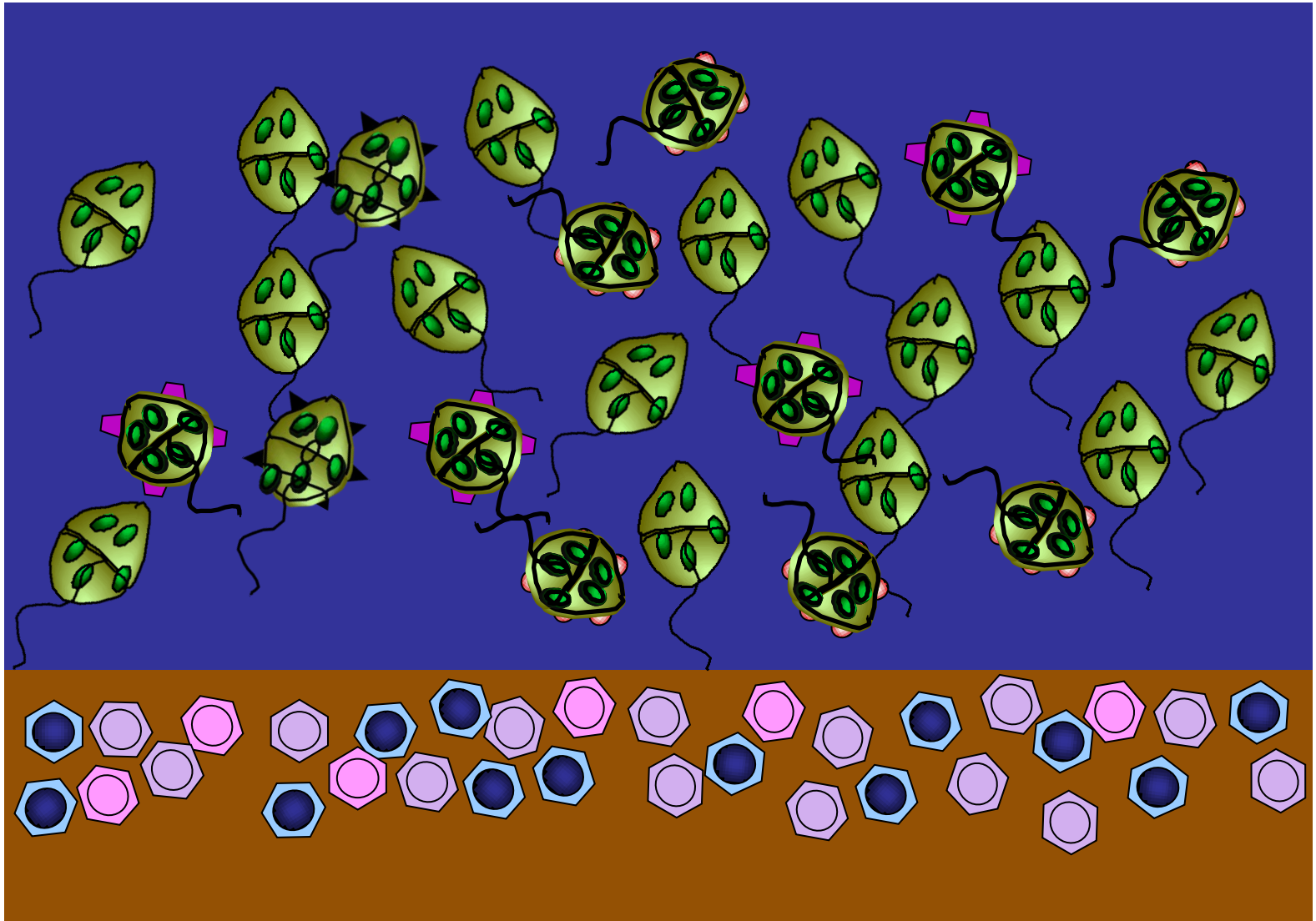
(Tomaru et. al 2004, Nakayama et al. 2013)

II.-② In natural sea water and sediment . . .

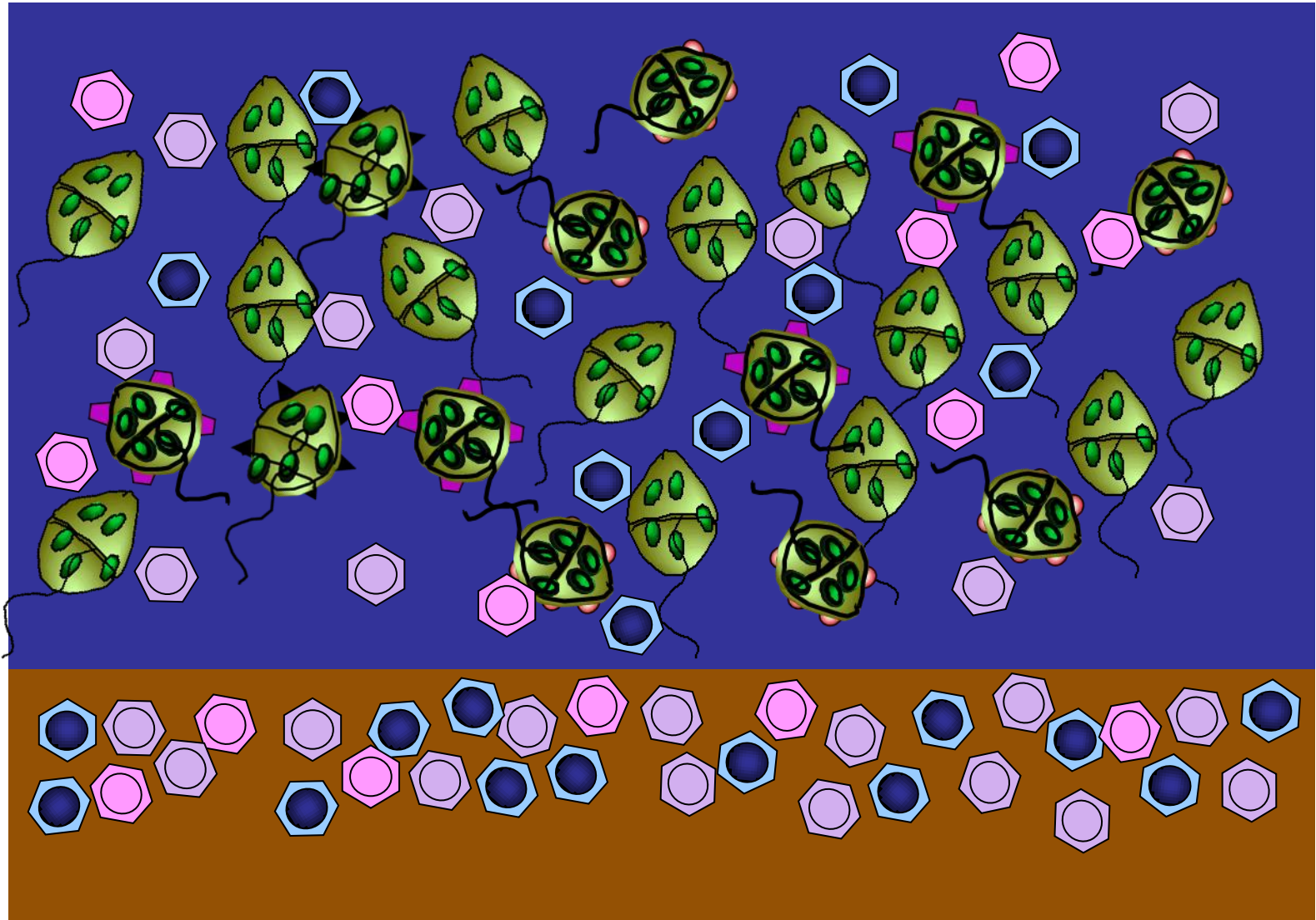
① before bloom



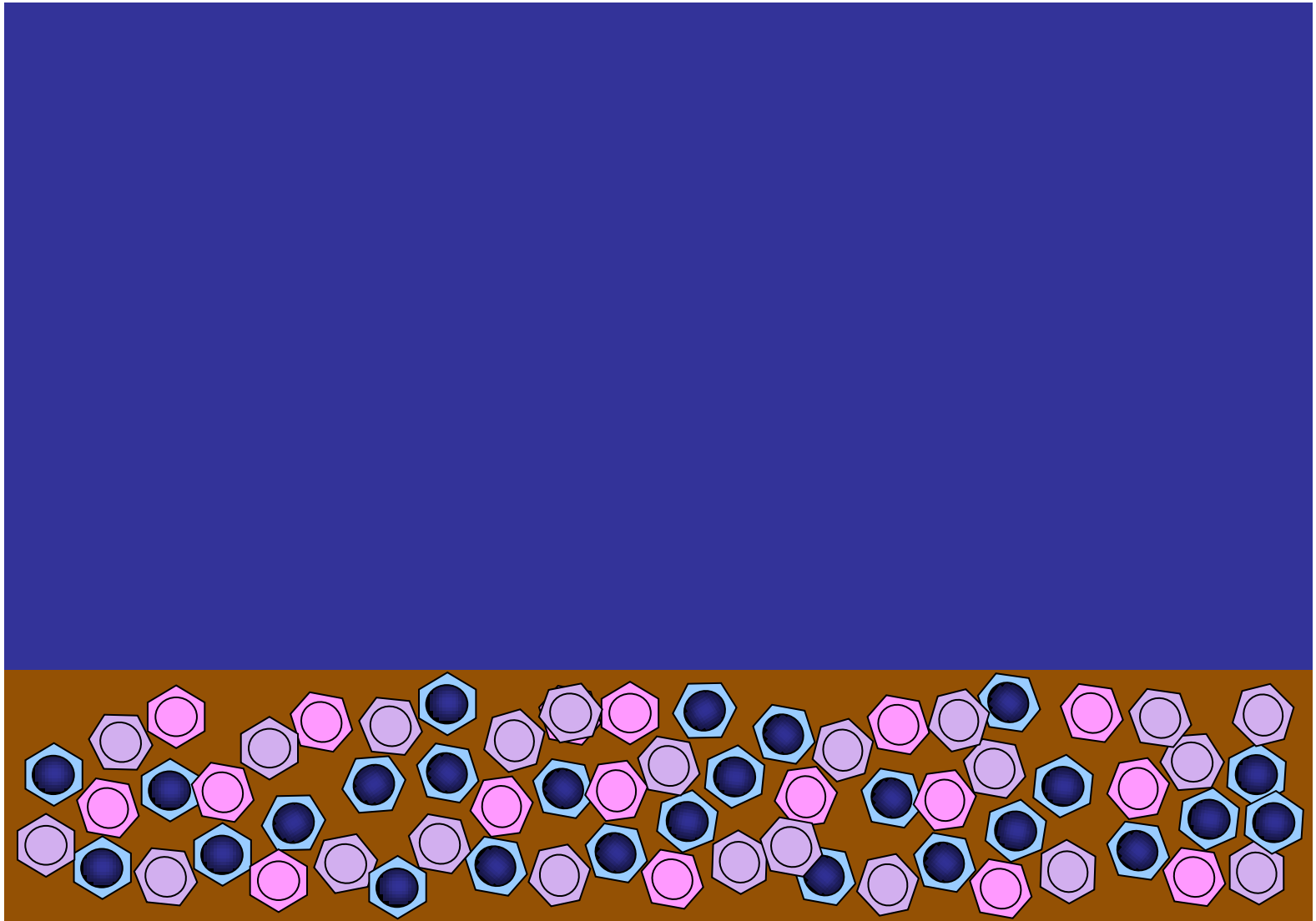
② bloom : Increasing variability of *H.circurarisquama*



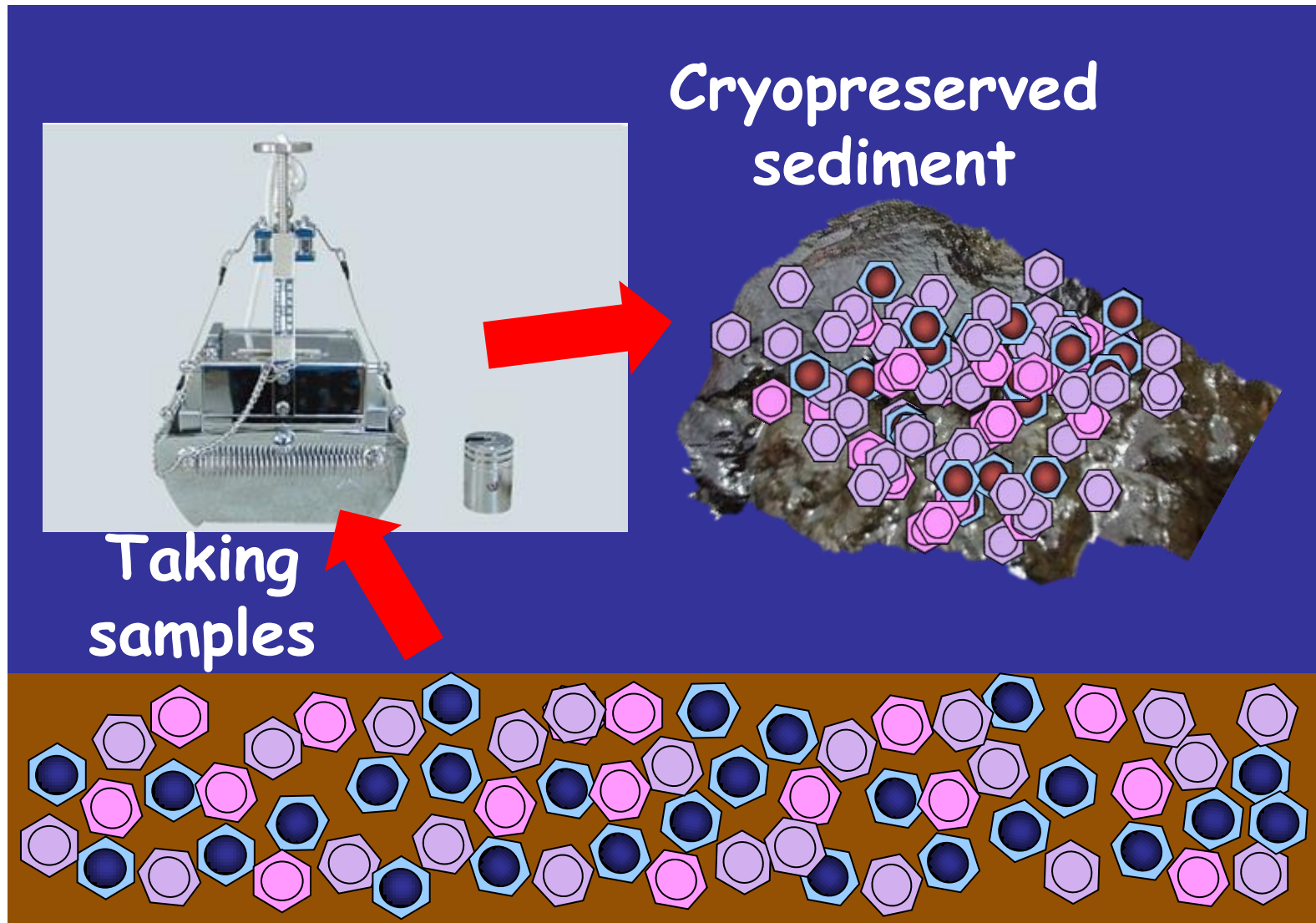
③ increase of variable viruses attacking the host



④ accumulation of viable viruses in sediment



⑤ sampling of the sediment, cryopreservation



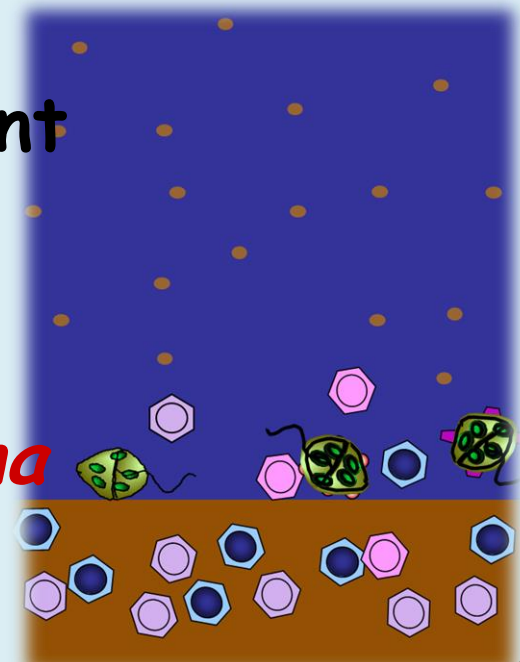
Advantages of using frozen sediment



- 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***



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

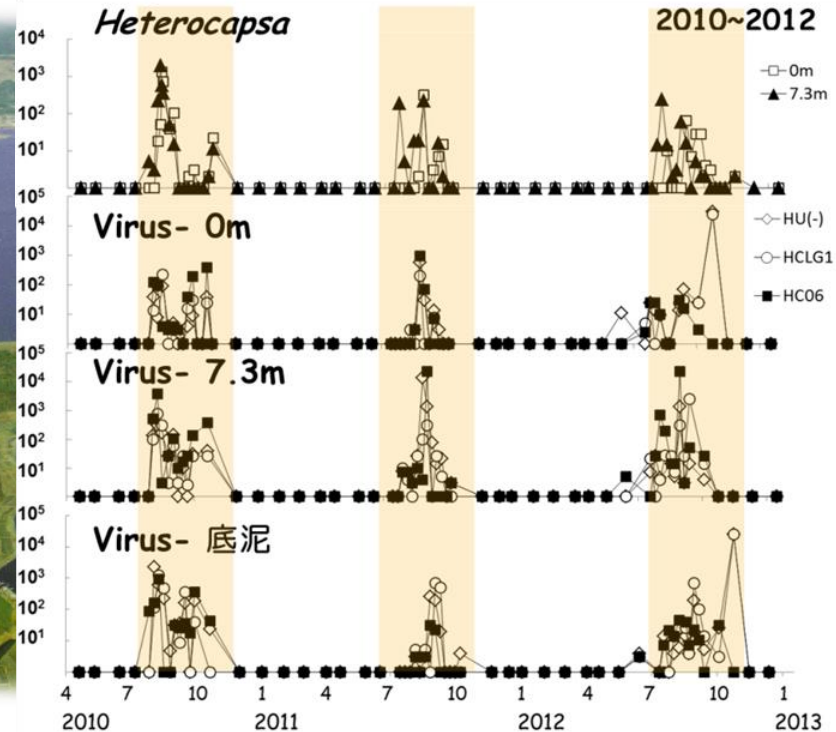
【Biological control Experiment】

- Study on the application of virus to diminish *H. circularisquama* bloom

Tentative

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



Red tide



natural sea water



natural sea water + sediment



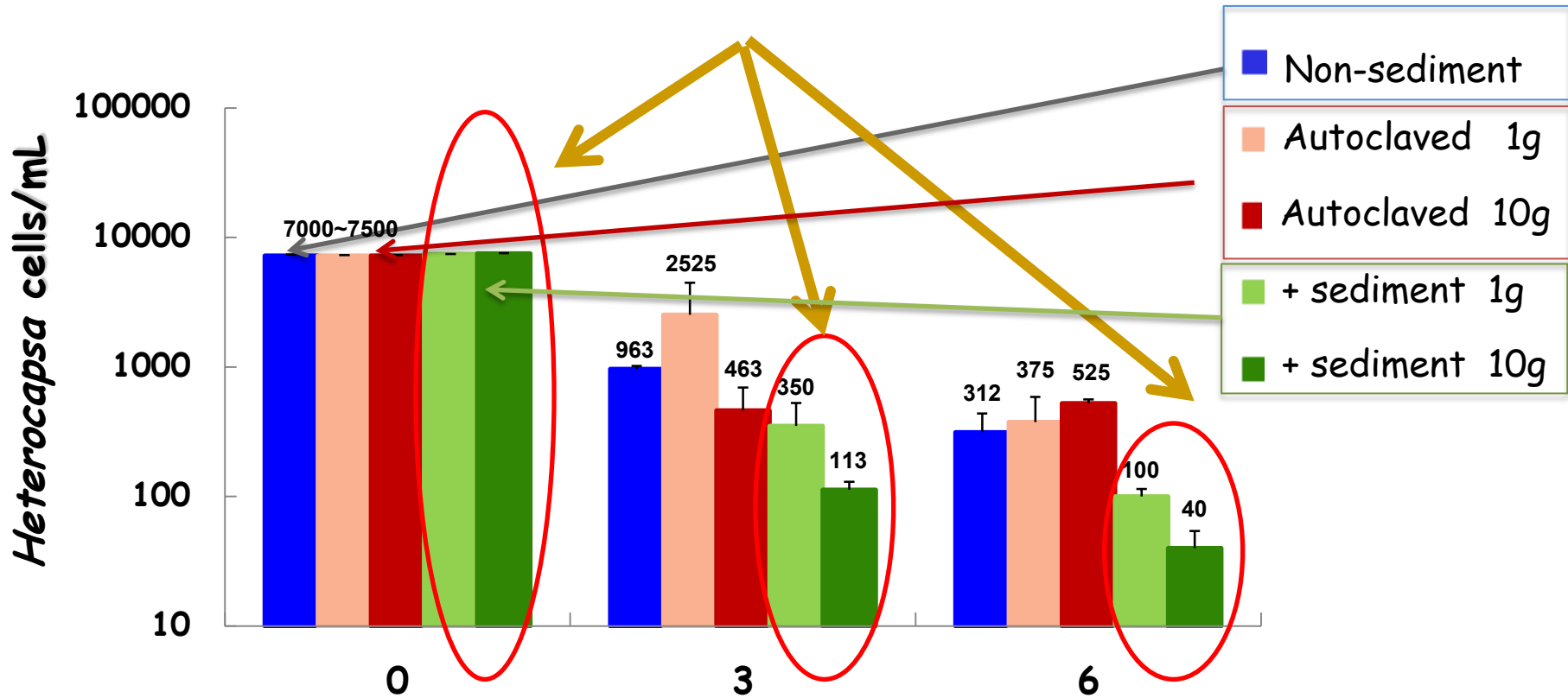
0.5m I

No sediment

Autoclaved sediment

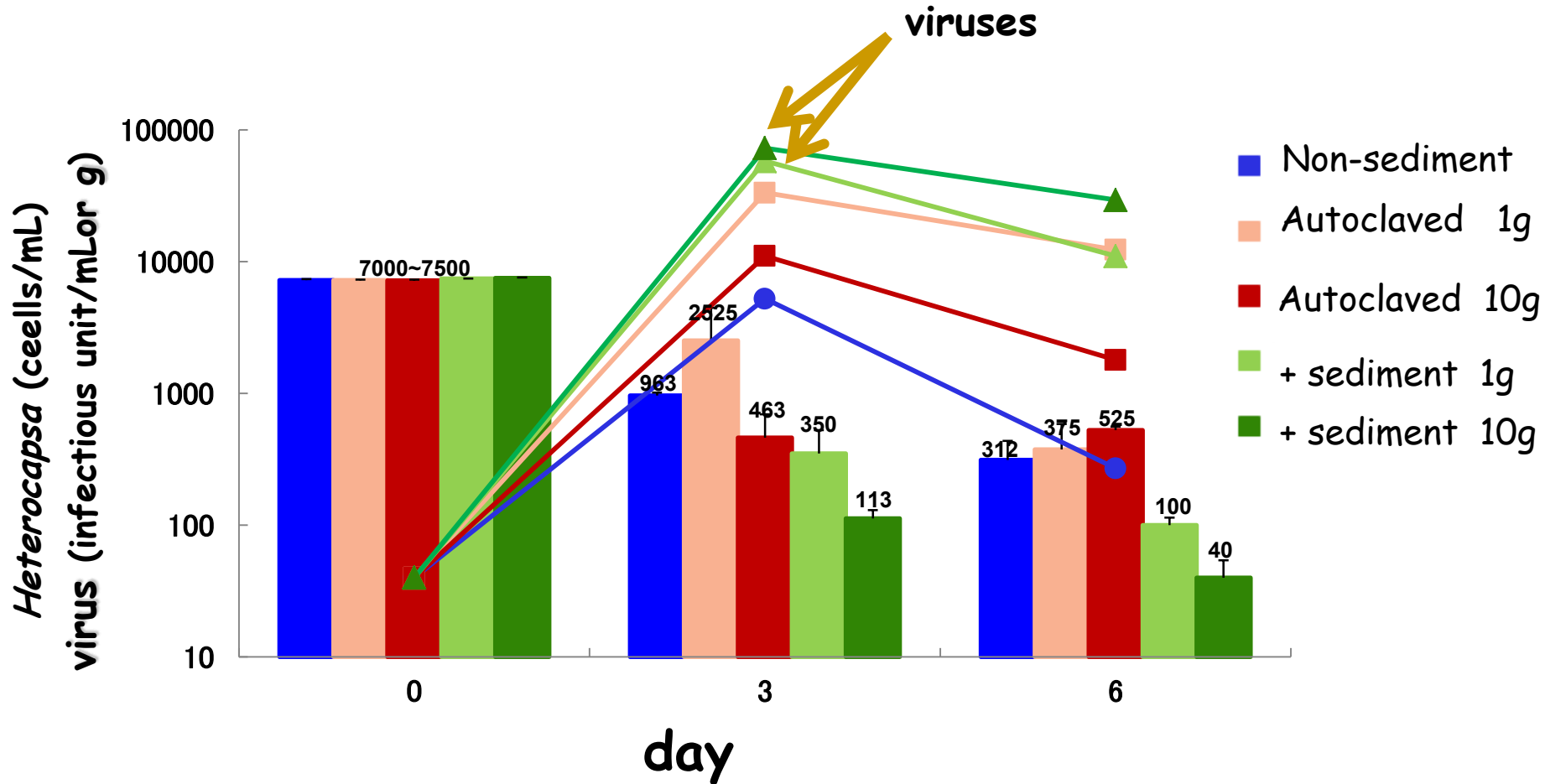
+ Sediment

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

-
-
-
-



Taking sediment samples

Field test

【Simulated experiment by large-scaled test】



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



Summery

- ☆ *H. circularisquama* and its virus (HcRNAV) showed a similar pattern of fluctuation in natural environments.
- ☆ There are large amount of HcRNAV in sediment.
- ☆ Inoculating frozen sediment is available to decrease *H.circularisquama* in cell density .

We will develop the application of preventing HABs.

The image features five cyan hexagons of varying sizes and positions. Two are in the top-left corner, one is in the center, one is in the bottom-right, and one is in the bottom-left. The text "Thank you all for your attention" is centered horizontally and partially overlaid by the central hexagon.

Thank you all for your attention