Annex X-1

Proposal of making a booklet of countermeasures to terminate and mitigate red tides

1. Background and Objectives

Through WG3 activities in the past two years, we obtained basic information and understand the situation of HABs (Harmful Algal Blooms) in the NOWPAP Region by the National Reports on HABs in the NOWPAP Region, the Integrated Report on HABs for the NOWPAP Region, HAB Reference Database, *Cochlodinium* Homepage and its pamphlet.

CEARAC is now ready to begin an activity for “Promotion of Mitigation”. CEARAC and WG3 shall collect information about countermeasures to terminate and mitigate red tide for the NOWPAP Region and make a booklet of case studies on countermeasures against red tides. “Booklet of countermeasures to terminate or mitigate red tides” aims to share information on countermeasures against red tides among NOWPAP Members, to contribute to establish policies and measures against red tides in stakeholders and related agencies. We expect that this booklet will be used to learn advantages and disadvantages of mitigation activities and invent better methods and applications to terminate and mitigate red tides.

Following the decision at the 4th CEARAC FPM, proposal of making a booklet of countermeasures to terminate and mitigate red tides was adopted at a main activity of NOWPAP WG3 in 2006. What follows are details of work within WG3.

2. Details of the booklet

CEARAC has made the draft table of contents of the booklet (See Chapter 3) and an example of a report of a countermeasure implemented in Japan (See Appendix A). In the 3rd WG3 Meeting to be held on 6-7 July 2006, the provisional contents and a format of reports will be discussed for improvement of the contents with advices and opinions from experts. Based on the agreement of the meeting, consultants who will be recommended by WG3 experts will start to collect information and make reports of countermeasures in their own countries with allocation of funds from CEARAC. The fund will be paid for not only gathering information about countermeasures but also collecting and categorizing reference information for HAB Reference Database.

WG3 will collect information on countermeasures to terminate and mitigate red tides and make a booklet based on the collected information in order to promote mitigation activities against red tides. CEARAC will ask WG3 experts of each country to collect information and make reports on countermeasures against red tides in each country in 2006 by allocating funds to a consultant in each country (US$2,000 for China; US$3,000 for Japan; US$3,000 for Korea; US$2,000 for Russia) and hire a consultant in 2007 to harmonize information (US$3,000), to integrate reports and to make a booklet (US$5,000). By the end of 2007, the booklet will be printed (US$4,000).

Although making the booklet is the main activity for WG3 for the next two years, maintenances and updates of HAB Reference Database and *Cochlodinium* Homepage will also be continued as WG3 activities (US$2,000 each year).

3. Draft table of Contents of booklet of countermeasures against red tides

Draft table of contents of the booklet is in Table 1, which tells what kinds of information are introduced in the booklet. This booklet introduces (1) countermeasures implemented in the past whether or not it was succeeded; (2) countermeasures conducted presently; (3) countermeasures under development and study. Those countermeasures conducted outside the NOWPAP Region are also to be collected.

Table 1. Outline of a booklet of countermeasures against red tides

Chapter/Section	Contents
1. Introduction	○ Purpose of this booklet. ○ Brief explanation of measures against harmful microalgae ○ Brief overview of countermeasures against red tides ○ Scope of the information in this booklet.
2. Countermeasures against red tides in the NOWPAP Region	
2.1 Situation of red tides in the NOWPAP Region and Necessity of development of countermeasures	○ Explanations of the situation of red tides in the NOWPAP Region, based on the National Reports and the Integrated Report. ○ Explanations of damage to aquaculture and fisheries in the NOWPAP Region referred to the National Reports, the Integrated Report and related literature. ○ Necessity of countermeasures against red tides → overview of features of each countermeasure → necessity of development of countermeasures ＊ <u>This section is not including measure against toxin-producing plankton (shellfish poisoning)</u> ＊ <u>New section can be introduced for measures against toxin-producing plankton if it is feasible and necessary, which will be discussed in the 3rd WG3 Meeting.</u>
2.2 Countermeasures against red tide in the NOWPAP Region	○ Brief explanations on termination and mitigation after red tides emergence (refer to difference between preventive measures and countermeasures)

	○ Introduction of each countermeasure (see Appendix 8-2) 2.2.1 Chemical methods (hydrogen peroxide, organic acid, surface-active agent, copper sulfate, ozone emergence, etc) 2.2.2 Physical methods (ultrasonic waves, cavitation, etc) 2.2.3 Biological methods (algacidal bacteria, pray on animals, etc) 2.2.4 Others (clay spraying, communication system after emergence, avoidance of culture rafts, feed withdrawal, ballast water treatment, recovery vessel of red tides, etc)
2.3 Countermeasures against each red tide causative species	○ Classification of countermeasures by red tides causative species in a chart (matrix by species names and countermeasures) －Red Tides causative species－ Genus *Chattonella* (*C. antique, C. marina*) *Cochlodinium polykrikoides* *Karenia mikimotoi* *Heterocapsa circularisquama* *Heterosigma akashiwo* Diatom red tides, etc
3. Countermeasures against red tides implemented in the world	○ Introduction of countermeasures against red tides in the world except for the NOWPAP Members. Countermeasures used in the Mediterranean and the United States could be introduced. ＊If possible, difference between methods in the world and those in the NOWPAP Region.
4. Summary (more countermeasures in the future)	○ Classification of features on case studies mentioned above in a chart ○ Showing problems and prospects of each method as much as possible ○ Proposing preferable methods which is environmental friendly and considering ecosystem
References	

4. Schedule

Future schedule is shown in Figure 1. The draft table of contents of the booklet was submitted at the 4th CEARAC FPM in March 2006, and then the necessary refinement to make it useful for each Member will be discussed at the 3rd CEARAC WG3 meeting, based on interim review of the report of countermeasure by the experts in each NOWPAP member. In early 2007, CEARAC will collect the reports from each NOWPAP Member. A consultant who will be hired by CEARAC will make a booklet based on the reports from each country. The booklet will be issued by the end of 2007.

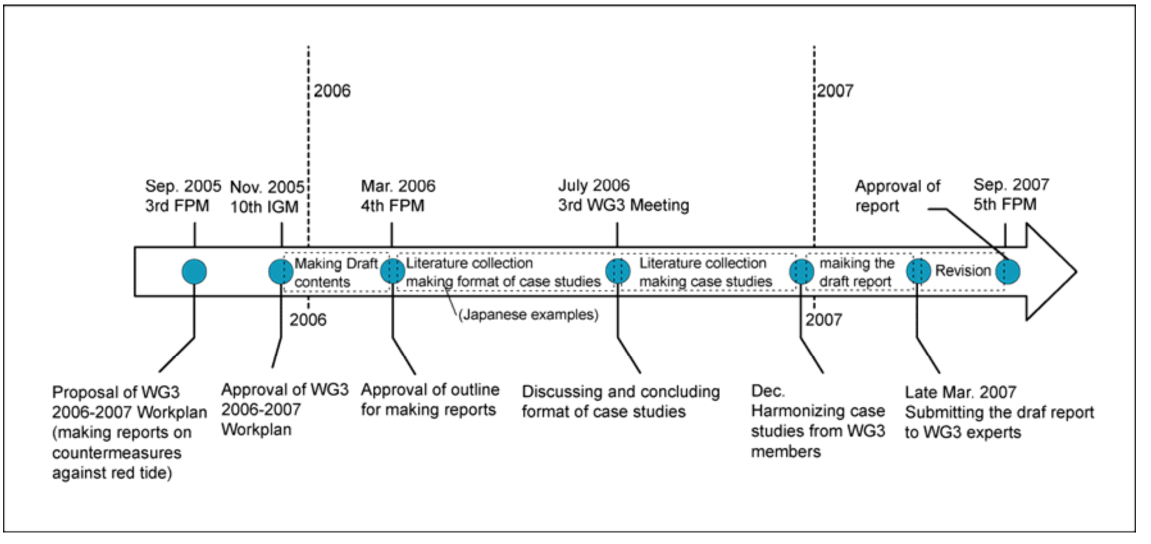


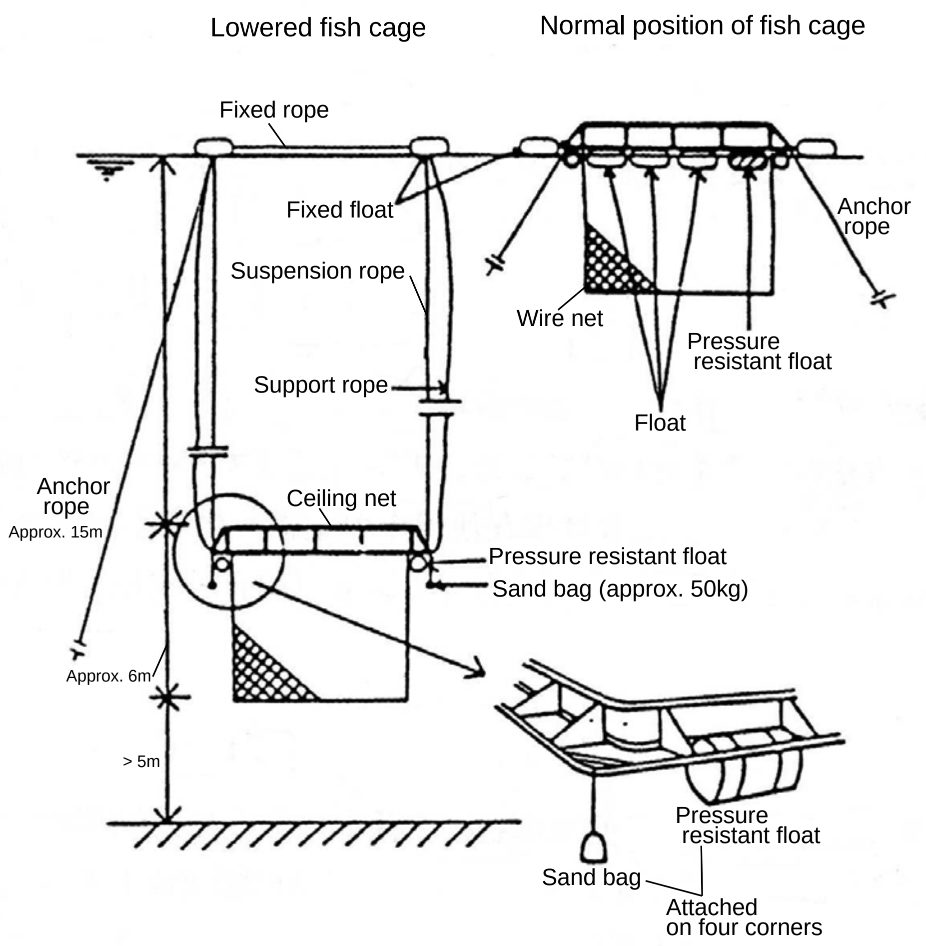
Figure 1. Provisional schedule of making a booklet

Appendix A Example of a report of countermeasure (under construction)

No.:

1) Title	Examination of the fish cage lowering system
2) Category	Others
3) Implementing organization	Kagawa Prefecture Fisheries Research Institute, Japan
4) Target species	Red-tide species
5) Implemented period	1980 – 1982
6) Experiment type	Field experiment
7) Application	Inner bay area (fish farm area)
8) Method / mechanism	➢ During red-tide events, cultured fish (e.g. yellowtail) are protected by intentionally lowering the fish cage to deeper waters. ➢ The system is economical and easy to operate (Figure-1). ➢ During red-tide events, the fish cage (8×8×6m) is lowered to a depth of 15m. The cage is lowered by removing the floats and attachment of weights (sand bags). The cage is returned to the surface by manually pulling up the support rope, and then the weights are removed and floats reattached (Figure-1).
9) Results	➢ No red-tide events occurred during the experimental period, thus the effectiveness of this system could not be evaluated.
10) Impact on environment / ecosystem	(1) Impact on cultured fish ➢ Fish cage with 2 year-old yellowtails was experimentally lowered for 35 days with no feeding. No yellowtail mortality was recorded. (2) Impact on the environment ➢ No description
11) Others	➢ The cost of installing this system on 10 cages was 741,000 yen (as of 1985). ➢ The appropriate timing and the optimum lowering depth of the fish cage during red-tide events are some of the future issues to be considered.

12) References	➢ Kagawa Prefecture Fisheries Research Institute (1980): Report on the development of countermeasures against red tides 1979, 11. Development of measures for the prevention of red-tide damages, Fisheries Agency. ➢ Kagawa Prefecture Fisheries Research Institute (1981): Report on the development of countermeasures against red tides 1980, 11. Development of measures for the prevention of red-tide damages, Fisheries Agency. ➢ Kagawa Prefecture Fisheries Research Institute (1982): Report on the development of countermeasures against red tides 1981, 11. Development of measures for the prevention of red-tide damages, Fisheries Agency.



Source: Kagawa Prefecture Fisheries Research Institute (1982)

Figure-1 Schematic diagram of fish cage lowering system

No.:

<table>
<tr><td>1) Title</td><td colspan="2">Experimental application of hydrogen peroxide for the elimination of red-tide species</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Oita Prefectural Fisheries Research Institute ,Japan (now Oita Prefectural Agriculture, Forestry and Fisheries Research Center, Fisheries Research Institute, Japan)</td></tr>
<tr><td rowspan="3">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Karenia mikimotoi (= Gymnodinium mikimotoi), Oxyrrhiis marina</td></tr>
<tr><td>others</td><td>Eutreptiella sp. (Euglenophyceae)</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1994 - 1995</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">Not described</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Cultured Karenia mikimotoi was exposed to hydrogen peroxide at concentrations of 0.33, 3.3 and 33 mg/L. The cell density of K. mikimotoi was measured after 2 hours and 4 days exposure.
➢ Cultured juvenile flounder and red-tide plankton (collected from the flounder fish farm) were exposed to five levels of hydrogen peroxide concentration, ranging between 0.3-300 mg/L. The motility of the cells was observed 15, 20, 39, 44 and 109.5 hours after the exposure. The experiment was conducted under room temperature and gentle ventilation.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ All K. mikimotoi cells were destroyed when hydrogen peroxide concentration was 3.3 and 33 mg/L. Possible inhibition to reproduction from hydrogen peroxide concentration of 0.33 mg/L (Table-1).
➢ After 15 hours exposure, reduction in cell number or motility was observed at hydrogen peroxide concentration of 3-300 mg/L. At hydrogen peroxide concentration of 300 and 30 mg/L, all cells were eliminated after 20 and 39 hours exposure, respectively (Table-2).</td></tr>
</table>

10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ All flounders died at hydrogen peroxide concentration of 300 and 30 mg/L. At hydrogen peroxide concentration of 0.3-6 mg/L, the survival rate of flounders was between 80-100% (Table-2). (2) Impact on the environment ➢ No description
11) Others	➢ The hydrogen peroxide concentration that eliminates red-tide species, but maintain flounder survival rate was estimated to range between 6-30 mg/L. ➢ The amount of hydrogen peroxide required for field application was estimated for an assumed area of 100 x 100 m. The estimated amount was 220 kg (200 L) with 30% hydrogen peroxide content. ➢ Hydrogen peroxide has strong oxidizing properties and is classified as a toxic substance. Therefore, a thorough investigation must be conducted prior to practical application.
12) References	➢ Nishimura, K. & Iwano, H., (1994): Experiment on the elimination of harmful red-tide plankton, Annual Report of Oita Prefectural Fisheries Research Institute 1994, pp.181-186, Oita Prefecture. ➢ Nishimura, K. & Iwano, H., (1995): Experiment on the elimination of harmful red-tide plankton, Annual Report of Oita Prefectural Fisheries Research Institute 1995, pp.212-218, Oita Prefecture.

Table-1 The change in number of swimming *Karenia mikimotoi* cells after exposure to different hydrogen peroxide concentrations

Date	Control		33mg／L		3.3mg／L		0.33mg／L		Note
	Lot1	Lot2	Lot1	Lot2	Lot1	Lot2	Lot1	Lot2	
3/18 16:00	173	123	185	109	18	202	171	148	Before exposure to H_2O_2
3/18 18:00	152	135	0	0	0	0	197	173	After 2 hrs. exposure Rupture of cell when 33 mg/L. Cell morphology became roundish when 3.3mg/L
3/22 14:00	331	231	0	0	0	0	331	169	After 4 days exposure All cells eliminated when 33mg/L and 3.3mg/L. Cell morphology became roundish when 0.33mg/L

Source：Nishimura & Iwano (1994)

Table-2 Observation of red-tide plankton and cultured flounder after exposure to different hydrogen peroxide concentration

Observation date Time Time after exposure (h)		4/14 10:00 15	4/14 15:00 20	4/15 10:00 39	4/15 15:00 44	4/18 8:30 109.5
Control	No. of surviving flounder	4	4	4	3	3
	Motility of plankton	＋	＋	±	＋	±
300 mg/L	No. of surviving flounder	0	0	0	0	0
	Motility of plankton	±	－	－	－	－
30 mg/L	No. of surviving flounder	5	3	1	1	0
	Motility of plankton	±	±	－	－	－
6 mg/L	No. of surviving flounder	5	5	5	5	5
	Motility of plankton	±	±	±	±	－
3 mg/L	No. of surviving flounder	5	5	5	5	4
	Motility of plankton	±	±	±	＋	－
0.3 mg/L	No. of surviving flounder	5	5	5	5	4
	Motility of plankton	＋	±	±	＋	－

Source: Nishimura & Iwano (1995)

Note 1: ＋ similar cell density and motility as during the start of the experiment, ± reduction in cell density and motility compared to the start of the experiment, － no cells observed

Note 2: No data available on the size of the flounders

No.:

<table>
<tr><td>1) Title</td><td colspan="2">Experimental application of clay spraying for the removal of red-tide species</td></tr>
<tr><td>2) Category</td><td colspan="2">Physical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Kagoshima Prefectural Fisheries Research Institute (now Kagoshima Prefectural Fisheries Technology and Development Center)</td></tr>
<tr><td rowspan="5">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Bacillariophyceae</td><td>Leptocylindrus danicus</td></tr>
<tr><td>Dinophyceae</td><td>Ceratiumu fusus, Cochlodinium polycrikoides (= Cochlodinium sp.(78' - type)), Karenia mikimotoi (= Gymnodinium sp. (65' - type)), Gyrodinium instriatum, Noctiluca scintillans, Prorocentrum micans, P. sigmoides, P. triestinum, Scrippsiella trochoidea, Alexandrium catenella (= Protogonyaulax catenella: Toxin Producing Plankton)</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella antiqua, Chattonella sp. (Kagoshima Bay), Heterosigma akashiwo (= Olisthoduscus sp.)</td></tr>
<tr><td>others</td><td>Mesodinium rubrum</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1980 – 1982</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Field experiment (Yatsushiro Sea/Kagoshima Bay, Kyushu Region), Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">Limited range in coast area</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Removal of red-tide species by spraying clay over the bloom.
➢ Red-tide species adhere onto the clay particles and sink. Also, when clay particles dissolve into seawater, Al ion is released and kills red-tide species.
➢ Examined clay types were kaolin, bentonite and montmorillonite. Montmorillonite was collected from Iriki town of Kagoshima Prefecture (hereinafter referred as Iriki montmorillonite).
➢ Lab and field experiments were conducted to examine the sinking rate of different plankton species by each clay type.
➢ During the field experiment, clay was sprayed either by hand or spraying pump (clay jet pump).</td></tr>
</table>

9) Results	➢ When kaolin and bentonite were applied, neither adhesion nor mortality of *Chattonella* was observed. On the other hand, when Iriki montmorillonite was sprayed at a concentration above 150 g/m^3, morphological change, cessation of swimming and cell damage of *Chattonella* were observed. ➢ Lab or field experiments were conducted on 15 different red-tide species (Table-1). Significant decrease of *Cochlodinium polycrikoides* cells was recorded when Iriki montmorillonite was applied. The sprayed concentration ranged between 110-400 g/m^3 (110-400 ppm between 0-1 m depth).
10) Impact on environment / ecosystem	(1) Impact on fish and shellfish ➢ The median tolerance limit* (TLm) of yellowtail (weight: 296-518g, ave. weight: 387g) against Iriki montmorillonite was 2,000 ppm after 24hrs exposure. ➢ No effects of Iriki montmorillonite on juvenile tiger prawn, egg and larvae of red seabream were observed after 4 hr. exposure at concentration of 2,000 ppm. *Median tolerance limit: concentration of some toxic substance at which just 50 percent of the test animals are able to survive for a specified period of exposure (2) Impact on the environment ➢ Elution test of Iriki montmorillonite was conducted with 3% Iriki montmorillonite-seawater weight percentage. The sample was shook for 6 hrs. at 200 rpm. The results showed decrease of pH, and increase in COD, DIN and soluble iron concentration (Table-2). However, the weight percentage of clay in field application will be less than 1/10 of the above elution test, thus the effect on pH and water quality should be insignificant compared to the above results.
11) Others	➢ Clay spraying was conducted on actual red-tide blooms, and has been effective with certain species such as *Cochlodinium polycrikoides*. ➢ The effects of clay spraying have been examined through field experiments and trial application by fish farmers. ➢ There is no detail description on the cost of clay spraying. However, according to the fish farmers, clay spraying is effective but high cost.

12) References	➢ Kagoshima Prefectural Fisheries Research Institute (1980): 2-(1) Experimental application of clay spraying for the removal of red-tide species, Report on the development of red tide countermeasures 1979, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Research Institute (1981): 2-(1) Experimental application of clay spraying for the removal of red-tide species, Report on the development of red tide countermeasures 1980, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Research Institute (1982): 1-(3) Experimental application of clay spraying for the removal of red-tide species, Report on the development of red tide countermeasures 1981, Fisheries Agency.

Table-1 Effects of Iriki montmorillonite on various red-tide species

Genus and Species	Experiment type	Clay concentration and results
Cochlodinium polycrikoides	Field (during red tide in Yachishiro Sea)	Showed significant removal between 110-400 g/m^3 (110-400 ppm between 0-1m depth). Also was effective in preventing fish mortality.
Chattonella sp. (Kagoshima Bay)	Lab (cultured strain)	The cell density of *Chattonella* sp. (Kagoshima Bay) was reduced to below lethal levels for fish (500 cells/mL/kg of fish) at 1,300-2,200 ppm. Also was effective in preventing fish mortality.
Chattonella antiqua	Lab (cultured strain)	The cell density of *Chattonella antiqua* was reduced to below lethal levels for fish (100 cells/mL/kg of fish) at 6,000-13,000 ppm. To remove *C. antiqua*, 3.3-6 times more clay spraying was required compared to the *Chattonella* sp. in Kagoshima Bay.
Noctiluca scintillans	Lab (samples collected from Kagoshima Bay)	Was effective when Iriki montmorillonite was mixed with seawater prior to spraying.
Mesodinium rubrum	Lab (samples collected from Harima-nada, Hyogo Prefecture)	At 7,500 ppm, 100% of the cells ruptured after 5 min.
Prorocentrum sigmoides	Lab (samples collected from Kagoshima Bay)	All cells ceased swimming at 2,000 ppm after 10 minutes (10L poly bucket). After 60 min., 90% of the cells sunk (2,360 cells/ml out of 2,600 cells/ml).
Leptocylindrus danicus	Field (during red tide in Kagoshima Bay)	No effects were observed at 90 g/m^3, probably due to low concentration.
Ceratium fusus	Lab (samples collected from Kagoshima Bay) and field (during red tide in Kagoshima Bay)	No effects were observed in lab and field up to 2,000 ppm.
Alexandrium catenella	Lab (cultured strain)	At 7,500 ppm, 89.3% (4,600 cells/ml out of 5,150 cells/ml) of the cells ceased swimming after 5 min.
Karenia mikimotoi	Lab (cultured strain)	At 7,500 ppm, 88.9%（9,250 cells/ml out of 10,400 cells/ml）of the cells ceased swimming after 5 min.
Heterosigma akashiwo	Lab (cultured strain)	At 7500ppm, 100% (6,700 cells/ml) of the cells show morphological change (shrinking) after 5min.
Prorocentrum micans	Lab (cultured strain)	At 7,500ppm, 100% (3,650 cells/ml) of the cells ceased swimming after 5 min.
Prorocentrum triestirum	Lab (cultured strain)	At 7500ppm, 100% (19,500 cells/ml) of the cells showed morphological change (shrinking) after 5min.
Gyrodinium instriatum	Lab (cultured strain)	At 7500ppm, 78.7% (6,450 cells/ml out of 8,200 cells/ml) of the cells showed morphological change (shrinking) after 5min.
Scrippsiella trochoidea	Lab (cultured strain)	At 7,500ppm, 100% (26,350 cells/ml) of the cells ceased swimming after 5 min.

Source: Kagoshima Prefectural Fisheries Research Institute (1982)

Table-2 Results of clay elution test

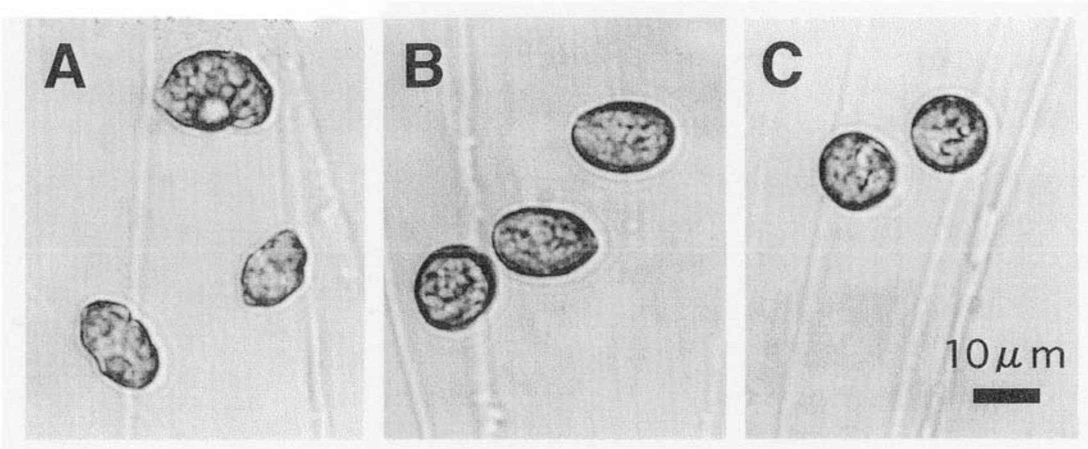
	pH	COD (ppm)	DIN (μ g-at・L^{-1})	DIP (μ g-at・L^{-1})	Soluble Fe (μ g-at・L^{-1})	Mn (μ g-at・L^{-1})
Extracted seawater	7.89	0.21	7.47	1.46	0.20	0.17
Iriki montmorillonite	4.08	1.24	36.12	1.11	6.97	3.17

Source: Kagoshima Prefectural Fisheries Research Institute (1980)
Note: Elution test was conducted with 3% Iriki montmorillonite-seawater weight percentage. The sample was shook for 6 hrs. at 200 rpm.

No.:

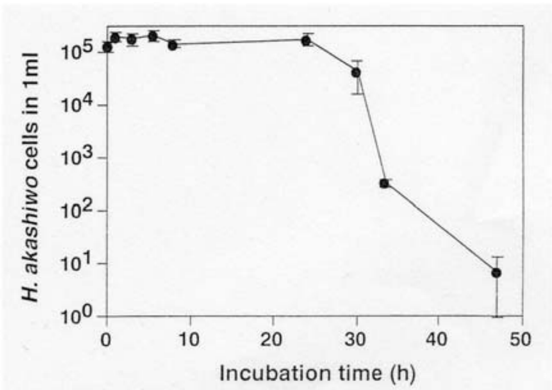
1) Title	Growth characteristics of *Heterosigma akashiwo* virus and its possible use as a microbiological agent for red tide control	
2) Category	Biological control	
3) Implementing organization	Nansei National Fisheries Institute, Japan (now National Research Institute of Fisheries and Environment of inland Sea, Fisheries Research Institute, Japan)	
4) Target species	**Class**	**Genus and Species**
	Raphidophyceae	*Heterosigma akashiwo*
5) Implemented period	1998 – 1999	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ *Heterosigma akashiwo* Virus (HaV01), which infects *H. akashiwo* was isolated from Unoshima Fishing Port (Fukuoka Prefecture) in 1996. The HaV01 stock was inoculated into a fresh culture of *H. akashiwo* and incubated at 20 ℃ for 3 days. ➢ The growth characteristics of HaV01 were examined by inoculation of HaV01 into *H. akashiwo* culture. The initial density of *H. akashiwo* was 1.27×10^5 cells/L, and inoculation density of HaV01 was 2.58×10^5 LCU*[1] (MOI*[2] was 2.04). ➢ The algicidal effects of HaV01 were examined by inoculation of HaV01 into a mixed algal culture containing 4 phytoplankton species (*H. akashiwo*, *Chattonella antiqua*, *Heterocapsa triquetra*, *Ditylum brightwellii*), with MOI levels of 3.2, 0.032, and 0. ➢ The algicidal effects of HaV01 on *H. akashiwo* were examined twice in natural seawater culture, which were collected from northern Hiroshima Bay. MOI level of the first test was 260, and 0.7, 0.07 and 0.007 for the second test. *[1]LCU: Lysis – Causing Units *[2]MOI: Multiplicity of infection	

9) Results	➢ After inoculation of HaV01, *H. akashiwo* cells became roundish within 8 hrs (Figure-1). At 47 hrs after inoculation, *H. akashiwo* density had decreased to less than 10^1 cells/mL (Figure-2). 7.7×10^2 infectious particles were produced by each *H. akashiwo* cell infected with HaV01. ➢ The rate of disappearance of *H. akashiwo* was affected by the MOI, *H. akashiwo* was specifically eliminated even with the lower MOI used in this experiment (0.03). In contrast, HaV01 had no conspicuous effect on the growth of the other three species of phytoplankton (Figure-3). ➢ HaV01 specifically affected *H. akashiwo* in unsterilized natural seawater cultures containing numerous natural microorganisms. In addition, HaV01 had no obvious effect on the growth of diatoms even at an MOI of 260. *H. akashiwo* was specifically eliminated even when the MOI was as low as 0.007 (Figures-4 & 5).
10) Impact on the environment / ecosystem	➢ No description
11) Others	➢ Although HaV could be a possible microbiological agent when scale, cost, and safety are considered, the effects of various HaV clones on natural populations of *H. akashiwo* must be assessed in more detail before this virus can be used for elimination of *H. akashiwo* red tides.
12) References	➢ Nagasaki, K., Tarutani, K. and Yamaguchi, M. (1999): Growth characteristics of *Heterosigma akashiwo* Virus and its possible use as a microbiological agent for red tide control, Applied and Environmental Microbiology, Vol. 63(3), 898-902. ➢ Nagasaki, K. and Yamaguchi, M. (1998): Effect of temperature on the algicidal activity and the stability of HaV (*Heterosigma akashiwo* Virus), Aquatic Microbial Ecology, Vol. 15, 211-216.



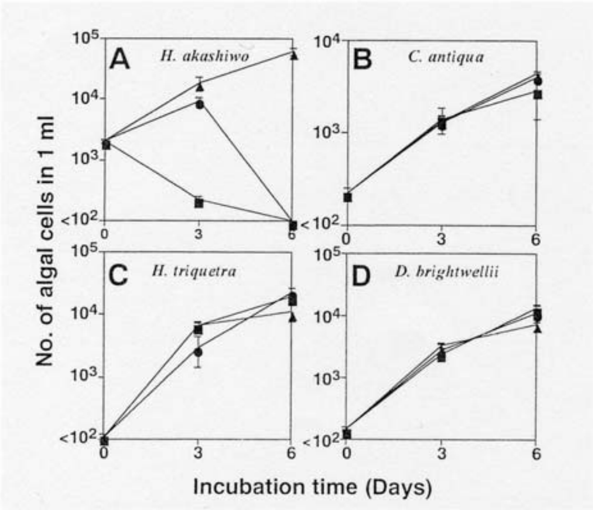
Source: Nagasaki et al (1999)

Figure-1 Optical microphotographs of *Heterosigma akashiwo* cells before inoculation (A) and 4h (B) 8h (C) after inoculation of HaV.



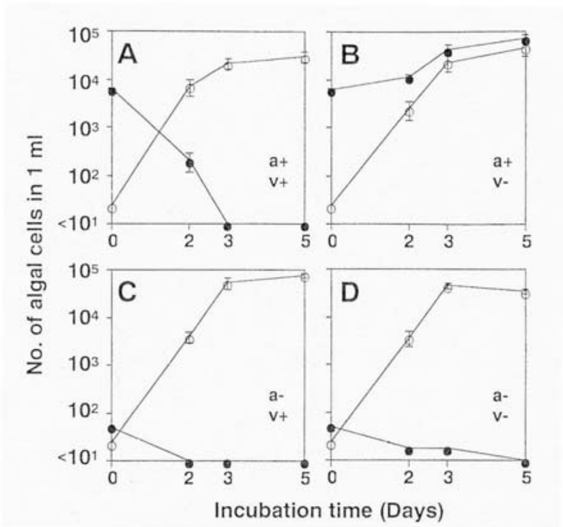
Source: Nagasaki et al (1999)

Figure-2 Changes in density of *Heterosigma akashiwo* cells in the one-step growth experiment in which the initial MOI of HaV was 2.04. The error bars indicate standard deviations.



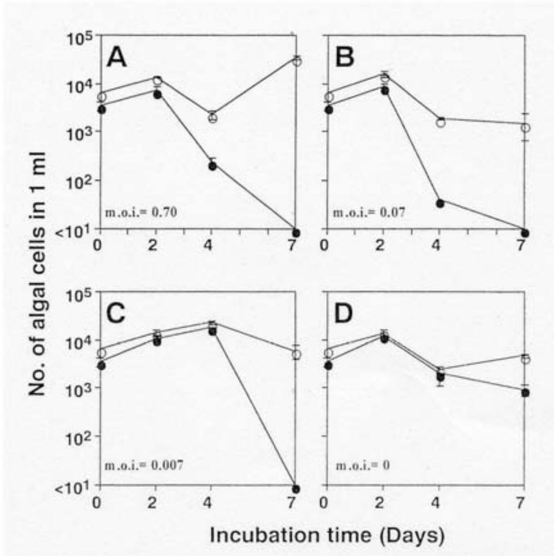
Source: Nagasaki et al (1999)

Figure-3 Changes in density of *Heterosigma akashiwo* (A), *C. antiqua* (B), *H. triquetra* (C), and *D. brightwellii* (D), cells in the mixed algal culture inoculated with HaV at MOI of 3.23 (■), 3.23 (●), and 0 (▲). The error bars indicate standard deviations.



Source: Nagasaki et al (1999)

Figure-4 Changes in densities of *Heterosigma akashiwo* (●) and diatoms (○) cells in the natural seawater sample collected at Kure port on 8 April 1998. The natural seawater was inoculated with a *H. akashiwo* culture (a+) and nontreated HaV (v+)(A), a *H. akashiwo* culture and heat-treated HaV(v-)(+)(B), a *H. akashiwo* culture filtrate (-a) and nontreated HaV(C), and a *H. akashiwo* culture filtrate and heat-treated HaV(D). The error bars indicate standard deviations.



Source: Nagasaki et al (1999)

Figure-5 Changes in densities of *Heterosigma akashiwo* (●) and diatoms (○) cells in the natural seawater collected at Kusatsu Fishing Port on 28 April 1998. The natural seawater samples were inoculated with *Heterosigma akashiwo* HaV at MOI of 0.7(A), 0.07(B), 0.007(C), and 0(D). The error bars indicate standard deviations.