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Booklet of Countermeasures against Harmful Algal Blooms (HABs) in the NOWPAP Region

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Preface

The objectives of Booklet of Countermeasures against HABs in the NOWPAP Region are to provide and to share information on countermeasures against HABs implemented in the NOWPAP member states, and to contribute to establishing policies and measures against HABs among stakeholders and related agencies. We expect that this booklet is used to learn advantage and disadvantage of mitigation activities and to invent better methods and applications in order to terminate and mitigate HABs.

This report was prepared by CEARAC in cooperation with experts and a collaborator of WG3 and CEARAC Focal Points. The CEARAC Secretariat would like to thank the CEARAC Focal Points, the experts of WG3 and their colleagues for great contributions to publishing this booklet of countermeasures against HABs in the NOWPAP region. CEARAC and WG3 would like to express special thanks to Dr. Chang-Kyu LEE (National Fisheries Research and Development Institute) for his provision of a photo to the front cover page.

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Countermeasures against HABs in the NOWPAP region

1. Introduction

In order to understand and share information on harmful algal blooms (HABs) in the NOWPAP region, each NOWPAP member has reported on the status of these blooms in their territorial waters by submitting National Reports (NOWPAP Working Group 3, 2004). Based on these reports, NOWPAP CEARAC compiled the 'Integrated Report on Harmful Algal Blooms for the NOWPAP region (Integrated Report)', which provides an overview of the status of HABs in the NOWPAP region. According to the Integrated Report, all NOWPAP members are experiencing HAB related environmental problems, despite variations in HAB magnitude and frequency among regions. The most commonly reported damages induced by HABs include mass mortality of aquaculture species and poisoning of fish/shellfish products that, as a result, have sometimes led to major economic losses and health hazards.

HABs can be classified broadly into two phenomena: red tides and fish/shellfish poisoning by toxin-producing phytoplankton (hereafter referred to as just plankton). There are basically two approaches to preventing or minimizing damage from red tides. One approach is to prevent red-tide blooms, such as by reducing nutrient levels in the water column. The other approach, which is the focus of this booklet, is to arrest red-tide blooms before they cause any significant damage. To prevent health hazards from fish/shellfish poisoning, regular safety inspections and shipping restrictions are vital, and these procedures will be detailed in the later chapters.

This booklet was compiled to assist organizations that are in need of effective HAB countermeasures by providing relevant information that has been implemented or considered by NOWPAP members and other countries. Another objective of this booklet is to identify the necessary future HAB-related activities of the Special Monitoring & Coastal Environmental Assessment Regional Activity Centre (CEARAC).

1.1 Definitions

Since each NOWPAP member has their own definition of a HAB, the first WG3 meeting in Busan, Korea, in October 2003 agreed on specific definitions, as follows.

HAB: A proliferation of unicellular phytoplankton that can cause massive fish or shellfish kills, contaminate seafood with toxins and alter aquatic ecosystems in ways that humans perceive as harmful. There are two phenomena, the so called red tide and toxin-producing plankton.

Red Tide: Water discoloration by vastly increased unicellular phytoplankton that induces deterioration of aquatic ecosystems and occasional fishery damage.

Toxin-producing Plankton: Phytoplankton species that produce toxins within their cells and contaminate fish and shellfish throughout the food chain.

Countermeasure: Measures that are implemented to prevent or minimize damage from HABs.

Some red-tide species have multiple scientific names due to past taxonomic amendments (e.g. the synonym of *Karenia mikimotoi*: *Gymnodinium nagasakiense*; basynonym of *K. mikimotoi*: *Gymnodinium mikimotoi*). This booklet mostly uses the same scientific names as in the Integrated Report, but in some cases scientific names from the source reference are used.

1.2 Countermeasures against HABs

Countermeasures against red tides can be broadly classified into either direct or indirect measures, as shown in Figure 1. Direct measures refer to countermeasures that are implemented directly against red-tide blooms. These countermeasures eliminate red-tide blooms through physical, chemical or biological control methods. Indirect measures, on the other hand, are not implemented against red-tide blooms but instead use other approaches to counter against red tides, such as by implementing effluent control and environmental improvement projects. Although this booklet generally focuses on direct measures, some indirect measures are also introduced because they may be useful for aquaculture operators. Measures bracketed by dashed lines in Figure 1 are introduced in this booklet.

There are currently no countermeasures available to prevent poisoning of fish/shellfish by toxin-producing plankton. Instead, countermeasures focus on preventing the poisoning of consumers by, for example, regular safety inspections and shipping restrictions. This booklet introduces such shellfish-poisoning countermeasures that are being implemented in the NOWPAP region.

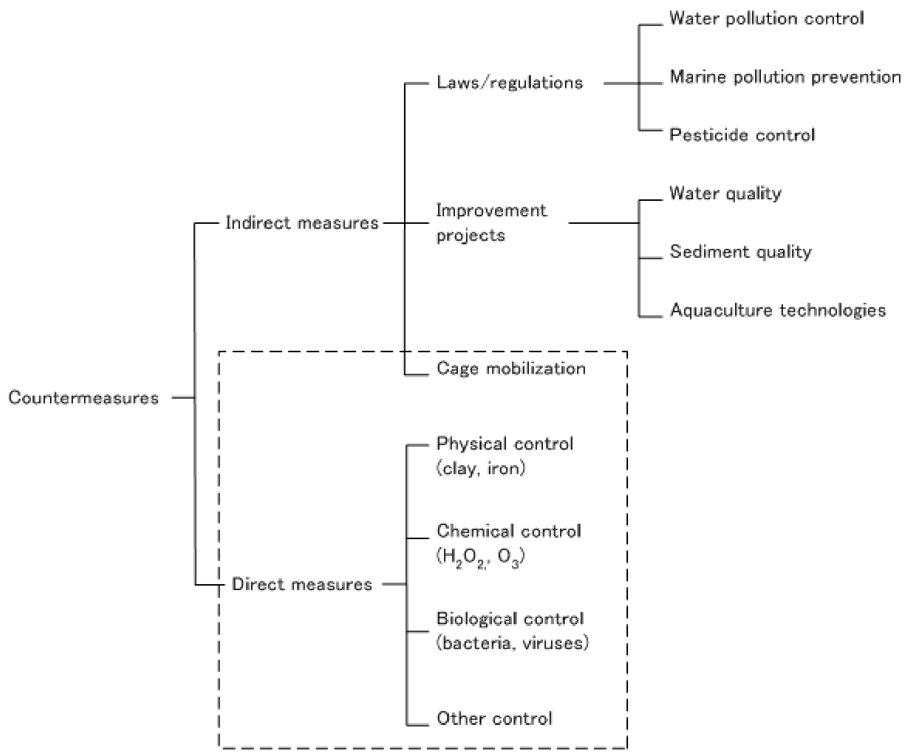

Figure 1 Classification of red-tide countermeasures

Source: modified from Shirota (1980) Red-tide mechanism and control, Kouseisha Kouseikaku, 105-123. (in Japanese)

1.3 Scope of the countermeasures included in the booklet

This booklet is targeted towards potential implementers of HAB countermeasures. Consequently, the following types of countermeasures were selected for this booklet.

- Countermeasures that have been implemented in the NOWPAP region
- Countermeasures that are under research and development, but have high potential for future application

Examples of HAB countermeasures were collected, mainly by literature searches of scientific papers and research reports published by research institutions in the NOWPAP region. Information from websites and abstracts are not included. Countermeasures implemented in non-NOWPAP countries were also collected, which are introduced in Sections 2.4 and 3.4.

2 Countermeasures against red tides in the NOWPAP region

2.1 Situation of red tides in the NOWPAP region and the necessity of developing countermeasures

2.1.1 Situation of red tides in the NOWPAP region

The situation of red tides in the NOWPAP region is summarized below. The information was extracted from Chapter 2 of the Integrated Report.

Table 1 summarizes the status of red-tide events in the NOWPAP region. To date, 75 red-tide species have been recorded in the NOWPAP region. Three flagellate species (*Heterosigma akashiwo, Noctiluca scintillans, Prorocentrum minimum*) and one diatom species (*Skeletonema costatum*) have been frequently recorded in the coastal waters of all NOWPAP members. All three flagellate species have caused extensive damage to local fisheries. Other common and damage-causing dinoflagellate (Dinophyceae) species include *Karenia mikimotoi*, *Gymnodinium sanguineum* and *P. micans*. In recent years, *Cochlodinium polykrikoides* has caused serious damage to fisheries in Japan and Korea.

The size of a red tide is usually less than 100 km^2 in Japanese, Korean and Russian waters, but in Chinese waters they often extend to over 100 km^2. More than 50% of the recorded blooms in China between 1990 and 2004 were larger than 100 km^2, and approximately 25% of them were larger than 1,000 km^2. One reason for these size differences between China and the other NOWPAP members could be due to the differences in observation methods. In China, bloom sizes were mostly recorded through aerial surveys, whereas other NOWPAP members mainly recorded bloom sizes from sea vessels.

Red tides are most frequent from spring to summer in the NOWPAP region. In China, the peak season is from June to August. In Japan, the peak is in April, June and July. In Korea, there is a prominent peak in August. In Russia, the peak appears in June and July. The dominant red-tide species during the peak months are shown below. All of these plankton species are known to cause damage to fisheries.

China: *Noctiluca scintillans* (June and July)
Japan: *Noctiluca scintillans* (April), *Heterosigma akashiwo* (June), *Karenia mikimotoi* (July)

Korea: *Cochlodinium polykrikoides* (August)
Russia: *Noctiluca scintillans* and *Heterosigma akashiwo* (June)

Most red-tide events in the NOWPAP region continue for about 1 week, although in rare cases they have lasted for 1−2 months (e.g. a *C. polykrikoides* bloom lasted for 62 days in Korea in 2003).

Table 1 (1) Summary of recorded red-tide events in the NOWPAP region

	China (Bohai and Yellow Sea)	Japan (Data from Kyushu region (1998–2002) unless stated)	Korea (1999–2003 unless stated)	Russia (1992–2003 unless stated)[1]
No. of events	84 from 1990–2004	150, of which 19 were harmful	304	23, all were harmless and caused no damage.
No. of causative species	24	36	41	13
Max. cell density of major species (cells/ml)	*Noctiluca scintillans* (49,000) *Skeletonema costatum* (72,000) *Ceratium furca* (1,250) *Gymnodinium* sp. (300,000)	*Karenia mikimotoi* (117,980)	*Cochlodinium polykrikoides* recorded the highest cell density each year. Maximum density was recorded in 2003 (48,000).	*Eutreptiella gymnastica* (30,900)
Location of occurrence	Mainly along the coast of Yellow Sea and Bohai Bay	Mainly along the coast of northern Kyushu	Along the entire coast except the northeast	Some areas in Peter the Great Bay
Size of bloom	Data from 1990–2004 <10 km^2: ≒18% 10–100 km^2: ≒29% 100–1,000 km^2: ≒30% >1,000 km^2: ≒23% Affected area generally larger in Bohai Sea than Yellow Sea[2]	<1 km^2: ≒51% 1–100 km^2: ≒48% >100 km^2: ≒1%	<1 km^2: ≒56% 1–100 km^2: ≒19% >100 km^2: ≒24% Large blooms were mostly by *C. polykrikoides*	*Noctiluca scintillans* and *Prorocentrum minimum* blooms > 1 km^2
Duration	Usually < 1 week. However, a *Ceratium furca* bloom lasted for 40 days in 1998. *Eucampia zodiacus* and *Chaetoceros socialie* blooms lasted for 20 days.	About 1 week, although there were variations. 18 of 150 events > 20 days.	Usually < 10 days, except for *C. polykrikoides*, which lasted for 1–2 months.	*N. scintillans* and *Oxyrrhis marina* blooms > 20 days

[1]No regular red-tide monitoring programs in Russia to date. Presented data are derived from *ad hoc* monitoring or research conducted by the IMB FEB RAS, 992–2002.
[2]Observation mainly through aerial surveys

Table 1 (2) Summary of recorded red-tide events in the NOWPAP region

	China (Bohai and Yellow Sea)	Japan (Data from Kyushu region (1998–2002) unless stated)	Korea (1999–2003 unless stated)	Russia (1992–2003 unless stated)[1]
Seasonal pattern	Most frequent in July and August (1990–2004).	High frequency April–September. Most frequent in June and July.	Recorded from January to November. Most frequent in August.	Usually observed March–September. Most frequent in June and July.
Damage	Mass mortality of fish and shellfish by *Ceratium furca*, *Exuviaella cordata*, *Gymnodinium* sp., *G. sanguineum*, *N. scintillans* and *Prorocentrum* sp. Most serious damage recorded in 1989 by *Gymnodinium* sp. in Bohai Bay (economic loss of US$38 million).	Mass mortality of fish and shellfish by *Heterosigma akashiwo*, *Heterocapsa circularisquama*, *G. mikimotoi*, *C. polykrikoides* and *N. scintillans*. Most serious damage recorded in 1999 by *C. polykrikoides* (economic loss of US$7 million)	*C. polykrikoides* has caused damage to fisheries for most years since 1993. Economic loss of US$95 million in 1995 and US$19 million in 2003.	No damage recorded

[1]No regular red-tide monitoring programs in Russia to date. Presented data are derived from *ad hoc* monitoring or research conducted by the IMB FEB RAS, 1992–2002.

Source: NOWPAP CEARAC (2005): Integrated Report on Harmful Algal Blooms (HABs) for the NOWPAP region

2.1.2 Necessity of developing red-tide countermeasures

As part of the HAB activities, NOWPAP CEARAC has established the CCG (*Cochlodinium* Corresponding Group) to study *Cochlodinium polykrikoides*, a highly controversial red-tide species in the NOWPAP region. However, since the NOWPAP region is also affected by other red-tide species, it is important to have many countermeasure options. Red-tide countermeasures are especially important for the growing aquaculture industries in the NOWPAP region.

2.2 Countermeasures against red tides in the NOWPAP region

In the following sections, red-tide countermeasures implemented or considered in the NOWPAP region are introduced. These countermeasures can be categorized into one of the following five categories. Table 2 lists and summarizes all of the introduced countermeasures (Russia has no red-tide countermeasures because they have not been affected by red tides to date).

Physical control

Countermeasures that control red-tide blooms by flocculation were categorized as 'physical control'. In the NOWPAP region, various clays, flocculants and synthetic polymers have been used or tested as flocculants. Other countermeasures categorized under physical control are magnetic separation, centrifugal separation and ultraviolet radiation.

Chemical control

Countermeasures that control red-tide blooms by using active chemical substances were categorized as 'chemical control'. In the NOWPAP region, chemical substances such as hydrogen peroxide, hydroxide radicals, ozone, copper sulfate, disinfectants, algicides and biologically derived substances have been considered.

Biological control

Countermeasures that control red-tide blooms using biological organisms were categorized as 'biological control'. In the NOWPAP region, bacteria, viruses and plankton grazers have been considered.

Avoidance measure

Unlike the above measures an ‘avoidance measure’ does not actively control red-tide blooms, but instead avoids their impacts by moving or protecting fish cages. In the NOWPAP region, fish-cage submergance and shield curtains have been considered.

Other control

The countermeasure of an automated HAB warning and oxygen supply system did not fit into the above categories and was thus was categorized as an ‘other control’.

Table 2　Outline of red-tide countermeasures implemented or considered in the NOWPAP region

Category	Countermeasure type	Method	Document no.*		
			China	Japan	Korea
Physical Control	Clays	Flocculation/settlement of red-tide plankton using clays	C-P-1～21	J-P-1, 2	K-P-1
	Flocculants	Flocculation/settlement of red-tide plankton using flocculants (PAC, PSAS)	C-P-22	J-P-3	
	Synthetic polymers	Flocculation/settlement of red-tide plankton using synthetic polymers		J-P-4	
	Magnetic separation	Flocculation/collection of red-tide plankton using iron powder/flocculant mixture and a magnetic separator		J-P-5	
	Centrifugal separation	Removal of red-tide plankton by pumping seawater through a centrifugal separator			K-P-2
	Ultraviolet radiation	Killing of red-tide plankton by exposure to UV radiation		J-P-6	
Chemical Control	Hydrogen peroxide	Killing of red-tide plankton by hydrogen peroxide		J-C-1～5	
	Hydroxide radicals	Killing of red-tide plankton by hydroxide radicals	C-C-1～4	J-C-6	
	Ozone	Killing of red-tide plankton by ozone		J-C-7	
	Copper sulfate	Killing of red-tide plankton by copper sulfate		J-C-8	
	Disinfectants	Killing of red-tide plankton by disinfectants (surfactant, povidone-iodine, chlorine dioxide)	C-C-5～10	J-C-9	
	Herbicides	Killing of red-tide plankton by herbicide	C-C-11～14		
	Biological secretion	Killing of red-tide plankton by biological secretion (wheat straw, seaweed etc.)	C-C-15～20	J-C-10-12	
	Other chemicals	Killing of red-tide plankton by other chemicals	C-C-21～23	J-C-13	
Biological Control	Algicidal bacteria	Killing of red-tide plankton by algicidal bacteria		J-B-1～13	
	Algicidal viruses	Killing of red-tide plankton by algicidal viruses		J-B-14～21	
	Plankton grazers	Killing of red-tide plankton by plankton grazers		J-B-22～28	
Avoidance measure	Submersion of fish cages	Submersion of fish cages during red-tide blooms		J-O-1	
	Perimeter skirt or shield curtain	Prevent intrusion of HAB species into fish cages by installing a perimeter skirt or shield curtain			K-O-1
Other Control	Automated HAB warning and oxygen supplying system	Automatic stoppage of water supply system when high concentrations of fish-killing dinoflagellates are recorded. Liquefied oxygen is supplied to the fish tank during stoppage.			K-O-2

*Numbers refer to the documents attached in the Appendix

2.2.1 Physical control

Physical control methods implemented or considered in the NOWPAP region are introduced in this section, and are summarized in Table 3.

Clays

Clays were initially employed in Japan in the 1970's through an initiative of the Japanese Fisheries Agency. Their effectiveness was first confirmed when clay was experimentally applied over a *Cochlodinium* bloom in the Yachishiro Sea in 1979. Since then, clay has been applied over several *Chattonella* spp. blooms in Kagoshima Bay, and more than a dozen times over *Cochlodinium polykrikoides*, *Chattonella marina* and *Karenia mikimotoi* blooms in the Yachishiro Sea (Wada, 2002). In recent years, clay has also been applied in China and Korea. Clay is commonly used in Korea to counter *C. polykrikoides* blooms.

Clay removes red-tide plankton through the flocculation of plankton with clay particles, which then sink toward the bottom. Metal ions in the clay particles also cause the shrinkage and rupture of plankton (Wada, 2002). Montmorillonite clay and yellow clay are commonly used in Japan and Korea, respectively. In China, Yu (1994) studied the theory on coagulation of algae with clay, and developed methods for the surface modification of clay to enhance its flocculation ability. For example, surfactants such as HDTMA (Hexadecyltrimethylammonium), AGQAC (Alkyl glucoside ammonium compounds) and DPQAC (Dialkyl-polyoxyethenyl-quaternary ammonium compound) have been experimentally applied, and shown to be highly efficienct in the removal of red-tide algae in laboratory experiments (Cao and Yu, 2003; Wu and Yu, 2006; Wu et al. 2006).

Clay is applied over red-tide blooms by first mixing it with seawater. The clay-seawater mixture is then sprayed over red-tide blooms, for example by using sprinkler-equipped vessels (Figure 2). In Korea, the removal ability of clay is enhanced by dissolving clay in electrolyzed seawater (Kim, 2006).

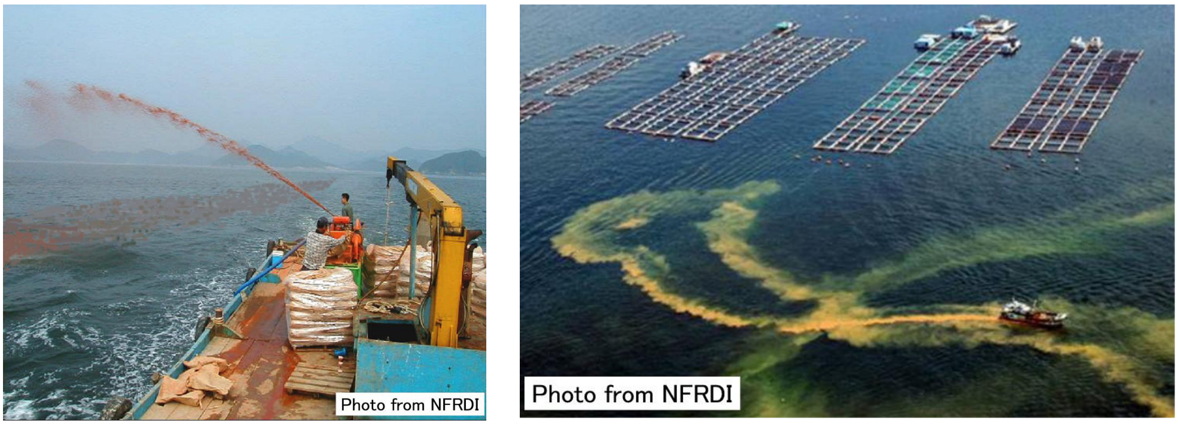

Figure 2 Clay sprayed around fish cages by sprinkler-equipped vessels (in Korea)

The following is an example of a clay-spraying procedure employed in a fish farm in Korea.

- Clay is first crushed into a powder (particle size < 50 μm), and then sprayed at concentrations of 100-400g/m^2. Spraying is usually conducted around midday, because red-tide species migrate to subsurface layers at this time.
- Taking into account the diffusion and sinking rate of clay, the area of spraying is about three times that of the cage area.
- The spraying interval is 30-40 minutes, taking into account the sinking rate of clay.
- Clay is sprayed so that the currents transport the clay in the direction of the fish cages.
- Effectiveness of clay improves when the density of red-tide plankton is high. Therefore, the Korean local government recommends clay spraying when the plankton density exceeds 1,000 cells/ml, to maximize the cost effectiveness of clay spraying.

According to laboratory experiments, the removal efficiency of *C. polykrikoides* was 80% at a clay concentration of 10 g/L. The modified clay method developed by Cao and Yu (2003) has an even higher removal efficiency of 95% for *Prorocentrum donghaiense* at a concentration of 0.01 g/L. No significant adverse impacts from clay spraying have been observed on aquatic organisms (e.g. yellowtails, tiger prawns and abalone) or the environment (NFRDI, 1999). Korea has also conducted surveys on the benthic organisms in clay-sprayed areas. No changes in species composition, diversity or biomass were recorded during the 5-year survey period (NFRDI, 1999).

Although there are no clear accounts on the cost of clay spraying in Japan, fishermen have commented on the high cost of clay. In Korea, there are specialized clay-spraying vessels that cost about US$210,000 per vessel. These vessels are equipped with a seawater electrolyzing system and a spraying gun.

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Flocculants

Flocculants remove red-tide plankton through flocculation and sinking. Flocculants such as polysilicate aluminum sulfate (PSAS) and aluminum sulfate (AS) have been considered by China, and polyaluminum chloride (PAC) by Japan.

In Japan, an onboard type of red-tide removal system composed of a flocculation tank and a pressure floatation system has been developed. This system removes red-tide plankton by pumping red-tide contaminated seawater into the flocculation tank. In the tank, plankton are flocculated by PAC and then collected as flocs through the pressure floatation system. The red-tide removal system achieved a 20-90% reduction in cell concentration and 75-93% reduction in chlorophyll concentration. However, since this system is usually installed on barges, it cannot operate in rough seas.

The removal efficiency of PSAS and AS were examined through laboratory experiments. The removal efficiency of PSAS was higher than AS for *Heterosigma akashiwo*, *Thalassiosira subtilis* and *Skeletonema costatum*.

The impacts of flocculants on the environment and ecosystem are unknown.

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Synthetic polymers

Synthetic polymers remove plankton through flocculation and sinking. To date, 15 types of synthetic polymers have been tested, which are listed below.

Tested synthetic polymers:
Petrosize J, Petrosize U, Polyethyleneimice, Polyoxyethylene Laurylamine, Polyoxyethylene Lauryl Alchohol Ether, Tween20, Tween40, Tween60, Tween80, Aminoethyl Amylose Acetate, FLONAC N[1], sodium alginate, KAYAFLOC C-533-1P[2], KAYAFLOC C-533-1O[2] and giant kelp

[1] product of KYOWA TECNOS CO., LTD (http://www.kyowatecnos.com/)
[2] product of KAYAFLOC CO., LTD (http://www.kayafloc.co.jp/)

According to laboratory experiments, some synthetic polymers caused cell lysis or deformation of *Chattonella marina* cells, even at low concentrations (< 10 ppm). However, synthetic polymers are currently not used, because they are toxic to other aquatic organisms and do not decompose in seawater (Kagoshima Pref., 1986, 1987).

-References-
(Japan)
・ Kagoshima Prefectural Fisheries Experimental Station (1986, 1987): Report on the development of red tide countermeasures, Fisheries Agency. (in Japanese)

Magnetic separation

Magnetic separation removes red-tide plankton by forming magnetized plankton-flocs. Magnetized plankton-flocs are formed by applying mixtures of iron oxide and chloride powders (Fe_3O_4, $FeCl_3$) and flocculants. The magnetized plankton-flocs are then removed from the water column when it is pumped it through a magnetic separator.

According to laboratory experiments, the removal efficiency of magnetic separation was over 80% with *Chattonella* sp. Efficiency was enhanced by adding at least 10 g of iron powder per liter of seawater. Removal efficiency also increased when small-sized iron particles were used (Suga, 1982). Impacts on the environment and ecosystem are unknown.

-References-
(Japan)
- Ichikawa, K. (1981): Report on red-tide species in the inner bay area 1980, Fisheries Agency.
- Suga, K. (1982, 1983, 1984): Report on the development of red-tide countermeasures, Fisheries Agency.

Centrifugal separation

Centrifugal separation removes red-tide plankton by pumping plankton-containing seawater into a land-based centrifugal separation system (Figure 3). This method is currently being developed by the Korean Ocean Research and Development Institute (KORDI). With this method, treatment of collected plankton and large quantities of supernatant are required, which has been an obstacle for field application. The price of this system is approximately US$21,000 for a small-scale aquaculture farm.

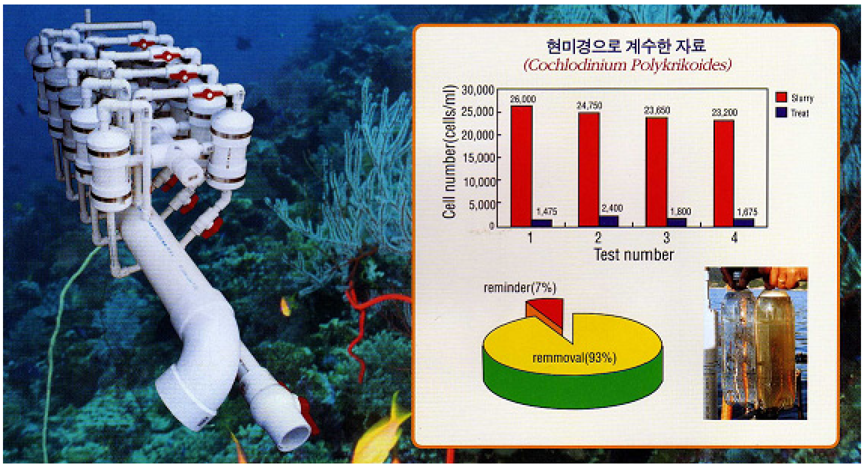

Figure 3　Centrifugal separation system

Ultraviolet radiation

Ultraviolet radiation kills red-tide plankton. According to laboratory experiments, resistance to UV radiation differs with plankton species. For example, the required UV intensity and duration to kill *Chattonella marina* was estimated to be above 3400 μW/cm^2 for 15 seconds. Other plankton species, such as *Heterosigma akashiwo* and *Karenia mikimotoi*, required less UV exposure. Impacts on the environment and ecosystem are unknown. The Ministry of Land, Infrastructure and Transport of Japan has developed an UV treatment system that could be installed on vessels.

-References-

(Japan)

- Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport (2002): Development of a red-tide removal system for deployment in anti-pollution vessels, KOBE, 1.
- Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport (2003): Development of a red-tide removal system for deployment in anti-pollution vessels, KOBE, 2.

Table 3 Summary of the physical control measures implemented or considered in the NOWPAP region

Methods	Implementing organization	Experiment type	Application	Sources
Clays	<China> ➢ Institute of Oceanology, Chinese Academy of Sciences ➢ Guangzhou Institute of Geochemistry ➢ Xiamen university ➢ Zhejiang University	➢ Lab experiment	➢ No description	➢ Yu et al. (1994a) ➢ Yu et al. (1994b) ➢ Yu et al. (1994c) ➢ Yu et al. (1994d) ➢ Yu et al. (1995a) ➢ Yu et al. (1995b) ➢ Li et al. (1998) ➢ Yu and Rao (1998) ➢ Yu et al. (1999) ➢ Zhou et al. (1999) ➢ Song et al. (2000) ➢ Wang et al. (2000) ➢ Song et al. (2003) ➢ Cao and Yu (2003) ➢ Deng et al. (2004) ➢ Yu et al. (2004) ➢ Cao et al. (2004) ➢ Cao et al. (2006) ➢ Wu and Yu (2006) ➢ Wu et al. (2006a) ➢ Wu et al. (2006b)
	<Japan> ➢ Kagoshima Prefectural Fisheries Technology and Development Center ➢ Kumamoto Prefectural Fisheries Research Center	➢ Field experiment (Ariake Sea, Yatsushiro Sea, Kagoshima Bay) ➢ Lab experiment	➢ Limited range in coastal areas	➢ Kagoshima Pref. (1980,1981,1982) ➢ Kumamoto Pref. (1980,1981,1982) ➢ Shirota (1980) ➢ Wada et al. (2002)
	<Korea> ➢ NFRDI and local municipal authorities	➢ Field experiment (Korean coastal water) ➢ Lab experiment	➢ Aquaculture farms	➢ Kim et al. (1999) ➢ NFRDI (2002) ➢ Kim (2006)
Flocculants	<China> ➢ Institute of Oceanology, Chinese Academy of Sciences	➢ Lab experiment	➢ No description	➢ Sun et al. (2002)
	<Japan> ➢ MODEC, Inc.	➢ Lab experiment	➢ No description	➢ MODEC (1976)
Synthetic polymers	<Japan> ➢ Kagoshima Prefectural Fisheries Technology and Development Center	➢ Lab experiment	➢ No description	➢ Kagoshima Pref. (1986, 1987)
Magnetic separation	<Japan> ➢ Osaka University	➢ Lab experiment	➢ No description	➢ Ichikawa (1981) ➢ Suga (1982, 1983, 1984)
Centrifugal separation	<Korea> ➢ KORDI and fish farmers	➢ Field experiment	➢ Land-based fish farms	(H.G. Kim, pers. comm.)
Ultraviolet radiation	<Japan> ➢ Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport	➢ Lab experiment	➢ No description	➢ Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport (2002, 2003)

2.2.2 Chemical control

Chemical control methods implemented or considered in the NOWPAP region are introduced in this section, and summarized in Table 4.

Hydrogen peroxide

Hydrogen peroxide kills red-tide plankton through its strong oxidizing properties. The effective concentration of hydrogen peroxide differs with plankton species (e.g. > 10 ppm for *Chattonella antique* and > 30 ppm for *Cochlodinium polykrikoides*). Hydrogen peroxide has also been tested against dinoflagellate cysts in ballast tanks. The effective concentration was 100 ppm (24 hrs) for *Polykrikos schwartzi* cysts and 50 ppm (48 hrs) for *Alexandrium catenella* cysts (Ichikawa et al., 1992).

Despite its effectiveness in killing red-tide plankton, the following are some of the negative aspects of hydrogen peroxide.

- Causes abnormalities in fish behaviour. Abnormalities in yellowtail swimming behaviour and gill movement were observed at concentrations above 150 ppm (Kagoshima Pref., 1988, 1989).
- Causes fish mortality. The 50% lethal concentration of rabbit fish, goby and horse mackerel were 224, 155 and 89 ppm, respectively (Kagoshima Pref., 1988, 1989).
- Invertebrates are more vulnerable to hydrogen peroxide than are fish.
- Low dilution rate in seawater (Kagoshima Pref., 1988, 1989).
- Causes fire when it reacts with flammable materials. Categorized as a deleterious substance in Japan.

The amount of hydrogen peroxide required for field application was estimated for an area of 100 x 100 m. The estimated amount for a 30% hydrogen peroxide concentration was 200 kg or 200 L (Oita Pref., 1994, 1995)

-References-

(Japan)
- Kagoshima Prefectural Fisheries Experimental Station (1988, 1989): Report on the development of red tide countermeasures, Fisheries Agency. (in Japanese)
- Murata H., T. Sakai, M. Endo, A. Kuroki, M. Kimura and K. Kumanda (1989): Screening of Removal Agents of a Red Tide Plankton *Chattonella marina*—with Special Reference to the Ability of the Free Radicals Derived from the Hydrogen Peroxide and Polyunsaturated Fatty Acids, Bulletin of the Japanese Society of Scientific Fisheries, 55(6), 1075-1082. (in Japanese)
- Shizuoka Prefectural Fisheries Experimental Station (1992): Removal effect of *Gymnodinium mikimotoi* with hydrogen peroxide red-tide removal agent, Annual

Report of Shizuoka Prefectural Fisheries Experimental Station FY 1991, pp.300-302. (in Japanese)
- Ichikawa, S., Y. Wakao, and Y. Fukuyo (1992): Extermination Efficacy of Hydrogen Peroxide against Cysts of Red Tide and Toxic Dinoflagellates, and Its Adaptability to Ballast Water, Nippon Suisan Gakkaishi, 58 (12), 2229-2233. (in Japanese)
- Kagoshima Prefectural Fisheries Experimental Station (1991, 1992, 1994): Report on the Development of Red-tide Countermeasures, Development of damage prevention measures against Chattonella red tides, Fisheries Agency. (in Japanese)
- Murata, H., T. Sakai, M. Endo, K. Yamauchi, S. Matsumoto, and A. Kuroki (1991): An attempt to save yellowtail from *Chattonella antiqua* red tide kill using Hydrogen Peroxide, Suisanzoshoku, 39(2), 189-193. (in Japanese)
- Nishimura, K. and H. Iwano (1994): Experiment on the elimination of harmful red-tide plankton, Annual Report of Oita Prefectural Fisheries Research Institute 1994, pp.181-186, Oita Prefecture. (in Japanese)
- Nishimura, K. and H. Iwano (1995): Experiment on the elimination of harmful red-tide plankton, Annual Report of Oita Prefectural Fisheries Research Institute 1995, pp.212-218, Oita Prefecture. (in Japanese)

Hydroxide radicals

Hydroxide radicals refer to chemical compounds with a hydroxide ion (OH^-), which have strong red-tide plankton elimination properties. China and Japan have examined their effectiveness.

When Bai et al. (2003), used hydroxide radicals against 31 dinoflagellate and diatom species, including *Karenia mikimotoi*, 99.8% were killed after 24 hours at a concentration of 0.68 mg/L.

In Japan, a product (Clear Water™) containing magnesium hydroxide was tested against various red-tide species. The elimination efficiency differed among the species tested, with a range of 64-99% at a concentration of 200 g/m^3 (= 0.2 mg/L). The elimination efficiency was high against *K. mikimotoi, Chattonella marina* and *Heterosigma akashiwo* (Marino-Forum 21, 2003).

The impacts of hydroxide radicals on the environment and ecosystem are unknown.

-References-
(China)
- Bai Xiyao, Bai Mindong, and Zhou Xiaojian (2002): Study on the treatment of red tide pollution using hydroxide radical Medicament, Ziran Zazhi, 26-32. (in Chinese)
- Bai Xiyao, Zhou Xiaojian, Lu Jibin, Zong Xu, and Huang Guibin (2003): Experiment of killing the microorganisms of red tide using hydroxyl radicals in the shore of Jiaozhou gulf, Journal of Dalian Maritime University, 29(2), 47-52. (in Chinese)
- Zhou Xiaojian, Bai Mindong, Deng Shufang, Dong Kebing, and Xing Lin (2004): Study on killing *Gymnodinium mikimotoi* with hydroxyl radical, Marine Environmental Science, 23(1), 64-66. (in Chinese)

・ Liu Xingwang, Zhou Xiaojian, Bai Xiao, and Xue Xiaohong (2004): Using of hydroxyl radical on oceanic biologic contamination prevention, Ocean Technology, 23(4), 39-43. (in Chinese)
(Japan)
・ Marino-Forum 21 (2003): Report on the Development of Red-tide Countermeasures and Practical Application Experiments. FY 2002, Fisheries Agency.

Ozone

Ozone has strong oxidizing properties, and is used as a water disinfectant in Europe and North America (Anderson, 2001). A possible application to red-tide plankton in the NOWPAP region has been considered by the Marino-Forum 21 (2003).

Ozone can kill red-tide plankton at very low concentrations. For example, *Prorocentrum triestinum, Karenia mikimotoi*, *Chattonella marina* and *Heterosigma akashiwo* were killed at concentrations under 0.1 ppm. However, ozone is also harmful to other marine organisms. Some fish species were killed when ozone concentrations were above 1 ppm. Impacts on zooplankton (*Paracalanus parvus* and *Artemia salina*) have also been confirmed at concentrations above 1 ppm.

The cost of an ozone treatment system for aquaculture farms was estimated to be approximately US$6 million per system (Marino-Forum 21, 2003).

-References-
(Japan)
・ Marino-Forum 21 (2003): Report on the Development of Red-tide Countermeasures and Practical Application Experiments. FY 2002, Fisheries Agency. (in Japanese)

Copper sulfate

Copper sulfate was first applied over a *Karenia mikimotoi* bloom in Gokasho Bay, Mie Prefecture in 1933 (Oda, 1935). It was also applied over a red-tide bloom in Florida in 1957 (Rounsefell and Evans, 1958).

The effectiveness of copper sulfate has been examined under laboratory conditions in Japan. In these experiments, *Gymnodinium* spp. were killed at a copper sulfate concentration of 1 mg/L (Sugawara and Sato, 1966). However, the use of copper sulfate is currently restricted in Japan through various laws.

-References-
(Japan)

- Oda, H. (1935): Red tide of *Gymnodinium mikimotoi* Miyake et Kominami n. sp.(MS) and inhibition by Copper sulfate, Douzatsu, 47, 35-48. (in Japanese)
- Sugawara, K. and M. Sato (1966): Red Tides of Tokyo Bay, Bulletin of the Japanese Society of Fisheries Oceanography, 9, 116-133. (in Japanese)

<u>Disinfectants</u>

In China, surfactants, povidone-iodine and chlorine dioxide have been considered as potential red-tide control methods. In Japan, acrinol has been considered. All of these chemical substances are considered as disinfectants because they are used for sterilization or washing in hospitals and water purification plants.

Surfactants are highly efficient in killing red-tide plankton. For example, biquaternary ammonium salt killed *Phaeoecystis globosa* and *Alexandrium tamarense* at a concentration of 0.4 mg/L (Zhang et al., 2003). This substance maintains its killing effects for a relatively long duration.

Povidone-iodine kills red-tide plankton at a concentration of 30 mg/L. Its killing efficiency is enhanced when used with insecticides such as isothiozolone (Hong et al., 2003, 2005).

Chlorine dioxide is commonly used in water purification plants in Europe and the United States, due to its strong oxidation and disinfection properties. Chlorine dioxide is considered to be effective against *Phaeoecystis globosa* blooms (Zhang et al., 2003).

The impacts of the above three disinfectants on the environment or ecosystem are unknown.

Acrinol is mainly used for sterilization in hospitals, and separation and refinement of organic compounds. Many experiments have been conducted to investigate the effectiveness of acrinol as a red-tide control agent. Following are some of the results obtained from these experiments.

- Acrinol killed *Gymnodinium pulchellum* at concentrations above 5 ppm and *Cochlodinium polykrikoides* at 4 ppm (Kagoshima Pref., 1987, 1988, 1989).
- When acrinol was applied to a water tank with *Chattonella marina* and three flounders at concentrations of 10 and 30 ppm, *C. marina* cells were destroyed but all of the flounder survived (Kagoshima Pref., 1987, 1988, 1989).

- In another experiment, the 50% lethal concentration of acrinol against minnows was estimated as 15-20 ppm. Yellowtails and flounders did not die at acrinol concentrations of 8-40 ppm.
- Acrinol decomposed after 2 hours under natural light conditions (Kagoshima Pref., 1987, 1988, 1989).
- When acrinol was sprayed over a sea area, acrinol mainly dispersed along the sea surface and did not reach below 1 m depth (Kagoshima Pref., 1987, 1988, 1989).

-References-
(China)
- Cao Xihua, Yu ZhiMing, and Wang Kui (2003): Mechanism of quaternary ammonium compounds extinguishing *Heterosigma akashiwo*, Oceanologia et Limnologia Sinica, 34(2), 201-207. (in Chinese)
- Zhang Heng, Liu Jiesheng, Yang Weidong, Gao Jie, and Li Jingxiong (2003): Studies on biquaternary ammonium salt algaecide for removing red tide, Marine Environmental Science, 22(4), 68-71. (in Chinese)
- Gong Liangyu, Wang Xiulin, Li Yanbin, Liang Shengkang, Han Xiurong, and Zhu Chenjian (2005): Inhibition and elimination of alkylpolyglycoside on red tide plankton, Marine Environment Science, 24(1), 1-4. (in Chinese)
- Hong Aihua, Yin pinghe, Zhao Ling, Huang Yunfeng, Qi Yuzhao, and Xie Longchu (2003): Povidone-iodine and isothiozolone for removing red tide algae *Phaeoecystis globosa*, Chinese Journal of Applied Ecology, 14(7), 1177-1180. (in Chinese)
- Hong Aihua, Yin Pinghe, Zhao Ling, Lu Songhui, Zhicheng, and Lin Chaoping (2005): Study of the extinguishing mechanism of povidone-iodine and isothiozolone, Journal of Jinan University (Natural Science), 26(3), 396-400. (in Chinese)
- Zhang Heng, Yang Weidong, Gao Jie, and Liu JieSheng (2003): Inhibition and elimination of chlorine dioxide on *Phaeoecystis globosa*. Chinese Journal of applied Ecology, 14(7), 1173-1176. (in Chinese)

(Japan)
- Kagoshima Prefectural Fisheries Experimental Station (1987, 1988, 1989): Report on the development of red-tide countermeasures, Fisheries Agency. (in Japanese)
- Muhammad, S. et al (1991): Control of Red-Tide Organisms, Especially the Genus *Chattonella* by Chemical Acrinol, Aquaculture Science, 39 (2), 141-145. (in Japanese)
- Kagoshima Prefectural Fisheries Experimental Station (1991, 1992, 1994): Report on the Development of Red-tide Countermeasures, Development of damage prevention measures against Chattonella red tides, Fisheries Agency. (in Japanese)

Herbicides

Herbicides are used by farmers to remove weeds. China has considered using herbicides as a red-tide removal agent. So far, herbicides such as bromogeramine, tertbutyl triazine and copper containing herbicides have been examined. Each herbicide showed different levels of effectiveness. Tertbutyl triazine killed *Phaeocystis globosa* at a concentration of 0.3 mg/L (Liu et al., 2004). The impacts of herbicides on the environment and ecosystem are unknown.

-References-
(China)

- Zhao Ling, Yin pinghe, Li Kunping, Yu Qiming, Xie Longchu, and Huang Changjiang (2001): Removal of red tide algae by a glass algaecide containing Cu (II), Marine Environmental Science, 20(1), 7-11. (in Chinese)
- Zhao Ling, Hong Aihua, Yin Pinghe, Qi Yuzao, and Xie Longchu (2002): Exploration of the algaecide zeolite carrying copper, China Environmental Science, 22(3), 207-209. (in Chinese)
- Hong Aihua, Yin Pinghua, Zhao Ling, Qi Yuzaoi, and Xie Longchu (2003): Studies on bromogeramine for removing and controlling prorocentrum micans red tide, Marine Environmental Science, 22(2), 64-67. (in Chinese)
- Liu Jiesheng, Zhang Heng, Yang Weidong, Gao Jie, and Ke Qiong (2004): Experimental study on algaecide Tertbutyl triazine for removing red tide, Journal ofTropical and Subtropical Botany, 12(5), 440-443. (in Chinese)

Biological secretion

Some biological organisms secrete chemical compounds that kill red-tide plankton. Phenazine pigment, wheat straw, jellyfish autolysate and seaweed have been considered as potential control methods.

Phenazine pigment is secreted by the bacterium *Pseudomonas aeruginosa*. It inhibits the growth of plankton, such as *Prorocentrum dentate* and *Heterosigma akashiwo* (Gong et al., 2004). Its impact on the environment and ecosystem is unknown.

Crushed wheat straw shows high plankton elimination effects through its adsorptive properties and growth-inhibition compounds. However, its impact on the environment and ecosystem is unknown.

The autolysate of jellyfish (*Aurelia aurita*) has shown algicidal effects against *Heterocapsa circularisquama* when added into seawater at a concentration of 5% (v/v) (Handa et al., 1998). Autolysate did not show any adverse impacts against pearl oysters or short-necked clams when exposed at the above concentration (Handa et al., 1998).

Algicidal effects of various seaweed species have been examined in China and Japan, as shown below.

China: green algae (*Ulva pertusa, Enteromorpha linza*), brown algae (*Laminaria japonica*) and red algae (*Gracilaria lemaneiformis*)
Japan: green algae (*U. fasciata, U. pertusa*) and brown algae (*Ecklonia kurome*)

Fresh tissue, dry powder and methanol extracts of *Ulva* species showed algicidal effects (Alamsjah, 2003). *Enteromorpha linza*, of the Ulvaceae family, also showed similar algicidal effects against *Heterosigma akashiwo*. However, the allelochemicals of *E. linza* are unstable and decompose at high temperatures (Xu et al., 2005). Phlorotannins extracted from the brown alga *Ecklonia kurome* showed algicidal effects against *Karenia mikimotoi* and *Cochlodinium polykrikoides* (Nagayama et al., 2003). No acute toxicity at 200 mg/L of phlorotannins was observed on red sea bream (ca. 13 g), tiger puffer (ca. 102 g) or blue crab (ca. 2 mm) (Nagayama et al., 2003).

-References-

(China)

・ Gong Liangyu, Wang Xiulin, Li Yanbin, Zhang Chuansong, Liang Shengkang, and Zhu Chenjian (2004): Isolation and Purification of Phenazine Pigments Produced by *Pseudomonas aeruginosa* and its Effects on the Growth of Red Tide Organisms, Journal of Fudan University (Natural Science), 43(4), 494-499, 506. (in Chinese)
・ Gao Jie, Yang weidong, Liu Jiesheng, Zhang Heng, and Tan Binghua (2005): Studies on wheat straw to inhibit the growth of *Phaeocystis globosa*, Marine Environmental Science, 24(1), 5-8, 31. (in Chinese)
・ Liang Xiang, Yin Pinghe, Zhao Ling, Yang Peihui, and Xie Longchu (2001): Removing red tide algae in the sea by biomass carrier as algaecide, China Environmental Science, 21(1): 15-17. (in Chinese)
・ Xu Yan, Dong ShuangLin, and Yu XiaoMing (2005): The allelopathic effects of *Enteromorpha linza* on *Heterosigma akashiwa*, ACTA Ecologica Sinica, 25(10), 2681-2685. (in Chinese)
・ Wang You, Yu Zhiming, Song Xiuxian, and Zhang Shandong (2006): Effects of Macroalgae on Growth of 2 Species of Bloom Microalgae and Interactions Between These Microalgae in Laboratory Culture, Environmental Science, 27(2), 274-280. (in Chinese)
・ Wang You, Yu Zhiming, song Xiuxian, and Zhang Shandong (2006): Effects of *Ulva pertusa* and *Gracilaria lemaneiformis* on Growth of *Heterosigma akashiwo* (Raphidophyceae) in Co-Culture, Environmental Science, 27(2), 246-252. (in Chinese)

(Japan)

・ Handa, S., J. Hiromi, and N. Uchida (1998): Algicidal effect of Autolysate of Jellyfish *Aurelia aurita* on New Type Red Tide Flagellate *Heterocapsa circularisquama*, Nippon Suisan Gakkaishi, 64(1), 123-124. (in Japanese)
・ Nagayama, K., T. Shibata, K. Fujimoto, T. Honjo and T. Nakamura (2003): Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae, Aquaculture, 218, 601-611.
・ Alamsjah, A. M., F. Ishimashi, H. Kitamura, and Y. Fujita (2006): The effectiveness of *Ulva fasciata* and *U. pertusa* (Ulvales, Chlorophyta) as algicidal substances on harmful algal bloom species, Aquaculture Science, 54(3), 325-334.

Other chemicals

Other chemicals, such as lime, coal ash and fatty acids, have been considered as red-tide control agents. For details of these chemicals please refer to the following literatures.

-References-

(China)

- Wang Huiqin and Du Guangyu (2000): The forecast and prevention and cure countermeasures of the red tide in Dalian along shore sea field. Environmental monitoring in China, 16(6), 42-45. (in Chinese)
- Lin Yi-an, Tang Renyou and Chen Quanzhen (2002): Development and preliminary test of a new material for prevention and control of red tide, Marine Sciences, 26(7), 7-12. (in Chinese)
- Lin Shengzhong and He Guangkai (2004): The technology of cleaning up red tide algae and nutrient by composite detergent, Marine Sciences, 23(4), 57-59. (in Chinese)

(Japan)

- Kagoshima Prefectural Fisheries Experimental Station (1987, 1988): Report on the development of red-tide countermeasures, Fisheries Agency. (in Japanese)
- Murata H., T. Sakai, M. Endo, A. Kuroki, M. Kimura and K. Kumanda (1989): Screening of Removal Agents of a Red Tide Plankton *Chattonella marina*—with Special Reference to the Ability of the Free Radicals Derived from the Hydrogen Peroxide and Polyunsaturated Fatty Acids, Bulletin of the Japanese Society of Scientific Fisheries, 55(6), 1075-1082. (in Japanese)

Table 4 Summary of chemical control measures implemented or considered in the NOWPAP region

Methods	Implementing organization	Experiment type	Application	Sources
Hydrogen peroxide	<Japan> ➢ Kagoshima Prefectural Fisheries Experimental Station ➢ Shizuoka Prefectural Fisheries Experimental Station ➢ Oita Prefecture	➢ Lab experiment ➢ Field experiment (Kagoshima Bay, Hamanako lake)	➢ No description	➢ Kagoshima Pref. (1988, 1989,1991, 1992, 1994) ➢ Murata et al.(1989) ➢ Murata et al.(1991) ➢ Shizuoka Pref. (1992) ➢ Ichikawa et al. (1992) ➢ Nishimura and Iwano (1994, 1995)
Hydroxide radicals	<China> ➢ Dalian Maritime University	➢ Lab experiment ➢ Field experiment (marine enclosure)	➢ No description	➢ Bai et al. (2002) ➢ Bai et al. (2003) ➢ Liu et al. (2004) ➢ Zhou et al. (2004)
	<Japan> ➢ Marino-Forum 21	➢ Lab experiment	➢ Aquaculture farms	➢ Marin-Forum 21(2003)
Ozone	<Japan> ➢ Marino-Forum 21	➢ Lab experiment	➢ Fish cage	➢ Marino-Forum 21(2003)
Copper sulfate	<Japan> ➢ Chiba Prefectural Fisheries Experimental Station	➢ Lab experiment	➢ No description	➢ Sugawara and Sato (1966)
Disinfectants <China> Surfactant Povidone-iodine Chlorine dioxide <Japan> Acrinol	<China> ➢ Institute of Oceanology, Chinese Academy of Sciences ➢ Jinan University ➢ Ocean University of China	➢ Lab experiment	➢ No description	➢ Cao et al. (2003) ➢ Hong et al. (2003) ➢ Zhang et al. (2003) ➢ Gong et al. (2005) ➢ Hong et al. (2005)
	<Japan> ➢ Kagoshima Prefectural Fisheries Experimental Station	➢ Lab experiment ➢ Field experiment (Kagoshima Bay)	➢ No description	➢ Kagoshima Pref. (1987, 1988, 1989,1991, 1992, 1994) ➢ Muhammad et al. (1991)
Herbicides	<China> ➢ Jinan University	➢ Lab experiment	➢ No description	➢ Zhao et al. (2001) ➢ Zhao et al. (2002) ➢ Hong et al. (2003) ➢ Liu et al. (2004)
Biological secretion <China> Phenazine pigments Wheat straw Seaweeds <Japan> Autolysate of jellyfish Seaweeds	<China> ➢ Jinan University ➢ Ocean University of China ➢ Institute of Oceanology, Chinese Academy of Sciences	➢ Lab experiment	➢ No description	➢ Liang et al. (2001) ➢ Gong et al. (2004) ➢ Gao et al. (2005) ➢ Xu et al. (2005) ➢ Wang et al. (2006) ➢ Wang et al. (2006)
	<Japan> ➢ Nihon University ➢ Kumamoto Prefectural Fisheries Experimental Station ➢ Nagasaki University	➢ Lab experiment	➢ No description	➢ Handa et al. (1998) ➢ Nagayama et al. (2003) ➢ Alamsjah et al. (2006)
Other chemicals <China> Lime Coal ash <Japan> Fatty acid	<China> ➢ The second institute of State Ocean Administration ➢ Jinan University ➢ National Marine Environmental Monitoring Center	➢ Lab experiment	➢ No description	➢ Wang and Du (2000) ➢ Lin et al. (2002) ➢ Hong et al.(2003) ➢ Lin and He (2004) ➢ Hong et al.(2005)
	<Japan> ➢ Kagoshima Prefectural Fisheries Experimental Station	➢ Lab experiment	➢ No description	➢ Kagoshima Pref. (1986, 1987, 1989)

2.2.3 Biological Control

Biological control methods implemented or considered in the NOWPAP region are introduced in this section.

Algicidal bacteria

Algicidal bacteria are known to play important roles in the natural elimination of red-tide blooms. Algicidal bacteria kill plankton by direct attack or by secreting toxic substances (Ishida, 1994).

Algicidal bacteria show algicidal effects only on their host plankton species. In the NOWPAP region, algicidal bacteria, such as *Alteromonas* sp., *Flavobacterium* sp. and *Cytophaga* sp., have been isolated from red-tide blooms of *Karenia mikimotoi*, *Heterocapsa circularisquama* and *Chattonella antique*. Table 5 summarizes algicidal bacteria isolated from the NOWPAP region and their host plankton species.

Although algicidal bacteria are considered to be highly effective in controlling red-tide blooms, they have not yet been applied in practice. For practical application, field application methods, as well as cost and safety issues, must be refined.

Table 5 Algicidal bacteria isolated from the NOWPAP region

Species and strains of algicidal bacteria	Host species	Sources
Alteromonas sp.	*Karenia mikimotoi*	Mie Pref. (1994), Yoshinaga (1997), Iwata et al. (2006), Marino-Forum 21 (2003)
	Chattonella antiqua	Imai et al. (1995), Imai (1997)
	Coscinodiscus wailesii	Nagai and Imai (1999)
Cytophaga sp.	*Heterocapsa circularisquama*	Imai et al. (1996), Nagasaki et al. (2000)
	C. antiqua	Imai et al. (1991), Imai (1997)
	Skeletonema costatum	Mitsutani et al. (1992)
Flavobacterium sp.	*K. mikimotoi*	Fukami et al. (1992), Yoshinaga (1997) Iwata et al. (2006)
γ-*proteobacterium* sp.	*H. circularisquama*	Marino-Forum 21 (2003)
Vivrio spp., *Acinetobacter* sp., *Pseudomonas* sp.	*K. mikimotoi*	Yoshinaga (1997)
Saprospira sp., *Vitreoscilla* sp., *Amoeba* sp., *Labyrinthula* sp.	*Chaetoceros ceraposporum*	Sakata (1991, 1992, 1993, 1994, 1995)

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Algicidal viruses

Algicidal viruses are known to play important roles in the natural elimination of red-tide blooms. Several algicidal viruses have been isolated from the NOWPAP region since the late 1990's, which are listed in Table 6.

These algicidal viruses show algicidal effects only on host plankton species. In the NOWPAP region, algicidal viruses of *Heterocapsa circularisquama* and *Heterosigma akashiwo* have been isolated.

Although algicidal bacteria are considered to be highly effective in controlling red-tide blooms, they have not yet been applied in practice.

Table 6 Algicidal viruses isolated from the NOWPAP region

Species and strains of algicidal virus	Host species	Sources
HcV (*Heterocapsa circularisquama* Virus: double-stranded DNA virus)	*Heterocapsa circularisquama*	Tarutani et al. (2001), Tomaru and Nagasaki (2004)
HcV (*H. circularisquam* Virus: single-stranded RNA virus)	*H. circularisquama*	Nagasaki et al. (2004)
HcRNAV (*H. circularisquama* Virus: single-stranded RNA virus)	*H. circularisquama*	Tomaru et al. (2004), Tomaru and Nagasaki (2004)
HaV (*Heterosigma akashiwo* Virus)	*Heterosigma akashiwo*	Nagasaki and Yamaguchi (1997), Nagasaki and Yamaguchi (1998), Yamaguchi (1998), Nagasaki et al. (1999), Tarutani et al. (2000), Tamaru et al. (2004)

-References-
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Plankton grazers

This method utilizes plankton grazers to control red-tide blooms.

To examine the effectiveness of plankton grazers, in the NOWPAP region, heterotrophic dinoflagellates, copepods and ciliates have been used against red-tide plankton, including *Karenia mikimotoi, Chattonella antiqua, C. marina* and *Heterocapsa circularisquama*. Table 7 summarizes some of the plankton grazers examined. According to these experiments, ciliates had a high grazing rate on red-tide plankton, which correlated with fluctuations in red-tide plankton populations (Kamiyama et al., 2001, Kamiyama and Matsuyama, 2005).

For the practical application of plankton grazing, methods must be developed on ways to control populations of grazers and their grazing ability.

Table 7 Plankton grazers examined in the NOWPAP region

Genus and Species of Grazer		References
Dinoflagellate	*Gyrodinium fissum*	Kagawa Prefecture Fisheries Research Institute / Red tide Research Institute (1992)
Copepod	*Paracalanus crassirostris, Oithona brevi-cornis, Acartia clausi, Pseudodiaptomus marinus, Calanus sinicus*	Nagasaki University (Shoji Iizuka) (1981-1984) Shin-Nippon Meteorological & Oceanographical Consultant Co., Ltd. (1986, 1987, 1988)
Ciliate	*Favella azorica, F. taraikaensis, F. ehrenbergii, Codonellopsis* sp., *Tintinopsis* sp., Ciliate assemblage (tintinnid ciliates aloricate ciliates)	Akashiwo Research Institute of Kagawa Prefecture (1986-1988) Kamiyama (1996) Kamiyama et al. (2001) Kamiyama and Matsuyama (2005)

-References-
(Japan)

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2.2.4 Avoidance measures

<u>Submersion of fish cages</u>

To prevent fish kills in aquaculture farms, this method submerges fish cages to a deeper depth to avoid red-tide blooms at the sea surface. Figure 4 shows the mechanism of this method.

The effectiveness of this method has been tested with a fish cage containing 2 year-old yellowtails. The experiment was conducted for 35 days without feeding. Although no red-tide blooms occurred during the experiment, no yellowtail mortalities were recorded during the 35-day experimental period (Kagawa Pref., 1980-1982). The installation cost of this system was estimated to be ¥741,000 for ten cages (as of 1982).

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

<u>Perimeter skirt or shield curtain</u>

This method prevents the intrusion of red-tide plankton into fish cages by installing perimeter skirts or shield curtains around the cages. Figure 5 is a photograph of a perimeter skirt. This method has been applied in Korea, and is often used during *C. polykrikoides* blooms in July-September. The cost of this system is approximately US$8,500 for ten cages.

-References-
(Korea)
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・ Kim, H. G. (2006): Mitigation and controls of HABs, 327-338. In: Ecology of Harmful Algae, Granéli, E., J.T. Turner (Eds.). Springer. 413pp.

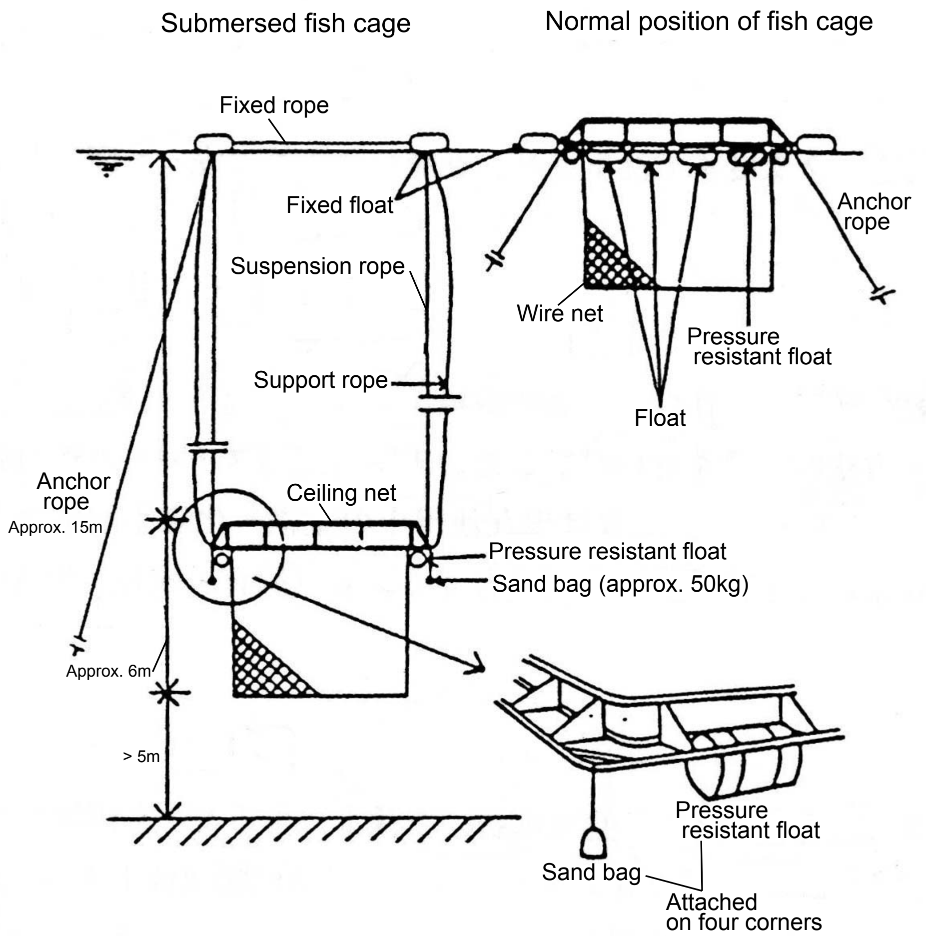

Figure 4　Schematic diagram of a fish-cage submersion system

Source: Kagawa Prefecture Fisheries Research Institute (1982)

Figure 5　Photograph of a perimeter skirt (shield curtain) (the perimeter skirt is wrapped around the fish cage to prevent the intrusion of HABs)

2.2.5 Other control

Automated HAB warning and oxygen supplying system

This system warns operators of land-based aquaculture farms, when fish-killing dinoflagellates, such as *C. polykrikoides*, are detected in the water supply system. The system detects dinoflagellate cells with a chlorophyll fluorescence sensor, and sends an alarm signal when the dinoflagellate density is high enough to kill the cultured fish (Figure 6). Once the alarm is triggered, the seawater supply to the fish tanks is automatically stopped and oxygen is supplied to the fish tanks.

-References-
(Korea)

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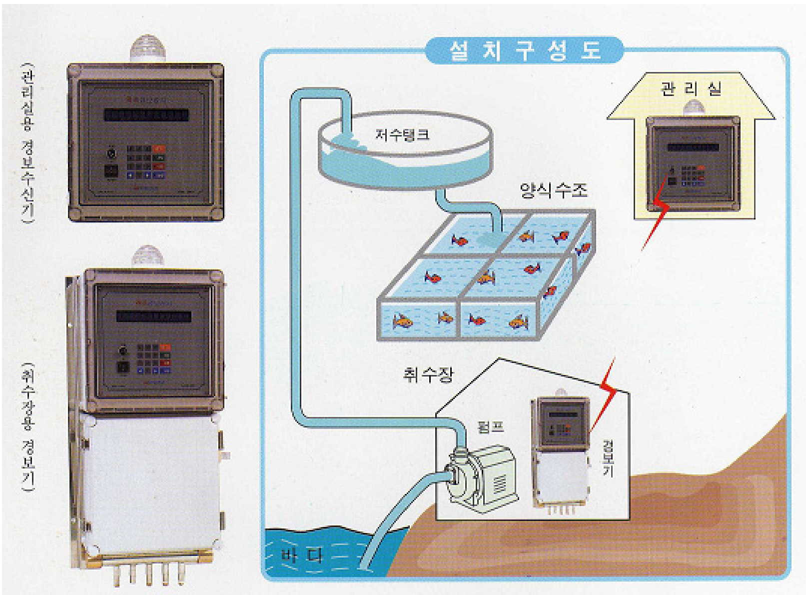

Figure 6 Automated HAB warning and oxygen supplying system for land-based fish tanks

Source: NFRDI (2002): The impacts of red tide and its mitigation techniques, 23pp. (in Korean)

2.3 Countermeasures against red-tide causative species in the NOWPAP region

Based on the red-tide countermeasures introduced in Section 2.2, Table 8 summarizes countermeasures that have been applied or considered against the following five common red-tide species in the NOWPAP region.

Dinophyceae: *Cochlodinium polykrikoides, Heterocapsa circularisquama, Karenia mikimotoi*
Raphidophyceae: *Chattonella* spp. (mainly *C. antiqua*/*marina*), *Heterosigma akashiwo*

C. polykrikoides blooms have been commonly reported from Japanese and Korean waters (NOWPAP CEARAC, 2005). Countermeasures implemented or considered against *C. polykrikoides* include physical control (clays), chemical control (hydrogen peroxide, ozone) and avoidance measures. No biological control measures have been considered to date. The effectiveness of clays has been proven through application in Japanese and Korean waters.

Recently in the NOWPAP region, *H. circularisquama* blooms have been reported only from Japanese waters (NOWPAP CEARAC, 2005). Countermeasures considered against *H. circularisquama* include biological secretion, algicidal bacteria/viruses and plankton grazing. *Heterocapsa circularisquama* specific algicidal bacteria/viruses have been isolated from the NOWPAP region (Tables 6 and 7).

K. mikimotoi blooms have been reported from Chinese, Japanese and Korean waters (NOWPAP CEARAC, 2005). Various physical, chemical and biological control measures, as well as avoidance and other control measures, have been implemented or considered against *K. mikimotoi*. In particular, ozone and algicidal bacteria have shown high effectiveness.

Chattonella spp. (mainly *C. antiqua*/*marina*) blooms have been reported from Chinese, Japanese and Russian waters (NOWPAP CEARAC, 2005). Various physical, chemical and biological control measures, as well as avoidance and other control measures, have been implemented or considered against *Chattonella* spp. Algicidal bacteria of *Chattonella* spp. have been isolated from the NOWPAP region (Tables 6 and 7).

Heterosigma akashiwo blooms have been reported from the waters of all NOWPAP members (NOWPAP CEARAC, 2005). Various control measures, such as clays, ozone, algicidal viruses and plankton grazing, have been applied or considered against *H. akashiwo*. An algicidal virus of *H. akashiwo* has been isolated from the NOWPAP region (Table 7).

Table 8(1) Countermeasures implemented or considered against red-tide species in the NOWPAP region

	Cochlodinium polykrikoides	*Heterocapsa circularisquama*	*Karenia mikimotoi*	*Chattonella* spp. (mainly *C. antiqua/marina)*	*Heterosigma akashiwo*	Other red-tide species
Physical Control						
Clays	◎		◎	◎	◎	*Prorocentrum donghaiense* *P. minimum* *Noctiluca scintillans* *Scrippsiella trochoidea* *Amphidinium carterae* *Gynmodinium* sp. *Gyrodinium* sp. *Aureococcus anophagefferens* *Skeletonema costatum* *Phaeodactylum tricornutum* *Pseudonitzschia pungens* var. *multiseries* *Cylindrotheca closterium*
Flocculants				○	○	*Skeletonema costatum* *Thalassiosira subtilis*
Synthetic polymers				○		
Magnetic separation				○		*Nannochloropsis oculata*
Centrifugal separation	○		○			*Gyrodinium* sp.
Ultraviolet radiation			○	○	○	
Chemical Control						
Hydrogen peroxide	○		○	○		*Oxyrrhiis marina* *Eutreptiella* sp. Dinoflagellate cyst
Hydroxide radicals			○			*Skeletonema costatum* *Chromulina* sp., *Dunaliella* sp. *Platymonas* sp. 36 spp. of dinoflagellates and diatoms
Ozone	○			○	○	*Prorocentrum minimum* *P. micans*
Copper sulfate						*Akashiwo sanguinea*?
Disinfectants	○			○	○	*Prorocentrum dentatum* *Gymnodinium pulchellum* *Cylindrotheca closterium* *Phaeoecystis globosa*
Herbicides						*Prorocentrum micans* *Phaeocystis globosa*
Biological secretion	○	○	○	○	○	*Prorocentrum dentatum*, *P. donghaiense*, *P. micans* *Phaeocystis globosa*
Other chemicals				○		*Prorocentrum micans*, *P.* sp. *Gymnodinium* sp. *Nitzschia* sp.
Biological Control						
Algicidal bacteria		○	○	○		*Skeletonema costatum* *Chaetoceros ceraposporum* *Coscinodiscus wailesii*
Algicidal viruses		○			○	
Plankton grazers		○	○	○	○	*Gyrodiniuminstriatum* *Heterocapsa triquetra*

◎: Countermeasure that has been practically applied in the NOWPAP region

○: Countermeasure that has been considered, but not yet practically applied in the NOWPAP region

Table 8(2) Countermeasures implemented or considered against red-tide species in the NOWPAP region

	Cochlodinium polykrikoides	*Heterocapsa circularisquama*	*Karenia mikimotoi*	*Chattonella* spp. (mainly *C. antiqua/marina*)	*Heterosigma akashiwo*	Other red-tide species
Avoidance measure						
Submersion of fish cages						No specific species
Perimeter skirt or shield curtain	◎		◎			*Gyrodinium* sp.
Other Control						
Automated HAB warning and oxygen supplying system	◎		◎			*Gyrodinium* sp.

◎: Countermeasure that has been practically applied in the NOWPAP region

○: Countermeasure that has been considered, but not yet practically applied in the NOWPAP region

2.4 Countermeasures against red tides around the world

Table 9 summarizes some countermeasures that have been applied or considered around the world. The countermeasures introduced in this section are mainly excerpted from Rensel and Martin (1999), Anderson et al. (2001) and Gobler et al. (2005).

The effectiveness of clays (physical control) has been laboratory tested against *Karenia brevis*, *Heterosigma akashiwo* and *Aureococcus anophagefferens* (Sengo et al., 2001; Sengo and Anderson, 2004).

Chemicals such as copper sulfate and aponin have been considered for chemical control measures. In 1957, copper sulfate was sprayed from an airplane over a *K. brevis* bloom (10,000 acres, ca. 40 km^2) that occurred along the Florida coast. As a result of the spraying, the initial *K. brevis* cell density of 1-10 x 10^6 cells/L was reduced to almost none. However, *K. brevis* cell density returned to its initial density after 2 weeks. Approximately 20 pounds (ca. 9 kg) of copper sulfate was spayed per acre and, as a result, the cost of spraying amounted to US$4/acre (as of 1957) (Rounsefell and Evans, 1958). Aponin, a sterol surfactant produced from the blue-green alga *Gomphosphaeria aponina*, has been used to eliminate *K. brevis* blooms (Taft and Martin, 1986; Martin and Taft, 1998).

Plankton grazers and algicidal viruses have been considered for biological control measures. The plankton grazing efficiency of filter feeders, such as bivalves and other benthic organisms, have been studied by Cloern (1982), Officer et al. (1982) and Caron and Lonsdale (1999). An algicidal virus was isolated from an *Aureococcus anophagefferens* bloom that occurred in 1992 along the New York coast, and its algicidal effects were examined under laboratory conditions by Milligan and Cosper (1994).

Most avoidance measures are developed to minimize fish-kills in aquaculture farms during red-tide blooms, and include mobilization of fish cages (Lindahl and Dahl, 1990), submersion of fish cages (Anderson et al., 2001), installation of perimeter skirts (Anderson et al., 2001) and aeration (Rensel and Martin, 1999).

Most red-tide countermeasures in the non-NOWPAP region have been implemented or considered by the U.S. The mobilization of fish cages was considered by Norway. Japan implemented fish-cage mobilization in the Seto Inland Sea, but this method is no longer used because fish-cages are now too large for easy mobilization (Fukuyo pers. comm.).

Table 9 Countermeasures against red tides implemented around the world

Category	Methods	Target species	Country	Sources
Physical Control	Clays	*Karenia brevis* *Aureococcus anophagefferens* *Alxandrium tamarense* *Heterosigma akashiwo*	US	Sengo et al. (2001)
		Karenia brevis *Heterocapsa triquetra*	US	Sengo and Anderson (2004)
Chemical Control	Copper sulfate	*Karenia brevis*	US	Rounsefell and Evans (1958)
	Aponin	*Karenia brevis*	US	Martin and Taft (1998)
		Karenia brevis	US	Taft and Martin (1986)
Biological Control	Plankton grazers	No description of target species	US	Cloern (1982)
		No description of target species	US	Officer et al. (1982)
		Aureococcus anophagefferens	US	Caron and Lonsdale (1999)
	Algicidal viruses	*Aureococcus anophagefferens*	Canada US	Milligan and Cosper (1994)
Avoidance measure	Mobilization of fish cage	*Chrysochromulina polylepis*	Norway	Lindahl and Dahl (1990)
	Submersion of fish cage	No description	US	Anderson et al. (2001)
	Perimeter skirts	No description	US	Anderson et al. (2001)
	Aeration or air-lift pumping	No description	US	Rensel and Martin (1999)

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3 Countermeasures against toxin-producing algal blooms in the NOWPAP region

3.1 The Situation of toxic species in the NOWPAP region and the necessity of development countermeasures

3.1.1 Situation of toxic species in the NOWPAP region

The situation of toxic species in the NOWPAP region is summarized below. The information is extracted from Chapter 2 of the Integrated Report.

Table 10 summarizes the situation of toxic species in the NOWPAP region. In this booklet, toxin-producing plankton are categorized into paralytic shellfish poisoning (PSP-), diarrhetic shellfish poisoning (DSP-) and amnesic shellfish poisoning (ASP-) inducing species.

A total of 20 toxin-producing plankton species have been recorded in the NOWPAP region. Six species were PSP-inducing species, and all except *Gymnodinium catenatum* belonged to the genus *Alexandrium*. The most commonly recorded PSP species in the NOWPAP region was *A. tamarense*.

Nine of the ten DSP species recorded in the NOWPAP region belong to the genus *Dinophysis*. The other was *Exuviaella marina* (= *Prorocentrum lima*), which was recorded only in China. Among the *Dinophysis* species, *D. fortii* and *D. acuminata* were recorded in all of the NOWPAP member seas.

Damage from ASP has not yet been recorded in the NOWPAP region, although ASP inducing *Pseudo-nitzschia* species have been recorded in Japan, Korea and Russia.

PSP has been recorded in the Shangdong Peninsula and Lianyungang area in China. Areas affected by PSP in Japan are found in the western Japan (Kyushu and Chugoku), Tohoku (Aomori Prefecture) and Hokkaido regions. In Korea, PSP has recently affected shellfish harvesting areas on the southeastern coast. Russia has not been affected by PSP to date.

DSP species have been recorded in the Shangdong Peninsula, the Lianyungang area and the Bohai Sea in China. In 1998, *Dinophysis ovata* blooms were recorded over an area of 5,000 km^2 in the Bohai Sea. Areas affected by DSP in Japan are mainly in the Hokkaido, Tohoku and Chugoku regions. In Korea, three *Dinophysis* species were recorded on the southeastern coast in 2002 and 2003, but it is uncertain if any damage was caused by these species. Russia has not been affected by DSP to date.

In Russia, observations of PSP-, DSP- or ASP-inducing species are conducted mainly in the aquaculture areas. Although incidents of shellfish poisoning have not been reported in these areas to date, the presence of toxin-producing plankton has been continuously monitored.

In China, more than 600 people have suffered from shellfish poisoning since 1967, of which 30 fatalities have resulted from PSP. In Japan, approximately 900 people have suffered from PSP or DSP since 1976, including several deaths from PSP. In Korea, shellfish harvesting was banned on the southeastern coast in 2002 (April–May) and 2003 (April–June) due to *A. tamarense*.

Table 10 Situation of toxic species in the NOWPAP region

	China	Japan	Korea	Russia
Main toxin-producing species	*Alexandrium catenella*, *Dinophysis forti*, *D. acuminata*, *D. ovata* and *Exuviaella marina*	*Alexandrium tamarense*, *A. catenella*, *A. tamiyavanichii*, *Gymnodinium catenatum, Dinophysis forti*, *D. acuminata*, *D. caudate, D. intundibra, D. mitra* and *D.rotundata*	*Alexandrium tamarense, Dinophysis forti*, *D. acuminata*, *D. caudate, D. rotundata* and *Pseudo-nitzschia pungens*	*Alexandrium tamarense*, *A. acatenella*, *A. pseudogonyaulax*, *Dinophysis forti*, *D. acuminata*, *D. acuta, D. norvegica, D. rotundata, Pseudo-nitzschia calliantha, P. multiseries, P. pseudodelicatissima* and *P. pungens*
Affected species	Information is available only for southern China (out of the NOWPAP region). PSP: Clam (*Soletellina diphos*; *Ruditapes phillipenensis*; *Pinna pectinata*); Mussel (*Perna viridis*)	PSP: Mediterranean blue mussel; Japanese oyster; noble scallop DSP: Mediterranean blue mussel; Japanese scallop	N/A	N/A
Affected area	Shangdong Peninsula, Lianyungang area	Mainly in Hokkaido, Tohoku and Chugoku regions	Southeast coast (Gosung, Tongyoung, Jinhaeman)	No shellfish poisoning reported. Potential causative species recorded in certain areas
Damage	More than 600 people have suffered from shellfish poisoning since 1967. There have been 30 fatalities from PSP across the nation.	Approximately 900 people have suffered from PSP or DSP since 1976, including several deaths from PSP. No fatalities since 1980.	Banning of shellfish harvest in 2002 and 2003 in the southeast coast due to PSP.	No damage recorded

Source: NOWPAP CEARAC, 2005

3.1.2 Necessity of countermeasures against toxic species

As mentioned previously, toxin-producing plankton are regularly recorded from the NOWPAP region, and these have caused shellfish poisoning incidents and seafood shipping restrictions in China, Japan and Korea.

Shellfish poisoning occurs when humans consume shellfish that are contaminated by toxin-producing plankton. Although shellfish poisoning can be prevented to a certain extent through regular monitoring of harvested shellfish and toxin-producing plankton occurrences, direct countermeasures against toxin-producing plankton are also necessary.

In the following sections, the status of toxin-producing plankton countermeasures and toxin-producing plankton and shellfish poisoning monitoring in the NOWPAP region is introduced. Future issues regarding the above topics are also discussed.

3.2 Countermeasures against toxic species in the NOWPAP region

There are no direct countermeasures against toxin-producing plankton currently established in the NOWPAP region. However, some research has been conducted, which is introduced below.

As a chemical control method, herbicides (Liu et al., 2004) and conifir woodchips (Zhang et al., 2005) have been tested against *Alexandrium tamarense*. Algicidal bacteria have also been considered as a biological control method of *A. tamarense* (Su et al., 2003, Zheng et al., 2005).

Countermeasures developed for red-tide blooms have also been experimentally applied to toxin-producing plankton. For example, algicidal bacteria of *Karenia mikimotoi* and *Chatonella antique* were tested against *Alexandrium* species by Imai (1997) and Yoshinaga (1997). Also, an algicidal virus of *H. circularisquama* was tested against *Alexandrium* species by Tarutani et al. (2001) and Tomaru et al. (2004). Ichikawa et al. (1992) tested hydrogen peroxide against *Alexandrium* cysts.

Since the NOWPAP region lacks effective direct countermeasures against toxin-producing plankton, regular monitoring of these plankton occurrences are important to minimize the risk of shellfish contamination. Table 11 summarizes the status of toxin-producing plankton monitoring in the NOWPAP region.

Monitoring of toxin-producing plankton is conducted in China, Japan and Korea, and usually by fisheries research organizations. In Japan, monitoring is conducted in selected shellfish-production areas.

In Japan and Korea, monitoring usually focuses on particular target species. In Japan, *Alexandrium* species and *Gymnodinium catenatum* are usually monitored for PSP, and *Dinophysis* species are monitored for DSP. In Korea, *A. tamarense* is monitored in the southeastern region near aquaculture farms.

Table 11 Status of toxin-producing plankton monitoring in the NOWPAP region

	China	Japan	Korea	Russia
Implementing organization	Some SOA laboratories and local fishery environmental laboratories. Monitoring network under construction.	Fishery laboratories of prefectural governments	NFRDI and Regional Maritime Affairs and Fisheries Office	No official regular monitoring program. However, IMB FEB RAS and SakhNIRO conduct observations on an *ad hoc* basis.
Method	N/A	Cell density of *Alexandrium* species and *Gymnodinium catenatum* are usually monitored for PSP, and *Dinophysis* species for DSP. However, the target species may differ among laboratories.	Cell density of *A. tamarense* is regularly monitored.	Cell density of certain toxin-producing plankton studied.
Location	N/A	Usually in shellfish production areas	Near the shellfish farms in the southeast coast.	Coastal waters of Primorye and South Sakhalin Island.
Frequency	N/A	Differs among laboratories.	N/A	*Ad hoc* basis

Source: NOWPAP CEARAC (2005)

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3.3 Countermeasures against shellfish poisoning by microalgal toxins

Although various fish and shellfish species can be poisoned by microalgal toxins, shellfish species are the more commonly affected in the NOWPAP region. Shellfish poisonings are mainly prevented by conducting regular monitoring of harvested shellfish. The monitoring status in the NOWPAP region and potential countermeasures against shellfish poisoning are introduced in the following sections.

3.3.1 Monitoring of harvested shellfish

Table 12 summarizes the status of shellfish monitoring in the NOWPAP region. Monitoring is conducted in China, Japan and Korea, usually by fisheries research organizations. In Japan and Korea, shellfish monitoring is implemented in shellfish-production areas.

All NOWPAP members have safety limits against harvested shellfish. When the toxin level exceeds the limit, shipping or harvesting of shellfish is stopped until the toxin level returns to acceptable levels. The limit for PSP in China, Korea and Russia is 80 μg (STX eq.) /100g of meat. Japan applies Mouse Units (MU) for expressing the toxin level. The Japanese standards are 4 MU/g of meat for PSP and 0.05 MU/g for DSP.

Table 12 Monitoring status of harvested shellfish in the NOWPAP region

	China	Japan	Korea	Russia
Implementing organization	Some SOA laboratories and local fishery environmental laboratories. Monitoring network under construction.	Fishery laboratories of prefectural governments	NFRDI and Regional Maritime Affairs and Fisheries Office	Monitoring not conducted
Method	N/A	Measurement of toxin level in the midgut gland.	Measurement of toxin level in the meat or midgut gland.	-
Location	N/A	Usually in shellfish production areas. See Figure 16 for monitored sites.	Shellfish farms in the western and southern coastal area. Over 100 stations. See Figure 16 for monitored sites.	-
Frequency	Varies with local harvest season.	At least monthly during the harvest season. Frequency increases to weekly if a high risk of poisoning is suspected.	At least more than once a month. Frequency increases when a toxin is detected in shellfish.	-
Shipping and/or harvest stoppage	Stoppage of harvesting and shipping when PSP toxin level exceeds the Department of Agriculture standard (80 µg/100g of whole meat). DSP toxin level must be undetectable.	Voluntary stoppage of shipping when toxin level exceeds the Fishery Agency standard (PSP: 4 MU/g; DSP: 0.05 MU/g). Shipping can recommence when toxicity level remains below the standard for 2 weeks.	Stoppage of harvesting when PSP toxin level > 80 µg/100 g meat.	Maximum permissible level. PSP: 80 µg/100 g wet mollusk tissue. DSP: No detection of ocadaic acid.

Source: NOWPAP CEARAC (2005)

3.3.2 Potential countermeasures against shellfish poisoning

New countermeasures against shellfish poisoning are being researched and developed by NOWPAP members. New detection methods of toxin-producing plankton and analysis methods of microalgal toxins are introduced in this section.

➢ Early detection of toxin-producing plankton by real-time PCR (polymerase chain reaction)

Compared to other PCR methods, real-time PCR can detect toxin-producing plankton with high accuracy and speed. Although this method is still under development, it is expected to become a widely used practice for microalgal detection.

➢ Analysis of microalgal toxins with high-performance liquid chromatography

The combination of high-performance liquid chromatography (HPLC) and mass spectroscopy enables highly sensitive and accurate analyses of toxic substances (Suzuki, 1994, Suzuki and Matsuyama, 1995, Suzuki et al., 2003). These analyses can detect PSP- and DSP-inducing toxic substances at very low concentrations.

➢ Analysis of microalgal toxins with enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked-immunosorbent assay (ELISA) is an easy and rapid analytical method for detecting DSP-inducing toxic substances. Since this method has not been officially authorized, it should be considered as a future potential alternative.

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3.4 Countermeasures against toxic species around the world

Similar to the NOWPAP region, there seem to be no effective direct countermeasures against toxin-producing plankton elsewhere. Sengo et al (2001) tested clay against *Alexandrium tararense* as a potential countermeasure.

Several countries monitor toxin-producing plankton and harvested shellfish. Table 13 summarizes some monitoring programs conducted around the world.

Each country has shipping and harvesting restriction standards for each shellfish-poisoning type (PSP, DSP and ASP). In addition to the shipping and harvesting restriction standards, Denmark and New Zealand also refer to toxin-producing plankton cell concentration. In the Philippines, PSP-inducing species and harvested shellfish are monitored through the Republic of Philippines Marine Biotoxins Monitoring Unit.

-References-

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- Republic of Philippines Marine Biotoxins Monitoring Unit, Frequently asked questions on Red Tide. http://www.bfar.gov.ph/download/redtide/RedTideFAQEn.pdf.

Table 13(1) Examples of monitoring toxin-producing plankton and harvested shellfish around the world

		US	Canada	Spain	Denmark
		PSP Toxins Monitoring Program	Shellfish Toxin Monitoring Program in Atlantic Canada	Toxin monitoring program in the R as Baixas of Galicia, NW Spain	The Danish monitoring program
Implementing Organization		➢ Atlantic US: State of Maine	➢ Atlantic Canada: Canadian Food Inspection Agency (CFIA)	➢ Autonomous Government of Galicia (Xunta de Galicia)	➢ The Danish Veterinary and Food Control Authority
Purpose/objectives		➢ To protect public health while providing for the harvest of susceptible species of marine molluscs in areas not affected by contamination ➢ To allow optimum utilization of local shellfish resources	➢ To provide public health protection ➢ To enhance the utilization of seafood resources for domestic and export markets by ensuring product safety	➢ Food safety ➢ To identify the causative agents of different toxic events, and therefore includes routine the collection of phytoplankton and oceanographic data	➢ To prevent toxic mussels from reaching the consumer ➢ To ensure that the effort of the mussel fishery is optimized by guiding boats to areas with a low risk of harvesting toxic mussels
Toxin-producing plankton	Target species	➢ *Alexandrium* species ➢ *Dinophysis* species ➢ *Pseudo-nitzschia* species ➢ *Prorocentrum* species	➢ *Alexandrium fundyense* ➢ *Pseudo-nitzschia pseudodelicatissima* (non-shellfish poisoning species: *Chaetoceros convolutus*, *Gyrodinium aureolum* and the ciliate *Mesodinium rubrum*)	➢ *Alexandrium minutum* ➢ *Gymnodinium catenatum* ➢ *Dinophysis acuminata* ➢ *Dinophysis acuta* ➢ *Dinophysis caudata* ➢ *Dinophysis sacculus*	➢ Toxic and potentially toxic algae reported from Danish waters
	Method	➢ Cell density of toxin-producing plankton are usually monitored between April and November	➢ Cell density of toxin-producing plankton are usually monitored	➢ Qualitative and quantitative phytoplankton analysis is conducted	➢ Qualitative and quantitative phytoplankton analysis is conducted using microscopy *Action limits on algal concentrations
	Location	➢ Coastal regions at 40 to 60 collection sites in Maine	➢ 4 sites in New Brunswick	➢ 35 primary stations and 14 secondary stations of sampling sites in Galicia	➢ Mussel fishing areas
	Frequency	➢ No description	➢ Weekly between June and September ➢ Biweekly during May and October ➢ Monthly from December through April	➢ Sampling frequency at primary stations is weekly all year-around.	➢ Biweekly

Source: Anderson et al. (2001)

Table 13(2) Examples of monitoring toxin-producing plankton and harvested shellfish around the world

		US	Canada	Spain	Denmark
		Maine PSP Toxins Monitoring Program	Shellfish Toxin Monitoring Program in Atlantic Canada	Toxin monitoring program in the R as Baixas of Galicia, NW Spain	The Danish monitoring program
Shellfish poisoning	Target Species	➢ Blue mussel (*Mytilus edulis*) ➢ Softshell clam (*Mya arenaria*)	➢ Blue mussel (*Mytilus edulis*) ➢ Softshell clam (*Mya arenaria*)	➢ Blue mussel (*Mytilus galloprovincialis)* ➢ Softshell clam (*Mya arenaria*)	➢ Blue mussel (*Mytilus edulis*) ➢ Cockles (*Cardium edule*) ➢ Surfclam (*Spesula* spp.)
	Method	➢ PSP toxins are analyzed by the standard AOAC mouse bioassay	➢ The AOAC mouse bioassay is used for routine analysis of PSP toxins ➢ Domoic acid was initially analyzed using the mouse bioassay and a more expanded observation period, but was subsequently replaced by HPLC methods	➢ PSP analysis using the AOAC mouse bioassay ➢ DSP analysis using Yasumoto et al.'s (1980) mouse bioassay ➢ Domoic Acid analysis using HPLC-UV detection	➢ PSP analysis using the AOAC mouse bioassay ➢ DSP analysis using modified Yasumoto et al.'s(1980) mouse bioassay ➢ Domoic Acid analysis using HPLC
	Location	➢ 18 coastal regions in Maine	➢ Coastal regions in Atlantic Canada	➢ 49 primary stations and 189 secondary stations of sampling sites in Galicia	➢ Mussel fishing areas
	Frequency	➢ Sampling takes place weekly in primary stations between early April and October	➢ Monitors throughout the year, weekly, bimonthly or monthly depending on the season and site	➢ Sample once a week at primary stations ➢ Sample weekly when neither toxic species, nor toxicity of bivalves is detected by mouse bioassay at secondary stations	➢ Biweekly
	Shipping and/or harvest stoppage	➢ Shellfish harvest area closed or stoppage of shipping when PSP toxin exceeds the regulatory level (80 μg STXeq/100 g) ➢ Shellfish harvesting can recommence when PSP toxin remains below the regulatory level for at least 2 weeks	➢ For PSP toxins, an action limit of 80μg STXeq/100g is used for raw shellfish tissues, and 160 μg STXeq/100 g is used for canned shellfish ➢ For domoic acid the action limit is 20 μg/g	➢ Closure of shellfish harvesting areas when toxin levels exceed the safety level (to fulfill the EC requirement)	➢ DSP toxins must be undetectable using the mouse bioassay ➢ PSP toxins, detected by the mouse bioassay must be < 80 μg/100 g ➢ ASP toxins, detected by HPLC must be < 2 mg/100 g (follow the guidelines outlined by EC Council directive No. L268, of 15 July 1991)

Source: Anderson et al. (2001)

4 Summary

4.1 Implementation status of HAB countermeasures

The majority of HAB countermeasures introduced in this booklet are still under research and development. However, these countermeasures could be practically applied in the future through technical advancements. In this chapter, the HAB countermeasures implemented or considered in the NOWPAP region are summarized.

4.1.1 Red tides

Table 14 summarizes the red-tide countermeasures implemented or considered in the NOWPAP region. Within these countermeasures, only clays, perimeter skirt/shield curtain and automated HAB warning and oxygen supplying system are practically applied.

Clay spraying has been implemented in Japan and Korea. Korea has enhanced the removal efficiency by mixing clay with electrolyzed water. The following are some of the advantages and disadvantages of clays.

Advantages

- High removal efficiency of red-tide blooms
- Limited impact on the environment and ecosystem because clays are natural material

Disadvantages

- High cost and complicated spraying procedure
- Not effective against certain red-tide species

Although further improvements are necessary, clay spraying is expected to remain as a popular red-tide countermeasure option.

A perimeter skirt/shield curtain protects cultured fish by blocking the intrusion of red-tide species, and is widely used by Korean aquaculture farms. The installation of perimeter skirt/shield curtains is relatively costly, and could be unfeasible for large fish cages. Also, its effectiveness declines when used for large-scale and long duration red-tide blooms.

The following are some other countermeasures that have high application potential.

Physical control: magnetic separation, ultraviolet radiation
Chemical control: synthetic and biological chemicals
Biological control: algicidal bacteria and viruses

Although magnetic separation showed high removal efficiency of *Chattonella* sp., the method is costly because a large amount of iron powder is necessary to achieve high removal efficiency. Also, a large capacity magnetic separator must be developed for field application.

Currently, the Ministry of Land, Infrastructure and Transport of Japan is developing an ultraviolet radiation system that could be installed on anti-pollution vessels. If the system shows high removal efficiency during field experiments, it could be a very effective countermeasure option.

Chemical control uses either synthetic or biological chemicals. Although synthetic chemicals are very effective in killing red-tide plankton, they also show toxicity towards harmless marine organisms. Also, the use of some synthetic chemicals, such as copper sulfate, is regulated. Their decomposition and dilution rate in seawater are also unknown. Therefore, for future application, the above issues must be solved through further research and development. Biological chemicals, on the other hand, are less harmful to other marine organisms, but their algicidal effects are lower compared to synthetic chemicals and thus require more volume. Also, since biological chemicals are derived from natural marine organisms, a constant supplying system must be established.

Research on algicidal bacteria and viruses have been conducted mainly in Japan. The advantages of algicidal bacteria and viruses are that they show high algicidal effects only towards their host species. However, they have not been applied in the field yet, because their impacts on the environment and ecosystem are unknown. Further research is required to clarify the effectiveness and safety of algicidal bacteria and viruses, which could be carried out in an enclosed environment, such as in a small-scale pond.

Table 14(1) Summary of red-tide countermeasures implemented or considered in the NOWPAP region

Countermeasures	Effectiveness	Application method / range	Field application	Impact on environment / ecosystem	Others
Physical Control					
Clays	Effective against red-tide plankton, especially *Cochlodinium polykrikoides*	Coastal area (around fish cages)	Implemented in China, Japan and Korea	Negligible impact on water quality and marine organisms	Cost of clays is high
Flocculants	Effectiveness confirmed against *Heterosigma akashiwo* and *Euglena* sp.	Installation on barge	Not applied yet	N/A	
Synthetic polymers	Effective against *Chattonella marina*	N/A	Not applied yet	Toxic to aquatic organisms	
Magnetic separation	High removal rate of *Chattonella* sp.	N/A	Not applied yet	N/A	10 g of iron powder required per 10 L of seawater for efficient removal
Centrifugal separation	Effective against *C. polykrikoides*	Land-based tank	Not applied yet	N/A	Difficult for field application
Ultraviolet radiation	Effective against *C. marina*, *H. akashiwo*, *Karenia mikimotoi*	Installation on anti-pollution vessels	Not applied yet	N/A	Onboard system under development
Chemical Control					
Hydrogen peroxide	Effective against *C. polykrikoides* and *Chattonella* spp.	Coastal area (around fish cages)	Limited past application in fish farms in Japan (not currently applied)	Toxic to fish and invertebrates	High concentration of resides in the water column are required to be effective
Hydroxide radicals	Effective against *K. mikimotoi, C. marina*, *H. akashiwo*	N/A	Not applied yet	N/A	Algicidal mechanism uncertain
Ozone	Effective against *Chattonella marina*, *K. mikimotoi*, *H. akashiwo*	Coastal area (around fish cages)	Not applied yet	Highly toxic to aquatic organisms	Approximately ¥6 million per ozone treatment system
Copper sulfate	Effective against *Gymnodinium* sp.	N/A	Not applied yet (records show trial application in Japan in the 1930's)	N/A (assumed to be highly toxic to aquatic organisms)	Use regulated in Japan
Disinfectant	Effective to *C. polykrikoides*, *Chattonella* sp., *H. akashiwo* and *Phaeocystis globosa*	N/A	Not applied yet (residual tests of acrinol have been conducted)	Toxic to fish	Under natural light conditions, acrinol decomposed after 2 hours
Biological secretion	Effective to *H. circularisquama*, *H. akashiwo* and *P. globosa*	N/A	Not applied yet	N/A (impact on other marine organisms unlikely)	Large volume required
Biological Control					
Algicidal bacteria	Effective only to certain red-tide species	N/A	Not applied yet	N/A	Further research required for field application
Algicidal viruses	Effective only to *H. circularisquama* and *H. akashiwo*	N/A	Not applied yet	N/A	Further research required for field application
Plankton grazers	Effective against most red-tide species	N/A	Not applied yet	N/A	Further research required for field application

Table 14(2) Summary of red-tide countermeasures implemented or considered in the NOWPAP region

Countermeasures	Effectiveness	Application method / range	Field application	Impact on environment / ecosystem	Others
Avoidance measure					
Submersion of fish cage	N/A	Installation on fish cages	Tested when red tide did not occur	No impact on cultured yellowtail	Installation cost on 10 cages was ¥741,000 (as of 1982)
Perimeter skirt or shield curtain	N/A	Installation on fish cages	Implemented in Korea	N/A	Installation cost on 10 cages was US$8,500
Other Control					
Automated HAB warning and oxygen supplying system	Effective against *C. polykrikoides*	Land-based tank	Implemented in Korea	N/A	Installation in aquaculture farms recommended by the Korean government

4.1.2 Toxin-producing plankton and shellfish poisoning

As mentioned in the previous chapters, there are no established direct countermeasures against toxin-producing plankton in the NOWPAP region. Therefore, countermeasures should focus on preventing shellfish poisoning through strengthening shellfish and toxin-producing plankton monitoring activities. The development of efficient and accurate monitoring technologies is important to spread these activities throughout the NOWPAP region (see Section 3.3.2).

4.2 Suggestions on future HAB countermeasures in the NOWPAP region

Coastal uses in the NOWPAP region are expected to increase in the future, which could lead to further increases in HAB events through environmental degradation. Under such scenarios, demands for effective HAB countermeasures will continue to grow. The development of effective HAB countermeasures is also important in terms of sustaining a safe and constant seafood supply to the growing population of the NOWPAP region.

Although various countermeasures have been developed and considered in the NOWPAP region, most of them are applicable only against HAB outbreaks. Future research and development efforts should also concurrently focus on the prevention of HAB outbreaks. Finally, impacts of the countermeasures on the environment and ecosystem should always be carefully considered prior to application.

Abbreviations

AGQAC: Alkyl glucoside ammonium compound

AOAC: Association of Analytical Communities

AS: Aluminum Sulfate

ASP: Amnesic Shellfish Poisoning

CCG: Cochlodinium Corresponding Group

CEARAC: Special Monitoring & Coastal Environmental Assessment Regional Activity Centre

DPQAC: Dialkyl-polyoxyethenyl-quaternary ammonium compound

DSP: Diarrhetic Shellfish Poisoning

ELISA: Enzyme-Linked Immunosorbent Assay

HAB: Harmful algal bloom

HPLC: High Performance Liquid Chromatography

IMB FEB RAS: The Institute of Marine Biology Far Eastern Branch Russian Academy of Sciences

IOC: Intergovernmental Oceanographic Commission

KORDI: Korean Ocean Research and Development Institute

LC-MS: Liquid Chromatography Mass Spectrometry

N/A: Not available

NFRDI: National Fisheries Research and Development Institute

NOWPAP: Northwest Pacific Action Plan

NPEC: Northwest Pacific Region Environmental Cooperation Center

PAC: Polyaluminum Chloride

PCR: Polymerase Chain Reaction

PSAS: Polysilicate Aluminum Sulfate

PSP: Paralytic Shellfish Poisoning

SOA: State Oceanic Administration

UNEP: United Nations Environment Programme

UV: Ultraviolet

WG3: Working Group 3

Appendix

Countermeasures against HABs in the NOWPAP region

China

Japan

Korea

Countermeasures against HABs in China

List of Countermeasures against HABs in China (1)

Study No.	Category	Methods	Title	Implementing organization (author)
C-P-1	Physical control	Clays	A new method to improve the capability of clays for removing red tide organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-2	Physical control	Clays	A more efficient clay for removing red tide organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-3	Physical control	Clays	Application of clays to removal of red tide organisms I: coagulation of red tide organisms with clays	Institute of Oceanology, Chinese Academy of Sciences
C-P-4	Physical control	Clays	Application of clays to removal of red tide organisms II: coagulation of different species of red tide organisms with montmorillonite and effect of clay pretreatment	Institute of Oceanology, Chinese Academy of Sciences
C-P-5	Physical control	Clays	Application of clays to removal of red tide organisms III: coagulation of Kaolin on red tide organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-6	Physical control	Clays	Study on the kinetics of clay removing red tide organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-7	Physical control	Clays	A study on optimum conditions for the removal of red tide organisms by modified clays	Institute of Oceanology, Chinese Academy of Sciences
C-P-8	Physical control	Clays	Impact of halloysite on growth of *Pseudonitzschia pungens* var. *multiseries* and production of algal toxins	Institute of Oceanology, Chinese Academy of Sciences
C-P-9	Physical control	Clays	Surface modification of the clay particles and its effect on the coagulation efficiency of red tide organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-10	Physical control	Clays	Effect of bentonite modified removing red tide organisms and DRP, COD of sea water	Xiamen university, Xiamen
C-P-11	Physical control	Clays	Study on the kinetics of clay-MMH system on coagulation of red-tide organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-12	Physical control	Clays	A preliminary study in controlling the red tide calamity by using pillared clay	Guangzhou Institute of Geochemistry, Cruangzhou
C-P-13	Physical control	Clays	Removal of different species of red tide organisms with an effective clay-complex system	Institute of Oceanology, Chinese Academy of Sciences
C-P-14	Physical control	Clays	Extinguishment of harmful algae by organo-clay	Institute of Oceanology, Chinese Academy of Sciences
C-P-15	Physical control	Clays	Removal of red tide organisms by organo-modified bentonite	Zhejiang University, Hangzhou
C-P-16	Physical control	Clays	Flocculation and removal of the brown tide organism, *Aureococcus anophagefferens* (Chrysophyceae), using clays	Institute of Oceanology, Academy of Sciences
C-P-17	Physical control	Clays	Removal efficiency of red tide organisms by modified clay and its Impacts on cultured organisms	Institute of Oceanology, Chinese Academy of Sciences
C-P-18	Physical control	Clays	Mechanisms of removing red tide organisms by organo-clays	Institute of Oceanology, Chinese Academy of Sciences
C-P-19	Physical control	Clays	A new type of clay modification agent-alkyl glucoside quaternary ammonium compound	Institute of Oceanology, Chinese Academy of Sciences
C-P-20	Physical control	Clays	Extinguishment of harmful algae by organo-clay modified by alkyl glucoside quaternary ammonium compound	Institute of Oceanology, Chinese Academy of Sciences
C-P-21	Physical control	Clays	Remediation from harmful algae bloom with organo-clay processed surfactant	Institute of Oceanology, Chinese Academy of Sciences

List of Countermeasures against HABs in China (2)

Study No.	Category	Methods	Title	Implementing organization (author)
C-P-22	Physical control	Flocculates	Preparation of PSAS (Polysilicate-aluminium sulfate) and its application in HAB prevention	Institute of Oceanology, Chinese Academy of Sciences
C-C-1	Chemical control	Hydroxide radicals	Study on the treatment of red tide pollution using hydroxide radical medicament	Dalian Maritime University, Dalian
C-C-2	Chemical control	Hydroxide radicals	Experiment of killing the microorganisms of red tide using hydroxyl radicals in the shore of Jiaozhou Gulf	Dalian Maritime University, Dalian
C-C-3	Chemical control	Hydroxide radicals	Study on killing *Gymnodinium mikimotoi* with hydroxyl radical	Dalian Maritime University, Dalian
C-C-4	Chemical control	Hydroxide radicals	Using of hydroxyl radical on oceanic biologic contamination prevention	Dalian Maritime University, Dalian
C-C-5	Chemical control	Disinfectants	Mechanism of quaternary ammonium compounds extinguishing *Heterosigma akashiwo*	Institute of Oceanology, Chinese Academy of Sciences
C-C-6	Chemical control	Disinfectants	Studies on biquaternary ammonium salt algaecide for removing red tide	Jinan University, Guangzhou
C-C-7	Chemical control	Disinfectants	Inhibition and elimination of alkylpolyglycoside on red tide plankton	Ocean University of China
C-C-8	Chemical control	Disinfectants	Povidone-iodine and isothiozolone for removing red tide algae *Phaeoecystis globosa*	Jinan University, Guangzhou
C-C-9	Chemical control	Disinfectants	Study of the extinguishing mechanism of povidone-iodine and isothiozolone	Jinan University, Guangzhou
C-C-10	Chemical control	Disinfectants	Inhibition and elimination of chlorine dioxide on *Phaeoecystis globosa*	Jinan University, Guangzhou
C-C-11	Chemical control	Herbocides	Removal of red tide algae by a glass algaecide containing Cu (II)	Jinan University, Guangzhou
C-C-12	Chemical control	Herbocides	Exploration of the algaecide zeolite carrying copper	Jinan University, Guangzhou
C-C-13	Chemical control	Herbocides	Studies on bromogeramine for removing and controlling *prorocentrum micans* red tide	Jinan University, Guangzhou
C-C-14	Chemical control	Herbocides	Experimental study on algaecide Tertbutyl triazine for removing red tide	Jinan University, Guangzhou
C-C-15	Chemical control	Biological secretion	Isolation and purification of phenazine pigments produced by *Pseudomonas aeruginosa* and its effects on the growth of red tide organisms	Ocean University of China
C-C-16	Chemical control	Biological secretion	Studies on wheat straw to inhibit the growth of *Phaeocystis globosa*	Jinan University, Guangzhou
C-C-17	Chemical control	Biological secretion	Removing red tide algae in the sea by biomass carrier as algaecide	Jinan University, Guangzhou
C-C-18	Chemical control	Biological secretion	The allelopathic effects of *Enteromorpha linza* on *Heterosigma akashiwa*	Ocean University of China
C-C-19	Chemical control	Biological secretion	Effects of macroalgae on growth of 2 species of bloom microalgae and interactions between these microalgae in laboratory culture	Institute of Oceanology, Chinese Academy of Sciences
C-C-20	Chemical control	Biological secretion	Effects of *Ulva pertusa* and *Gracilaria lemaneiformis* on growth of *Heterosigma akashiwo* (Raphidophyceae) in co-culture	Institute of Oceanology, Chinese Academy of Sciences
C-C-21	Chemical control	Other chemicals	Removal of red tide in Tahe, Lvshun by simple physical and chemical methods	(Wang Huiqin, Du Guangyu)
C-C-22	Chemical control	Other chemicals	Development and preliminary test of a new material for prevention and control of red tide	The second institute of State Ocean Administration

List of Countermeasures against HABs in China (3)

Study No.	Category	Methods	Title	Implementing organization (author)
C-C-23	Chemical control	Other chemicals	The technology of cleaning up red tide algae and nutrient by composite detergent	National Marine Environmental Monitoring Center
**	(Toxic species)	Biological secretion	Effect of chinese fir wood meals on the growth of *Alexandrium tamarense*	Jinan University, Guangzhou
**	(Toxic species)	Algicidal bacteria	Effect of marine bacteria on the growth and PSP procuction of the red-tide algae	Xiamen University, Xiamen
**	(Toxic species)	Algicidal bacteria	Microbial modulation in the biomass and toxin production of a red-tide causing alga	Xiamen University, Xiamen
**	Indirect measure	**	A preliminary study on prediction of dissolved oxygen lack after near shore red tide occurrence and biological prevention of red tide	Xiamen University, Xiamen
**	Indirect measure	**	Competition about nutrients between Gracilaria lemaneiformis and Prorocentrum donghaiense	Institute of Oceanology Chinese Academy of Sciences
**	Indirect measure	**	Competition on nutrients between Gacilria Lemaneiformis and Scrippsiella Trochoidea (Stein) loeblich III	Institute of Oceanology, Chinese Academy of Sciences
**	Indirect measure	**	Influences of adding macroalgae Gracilaria lemaneiformis to Skeletonema costatum´s bloom	Xiamen University, Xiamen

Physical Control:

Clays:

No.: C-P-1

1) Title	A new method to improve the capability of clays for removing red tide organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum minimum*
5) Implemented period	/
6) Experiment type	Laboratory study
7)Application	Not applied
8)Method/ mechanism	The improved method of adding PACS (polyhydroxy aluminum chloride)in clays is studied.
9)Results	(1) A theoretical based on the study of coagulation of red tide organisms with clays showed that the surface modification of the clay the main way to improve the capability for clays to remove red tide organism. (2) The amount of kaolin needed for removing more than 90% of red tide organisms reduced from 2g/L to 0.1g/L. (3) The condition for preparation of PACS modified clays was optimized, the effects of concentration of clay, alkalinity, and the Al/sulfate ratio on the removal efficiency was studied.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu Zhiming, Zou Jingzhong, Ma Xinian, 1994,A new method to improve the capability of clays for removing red tide organisms, Oceanologia et Liminologia Sinica,25(2):226-232.

No.: C-P-2

1) Title	A more Efficient clay for removing red tide organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum minimum,Skeletonema costatum, Noctiluca scintillans*
5) Implemented period	/
6) Experiment type	Laboratory study
7)Application	Not applied
8)Method/ mechanism	(1) The efficiency of a Kaolin for removal of red tide species was tested. (2) The effects of pH and acid - modifying on coagulation was studied.
9)Results	(1) A kaolin with higher efficiency for removal of red tide species than montmorillonite was found; (2) Acid treatment was not good for this kaolin, the mechanism was studied and elucidated.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Yu Zhiming, Zou Jingzhong, Ma Xinian, 1994,A more effective clay for removing red tide organisms, JOURNAL OF NATURAL DISASTERS, 3(2):105-108.

No.: C-P-3

1) Title	Application of clays to removal of red tide organisms I: coagulation of red tide organisms with clays
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum minimum, Noctiluca scintillance*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	To develop the theory on coagulation of algae with clay, and compare the theory with the experimental results.
8)Method/ mechanism	(1) Coagulation experiment; (2) pH titrating experiment
9)Results	(1) A quantitative model was presented to describe how the coagulation varies with pH in solution, it was indicated that when the $pH=(pH_{zpca}+pH_{zpcb})$, the coagulation is the strongest; (2) It was indicated that when the diameter of clay particles is close to that of cells, the coagulation is weaker by the model analysis.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu ZhiMing, Zou Jingzhong, Ma Xinian,1994, Application of clays to removal of red tide organisms I: coagulation of red tide organisms with clays, Chinese Journal of Oceanology and Limnology, 12(3): 193-200.

No.: C-P-4

1) Title	Application of clays to removal of red tide organisms II: coagulation of different species of red tide organisms with montmorillonite and effect of clay pretreatment
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Nitzschia pungens, Skeletonema constatum, Prorocentrum minimum and Noctiluca scintillans*.
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	Montmorillonite were used to remove different species of red tide causative species. Acid treatment was tried to improve the coagulation efficiency of montmorillonite.
8)Method/ mechanism	(1) Algal removal experiment using montmorillonite. The tested algal species include: *Nitzschia pungens, Skeletonema constatum, Prorocentrum minimum and Noctiluca scintillans*. (2) Effect of acid pretreatment on the removal efficiency of montmorillonite was studied, using *Noctiluca scintillans* as test organism.
9)Results	(1) It was found that the capability for montmorillonite to coagulate the red tide causative species in the following order: *N.pungens*>*S. costatum*>*P. minimum*>*N. scintillans*. The difference was discussed from the aspects of the structure, shape, size, movement and habit of the test organisms etc. (2) The acid pretreatment of montmorillonite could enhance its coagulation efficiency.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu Zhiming, Zou Jingzhong and Ma Xinian, 1994, Application of clays to removal of red tide organisms II: coagulation of different species of red tide organisms with montmorillonite and effect of clay pretreatment, Chinese Journal of Oceanology and Limnology, 12(4): 316-324.

No.: C-P-5

1) Title	Application of clays to removal of red tide organisms III: coagulation of Kaolin on red tide organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Skeletonema constatum, Prorocentrum minimum and Noctiluca scintillans*.
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The kaolin was tested for its coagulation on various red tide organisms.
8)Method/ mechanism	(1) Algal removal experiment using kaolin. The tested algal species include: *Skeletonema constatum, Prorocentrum minimum and Noctiluca scintillans*. (2) Effect of acid pretreatment on the removal efficiency of kaolin was studied.
9)Results	(1) It was firstly found that the coagulation of kaolin was much greater than that of montmorillonite so that the kaolin is a more effective clay for removing red tide organisms; (2) The acid treatment does not have much influence on the kaolin system, whereas the effect of pH on the kaolin system is the same as that on the montmorillonite system.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu Zhiming, Zou Jingzhong, Ma Xinian, 1995, Application of clays to removal of red tide organisms III: coagulation of Kaolin on red tide organisms, Chinese Journal of Oceanology and Limnology, 13(1): 62-70.

No.: C-P-6

1) Title	Study on the kinetics of clay removing red tide organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum minimum*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The kinetics of clay coagulation of red tide organism *Prorocentrum minimum* was studied. The effects of clay type, concentration, second component PACS and pH was also studied. Potential methods for increasing the coagulation efficiency of clay was discusses based on a theoretical model developed.
8)Method/ mechanism	The kinetics of clay coagulation and the effects of clay type, concentration, second component PACS and pH on the coagulation rate was studies using the transparency measurement at the wave length of 420nm. A theoretical model was developed to understand and predict the potential methods for increasing the coagulation efficiency.
9)Results	(1) The result indicated that kaolin has a higher coagulation rate than montmorillonite, which can be explained by the model developed. It was indicated that the potential energy and radius of interaction between clay particles and organism cells are the major factors controlling the coagulation rate. (2) It was found that the increase of clay concentration can accelerate coagulation, but it is not the most effective way. Adding PACS in clays appears to be the most effective way of increasing the coagulation rate.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu ZhiMing, Zou Jingzhong, Ma Xinian, Study on the kinetics of clay removing red tide organisms, 1995, 26(1): 1-5.

No.: C-P-7

1) Title	A study on optimum conditions for the removal of red tide organisms by modified clays
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Phaeodactylum tricornutum*, *Nitzschia closterium*, *Gynmodinium* sp
5) Implemented period	May-Aug, 1995
6) Experiment type	Laboratory simulation study
7)Application	The algae concentration in the shrimp culturing ponds in Shangma, Qingdao was above the criteria for red tide, the algae samples were then collected and the predominant species were identified. The collected algae samples were treated with modified clays in the lab to test the efficiency for removal of red tide organisms.
8)Method/ mechanism	The collected algae samples from the shrimp ponds were treated with modified clays to remove the red tide organisms, and the optimum conditions for treatment was tested with a 4-factor, 3-level orthogonal experiment.
9)Results	(1) The modified clay prepared by Kaolin and PACS had high efficiency in removing red tide organisms in the shrimp ponds. The removal rate could reach 80-90% in less than 12 hours. (2) The best formula for Kaolin, PACS, component A and pH was optimized to remove the red tide organism. The formulae were different for the different species of red tide organisms.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Li Quansheng, Yu Zhiming, Zhang Bo, Zhang Yongshan, Ma Xinian, 1998, A study on optium conditions for the removal of red tide organism by modified clays. Oceanologia et Liminologia Sinica, 29(3):313-317.

No.: C-P-8

1) Title	Impact of halloysite on growth of *Pseudonitzschia pungens* var. *multiseries* and production of algal toxins
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Pseudonitzschia pungens* var. *multiseries*
5) Implemented period	1994
6) Experiment type	Laboratory simulation study
7)Application	The clay was applied to the culture of *Pseudonitzschia pungens* var. *multiseries* to study its effects on the growth and toxin production of the algae.
8)Method/ mechanism	Clay was applied to the batch culture of *Pseudonitzschia pungens* var. *multiseries,* the growth and domoic acid production were monitored by cell counting and HPLC analysis.
9)Results	(1) The growth of *Pseudonitzschia pungens* var. *multiseries* was inhibited by the addition of clay, probably due to the shading effect of clay; (2) The domoic acid production was affected by the addition of clay, the cellular toxin content decreased about one third compared to the control.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu ZhiMing, D.V. Subba Rao, 1998, Impact of halloysite on growth of *Pseudonitzschia pungens* var. *multiseries* and production of algal toxins, Oceanologia et Liminologia Sinica, 19(1): 47-52.

No.: C-P-9

1) Title	Surface modification of the clay particles and its effect on the coagulation efficiency of red tide organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Heterosigma akashiwo*
5) Implemented period	Not mentioned
6) Experiment type	Laboratory simulation study
7)Application	A technique for surface modification of the clay particles was developed and the preparation protocol was optimized to increase the efficiency for algal removal.
8)Method/ mechanism	An “inserting” method was developed to reverse the surface charge of the clay particles. The preparation protocol was optimized by adjusting the parameters such as temperature and Mg^{2+} concentration. The surface modified clay particles have a high efficiency in coagulation of red tide organism *Heterosigma akashiwo.* The amount of modified clays needed for algal removal was reduced to 10-20% compared to the original clay.
9)Results	(1) An “inserting” method was developed for surface modification of clay particles. (2) The parameters were optimized for the preparation protocol. (3) The surface-modified clay has a high efficiency in removing red tide organism, The amount of modified clays needed for algal removal was reduced to 10-20% compared to the original clay. (4) The method further confirmed the theory developed previously.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Yu Zhiming, Song Xiuxian, Zhang Bo, Sun Xiaoxia, 1999, Clay surface modification and its coagulation of red tide organisms, Chinese Science Bulletin, 43(24): 2091-2094.

No.: C-P-10

1) Title	Effect of bentonite modified removing red tide organisms and DRP、COD of sea water
2) Category	Physical control
3) Implementing organization	Xiamen university, Xiamen
4) Target species	*Skeletonema costatum*
5) Implemented period	Not mentionable
6) Experiment type	Laboratory simulation study
7)Application	Laboratory research has been studied about the effect of bentonite modified to remove red tide organisms and DRP, COD of the sea water under various conditions.
8)Method/ mechanism	A series of concentrated modified bentonite was prepared, then dispersed in *S. costatum* cultures.
9)Results	(1) The effect of bentonite containing efficacious Al 15% had the highest efficiency in algal removing. (2) The bentonite modified by Na_2SO_4 and $Al_2(SO_4)_3$ at pH 5.5 was the best choice for preparation. (3) The efficiency of modified bentonite increased with a rise of Al/SO_4 ratio. (4) The addition of coagulants chitin and $Ca(OH)_2$ increased removal efficiency of modified bentonite.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhou Ciyou, Fang Zhishan, Zheng Airong, Li Ying, 1999, Effect of bentonite modified rem oving red tide organism s and DRP, COD of sea water, Acta Oceanologica Sinica, 21(2): 49-55.

No.: C-P-11

1) Title	Study on the kinetics of clay-MMH system on coagulation of red-tide organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Heterosigma akashiwo* and *Nitzschia closterium*
5) Implemented period	Not mentioned
6) Experiment type	Laboratory simulation study
7)Application	The kinetics in coagulation of red tide organisms by clay-MMH system was tested with *Heterosigma akashiwo* and *Nitzschia closterium.*
8)Method/ mechanism	The surface charge of clay was modified after adding a second component MMH (Mixed Metal Layered Hydroxide). The effects of MMH ratio, clay-MMH concentration, and pH on the coagulation rate were tested with *Heterosigma akashiwo* and *Nitzschia closterium.*
9)Results	(1) The addition of a second component MMH to the clay will invert the surface charge of the clay particles and increase the efficiency for algal removal. (2) The coagulation rate increased with the increasing ratio of MMH and the concentration of clay-MMH system. pH also affected the coagulation rate.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Song Xiuxian, Yu Zhiming, Sun Xiaoxia, 2000, Study on the kinetics of clay-MMH system on coagulation of red-tide organisms, 31(4): 434-439.

No.: C-P-12

1) Title	A Preliminary study in controlling the red tide calamity by using pillared clay
2) Category	Physical control
3) Implementing organization	Guangzhou Institute of Geochemistry, Cruangzhou
4) Target species	*Anabaena spiroides, Microcystis sp.*
5) Implemented period	Not mentioned
6) Experiment type	Field study
7)Application	The effect of pillared clay on controlling red tide in the reservoir was studied.
8)Method/ mechanism	The pillared clay was added into the water directly
9)Results	(1) The algae was killed by pillared clay added within ten minutes. (2) The pillared clay has quick removing effects on diatom and a strain of dinoflagellate. (3) The pillared clay has same removing effects on *Platymonas subcordiformis*.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Wang Faya, Zhang Huifen, Feng Huang, Guo Jiugao, Wang Deqiang, 2000, A Preliminary study in controlling the red tide calamity by using pillared clay, GeoloRicaI JoumaI of China Universities, 6(2): 366.

No.: C-P-13

1) Title	Removal of different species of red tide organisms with an effective clay-complex system
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Scrippsiella trochoidea, Amphidinium carterae* and *Heterosigma akashiwo*
5) Implemented period	/
6) Experiment type	Laboratory study
7)Application	Not applied
8)Method/ mechanism	(1) A clay-complex system was prepared by the addition of component A and B to the clay; (2) The conditions for preparation of the clay-complex system were optimized, using a 3-factor, 3-level orthogonal test.
9)Results	(1) The prepared clay-complex system was efficient in removing red tide organisms, and the clay was the most important factor in coagulation of red tide organisms. (2) The removal efficiency on three species was in order of *Scrippsiella trochoidea > Amphidinium carterae >Heterosigma akashiwo*. (3) A bioassay experiment showed that the clay-complex system decreased the mortality rate of *Penaeus japonicus,* suggesting that the clay-complex system has little toxic effects on the shrimps.
10)Impact on environment ecosystem	The bioassay experiment showed that the clay-complex system decreased the mortality rate of *Penaeus japonicus,* suggesting that the clay-complex system has little toxic effects on the test shrimps.
11)Others	
12)Reference	Song Xiuxian, Yu Zhiming, Gao Yonghui, 2003, Removal of different species of red tide organisms with an effective clay-complex system. Chinese Journal of Applied Ecology, 14(7):1165-1168.

No.: C-P-14

1) Title	Extinguishment of harmful algae by organo-clay
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense*
5) Implemented period	Not mentioned
6) Experiment type	Laboratory simulation study
7)Application	Hexadecyltrimethyleamine bromide (HDTMAB), a kind of cationic organo-surfactants, was chosen to improve the efficiency of kaolin in removing red tide algae by surface sorption and cationic exchange. The efficiency of organo-modified clay was tested with *Prorocentrum donghaiense*, a large-scale red tide causative species in East China Sea.
8)Method/ mechanism	The organo clay was prepared by mixing HDTMAB with kaolin based on the clay's cationic exchanging capacity for 5 days at 40 centigrade. The removing efficiency was tested with cultured *P. donghaiense*. The offect of HDTMAB amount used on the removing efficiency was tested and the mechanism for algal removal by organo-clay was discussed.
9)Results	(1) The organo-clay prepared had a high efficiency in removing *P. donghaiense*. The removal rate could reach 95% when 0.01g/L organo-clay was used. (2) The efficiency of organo-clay has a direct relationship with the amount of HDTMAB used. The more HDTMAB used, the high efficiency of clay was found. (3) The change of surface electric charge of clay particles, the "net capture" effect by the long lipoid chains of HDTMAB on algal cells, and the toxic effect of local high concentration of HDTMAB on the surface of clay particles on the captured cells, were believe to be associated with the high efficiency of ogano-clays.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	CAO Xihua,YU Zhiming, 2003, Extinguishment of harmful algae by organo-clay. Chinese Journal of applied ecology, 14(7): 1169-1172

No.: C-P-15

1) Title	Removal of red tide organisms by organo-modified bentonite
2) Category	Physical control
3) Implementing organization	Zhejiang University, Hangzhou
4) Target species	*Skeletonema costatum*
5) Implemented period	From March, 2001 to June, 2001
6) Experiment type	Laboratory simulation study
7)Application	A series of organo-bentonites were synthesized by exchanging cation surfactants
8)Method/ mechanism	Organo-bentonites were prepared and added into the algal cultures directly to test their efficiency.
9)Results	(1) the removal rate of *S. costatum* by the bentonites was in the order of cyltrimethylammonium surfactant modified iron pillared bentonite> cetyltrimethylammoium surfactant modified iron pillared bentonite>iron pillared bentonite> cyltrimethylammonium surfactant modified sodium bentonite> cetyltrimethylammoium surfactant modified>sodium bentonite. (2) T'he remova1 rate of *S. costatum* was related to the length of alkyl chains and the amount of cation surfactants exchanged on bentonites.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Deng Yuesong, Xu Zirong, Xia Meisheng, Ye Ying, Hu Caihong, 2004, Removal of red tide organisms by organo-modified bentonite, Chinese Journal of Applied Ecology, 15(1):116-118.

No.: C-P-16

1) Title	Flocculation and removal of the brown tide organism, *Aureococcus anophagefferens* (Chrysophyceae), using clays
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Academy of Sciences
4) Target species	*Aureococcus anophagefferens*
5) Implemented period	Not mentionable
6) Experiment type	Laboratory simulation study
7)Application	the removal efficiency of Aureococcus anophagefferens by clays was studied not only with the mineral used, but also with the way the slurry is prepared (salinity and initial concentration of the stock slurry) and dispersed into the culture (layered, pulsed or mixed).
8)Method/ mechanism	A series of concentrated clay stocks was prepared, then dispersed in A. anophagefferens cultures.
9)Results	(1) phosphatic clay (IMC-P2) had a higher cell removal efficiency (RE) than kaolinite (H-DP) when seawater was used to disperse the clay, but H-DP removed cells more efficiently when suspended in distilled water prior to application. (2) Mixing after dispersal approximately doubled RE for both clays compared to when the slurry was layered over the culture surface. (3) Lowering the concentration of clay stock and pulsing the clay loading increased RE. (4) These empirical studies demonstrated that clays might be an important control option for the brown tide organism, given the proper attention to preparation, dispersal methods, environmental impacts, and the hydrodynamic properties of the system being treated.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Yu Zhiming, Mario R. Sengco, Donald M. Anderson, 2004, Flocculation and removal of the brown tide organism, *Aureococcus anophagefferens* (Chrysophyceae), using clays, Journal of Applied Phycology, 16: 101–110.

No.: C-P-17

1) Title	Removal efficiency of red tide organisms by modified clay and its Impacts on cultured organisms
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense, Heterosigma akashiwo*
5) Implemented period	April, May, 2003
6) Experiment type	Laboratory simulation study and field trial
7)Application	The removal efficiencies of *Prorocentrum donghaiense* by Hexadecyltrimethylammonium (HDTMA) bromide and the organo-clay prepared with HDTMA were studied. The toxic effects of HDTMA and the organo-clay prepared were tested with shrimp larval. The organo-clay was applied to remove *Prorocentrum donghaiense* in a field trial in the East China Sea.
8)Method/ mechanism	(1) Preparation of organo-clay by mixing the clay and HDTMA solution. (2) The acute toxicity of HDTMA and clay was tested with *Penaeus japonicus*; (3) Removal efficiency of *P. donghaiense* was tested in the laboratory simulation experiment and in an *in situ* field experiment.
9)Results	(1) The organo-clay has a high efficiency in removing red tide organism. The concentration for removing 100% *P.donghaiense* was 0.03g/L, and that for *H.akashiwo* was 0.09g/L. (2) The clay could significantly reduce the acute toxicity of HDTMA, no mortality of the *Penaeus japonicus* larvae was observed at the effective concentration of organo-clay for algal removal. (3) Both the in-door simulation experiment and the field experiment indicated that the organo-clay has a high efficiency in removing *P. donghaiense*, the large scale bloom causative species.
10)Impact on environment ecosystem	No acute toxicity was found for the organo-clay.
11)Others	
12)Reference	Cao Xihua, Song Xiuxian, Yu Zhiming, 2004, Removal efficiency of red tide organisms by modified clay and its Impacts on cultured organisms, Environmental Science, 25(5):148-152.

No.: C-P-18

1) Title	Mechanisms of removing red tide organisms by organo-clays
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense, Heterosigma akashiwo*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The mechanism for the prepared organo-clay in removing red tide organism was studied, and the factors affecting the coagulation efficiency was discussed.
8)Method/ mechanism	The type and amount of quaternary ammonium, clay, aging time, ion strength, temperature, pH were studied for their effects on the coagulation efficiency of prepared organo-clay.
9)Results	(1) Surface modification by HDTMA will increase the coagulation efficiency of the clay, and it also increase the killing efficiency of the organo-clay system; (2) The existence of HDTMA in the metastable state is critical for the removing efficiency of the organo-clay. The increasing amount of HDTMA in the metastable state will increase the removing efficiency. (3) The factors such as amount of HDTMA adsorbed, type of clay, aging time, reaction media and temperature could affect the HDTMA in the metastable state.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Cao Xihua, Song Xiuxian, Yu ZhiMing, Wang Kui, 2006, Mechanisms of removing red tide organisms by organo-clays, Environmental Science, 27(8): 1522-1530.

No.: C-P-19

1) Title	A new type of clay modification agent-alkyl glucoside quaternary ammonium compound
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense, Amphidinium carterae, Scrippsiella trochoidea*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	This paper studied the adsorption behavior of two kinds of alkyl glucoside ammonium compounds (AGQAC) on kaolin and bentonite. The algal removing efficiency and the acute toxicity of the alkyl glucoside ammonium compounds were also studied. The coagulation dynamics of the prepared organo-clays was studied.
8)Method/ mechanism	(1) Adsorption experiment of AGQAC on different clays; (2) Algal removing experiment by AGQAC; (3) Algal coagulation experiment by prepared organo-clay; (4) Acute toxicity experiment with *Neomysis awatschensis*
9)Results	(1) The adsorption behavior of AGQAC on the clay coincide the Langmuir adsorption isotherm. The sorption amount on bentonite was greater than that on kaolin. The amount of C8-AGQAC adsorbed was smaller than the C12-AGQAC; (2) To eradicate 90% of the three red tide causative species in 24hs, the amount of C8-AGQAC needed is 2.4mg/L, while the amount of C12- AGQAC needed is 1.5mg/L. (3) The 48 LC50 C12- AGQAC in the acute toxicity experiment with *Neomysis awatschensis* was 17.5mg/L. It was supposed that the application of this new organo-clay would not affect the cultured organisms. The application of organo-clay could reduce the impacts of red tide on the test organisms.
10)Impact on environment ecosystem	Acute toxicity experiment suggested that the application of organo-clay wouldn't affect the cultured organisms.
11)Others	
12)Reference	Wu Ping, Yu Zhiming, 2006, A new type of clay modification agent-alkyl glucoside quaternary ammonium compound, China Environmental Science, 26(6): 680-684.

No.: C-P-20

1) Title	Extinguishment of harmful algae by organo-clay modified by alkyl glucoside quaternary ammonium compound
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense, Amphidinium carterae, Scrippsiella trochoidea*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The adsorption characteristics of alkyl glucoside quaternary ammonium compounds (AGQAC) on different clays were studied; The removing efficiency and coagulation dynamics of prepared organo-clays were also studied.
8)Method/ mechanism	(1) Adsorption experiment of AGQAC on the clay; (2) Removing efficiency of prepared organo-clay on different red tide causative species; (3) Coagulation kinetics experiment.
9)Results	(1) It was found that the adsorption rate of AGQAC on the clay was very fast, the adsorption equilibration could be reached in 1-2 mintues; (2) The organo-clay could significantly increase the efficiency in removing red tide algal species, the same amount of organo-clay could increase the algal removing efficiency from 20% (original clay) to 90%; (3) The coagulation kinetic experiment indicated that the type and concentration of clay, and the addition of a second component, could significantly affect the algal removing efficiency.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Wu Ping, Yu Zhiming, Song Xiuxian, 2006, Extinguishment of harmful algae by organo-clay modified by alkyl glucoside quaternary ammonium compound, Environmental Science, 27(8): 1522-1530.

No.: C-P-21

1) Title	Remediation from harmful algae bloom with organo-clay processed surfactant
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Heterosigma akashiwo, Amphidinium carterae, Scrippsiella trochoidea*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The algal removing efficiency and the acute toxicity of a new organo-clay prepared by dialkyl-polyoxyethenyl-quaternary ammonium compound (DPQAC) were studied.
8)Method/ mechanism	(1) Experiment on algal removing efficiency of the prepared organo-clay; (2) Coagulation kinetic experiment; (3) Acute toxicity experiment with shrimp larvae.
9)Results	(1) It was found that the prepared organo-clay at the concentration of 0.03g/L (the DPQAC concentration 3mg/L) could remove nearly 100% of the tested algae in 24 hours; (2) The prepared organo-clay had a relatively low coagulation rate. But the coagulation rate of Kaolinite was higher than bentonite. Increasing clay concentration could accelerate the coagulation rate. (3) The acute toxicity experiment indicated that the toxicity of DPQAC was 50 times lower that the traditionally used hexadecyltrimethyleamine bromide, the LC50 of DPQAC was 61.9mg/L. The combination of DPQAC with clay could significantly decrease the toxicity of DPQAC. Treatment with prepared organo-clay could significantly decrease the impacts of algae *Amphidinium carterae* on the shrimp larvae.
10)Impact on environment ecosystem	DPQAC prepared organo-clay had no obvious acute toxicity on the shrimp larvae.
11)Others	
12)Reference	Wu Ping, Yu Zhiming, Yang Guipeng, Song Xiuxian, 2006, Remediation from harmful algae bloom with organo-clay processed surfactant, Oceanologia et Limnologia Sinica, 37(6): 511-516.

Flocculates:

No.: C-P-22

1) Title	Preparation of PSAS（Polysilicate-aluminium sulfate) and its application in HAB prevention
2) Category	Physical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Heterosigma akashiwo, Thalassiosira subtilis Skeletonema costatum*
5) Implemented period	/
6) Experiment type	Laboratory study
7)Application	Not applied
8)Method/ mechanism	(1) Remove the red tide algae by coagulation with prepared PSAS; (2) The preparation conditions of PSAS were optimized using 3-factor and 3-level orthogonal experiment. Concentration of SiO_2, Al^{3+}/SiO_2 molar ratio and pH were chosen as the three chief factors in PSAS preparation.
9)Results	(1) The removal rates of PSAS were much higher than those of AS (Aluminium sulfate), and the dosages of PSAS were 30~40% lower than those of AS when they achieved the same removal rates. (2) The anti-coagulation ability of HAB organisms varied with algal species, which was related to different physiological and ecological features of various algal cells.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Sun Xiaoxia, Zhang Bo, Yu Zhiming, 2002, Preparation of PSAS and its application in HAB prevention. Chin.J. Appl. Ecol., 13(11): 1468-1470.

Chemical Control:
Hydroxide radicals:

No.: C-C-1

1) Title	Study on the treatment of red tide pollution using hydroxide radical medicament
2) Category	Chemical control
3) Implementing organization	Dalian Maritime University, Dalian
4) Target species	*Chromulina* sp., *Platymonas* sp., *Dunaliella* sp.
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The study on killing *Chromulina* sp., *Platymonas* sp., and *Dunaliella* sp. with hydroxyl radical was investigated.
8)Method/ mechanism	The different concentrations of hydroxyl radical liquid were prepared and added into the algal cultures.
9)Results	(1) the three species of algae were killed when the concentration of hydroxyl radical was 1.1×10^{-6}g/L within ten seconds. (2) The experimental data show that it is a green and effective means to apply hydroxyl radical to treat red tide.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Bai Xiyao, Bai Mindong, Zhou Xiaojian, 2002, Study on the treatment of red tide pollution using hydroxide radical medicament, Ziran Zazhi, 26-32.

No.: C-C-2

1) Title	Experiment of killing the microorganisms of red tide using hydroxyl radicals in the shore of Jiaozhou gulf
2) Category	Chemical control
3) Implementing organization	Dalian Maritime University, Dalian
4) Target species	Thirty-six species of dinoflagellates and diatoms
5) Implemented period	August 20, 2002
6) Experiment type	marine enclosure experiment
7)Application	The enclosure experiment of killing microorganism by hydroxyl radicals was carried out in the shore of Jiaozhou Gulf, China.
8)Method/ mechanism	Hydroxyl medicament was sprayed to the enclosure water surface.
9)Results	(1) The concentration of hydroxyl radical reached 0.68 mg/L, the killing efficiency reached 99.8% after 24 h. (2) hydroxyl medicament is a new effective and feasible method in treatment of the red tide.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Bai Xiyao, Zhou Xiaojian, Lu Jibin, Zong Xu, Huang Guibin, 2003, Experiment of killing the microorganisms of red tide using hydroxyl radicals in the shore of Jiaozhou gulf, Journal of Dalian Maritime University, 29(2): 47-52.

No.: C-C-3

1) Title	Study on killing *Gymnodinium mikimotoi* with hydroxyl radical
2) Category	Chemical control
3) Implementing organization	Dalian Maritime University, Dalian
4) Target species	*Gymnodinium mikimotoi*
5) Implemented period	June, 1998
6) Experiment type	Laboratory simulation study
7)Application	The study on hydroxyl radical in killing *Gymnodinium mikimotoi* was studied
8)Method/ mechanism	Different concentrations of hydroxyl radical liquid were prepared and added into the algal cultures.
9)Results	(1) At the concentration of 0.68mg/L of hydroxyl radical, algae and bacteria were decreased to undetectable level. (2) At the concentration of 0.6mg/L of hydroxyl radical, chlorophyll a and carotene are undetectable. (3) The experimental data show that it is a green and effective means to apply hydroxyl radical to treat red tide.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhou Xiaojian, Bai Mindong, Deng Shufang, Dong Kebing, Xing Lin, 2004, Study on killing *Gymnodinium mukimotoi* with hydroxyl radical, Marine Environmental Science, 23(1): 64-66.

No.: C-C-4

1) Title	Using of hydroxyl radical on oceanic biologic contamination prevention
2) Category	Chemical control
3) Implementing organization	Dalian Maritime University, Dalian
4) Target species	*Chromulina* sp., *Platymonas* sp., *Dunaliella* sp.
5) Implemented period	Not available
6) Experiment type	The pilot experiment of ballast water and enclosure experiment
7)Application	The algaecide effects of hydroxyl radical production were introduced
8)Method/ mechanism	The different concentrations of hydroxyl radical liquid were prepared, then were added into the algal cultures.
9)Results	(1) In the 20t/h pilot experiment of ballast water and enclosure experiment of red ride, the killing efficiency reached 100% for the ballast water, and 99.89% for the enclosure experiment. (2) Experimental results indicate that hydroxyl radical is a feasible method to treat biological contamination in the sea.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Liu Xingwang, Zhou Xiaojian, Bai Xiao, Xue Xiaohong, 2004, Using of hydroxyl radical on oceanic biologic contamination prevention, Ocean Technology, 23(4): 39-43.

Disinfectants:

No.: C-C-5

1) Title	Mechanism of quaternary ammonium compounds extinguishing *Heterosigma akashiwo*
2) Category	Chemical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Heterosigma akashiwo*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The removing efficiency and mechanism of quaternary ammonium compounds (QACs) in extinguishing *Heterosigma akashiwo* was studied.
8)Method/ mechanism	(1) The experiment on removing efficiency using different QACs; (2) Physiological study on the mechanism of hexadecyltrimethyleamine bromide (HDTMAB) in removing *H. akashiwo*.
9)Results	(1) It was found that QACs with a single long-chain alkyl has higher removing efficiency for *H. akashiwo* than those with double long-chain alkyls. (2) it was suggested that the high removing efficiency of QACs was mainly due to their effects in destroying the structure and function of quasi-membrane configuration in the algal cells.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Cao Xihua, Yu ZhiMing, Wang Kui, 2003, Mechanism of quaternary ammonium compounds extinguishing *Heterosigma akashiwo*, Oceanologia et Limnologia Sinica, 34(2): 201-207.

No.: C-C-6

1) Title	Studies on biquaternary ammonium salt algaecide for removing red tide
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Phaeoecystis globosa*, *Alexandrium tamarense*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The effects of biquatemary ammonium salt on red tide algae *P. globosa* and *A. tamarense* were studied.
8)Method/ mechanism	Different concentrations of biquatemary ammonium salt were added into the algae cultures directly.
9)Results	(1) The biquatemary ammonium salt could kill the two algae efficiently in 96h at the concentration of $0.4 mg \cdot L^{-1}$. (2) Biquaternary ammonium salt has the features of high effectiveness, long acting time. (3) Biquatemary ammonium salt might be an excellent algaecide.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhang Heng, Liu Jiesheng, Yang Weidong, Gao Jie, Li Jingxiong, 2003, Studies on biquaternary ammonium salt algaecide for removing red tide, Marine Environmental Science, 22(4): 68-71.

No.: C-C-7

1) Title	Inhibition and elimination of alkylpolyglycoside on red tide plankton
2) Category	Chemical control
3) Implementing organization	Ocean University of China
4) Target species	*Prorocentrum dentatum, Hererosigma akashiwo*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	To investigate the algicidal activity of alkylpolyglycoside against the growth of *Prorocentrum dentatum* and *Heterosigma akashiwo.*
8)Method/ mechanism	Different concentrations of alkylpolyglycoside were added into the algal culture mediums at the different growth phases respectively.
9)Results	(1) The growth of *P. dentatum* and *H. akashiwo* was strongly inhibited in medium contained alkylpolyglycosid. (2) alkylpolyglycosid was lethal to the algae tested in the relatively higher concentrations, and could be considered as a potential algaecide.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Gong Liangyu, Wang Xiulin, Li Yanbin, Liang Shengkang, Han Xiurong, Zhu Chenjian, 2005, Inhibition and elimination of alkylpolyglycoside on red tide plankton, Marine Environment Science, 24(1): 1-4.

No.: C-C-8

1) Title	Povidone-iodine and isothiozolone for removing red tide algae *Phaeoecystis globosa*
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Phaeoecystis globosa*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The removal and control effects of povidone-iodine and isothiozolone on *Phaeoecystis globosa* were studied
8)Method/ mechanism	The water solutions of Povidone-iodine and isothiozolone were put into the algae culture respectively.
9)Results	(1) *P. globosa* could be killed and controlled by povidone-iodine and isothiozolone. (2) The effective concentration of povidone-iodine was 30 mg/L and that of isothiozolone was 0.30 mg/L. (3) Using povidone-iodine and isothiozolone together could improve the efficiency, and the ideal composite ratio of povidone-iodine and isothiozolone was 1.0:0.15.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Hong Aihua, Yin pinghe, Zhao Ling, Huang Yunfeng, Qi Yuzhao, Xie Longchu, 2003, Povidone-iodine and isothiozolone for removing red tide algae *Phaeoecystis globosa*, Chinese Journal of Applied Ecology, 14(7): 1177-1180.

No.: C-C-9

1) Title	Study of the extinguishing mechanism of povidone-iodine and isothiozolone
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Phaeoecystis globosa*
5) Implemented period	Not available
6) Experiment type	Laboratory study
7)Application	The mechanism for povidone-iodine and isothiozolone to remove *P. globosa* was studied
8)Method/ mechanism	The effects of povidone-iodine and isothiozolone On the *P. globosa*'s chlorophyl a, protein and SOD enzyme were studied.
9)Results	povidone-iodine and isothiozolone can destroy the *P. globosa*'s chlorophyll a, protein and SOD enzyme, and do harm to the algae.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Hong Aihua, Yin Pinghe, Zhao Ling, Lu Songhui, Zhicheng, Lin Chaoping, 2005, Study of the extinguishing mechanism of povidone-iodine and isothiozolone, Journal of Jinan University (Natural Science), 26(3): 396-400.

No.: C-C-10

1) Title	Inhibition and elimination of chlorine dioxide on *Phaeoecystis globosa*
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Phaeoecystis globosa*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The inhibition and elimination effects of chlorine dioxide on *Phaeoecystis globosa* were studied.
8)Method/ mechanism	Different densities of *Phaeoecystis globosa* (ST strain) were exposed to different concentrations of chlorine dioxide.
9)Results	(1) chlorine dioxide could effectively control the growth of algae. (2) Chlorine dioxide could be considered as a potential algaecide to control red tide.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhang Heng, Yang Weidong, Gao Jie, Liu JieSheng, 2003, Inhibition and elimination of chlorine dioxide on *Phaeoecystis globosa*. Chinese Journal of applied Ecology, 14(7): 1173-1176.

Herbocides:

No.: C-C-11

1) Title	Removal of red tide algae by a glass algaecide containing Cu (II)
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Prorocentrum micans*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The removal and control effects on tide algae by a water soluble glass algaecide containing copper were studied.
8)Method/ mechanism	A water soluble glass algaecide was put into the algae culture directly.
9)Results	(1) The concentration level of copper ions was gradually eluted from the surface of the algaecide as it was dissolved slowly when it was put into water. The level of copper can kill the red tide algae and keep the level within 7 days. (2) The removal efficiency of *P. micans* was more than 96.8% within 12 hours when the dose the algaecide was 2.0 mg/L.
10)Impact on environment ecosystem	The method could reduce the defect of direct addition of $CuSO_4$ which causes too high concentration of partial ion and hurt of the fish.
11)Others	
12)Reference	Zhao Ling, Yin pinghe, Li Kunping, Yu Qiming, Xie Longchu, huang Changjiang, 2001, Removal of red tide algae by a glass algaecide containing Cu (II), Marine Environmental Science, 20(1): 7-11.

No.: C-C-12

1) Title	Exploration of the algaecide zeolite carrying copper
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Prorocentrum micans*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	FZT (the zeolite carrying copper) as an algaecide to kill and control the red tide organisms was studied.
8)Method/ mechanism	FZT was put into the algae culture directly.
9)Results	(1) The FZT could release copper ion to kill *Prorocentrum micans* slowly with prolonged effects. (2) Adding $FeCl_3$ as a synergist could strengthen the capability and reduce the dose of the FZT.
10)Impact on environment ecosystem	The method could reduce the defect of direct addition of $CuSO_4$ which causes adverse effects on marine organisms.
11)Others	
12)Reference	Zhao Ling, Hong Aihua, Yin Pinghe, Qi Yuzao, Xie Longchu, 2002, Exploration of the algaecide zeolite carrying copper, China Environmental Science, 22(3): 207-209.

No.: C-C-13

1) Title	Studies on bromogeramine for removing and controlling *prorocentrum micans* red tide
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*prorocentrum micans*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The effects of organic algaecide Glutaraldehyde and bromogeramine on red tide algae were studied.
8)Method/ mechanism	Glutaraldehyde and bromogeramine were used separately and in combination to test their effects.
9)Results	Bromogeramine can kill and control *P. micans*.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Hong Aihua, Yin Pinghua, Zhao Ling, Qi Yuzaoi, Xie Longchu, 2003, Studies on bromogeramine for removing and controlling *prorocentrum micans* red tide, Marine Environmental Science, 22(2): 64-67.

No.: C-C-14

1) Title	Experimental study on algaecide Tertbutyl triazine for removing red tide
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Phaeocystis globosa, A lexandrium tamarens*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	Herbicide tertbutyl triazine was used for removal and control of red tide caused by *P. globosa* and *A. tamarense* under laboratory condition.
8)Method/ mechanism	Different concentrations of biquatenary tertbutyl triazine were added into the algae cultures directly.
9)Results	(1) The effective concentration of tertbutyl triazine for killing *P. globosa* and *A. tamarense* in 96h were 0.3 mg/L and 0.2 mg/L, respectively. (2) Tertbutyl triazine might be a good algaecide with high efficiency and long duration.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Liu Jiesheng, Zhang Heng, Yang Weidong, Gao Jie, Ke Qiong, 2004, Experimental study on algaecide Tertbutyl triazine for removing red tide, Journal ofTropical and Subtropical Botany, 12(5): 440-443.

Biological secretion:
No.: C-C-15

1) Title	Isolation and purification of Phenazine pigments produced by *Pseudomonas aeruginosa* and its effects on the growth of red tide organisms
2) Category	Chemical control
3) Implementing organization	Ocean University of China
4) Target species	*Heterosigma akashiwo, Prorocentrum dentatum*
5) Implemented period	Not available
6) Experiment type	Laboratory study
7)Application	The effects of the pigments produced by bacteria *Pseudomonas aeruginosa* on the control of harmful algal bloom species were discussed
8)Method/ mechanism	The pigments separated form bacteria *Pseudomonas aeruginosa* were added into the algal culture mediums directly.
9)Results	(1)The yellow pigment had potential for the selective control of harmful algal bloom species. The blue pigment exhibited no apparent growth inhibitory effect on *H. akashiwo*. (2)The yellow pigment could generate from the blue pigment by alkaline hydrolysis.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Gong Liangyu, Wang Xiulin, Li Yanbin, Zhang Chuansong, Liang Shengkang, Zhu Chenjian, 2004, Isolation and Purification of Phenazine Pigments Produced by Pseudomonas aeruginosa and its Effects on the Growth of Red Tide Organisms, Journal of Fudan University(Natural Science), 43(4): 494-499, 506.

No.: C-C-16

1) Title	Studies on wheat straw to inhibit the growth of *Phaeocystis globosa*
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Phaeocystis globosa*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The possibility of wheat straws were used to control HABs and the inhibition of Wheat straws after physical disruption on the growth of *Phaeocystis globosa* was investigated
8)Method/ mechanism	The mechanism was put forward though assessment of the roles of microorganism and adsorption of chopped straws, and morphological observation by SEM in the growth inhihition.
9)Results	(1) Finely chopped straws have an excellent algae removing activity, adsorption of straws and inhibition compounds from the straws might be responsible for the inhibition. (2) Wheat straws may be a potential candidate for HABs control.
10)Impact on environment ecosystem	Finely chopped straws had little effects on fish and other hydrophytic plants.
11)Others	
12)Reference	Gao Jie, Yang weidong, Liu Jiesheng, Zhang Heng, Tan Binghua, 2005, Studies on wheat straw to inhibit the growth of *Phaeocystis globosa*, Marine Environmental Science, 24(1): 5-8, 31.

No.: C-C-17

1) Title	Removing red tide algae in the sea by biomass carrier as algaecide
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Prorocentrum micans*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The capacities of some biomass carriers copper and the removing effects of biomass carrier carried copper on *Prorocentrum micans* were studied.
8)Method/ mechanism	Different concentrations biomass carrier with copper were put into the algae culture directly.
9)Results	(1) The biomass carrier has a prolonged time in removing red tide algae. (2) The biomass of the *Laminaria japonica* is not only suitable for the development of efficient biosorbents for the removal of heavy metals (copper) from waste water, but also for the carrier to control red tide.
10)Impact on environment ecosystem	The method could reduce the defect of direct addition of $CuSO_4$ which causes too high concentration of partial ion and hurt of the fish.
11)Others	
12)Reference	Liang Xiang, Yin Pinghe, Zhao Ling, Yang Peihui, Xie Longchu, 2001, Removing red tide algae in the sea by biomass carrier as algaecide, China Environmental Science, 21(1): 15-17.

No.: C-C-18

1) Title	The allelopathic effects of *Enteromorpha linza* on *Heterosigma akashiwa*
2) Category	Chemical control
3) Implementing organization	Ocean University of China
4) Target species	*Heterosigma akashiwo*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The allelopathic effects of fresh tissue and dry powder of *Enteromorpha linza* on *H. akashiwa* were studied using coexistence culture systems.
8)Method/ mechanism	The allelopathic effects of fresh tissue and dry powder of *E. linza* on *H. akashiwa* were studied using coexistence culture systems.
9)Results	(1) The fresh tissue and dry powder of *Enteromorpha linza* have allelopathic effects on *Heterosigm akashiwa*. (2) The growth of *H. akashiwo* was strongly inhibited by the culture medium filtrate of macroalgae. (3) The allelochemicals from the fresh tissue of *Enteromorpha linza* were unstable and degradable at higher temperature.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Xu Yan, Dong ShuangLin, Yu XiaoMing, 2005, The allelopathic effects of *Enteromorpha linza* on *Heterosigma akashiwa*, , ACTA Ecologica Sinica, 25(10): 2681-2685.

No.: C-C-19

1) Title	Effects of macroalgae on growth of 2 species of bloom microalgae and interactions between these microalgae in laboratory culture
2) Category	Chemical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense*, *Alexandum tamarense*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The effects of fresh tissue and culture medium filtrate of *Ulva pertusa* and *Gracilaria lemaneiformis* on the growth of *Prorocentrum donghaiense* and *Alexandum tamarense* in the laboratory were studied.
8)Method/ mechanism	The macroalgae and red tide algae were cultured together to see their interactions.
9)Results	(1) Both *U. pertusa* and *G. lemaneiformis* significantly interfered the growth of the co-cultured microalgae. *P. donghaiense* could be completely killed in the bialgal culture, but the growth of *A. tamarense* was not significantly affected. (2) The culture filtrate of *A. tamarense* had algicidal effect on *P. donghaiense*, while that of *P. donghaiense* had little effect on the growth of *A. tamarense*.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Wang You, Yu Zhiming, Song Xiuxian, Zhang Shandong, 2006, Effects of macroalgae on growth of 2 species of bloom microalgae and interactions between these microalgae in laboratory culture, Environmental Science, 27(2): 274-280.

No.: C-C-20

1) Title	Effects of *Ulva pertusa* and *Gracilaria lemaneiformis* on growth of *Heterosigma akashiwo* (Raphidophyceae) in co-culture
2) Category	Chemical control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Heterosigma akashiwo*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The effects of fresh tissue and culture medium filtrate of *Ulva pertusa* and *Gracilaria lemaneiformis* on growth of *Heterosigma akashiwo* were studied.
8)Method/ mechanism	The seaweed and the red tide algae were cultured together to see their interaction.
9)Results	(1) Fresh tissues and culture medium filtrate of the two species of seaweeds significantly impede the growth of *H. akashiwo*. (2) Nitrate and phosphate are almost exhausted in the *G. lemaneiformis* co-culture system. (3) The results show a positive correlation between the initial seaweed concentration and the negative effects they exert on the co-cultured microalgae. (4) Results suggest that the allelopathic effects of *U. pertusa* may be essential for its negative effects on *H. akashiwo*. But the combined roles of allelopathy and nutrient competition may be responsible for the negative effect of *G. lemaneiformis*.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Wang You, Yu Zhiming, song Xiuxian, Zhang Shandong, 2006, Effects of *Ulva pertusa* and *Gracilaria lemaneiformis* on growth of *Heterosigma akashiwo* (Raphidophyceae) in co-culture, Environmental Science, 27(2): 246-252.

Other chemicals:

No.: C-C-21

1) Title	Removal of red tide in Tahe, Lvshun by simple physical and chemical methods
2) Category	Chemical control
3) Implementing organization	Not reported
4) Target species	*Prorocentrum micans, Nitzschia* sp.
5) Implemented period	May, 26, 1994
6) Experiment type	Field treatment
7)Application	Application of straw, coal ash, montmorillonite, lime and copper sulfate were used to treat the red tide in Tawan Bay, Lvsun.
8)Method/ mechanism	The treatment agents were directly sprayed into the water.
9)Results	(1) All the five treatment agents were efficient in treatment of red tides. The lime had the highest removal efficiency. (2) It was suggested that the algae adsorbed on the straw could be re-collected and dried to burn. Therefore, the method is an environmental-friendly method.
10)Impact on environment ecosystem	Not mentioned.
11)Others	
12)Reference	Wang Huiqin, Du Guangyu, 2000, The forecast and prevention & cure countermeasures of the red tide in Dalian along shore sea field. Environmental monitoring in China, 16(6): 42-45.

No.: C-C-22

1) Title	Development and preliminary test of a new material for prevention and control of red tide
2) Category	Chemical control
3) Implementing organization	The second institute of State Ocean Administration
4) Target species	*Prorocentrum* sp.,*Gymnodinium* sp.
5) Implemented period	June, 1998
6) Experiment type	Field treatment
7)Application	Application of prepared new material to get rid of the red tide organisms in an abalone breeding plant in Fujian, China.
8)Method/ mechanism	The treatment agent was made from the coal ash. Materials prepared were mixed with seawater at the concentration of 15g/L and 30g/L to get rid of the red tide organisms.
9)Results	(1) About 91-95% red tide organisms were removed in less than 15 minutes. (2) pH would affect the efficiency of algal removal. (3) The amount of treatment agents had no significant effects on algal removal efficiency.
10)Impact on environment ecosystem	No significant effects on DO and pH of seawater were observed after the addition of treatment agent.
11)Others	The material prepared also had high efficiency in reducing COD level and turbidity in water.
12)Reference	Lin Yi-an, Tang Renyou and Chen Quanzhen, 2002, Development and preliminary test of a new material for prevention and control of red tide. Marine Sciences, 26(7): 7-12.

No.: C-C-23

1) Title	The technology of cleaning up red tide algae and nutrient by composite detergent
2) Category	Chemical control
3) Implementing organization	National Marine Environmental Monitoring Center
4) Target species	*Prorocentrum micans*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation experiment
7)Application	Application of prepared composite detergent to remove the algae and nutrients in seawaters.
8)Method/ mechanism	The composite detergent was prepared by mixing coal ash and lime, with a grey color. The sizes of the particles were about 1-50μm. The prepared composite detergent was then added to the seawater to remove the algae and nutrients.
9)Results	(1) The composite detergent had a high efficiency in removing algae. The removing rate could reach 95% at the concentration of 1g/L. (2) The composite detergent also had a high efficiency in removing nutrients, such as ammonium and phosphate in seawater.
10)Impact on environment ecosystem	Not available
11)Others	
12)Reference	Lin Shengzhong, He Guangkai, 2004, The technology of cleaning up red tide algae and nutrient by composite detergent. Marine Sciences, 23(4): 57-59.

Toxic species:
Biological secretion:
No.1:

1) Title	Effect of chinese fir wood meals on the growth of *Alexandrium tamarense*
2) Category	Chemical control
3) Implementing organization	Jinan University, Guangzhou
4) Target species	*Alexandrium tamarense*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	To assess the ability of fir wood meals to control the growth of *Alexandrium tamarense*
8)Method/ mechanism	The fir wood meals and the extract were added into the algal clutures.
9)Results	(1) The inhibitory efficiency of fir wood meals on *A. tamarense* was above 80% in 3 days when the cell density was 2.88×10^6 and 6.08×10^6/L. (2) These studies shown that the wood meals from fir might be potential candidate for HAB control.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhang Xinlian, Yang Weidong, Liu Jiesheng, Shen Mingfeng, 2005, Effect of chinese fir wood meals on the growth of *Alexandrium tamarense*, Marine Environmental Science, 24(2): 23-25.

Algicidal bacteria:

No.2:

1) Title	Effect of marine bacteria on the growth and PSP procuction of the red-tide algae
2) Category	Biological control
3) Implementing organization	Xiamen University,
4) Target species	*Alexandrium tamarense*
5) Implemented period	Not available
6) Experiment type	Laboratory study
7)Application	The effects of two strains of marine bacteria isolated from sediment of Xiamen West Sea Area on the growth and PSP production of *Alexandrium tamarense* were studied under controlled experimental conditions.
8)Method/ mechanism	Different amounts of bacteria were added into the algal culture medium directly.
9)Results	(1) The growth of *A. tamarense* was inhibited more obviously by strain S_{10} at high concentration than at low concentration. PSP production of *A . tamarense* was also inhibited by the strain S_{10} at different concentration especially at low concentration. (2) The function of the strain P_{42} was contrary to the strain S_{10}, the growth of *A. tamarense* was inhibited obviously by the strain P_{42} at low concentration, but PSP toxin production of *A . tamarense* was inhibited by P_{42} at at high concentration.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Su jianqiang, Zheng Tianling, Yu Zhiming, Song Xiuxian, 2003, Effect of marine bacteria on the growth and PSP procuction of the red-tide algae, Oceanologia ET Limnologia Sinica, 34(1): 44-49.

No.3:

1) Title	Microbial modulation in the biomass and toxin production of a red-tide causing alga
2) Category	Biological control
3) Implementing organization	Xiamen University,
4) Target species	*Alexandrium tamarense*
5) Implemented period	Not available
6) Experiment type	Laboratory study
7)Application	The mechanism involved in the inhibition of growth and PSP production of *A. tamarense* by this strain of marine bacteria, and the prospect of using it and other marine bacteria in the bio-control of red-tides was discussed.
8)Method/ mechanism	The effects of marine bacteria on the growth and toxin production of red-tide algae under different pH and salinities were studied.
9)Results	(1) Bacterium S_{10} inhibited the growth and the PSP production of *A. tamarense* at different pH and salinities. The inhibitory effect was the highestfunction on the growth of *A. tamarense* at pH 7 and salinity of 34. (3) The best inhibitory function on the PSP production of *A. tamarense* was at pH 7, but this inhibitory function was not related to salinity.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zheng Tianling, Su jianqiang, K. Maskaoui Yu Zhiming Hu Zhong, Xu Jinsen, Hong Huasheng, 2005, Microbial modulation in the biomass and toxin production of a red-tide causing alga, Marine Pollution Bulletin, 51:1018–1025.

Indirect measures:

No.1:

1) Title	A Preliminary study on prediction of dissolved oxygen lack after near shore red tide occurrence and biological prevention of red tide
2) Category	Biological control
3) Implementing organization	Xiamen University, Xiamen
4) Target species	*Skeletonema costatum*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	*Gracilaria* was applied to the red tide water to investigate its influence on DO after red tide bloom.
8)Method/ mechanism	The indoor simulation method was adopted
9)Results	(1) The seaweed could ease the hypoxia caused by red tide happened. (2) The indoor mimic method might be adopted to predict the trends of DO concentration *in situ* after red tide occurred.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Tang Kunxian, Yuan Dongxing, Lin Yasen, Chen Miner Hong Wanshu, 2004, A Preliminary study on prediction of dissolved oxygen lack after near shore red tide occurrence and biological prevention of red tide, Journal of Xiamen University (Natural Science), 43(6): 886-888.

No.2:

1) Title	Competition about nutrients between *Gracilaria lemaneiformis* and *Prorocentrum donghaiense*
2) Category	Biological control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Prorocentrum donghaiense*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The seaweed *Gracilaria lemaneiformis* was used to estimate its interference with *Prorocentrum donghaiense* under controlled laboratory conditions from view of nutrient competition
8)Method/ mechanism	The co-cultured Method between *Gracilaria lemaneiformis* and *Prorocentrum donghaiense* was used.
9)Results	(1) *G. lemaneiformis* had obviously algicidal effects on *P. donghaiense* in the coexisting system and the cells of *P. donghaiense* could be entirely extinguished at the nd of experiments. (2) *P. donghaiense* had little effects on growth of G. *lemaneiformis*. (3) G. *lemaneiformis* absorbed nitrate and phosphate more efficiently and played dominant role in nutrition competition compared with *P. donghaiense* in the coexisting system.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhang Shandong, Yu Zhim ing, Song Xiuxian, Song Fei, Wang You, 2005, Competition about nutrients between *Gracilaria lemaneiformis* and *Prorocentrum donghaiense*, Acta Ecologia Sinca, 25(10): 2676-2680.

No.3:

1) Title	Competition on nutrients between *Gacilria Lemaneiformis* and *Scrippsiella Trochoidea* (Stein) loeblich III
2) Category	Biological control
3) Implementing organization	Institute of Oceanology, Chinese Academy of Sciences
4) Target species	*Scrippsiella Trochoide*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	The seaweed *Gracilaria lemaneiformis* was used to estimate its interference with *Scrippsiella Trochoidea* under controlled laboratory conditions from view of nutrient competition
8)Method/ mechanism	The co-cultured Method between *Gracilaria lemaneiformis* and *Scrippsiella Trochoidea* was used.
9)Results	(1) *G. lemameiformis* had obvious algicidal effects on *S. trochoidea* in the coexisting system. (2) Both growth period and maximum cell density of *S. trochoidea* were decreased, and the degree of the decrease was positively related to the initial density of *G . lemameiformis*. (3) *S. trochoidea* had little effects on growth of *G. lemoneiformis*. (4) Predominance of *G. lemaneiformis* in competing for the available nutrient supply was the major reason for the depression *of S. trochoidea*. *G. lemaneiformis* may become a promising candidate in HABs mitigating.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Zhang Shandong, Song Xiuxian, Wang You, Yu Zhiming, 2005, Competition on nutrients between *Gacilria Lemaneiformis* and *Scrippsiella Trochoidea* (Stein) loeblich III, Oceanologia Et Limnologia Sinca, 36(6): 556-561.

No.4:

1) Title	Influences of adding macroalgae *Gracilaria lemaneiformis* to *Skeletonema costatum*´s bloom
2) Category	Biological control
3) Implementing organization	Xiamen University, Xiamen
4) Target species	*Skeletonema costatum*
5) Implemented period	Not available
6) Experiment type	Laboratory simulation study
7)Application	Investigating the function of *G. lemaneiformis* on *S. costatum* bloom
8)Method/ mechanism	*G. lemaneiformis* and *S. costatum* bloom was adopted.
9)Results	*G. lemaneiformis* of 2 kg/m is enough to provide water bodies with dissolved oxygen and stabilize pH value. The seaweed also can uptake nutrients and control the bacteria.
10)Impact on environment ecosystem	Not mentioned
11)Others	
12)Reference	Xu Yongjian, Qian Lumin, Jiao Nianzhi, 2005, Influences of adding macroalgae *Gracilaria lemaneiformis* to *Skeletonema costatum*´s bloom, Journal of Oceanolography in Taiwan Strait, 24(4): 533-539.

Countermeasures against HABs in Japan

List of Countermeasures against HABs in Japan (1)

Study No.	Category	Methods	Title	Implementing organization (author)
J-P-1	Physical Control	Clays	Experimental application of clay spraying for the removal of red-tide species	Kagoshima Prefectural Fisheries Experimental Station (now Kumamoto prefectural Fisheries Research Center)
J-P-2	Physical Control	Clays	Red-tide removal by clay spraying	Kumamoto Prefectural Fisheries Experimental Station (now Kumamoto Prefectural Fisheries Research Center)
J-P-3	Physical Control	Flocculants	Application experiments of red-tide removal technologies	MODEC, Inc.
J-P-4	Physical Control (Chemical Control)	Synthetic polymer	Effects of synthetic polymer coagulants on Chattonella marina	Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)
J-P-5	Physical Control	Magnetic separation	Red-tide removal through magnetic separation	Osaka University, Japan (Ichikawa, K. & Suga, K.)
J-P-6	Physical Control	Ultraviolet treatment	Development of a red-tide removal system for deployment in anti-pollution vessels	Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport
J-C-1	Chemical Control	Hydrogen peroxide	Experimental application of hydrogen peroxide for the elimination of red-tide species	Oita Prefectural Agriculture, Forestry and Fisheries Research Center, Fisheries Research Institute
J-C-2	Chemical Control	Hydrogen peroxide	Effects of hydrogen peroxide on *Chattonella marina*	Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)
J-C-3	Chemical Control	Hydrogen peroxide	Removal of *Gymnodinium mikimotoi* with hydrogen peroxide	Shizuoka Prefectural Fisheries Experimental Station
J-C-4	Chemical Control (include Toxic species)	Hydrogen peroxide	Extermination efficacy of hydrogen peroxide against cysts of red tide and toxic dinoflagellates, and its adaptability to ballast water	Seiichi Ichikawa, Yoshiharu Wakao, Yasuwo Fukuyo
J-C-5	Chemical Control	Hydrogen peroxide Acrinol	Development of damage prevention measures against Chattonella red tides	Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)
J-C-6	Chemical Control (Physical Control)	Hydroxide radicals	Development of red-tide killing and growth inhibition methods using hydroxide ion releasing material	Marino-Forum 21

List of Countermeasures against HABs in Japan (2)

Study No.	Category	Methods	Title	Implementing organization (author)
J-C-7	Chemical Control	Ozone	Development of red-tide countermeasures using ozone	Marino-Forum 21
J-C-8	Chemical Control	Copper sulfate	Red-tide removal effects of calcium nitrate $Ca(NO_3)_2$ and copper (II) sulfate $CuSO_4$	Sugawara, K. & Sato, M. Chiba Prefectural Fisheries Research Institute (now Chiba Prefectural Fisheries Research Center)
J-C-9	Chemical Control	Disinfectants	Effects of acrinol on red-tide plankton	Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)
J-C-10	Chemical Control	Biological secretion	Algicidal effect of autolysate of jellyfish *Aurelia aurita* on new type red tide flagellate *Heterocapsa circularisquama*	Shinya Handa, Juro Hiromi, and Naoyuki Uchida (Nihon University, Japan)
J-C-11	Chemical Control	Biological secretion	Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae	Koki Nagayama, Toshiyuki Shibata, Ken Fujimoto, Tuneo Honjo, and Takashi Nakamura (Kumamoto Prefectual Fisheries Research Center etc., Japan)
J-C-12	Chemical Control	Biological secretion	The effectiveness of *Ulva fasciata* and *U. pertusa* (Ulvales, Chlorophyta) as algicidal substances on harmful algal bloom species	Mochammad Amin Alamsjah, Fumito Ishimashi, Hitoshi Kitamura, and Yuji Fujita (Nagasaki Univ., Japan)
J-C-13	Chemical Control	Other chemicals	Effects of fatty acids on *Chattonella marina*	Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)
J-B-1	Biological Control	Algicidal bacteria	Isolation and properties of a bacterium inhibiting the growth of *Gymnodinium nagasakiense*	Kimio Fukami, Atsushi Yuzawa, Toshitaka Nishijima, and Yoshihiko Hata (Kochi University, Japan)
J-B-2	Biological Control	Algicidal bacteria	The algicidal effects of *Alteromonas* sp. (6/6-46 strain) on *Gymnodinium mikimotoi*	Mie Prefectural Fisheries Technology Center, Japan
J-B-3	Biological Control	Algicidal bacteria	Analysis of algicidal ranges of the bacteria killing the marine dinoflagellate *Gymnodinium mikimotoi* isolated from Tanabe Bay, Wakayama pref., Japan	Ikuo Yoshinaga (Kyoto University, Japan)
J-B-4	Biological Control	Algicidal bacteria	Distribution and fluctuation of algicidal bacterium in the decay process of *Karenia mikimoti* in cylindrical culture instrument	Yuzo Iwata, Isao Sugahara, Hiroto Maeda, Toshio Kimura, Kentaro Noritake, and Hiroe Kowa (Mie university, Japan)
J-B-5	Biological Control	Algicidal bacteria	Development of red-tide removal technologies using algicidal bacteria fixed carriers	Marino-Forum 21
J-B-6	Biological Control	Algicidal bacteria	Detection and isolation of micro-organisms that inhibit the growth of noxious red-tide dinoflagellate Heterocapsa circularisquama	Imai, I., et al. (Kyoto University, Japan)

List of Countermeasures against HABs in Japan (3)

Study No.	Category	Methods	Title	Implementing organization (author)
J-B-7	Biological Control	Algicidal bacteria	Algicidal activity of a killer bacterium against the Harmful red tide dinoflagellate *Heterocapsa circularisquama* isolated from Ago Bay, Japan	Keizo Nagasaki, Mineo Yamaguchi, and Ichiro Imai (National Research Institute of Fisheries and Environment of Inland Sea, Kyoto University, Japan)
J-B-8	Biological Control	Algicidal bacteria	Isolation of a marine gliding bacterium that kills *Chattonella antique*	Ichiro Imai, Yuzaburo Ishida, Shigeki Sawayama, and Yoshihiko Hata (Kyoto University etc., Japan)
J-B-9	Biological Control	Algicidal bacteria	Algicidal marine bacteria isolated from northern Hiroshima Bay, Japan	Ichiro Imai, Yuzaburo Ishida, Keiichi Sakaguchi, and Yoshihiko Hata (Kyoto University, Japan)
J-B-10	Biological Control	Algicidal bacteria	Algicidal ranges in killer bacteria of direct attack type for marine phytoplankton	Imai, I. (Kyoto University, Japan)
J-B-11	Biological Control	Algicidal bacteria	Lysis of *Skeletonema costatum* by *Cytophaga* sp. isolated from the coastal water of the Ariake Sea	Atsushi Mitsutani, Kaoru Takesue, Masanori Kirita, and Yuzaburo Ishida (Shimonoseki University of Fisheries etc., Japan)
J-B-12	Biological Control	Algicidal bacteria	Growth inhibition of diatoms with algicidal bacteria	Sakata T. (Kagoshima University, Japan)
J-B-13	Biological Control	Algicidal bacteria	Possibility for bio-control of harmful diatom blooms in *Coscinodiscus wailesii* by marine bacteria	Satoshi Nagai and Ichiro Imai
J-B-14	Biological Control	Algicidal virus	Isolation of a virus infecting the novel shellfish-killing dinoflagellate *Heterocapsa circularisquama*	Kenji Tarutani, Keizo Nagasaki, Shigeru Itakura, Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea, Japan)
J-B-15	Biological Control	Algicidal virus	Dynamics of *Heterocapsa circularisquama* (Dinophyceae) and its viruses in Ago Bay, Japan	Keizo Nagasaki, Yuji Tamaru, Katsuya nakanishi, Naotsugu Hata, Noriaki Katanozaka. Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea, Japan etc.)
J-B-16	Biological Control	Algicidal virus	Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*	Yuji Tomaru, Noriaki Katanozaka, Kensho Nishida,Yoko Shirai, Kenji Tarutani, Mineo Yamaguchi, Keizo Nagasaki (National Research Institute of Fisheries and Environment of Inland Sea, Japan, etc)

List of Countermeasures against HABs in Japan (4)

Study No.	Category	Methods	Title	Implementing organization (author)
J-B-17	Biological Control	Algicidal virus	Widespread occurrence of viruses lytic to the bivalve-killing dinoflagellate *Heterocapsa circularisquama* along the western coast of Japan	Yuji Tomaru and Keizo Nagasaki (National Research Institute of Fisheries and Environment of Inland Sea, Japan)
J-B-18	Biological Control	Algicidal virus	Effect of temperature on the algicidal activity and the stability of HaV (*Heterosigma akashiwo* virus)	Keizo Nagasaki and Mineo Yamaguchi (Nansei National Fisheries Research Institute, now National Research Institute of Fisheries and Environment of Inland Sea, Japan)
J-B-19	Biological Control	Algicidal virus	Growth characteristics of *Heterosigma akashiwo* virus and its possible use as a microbiological agent for red tide control	Nansei National Fisheries Institute, Japan (now National Research Institute of Fisheries and Environment of inland Sea, Fisheries Research Institute, Japan)
J-B-20	Biological Control	Algicidal virus	Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo*	Kenji Tarutani, Keizo Nagasaki, Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea, Japan)
J-B-21	Biological Control	Algicidal virus	Quantitative and qualitative impacts of viral infection on a *Heterosigma akashiwo* bloom in Hiroshima Bay, Japan	Yuji Tamaru, Kenji Tarutani, Mineo Yamaguchi, Keizo Nagasaki (National Research Institute of Fisheries and Environment of Inland Sea, Japan)
J-B-22	Biological Control	Plankton grazers	Experiment on *Gymnodinium mikimotoi* prey-predation relationship	Kagawa Prefecture Fisheries Research Institute / Red Tide Research Institute（Yoshimatsu, S. and N. Tatsumitsu）
J-B-23	Biological Control	Plankton grazers	Studies on the effects of grazing pressure on red-tide development	Nagasaki University (Shoji Iizuka)
J-B-24	Biological Control	Plankton grazers	Investigation and identification of zooplankton that graze on red-tide species	Shin-Nippon Meteorological & Oceanographical Consultant Co., Ltd. (now IDEA Consultants, Inc.)
J-B-25	Biological Control	Plankton grazers	Rearing technologies of zooplankton for use as a red-tide control agent	Akashiwo Research Institute of Kagawa Prefecture (Kagawa Pref., Japan)
J-B-26	Biological Control	Plankton grazers	The growth and grazing rate of tintinnid ciliates on the red-tide species *Heterocapsa circularisquama*	Kamiyama, T. (Nansei National Fisheries Research Institute)
J-B-27	Biological Control	Plankton grazers	Grazing impact of the field ciliate assemblage on a bloom of the toxic dinoflagellate *Heterocapsa circularisquama*	Takashi Kamiyama, Haruyoshi Takayama, Yoshinori Nishii & Takuji Uchida (National Research Institute of Fisheries and Environment of Inland Sea, etc.)

List of Countermeasures against HABs in Japan (5)

Study No.	Category	Methods	Title	Implementing organization (author)
J-B-28	Biological Control	Plankton grazers	Temporal changes in the ciliate assemblage and consecutive estimates of their grazing effect during the course of a *Heterocapsa circularisquama* bloom	Takashi Kamiyama and Yukihiko Matsuyama (Tohoku National Fisheries Research Institute, Japan etc.)

Physical control:
Clays:

No.: J-P-1

<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">1979 – 1981 (published year: 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>
<tr><td>9) Results</td><td colspan="2">➢ 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).</td></tr>
</table>

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 (μg-at・L^{-1})	DIP (μg-at・L^{-1})	Soluble Fe (μg-at・L^{-1})	Mn (μg-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.: J-P-2

<table>
<tr><td>1) Title</td><td colspan="2">Red-tide removal by clay spraying</td></tr>
<tr><td>2) Category</td><td colspan="2">Physical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Kumamoto Prefectural Fisheries Experimental Station
(now Kumamoto Prefectural Fisheries Research Center)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Cochlodinium sp. 78 type (= Cochlodinium polycrikoides)</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1979–1981 (published year: 1980-1982)</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment, field experiment (coast of Amakusa, Kyushu region)</td></tr>
<tr><td>7) Application</td><td colspan="2">Applicable to local-scale red tides in the coastal area</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ The effectiveness of clay spraying for removing red-tide species was examined.
➢ The tested clays were kaolin and clay from the Amakusa area (Amakusa clay).
➢ The sinking rate of Cochlodinium sp. 78 type was examined with and without kaolin, by pouring the samples into an approximately 3m long acrylic pipe.
➢ Kaolin was sprayed over a fish cage (size: 4.0×4.0×4.0 m) installed in a sea area with Cochlodinium sp. 78 type. The sprayed amount was 8.0 kg / 16 m^2 (500 g/m^2). The experiment was conducted twice.
➢ Kaolin and Amakusa clay were sprayed over a Cochlodinium sp. 78 type bloom, to examine their effectiveness as Cochlodinium sp. 78 type removal agents. The sprayed amount was 60 kg / 300 m^2 (200 g/m^2) for both clays.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Flocculation and sinking pattern of Cochlodinium sp. 78 type were similar with or without the addition of kaolin. Thus, kaolin was considered as not having any significant flocculation / sinking effects on Cochlodinium sp. 78 type.
➢ With the fish cage field experiment, no obvious flocculation / sinking of Cochlodinium sp. 78 type were observed, partly due to its low initial cell density in the water column.
➢ When kaolin and Amakusa clay were sprayed over a Cochlodinium sp. 78 type bloom, the cell density of Cochlodinium sp. 78 type decreased in some cases. However, it could not be concluded as being caused by kaolin or Amakusa clay.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish
➢ Amakusa clay was sprayed over a fish cage (size: 3.5×3.5×3.5 m), to examine its effects on cultured Japanese amberjack and red seabream. For both fish, 52 individuals were present in the fish cage. The Amakusa clay was sprayed 3 times per day for 5 days, at 8 kg per spray (total sprayed amount: 120 kg). No mortality was observed with both fish.
(2) Impact on the environment
➢ Elution of nutrients or hazard substances from kaolin or Amakusa clay was negligible.</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ Several clay-spraying methods were tested. The most practical method was by spraying from a boat with a pump. With this method, prior to spraying, clay is mixed with seawater onboard.</td></tr>
</table>

12) References	➢ Kumamoto Prefectural Fisheries Experimental Station (1980): Report on the development of red-tide countermeasures Year 1979, Fisheries Agency. ➢ Kumamoto Prefectural Fisheries Experimental Station (1981): Report on the development of red-tide countermeasures Year 1980, Fisheries Agency. ➢ Kumamoto Prefectural Fisheries Experimental Station (1982): Report on the development of red-tide countermeasures Year 1981, Fisheries Agency.

Flocculants:

No. : J-P-3

<table>
<tr><td>1) Title</td><td colspan="2">Application experiments of red-tide removal technologies</td></tr>
<tr><td>2) Category</td><td colspan="2">Phisical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">MODEC, Inc.</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Ceratium furca</td></tr>
<tr><td>Raphidophycea</td><td>Olithodiscus sp.(= Heterosigma akashiwo?), Hornelia sp. (=Chattonella sp.)</td></tr>
<tr><td>others</td><td>Euglena sp.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1976</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Field experiment (Tokuyama Bay, Yamaguchi Prefecture), lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Red-tide removal experiments were conducted using a red-tide removal system. The red-tide removal system was installed on a barge, and towed to a red-tide sea area (predominantly Ceratium furca) for field experiment. The removal rate was calculated by comparing the plankton cell and chlorophyll concentrations in the pre-treatment and post-treatment stages.
➢ The red-tide removal system is composed of a coagulation tank and pressure floatation system. The planktons are coagulated in the coagulation tank by using Poly Aluminum Chloride (PAC) as the coagulant. The coagulated planktons are then collected as scum via the pressure floatation system.
➢ Cell lysis and coagulation effects of ultrasonic waves on red-tide planktons were examined through lab experiments.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ The red-tide removal system achieved a 20-90% reduction in cell concentration and 75-93% reduction in chlorophyll concentration.
➢ Cell lysis of Olithodiscus sp., Hornelia sp. and Euglena sp. was observed after ultrasonic wave irradiation (wave frequency unknown).
➢ Coagulation of Olithodiscus sp. and Euglena sp. was observed after ultrasonic wave irradiation at frequency of 400 kHz.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on the ecosystem
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ The removal of planktons was not possible when the wind wave induced pitching and rolling angle of the barge was above 1 and 2-3 degrees, respectively.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ MODEC, Inc. (1976): Measures against sludge and red tide marine pollution, Application experiments of red-tide removal technologies, OCEAN AGE, May Issue, 17-23.</td></tr>
</table>

Synthetic polymers:

No.: J-P-4

<table>
<tr><td>1) Title</td><td colspan="2">Effects of synthetic polymer flocculants on Chattonella marina</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control (also includes Chemical control)</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella marina</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1985-1986 (published year: 1986-1987)</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Cultured Chattonella marina were exposed to various synthetic polymer flocculants to examine their efficacy as a removal agent. The initial cell concentration of C. marina was set between 3,500-5,000 cells/mL, with water temperature at 23±2°C.
➢ A total of 15 synthetic polymer coagulants were tested: Petrosize J, Petrosize U, Polyethyleneimice, Polyoxyethylene Laurylamine, Polyoxyethylene Lauryl Alchohol Ether, Tween20, Tween40, Tween60, Tween80, Aminoethyl Amylose Acetate, FLONAC N[*1], sodium alginate, KAYAFLOC C-533-1P[*2], KAYAFLOC C-533-1O[*2] and giant kelp.

*[1]: product of KYOWA TECNOS CO., LTD (http://www.kyowatecnos.com/)
*[2]: product of KAYAFLOC CO., LTD (http://www.kayafloc.co.jp/)</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ C. marina cells were either destroyed or morphologically modified at exposure concentrations below 10 ppm, when tested with the following five flocculants: Polyethyleneimice, Polyoxyethylene Laurylamine, Polyoxyethylene Lauryl Alchohol Ether, Tween 20 and Aminoethyl Amylose Acetate (Table-1).
➢ The five flocculants, Petrosize J, Petrosize U, Tween40, Tween60 and Tween80 did not destroy or morphologically modify C. marina cells, except when the exposure concentration was above 100 ppm (Table-1).
➢ FLONAC N showed some flocculation effect between 50-100 ppm, and destroyed all C. marina cells at 100 ppm. KAYAFLOC C-533-1P showed some flocculation effect at 50 ppm, and destroyed all C. marina cells at 100 ppm. Coagulation of C. marina cells were not observed with sodium alginate, KAYAFLOC C-533-1O and giant kelp, even at 100 ppm.</td></tr>
</table>

10) Impact on the environment / ecosystem	(1) Impact on fish ➢ Exposure tests were conducted on Japanese rice fish and Japanese amberjack with flocculants that have negative effects on *C. marina*. The 50% lethal concentration of Polyoxyethylene Lauryl Alchohol Ether and Tween 20 was high, but was low for Polyethyleneimice, Polyoxyethylene Laurylamine and Aminoethyl Amylose Acetate (Table 2). The 50% lethal concentration of FLONAC N and KAYAFLOC C-533-1P for Japanese rice fish were above 100 ppm and below 10 ppm, respectively. (2) Impact on the environment ➢ The above flocculants were tested for their resistance against bacterial decomposition by using BOD as an indicator. Polyoxyethylene Laurylamine, Polyoxyethylene Lauryl Alchohol Ether and Tween 20 decomposed quickly, but Polyethyleneimice and Aminoethyl Amylose Acetate showed very little decomposition.
11) Others	➢ The 50% lethal concentration of Polyoxyethylene Laurylamine for Japanese amberjack was 2.6 ppm. Polyoxyethylene Laurylamine also morphologically modified the *C. marina* cells at 0.5 ppm. ➢ Japanese amberjack did not die after been exposed to 100 ppm of Tween 20 for 24 hours, if enough oxygen was supplied. ➢ Although FLONAC N is made from natural substances (crab shells) and will eventually decompose, it is not practical for field application due to its low solubility in seawater. ➢ In conclusion, the most appropriate flocculants for red-tide removal were determined as Polyoxyethylene Laurylamine and Tween 20.
12) References	➢ Kagoshima Prefectural Fisheries Experimental Station (1986): Report on the development of red tide countermeasures Year 1985, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1987): Report on the development of red tide countermeasures Year 1986, Fisheries Agency.

Table－1　Effects of synthetic polymer coagulants on *Chattonella marina*

	Concentration (ppm)	Exposure time (min)	Cell density (cells / mL)			
			Swimming	Non-swimming	Round	Destroyed
Petro. J	1000	10	4000	-	-	-
		30	4000	-	-	-
Petro. U	1000	10	4000	-	-	-
		30	4000	-	-	-
Polyethyleneimice	1000	5	-	-	-	Uncountable
	100	5	-	-	-	3850
	10	5	-	-	-	3950
	1	5	-	-	950	3050
	0.1	5	-	1650	2200	-
	0.05	5	400	3500	-	-
	0.01	5	2600	1200	-	-
Polyoxyethylene Laurylamine	100	10	-	-	-	Uncountable
	10	10	-	-	-	3000
	1	10	-	30	130	2860
	0.5	10	30	80	390	2520
	0.1	10	2800	70	140	40
		30	500	1000	1500	100
	0.05	10	3000	-	-	-
		30	2000	140	260	170
Polyoxyethylene Lauryl Alchohol Ether	1000	10	-	-	-	Uncountable
	100	10	-	-	-	3500
	10	10	250	400	2000	2500
		30	-	-	1000	4000
	1	10	4000	-	-	-
		30	4000	-	-	-
Tween 20	1000	10	-	-	-	5000
	100	5	200	300	4500	-
		10	-	-	3460	2080
	10	10	400	200	2500	-
	1	30	2900	150	-	-
	0.1	30	3000	-	-	-
Tween 40	1000	5	320	-	3150	-
		10	110	-	3000	500
	100	5	1000	2000	-	-
		10	-	2500	500	-
	10	5	3500	360	-	-
		10	2900	230	460	-
		30	2500	200	360	100
	1	5	4500	-	-	-
		10	4000	200	50	-
		30	3200	560	700	-
	0.1	30	4500	-	-	-
Tween 60	1000	10	3500	500	-	-
		30	580	1280	1750	460
	100	10	4000	-	-	-
		30	1560	970	880	50
	10	30	4000	-	-	-
Tween 80	1000	5	600	-	2400	-
		10	-	-	2700	300
	100	10	1250	2100	570	-
		30	430	1160	1180	1140
	10	10	3000	160	280	-
		30	2500	600	950	-
	1	30	3500	-	-	-

Aminoethyl Amylose Acetate	1000	10	-	-	-	Uncountable
	100	10	-	-	3000	500
	10	10	-	-	3400	100
	1	10	2000	1500	500	-
		30	1200	2000	800	-
	0.1	30	3500	-	-	-

Source：Kagoshima Prefectural Fisheries Experimental Station (1986)

Table－2　The 50% lethal concentration of synthetic polymer coagulants on fish

	24h LC50 (ppm)	
	Japanese rice fish	Japanese amberjack
Polyethyleneimice	0.50	-
Polyoxyethylene Laurylamine	1.23	2.66
Polyoxyethylene Lauryl Alchohol Ether	42.21	5.62
Tween 20	89.11	82.24
Aminoethyl Amylose Acetate	5.19	-

Source：Kagoshima Prefectural Fisheries Experimental Station (1986)

Magnetic separation:

No.: J-P-5

1) Title	Red-tide removal through magnetic separation	
2) Category	Physical control	
3) Implementing organization	Osaka University, Japan (Ichikawa, K. & Suga, K.)	
4) Target species	**Class**	**Genus and Species**
	Raphidophyceae	*Chattonella* sp.
	others	*Nannochloropsis oculata*
5) Implemented period	1980 – 1983 (published year: 1981-1984)	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ An efficient removal method of red-tide plankton was developed, by combining the process of flocculation (with iron powder) and magnetic separation (Figure-1). To enhance flocculation, iron powder (Fe_3O_4, $FeCl_3$) was used together with Poly Aluminum Chloride (PAC) or Sumifloc* (polymer flocculant), and also was aided by silicic acid. ➢ Iron powder and flocculants were added at different ratios, and the removal rates of the red-tide planktons were examined. *SUMITOMO CHEMICAL (http://www.sumitomo-chem.co.jp/english/index.html)	
9) Results	➢ For *Chattonella* sp. (cell density of 1×10^4 cells/mL), the removal efficiency was above 80% when the concentration of SUMIFLOC was 0.1 ppm, $FeCl_3$ 200 ppm and Fe_3O_4 50 ppm. ➢ The floc size of the planktons after flocculation treatment was approximately 150 µm for *Chattonella* sp. and 10 µm for *Nannochloropsis oculata*. ➢ For the efficient removal of *Chattonella* sp., 10 g of iron powder was required per 1 L of seawater.	
10) Impact on the environment / ecosystem	(1) Impact on the ecosystem ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ The removal of red-tide planktons was more effective with smaller iron powder particle size. ➢ To achieve any effectiveness with small quantity of iron powder, simultaneous use of other coagulants were required.	

12) References	➢ Ichikawa, K. (1981): Report on red-tide species in the inner bay area Year 1980, Fisheries Agency. ➢ Suga, K. (1982): Report on the development of red-tide countermeasures Year 1981, Fisheries Agency. ➢ Suga, K. (1983): Report on the development of red-tide countermeasures Year 1982, Fisheries Agency. ➢ Suga, K. (1984): Report on the development of red-tide countermeasures Year 1983, Fisheries Agency.

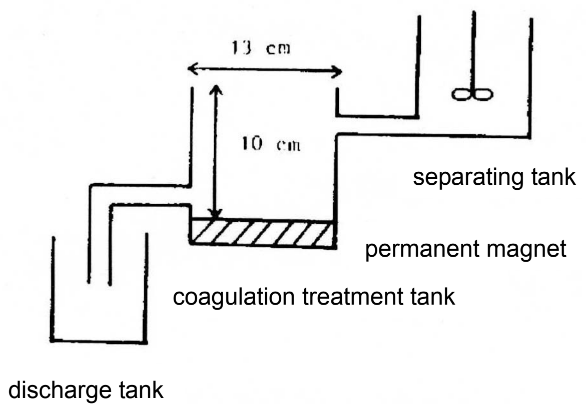


Figure-1 Magnetic separation system of coagulated floc

Source : Suga, K. (1982)

Ultraviolet radiation:

No.: J-P-6

1) Title	Development of a red-tide removal system for deployment in anti-pollution vessels	
2) Category	Physical control	
3) Implementing organization	Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport	
4) Target species	Class	Genus and Species
	Dinophyceae	*Karenia mikimotoi* (= *Gymnodinium mikimotoi*)
	Raphidophyceae	*Chattonella marina, Heterosigma akashiwo*
5) Implemented period	2002-2003	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ An anti-pollution vessel with an ultraviolet treatment system was considered as a possible red-tide removal method. The effects of ultraviolet treatment on red-tide planktons were examined through lab experiments. ➢ Red-tide plankton culture mediums were exposed to ultraviolet radiation (wavelength of 254 nm), with varying intensity and duration. The effects of the ultraviolet radiation on red-tide planktons were evaluated by observing their cell motility. ➢ The tested red-tide planktons were *Gymnodinium mikimotoi, Chattonella marina* and *Heterosigma akashiwo*.	
9) Results	➢ Resistance to ultraviolet radiation increased in the order of *Heterosigma akashiwo*, *Gymnodinium mikimotoi* and *Chattonella marina*. The required ultraviolet intensity and duration for *Chattonella marina* removal, i.e. all the tested planktons, was estimated to be above 3400 $\mu W/cm^2$ and 15 seconds, respectively.	
10) Impact on the environment / ecosystem	(1) Impact on the ecosystem ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ Based on the results of the experiments, an ultraviolet treatment system was produced for the deployment in anti-pollution vessels. The specifications of the system are shown on Table-1.	

12) References	➢ Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport (2002): Development of a red-tide removal system for deployment in anti-pollution vessels, KOBE, Vol.1. ➢ Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport (2003): Development of a red-tide removal system for deployment in anti-pollution vessels, KOBE, Vol.2. Website of Ministry of Land, Infrastructure and Transport, Kinki Regional Development Bureau, Kobe Research and Engineering Office for Port and Airport (http://www.pa.kkr.mlit.go.jp/kobegicyo/sempaku/akasio.html)

Table-1 Specifications of the ultraviolet treatment system for anti-pollution vessel deployment

Treatment method	UV treatment	Five UV lamps (straight type)
	Treatment type	Batch and continuous treatment possible
UV treatment system	UV lamp	40 W (UV-C power: 12 W), ϕ18.5 mm x 700 mm, quartz glass tube: ϕ30 mm x 730 mm
	Configuration	Cylinder type chamber, parallel alignment of 5 UV lamps, 2 types of inner cylinder for testing
	Stirrer	Motor (90W), 2 blades, sealed type
	Control panel	On/off switch of each instrument, built-in UV lamp ballast
	Power source	AC100V
	Dimensions etc.	Diameter: 400 mm, height: 730 mm, volume: 90 L, weight: 70 kg

Chemical control:
Hydrogen peroxide:

No.:J-C-1

<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.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1993 - 1994 (published year: 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">No description</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>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(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</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ 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.</td></tr>
</table>

12) References	➢ Nishimura, K. & Iwano, H., (1994): Experiment on the elimination of harmful red-tide plankton, Annual Report of Oita Prefectural Fisheries Research Institute 1993, 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 1994, 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. : J-C-2

1) Title	Effects of hydrogen peroxide on *Chattonella marina*	
2) Category	Chemical control	
3) Implementing organization	Kagoshima Prefectural Fisheries Experimental Station (now Kagoshima Prefectural Fisheries Technology and Development Center)	
4) Target species	Class	Genus and Species
	Raphidophyceae	*Chattonella marina*
5) Implemented period	1987-1988 (published year: 1988–1989)	
6) Experiment type	Lab and field experiment	
7) Application	No description	
8) Method / mechanism	➢ To examine the coagulation and removal effect of hydrogen peroxide, cultured *Chattonella marina* were exposed to 4 different concentrations of hydrogen peroxide solutions*[1] (0.15-150 ppm) for 1 and 30 minutes. ➢ To examine the coagulation and removal effect of hydrogen peroxide and silicic acid compound mixtures, cultured *C. marina* were exposed to varying combinations of hydrogen peroxide and silicic acid compound (silica gel or silica sol) mixtures for 1 and 30 minutes. The hydrogen peroxide and silica gel / sol mixtures were prepared with the following combinations: - Hydrogen peroxide (3-300 ppm) and silica gel (10-1,000 ppm) - Hydrogen peroxide (1-100 ppm) and silica sol (4-800 ppm) *1 : Fuji Silysia Chemical Ltd. (http://www.fuji-silysia.co.jp/)	
9) Results	➢ The rate of cell lysis and non-swimming *C. marina* cells generally increased with increasing concentration of hydrogen peroxide. All cells ceased swimming when the hydrogen peroxide concentrations were 15 and 150 ppm. Of these cells, approximately 60% became roundish and approximately 40% showed cell lysis (Table-1). ➢ The hydrogen peroxide and silica gel / sol mixtures were less effective in removing *C. marina* cells, in comparison to using solely hydrogen peroxide.	

10) Impact on the environment / ecosystem	(1) Impact on fish ➢ To examine the fish toxicity of hydrogen peroxide, Japanese amberjack (200-365 g) were exposed to four different concentrations of hydrogen peroxide (0, 15, 50, 150 ppm) for 3 hours. Although no individuals died at all concentrations, abnormal swimming behavior and damage to gill tissues were observed at concentrations above 150 ppm. When Japanese amberjack were exposed to both *C. marina* and hydrogen peroxide, no mortality was observed at 50 ppm after 180 minutes, but all individuals died at 150 ppm after 80 minutes. ➢ To examine the fish toxicity of hydrogen peroxide, mottled spinefoot (10.69 ±3.21g), chameleon goby (0.91±0.36g) and Japanese horse mackerel (21.68±4.96g) were exposed to four different concentrations (10-1,000 ppm) of hydrogen peroxide for 24 hours. The 50% lethal concentration (24 hrs.) of hydrogen peroxide for mottled spinefoot, chameleon goby and Japanese horse mackerel were 224 ppm, 155 ppm and 89 ppm, respectively. (2) Impact on the environment ➢ The decomposition rate of hydrogen peroxide in test seawater was relatively slow, but increased slightly when exposed to sunlight or planktons. ➢ The diffusion pattern of hydrogen peroxide solution was examined in an indoor test tank and natural sea area, using solutions of three different densities: denser than seawater, same density with seawater and less dense than seawater. The low-density solution maintained its concentration at the surface layer even after 30 minutes. The high-density solution quickly sank towards the bottom layer, while still maintaining its high concentration. This result shows some possible negative effects to benthic species and planktons in the mid and deep layers.
11) Others	➢ Removal of *C. marina* by hydrogen peroxide is considered to be more effective, if hydrogen peroxide is only used and not with silicic acid compounds.
12) References	➢ Kagoshima Prefectural Fisheries Experimental Station (1988): Report on the development of red tide countermeasures Year 1987, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1989): Report on the development of red tide countermeasures Year 1988, Fisheries Agency. ➢ Murata H., Sakai T., Endo M., Kuroki A., Kimura M. & Kumanda K. (1989): Screening of Removal Agents of a Red Tide Plankton *Chattonella marina*— with Special Reference to the Ability of the Free Radicals Derived from the Hydrogen Peroxide and Polyunsaturated Fatty Acids, Bulletin of the Japanese Society of Scientific Fisheries, 55(6), 1075-1082.

Table－1　Cell concentration of *C. marina* after exposure to hydrogen peroxide

Concentration (ppm)	Time (min)	*C. marina* (cells/mL)				
		Normal cell	No swimming		Cell lysis	Total
			Spindle	Round		
0.15	1	1500	3300	150	450	6400
	30	2000	3550	0	500	6050
1.5	1	2500	3400	150	800	6350
	30	1500	3300	150	450	5400
15	1	1050	4450	300	160	6050
	30	0	0	3350	2650	6000
150	1	1000	2200	600	1600	5200
	30	0	0	3500	2250	6750

Source：Kagoshima Prefectural Fisheries Experimental Station (1988)

No. : J-C-3

1) Title	Removal of *Gymnodinium mikimotoi* with hydrogen peroxide	
2) Category	Chemical control	
3) Implementing organization	Shizuoka Prefectural Fisheries Experimental Station	
4) Target species	Class	Genus and Species
	Dinophyceae	*Gymnodinium mikimotoi* (= *Karenia mikimotoi*)
5) Implemented period	1991 (published year: 1992)	
6) Experiment type	Field experiment (Hamanako, Shizuoka Prefecture, 10m x 10m area in the central area of Lake Hosoe)	
7) Application	No description	
8) Method / mechanism	➢ A red-tide removal agent (porous calcium silicate granules absorbed with hydrogen peroxide*) was experimentally sprayed over an area with *Gymnodinium mikimotoi* distribution. The cell density of *G. mikimotoi* was measured after 30 and 60 minutes by taking water samples from five depths (0-6 m). The pH and hydrogen peroxide concentrations were also measured. * : product of KATAYAMA CHEMICAL INDUSTRIES Co., Ltd. (http://www.katayama-chem.co.jp/product/index.html)	
9) Results	➢ No significant changes in *G. mikimotoi* cell density were observed (Fig-1).	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ The highest hydrogen peroxide concentration was measured at 2 m depth, at a value of 0.7 mg/L (30 min. after spraying), which is lower than the effective concentration of 3-6 mg/L. ➢ Appropriate spraying methods and spraying concentration, and the diffusion characteristics of hydrogen peroxide in the field should be investigated in the future.	
12) References	➢ Shizuoka Prefectural Fisheries Experimental Station (1992): Removal effect of *Gymnodinium mikimotoi* with hydrogen peroxide red-tide removal agent, Annual Report of Shizuoka Prefectural Fisheries Experimental Station Year 1991, pp.300-302.	

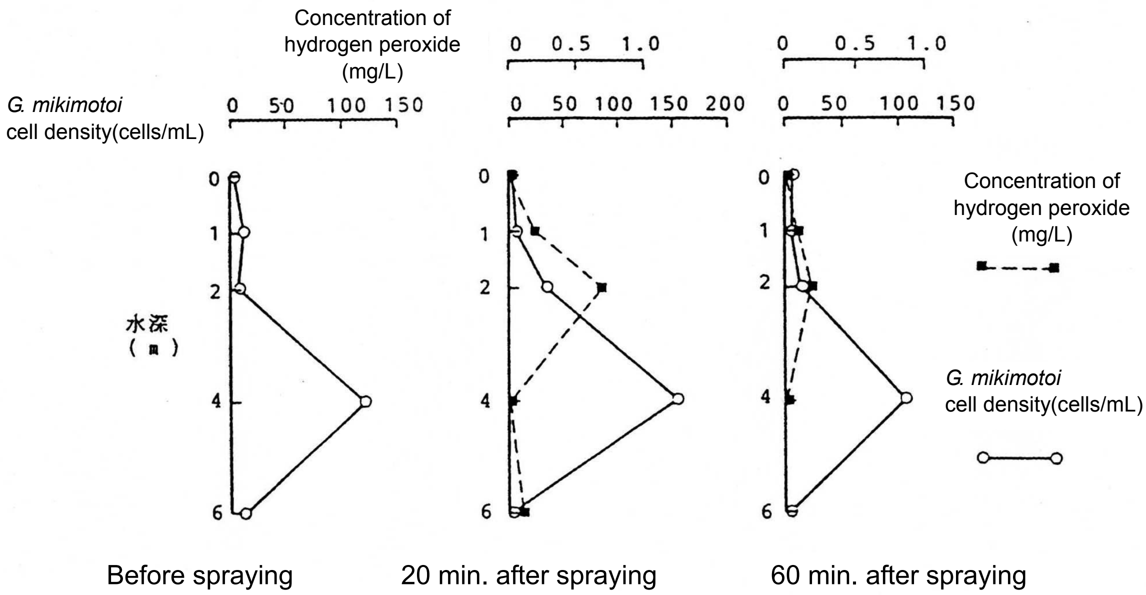


Fig-1　Fluctuation of hydrogen peroxide concentration and *G. mikimotoi* cell density

No. : J-C-4

1) Title	Extermination Efficacy of Hydrogen Peroxide against Cysts of Red Tide and Toxic Dinoflagellates, and Its Adaptability to Ballast Water	
2) Category	Chemical control	
3) Implementing organization	Seiichi Ichikawa, Yoshiharu Wakao, Yasuwo Fukuyo	
4) Target species	Class	Genus and Species
	Dinophyceae	*Polykrikos schwartzi* Cysts, *Alexandrium catenella or A. tamarense* Cysts
5) Implemented period	1992	
6) Experiment type	Lab experiment	
7) Application	Applicable to Inside ballast tank	
8) Method / mechanism	➢ The germination ability of *Polykrikos schwartzii*, *Alexandrium catenella* and *A. tamarense* cysts were examined when exposed to hydrogen peroxide solution. The cysts were collected from the seafloor. ➢ The test conditions were set for each species as follows: The initial exposure concentrations of hydrogen peroxide: *P. schwartzi* (10, 100, 1000 ppm), *A. catenella* (10, 30, 50, 100, 200 ppm), *A. tamarense* (10, 30, 50 ppm) Exposure time: *P. schwartzi* (3, 24, 48 hrs.), *A. catenella* (48 hrs.), *A. tamarense* (48 hrs.) ➢ The exposed cysts were rinsed 3 times with filtered seawater, then incubated individually for 10 days in sterile filtered seawater under the following conditions: water temperature of 22 °C, 3,000 lux and 12h light 12h dark photo-cycle. For *A. tamarense*, incubation was conducted for 56 days. The germination status of the incubated cysts was observed with an inverted microscope.	
9) Results	➢ The germination ability of the cysts were lost when under the following hydrogen peroxide exposure conditions: *P. schwartzi*: 24 hrs. exposure at 100 ppm (Table-1), *A. catenella*: 48 hrs. exposure at 50 ppm (Table-2), *A. tamarense*: no germination observed for all exposure conditions	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ The decomposition rate of hydrogen peroxide in non-filtered seawater was examined. The detection limit was reached inside 4 days at the initial concentration of 10 ppm, inside 10 days at 30 ppm and inside 30 days at 100 ppm.	
11) Others	➢ Corrosion of iron plate in hydrogen peroxide seawater solution was examined. The weight reduction of the test iron plate was similar to the control iron plate (no hydrogen peroxide added). Therefore, hydrogen peroxide is considered to have no corrosion effects on ballast tanks.	

12) References	➢ Seiichi Ichikawa, Yoshiharu Wakao, Yasuwo Fukuyo (1992): Extermination Efficacy of Hydrogen Peroxide against Cysts of Red Tide and Toxic Dinoflagellates, and Its Adaptability to Ballast Water, Nippon Suisan Gakkaishi, Vol.58 (12), 2229-2233.(in Japanese)

Table-1 Effect of hydrogen peroxide on germination of *Polykrikos schwartzii* cysts

H_2O_2 (mg/l)	Exposed time (h)	Number of incubated cysts	Cumulative number of germinated cysts (germination rate; %)					
			Incubation days					
			0	1	2	3	4	10
0	3	11	0	1(9.1)	3(27.3)	3(27.3)	3(27.3)	3(27.3)
	24	27	5(18.5)	7(25.9)	7(25.9)	7(27.9)	7(27.9)	7(27.9)
	48	27	12(44.4)	12(44.4)	12(44.4)	12(44.4)	12(44.4)	12(44.4)
10	3	11	0	0	0	0	1(9.1)	1(9.1)
	24	13	1(7.7)	1(7.7)	1(7.7)	1(7.7)	1(7.7)	1(7.7)
	48	13	3(23.1)	3(23.1)	3(23.1)	3(23.1)	3(23.1)	3(23.1)
100	3	12	0	1(8.3)	1(8.3)	3(25.0)	4(33.3)	4(33.3)
	24	13	0	0	0	0	0	0
	48	13	0	0	0	0	0	0
1,000	3	12	0	0	0	0	0	0
	24	12	0	0	0	0	0	0
	48	13	0	0	0	0	0	0

Source : Ichikawa et al (1992)

Table-2 Effect of hydrogen peroxide on germination of *Alexandrium catenella* cysts

H_2O_2 (mg/l)	Number of incubated cysts	Cumulative number of germinated cysts (germination rate; %)				
		Incubation days				
		0	1	2	3	10
0	40	13(32.5)	25(62.5)	31(77.5)	33(82.5)	33(82.5)
10	13	1(7.7)	1(7.7)	1(7.7)	1(7.7)	1(7.7)
30	15	0	0	0	0	0
50	13	0	0	0	0	0
100	13	0	0	0	0	0
200	12	0	0	0	0	0

Source : Ichikawa et al (1992)

No. : J-C-5

<table>
<tr><td>1) Title</td><td colspan="2">Development of damage prevention measures against Chattonella red tides</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Kagoshima Prefectural Fisheries Experimental Station ,Japan(now Kagoshima Prefectural Fisheries Technology and Development Center, 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>Cochlodinium sp. type-’78 (= Cochlodinium polykrikoides)</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella antiqua , Chattonella marina</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1990-1993 (published year: 1991-1994)</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab and field experiment (Kagoshima Bay)</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Cultured Chattonella antiqua and C. marina were exposed to hydrogen peroxide to examine its efficacy as a removal agent. Cultured Japanese amberjack (2-3 individuals) were also simultaneously exposed, to examine any negative effects of hydrogen peroxide on fish.
➢ The removal effect of hydrogen peroxide and acrinol were examined by exposing these agents to Cochlodinium sp. type-’78 (isolated from Yachiyo Sea).
➢ The exposure concentrations of hydrogen peroxide and acrinol were between 1-50 ppm and 1-30 ppm, respectively.
➢ An experimental hydrogen peroxide spraying device was tested in the field for practical application. The field test was conducted by installing a fish cage in the inner area of Kagoshima Bay.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ The effective removal concentrations of hydrogen peroxide against C. antiqua and C. marina were estimated to be above 10 ppm.
➢ The cell morphology of Cochlodinium sp. type-’78 changed when the hydrogen peroxide concentration was 30 ppm. In the field, the concentration of Cochlodinium sp. type-’78 in the surface layer (0 m) decreased 2 minutes after spraying hydrogen peroxide.
➢ The 100% lethal concentration (1 min.) of acrinol against Cochlodinium sp. type-’78 was estimated at 4 ppm. In the field, the concentration of Cochlodinium sp. type-’78 between 0-5 m depth decreased 4 minutes after spraying acrinol.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and other marine species
➢ During the experiment, no mortality of Japanese amberjacks were recorded when the concentration of hydrogen peroxide and acrinol were both 30 ppm. According to other studies, the 50% lethal concentration of hydrogen peroxide is 70-80 ppm for juvenile red seabream, 14.0 ppm for post-larva tiger prawn, 1.1 ppm for Artemia larva and 3.3 ppm for Daphnia sp. This shows that hydrogen peroxide is more toxic towards invertebrates.

(2) Impact on the environment
➢ No description</td></tr>
</table>

11) Others	➢ Field experiments showed that, when hydrogen peroxide spraying device was equipped with organic absorbent and super absorbent polymer, it was possible to maintain hydrogen peroxide concentration of 30-40 ppm in the water column, even 60 minutes after operation.
12) References	➢ Kagoshima Prefectural Fisheries Experimental Station (1991): Report on the Development of Red-tide Countermeasures Year 1990, Development of damage prevention measures against *Chattonella* red tides, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1992): Report on the Development of Red-tide Countermeasures Year 1991, Development of damage prevention measures against *Chattonella* red tides, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1994): Report on the Development of Red-tide Countermeasures Year 1993, Development of damage prevention measures against *Chattonella* red tides, Fisheries Agency. ➢ Hisashi Murata, Tadashi Sakai, Makoto Endo, Kyoshi Ymauchi, Shokou Matsumoto, and Akira Kuroki (1991): An attempt on save yellowtail from Chattonella antiqua red tide kill Using Hydrogen Peroxide, Suisanzoshoku, Vol. 39(2), 189-193.

Hydrogen radicals:

No. : J-C-6

1) Title	Development of red-tide killing and growth inhibition methods using hydroxide ion releasing material	
2) Category	Chemical Control (also includes Physical control)	
3) Implementing organization	Marin-Forum 21	
4) Target species	**Class**	**Genus and Species**
	Dinophyceae	*Gymnodinium mikimotoi* (= *Karenia mikimotoi*)
	Raphidophyceae	*Chattonella marina, Heterosigma akashiwo*
	Bacillariophyceae	*Skeletonema costatum*
5) Implemented period	2003	
6) Experiment type	Lab experiment	
7) Application	Aquaculture farms	
8) Method / mechanism	➢ The algicidal / removal effect of a hydroxide ion releasing material against red-tide plankton was examined. Small and large-scale experiments were conducted. ➢ For the hydroxide ion releasing material, a product called Clear Water* was used. This product is mainly composed of magnesium hydroxide, which is produced by concentrating the trace minerals in seawater and removing the excess salts. ➢ The experiments were tested with 4 red-tide plankton species: *Gymnodinium mikimotoi, Chattonella marina, Heterosigma akashiwo* and *Skeletonema costatum.* ➢ Small scale experiment: culture medium of red-tide plankton and Clear Water (50-200 g/m^2) were added into a 50 mm (diameter) x 800 mm (length) acrylic pipe, and exposed for a certain period. Two types of Clear Water were used: powder and granule type. ➢ Large scale experiment: culture medium of red-tide plankton and Clear Water (50 g/m^2) were added into a 1000 mm (diameter) x 1200 mm (length) polyethylene tank, and exposed for a certain period. Powder type Clear Water was used. *Ube Material Industries, Ltd. (http://www.ubematerial.com/index2.html)	
9) Results	➢ With the acrylic pipe experiment, Clear Water successfully coagulated and removed the red-tide planktons. Powder type was more effective, which removed 64-99% of the red-tide planktons within 60 minutes, with Clear Water concentration of 200 g/m^2. ➢ The removal effects of Clear Water differed with the plankton species. Removal was most effective in order of *Gymnodinium mikimotoi*, *Chattonella marina* and *Heterosigma akashiwo*. ➢ Clear Water also successfully coagulated and removed the red-tide planktons with the tank experiment. Coagulation of the planktons were observed immediately after the addition of Clear Water and showed maximum effects after 5-6 minutes.	

10) Impact on the environment / ecosystem	(1) Impact on the ecosystem ➢ No description (2) Impact on the environment ➢ It was confirmed that the removed red-tide planktons and Clear Water did not have any adverse impacts on the bottom sediments.
11) Others	➢ When Clear Water was exposed to *Heterosigma akashiwo* cysts, it inhibited its germination. ➢ Understanding the coagulation and removal mechanism of Clear Water, and the impacts on marine species and the environment were raised as future issues.
12) References	➢ Marino-Forum 21 (2003): Report on the Development of Red-tide Countermeasures and Practical Application Experiments Year 2002, Fisheries Agency.

Ozone:

No. : J-C-7

<table>
<tr><td>1) Title</td><td colspan="2">Development of red-tide countermeasures using ozone</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Marino-Forum 21</td></tr>
<tr><td rowspan="3">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Prorocentrum minimum, P. triestinum, Gymnodinium mikimotoi (= Karenia mikimotoi)</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella marina, Heterosigma akashiwo</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2003</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">Fish cage</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Red-tide planktons were exposed to ozone, to examine its removal effects.
➢ Ozone was dissolved into seawater containing Prorocentrum minimum and P. triestinum, and the effects on these species were examined.
➢ Cultured red-tide plankton (Prorocentrum triestinum, Gymnodinium mikimotoi、Chattonella marina, Heterosigma akashiwo) were exposed to different ozone concentration solutions, and the effects on these species were observed with a microscope at regular intervals.
➢ Methods for field application were examined.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ The movement of red-tide planktons stopped, at ozone concentration below 0.1 ppm.
➢ The effects of ozone on red-tide planktons differed with the species.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish
➢ When ozone was exposed to several fish species, mortality was observed when the ozone concentration was above 1 ppm.
(2) Impact on the environment
➢ Impacts of ozone on zooplanktons (Paracalanus parvus and Artemia salina) were observed when the ozone concentration was above 1 ppm.</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ Practical application of the ozone method was considered for use in a fish cage. If a 20 g/h ozone generation system is used, the approximate cost was estimated at 6 million yen. The running cost of this system was considered to be low.
➢ To compare with ozone, the effects of copper sulfate and hydrogen peroxide on red-tide planktons were examined. Both chemicals required high concentration (> 100 mg / L), before having any impacts on red-tide planktons.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Marino-Forum 21 (2003): Report on the Development of Red-tide Countermeasures and Practical Application Experiments Year 2002, Fisheries Agency.</td></tr>
</table>

Cupper sulfate:

No. : J-C-8

1) Title	Red-tide removal effects of calcium nitrate $Ca(NO_3)_2$ and copper (II) sulfate $CuSO_4$	
2) Category	Chemical control	
3) Implementing organization	Sugawara, K. and Sato, M. (Chiba Prefectural Fisheries Research Institute, now Chiba Prefectural Fisheries Research Center)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Gymnodinium splendens*? (= *Akashiwo sanguinea*?)
5) Implemented period	1966	
6) Experiment type	Lab and field experiment (Tokyo Bay: offshore of the mouth of Miyako River, Chiba Prefecture)	
7) Application	No description	
8) Method / mechanism	➢ Calcium nitrate and copper (II) sulfate solutions of varying concentrations were added to *Gymnodinium splendens* containing seawater samples (500 ml), to examine their red-tide removal effects. Visual observations of the samples were conducted after 1 and 24 hours. ➢ Calcium nitrate solution (0.3-0.4 mg/L) was uniformly sprayed over an area of 5m^2 in a red-tide area, and its removal effects were visually observed.	
9) Results	➢ With the lab experiment, effects on *G. splendens* were observed from concentrations of approximately 0.1 g/L (1 drop of 5 mg/L solution over 1 cm^2) for calcium nitrate and 1mg/L (1 drop of 5000 mg/L solution over 2 cm^2) for copper (II) sulfate. ➢ After spraying calcium nitrate solution over a red-tide area, the red and brownish color of the sea gradually faded, and the sea became more transparent after 2-3 minutes.	
10) Impact on the environment / ecosystem	➢ No description (In Japan, copper (II) sulfate and calcium nitrate are regulated by laws)	
11) Others	➢ No description	
12) References	➢ Sugawara, K. & Sato, M. (1966): Red Tides of Tokyo Bay, Bulletin of the Japanese Society of Fisheries Oceanography, 9, 116-133.	

Disinfectants:

No. : J-C-9

<table>
<tr><td>1) Title</td><td colspan="2">Effects of acrinol on red-tide planktons</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Kagoshima Prefectural Fisheries Experimental Station, Japan(now Kagoshima Prefectural Fisheries Technology and Development Center, Japan)</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Gymnodinium sp. Type' 84K (=Gymnodinium pulchellum)</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella antiqua, C. marina</td></tr>
<tr><td>Bacillariophyceae</td><td>Cylindrotheca closterium</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1986-1988 (Published year: 1987-1989)</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Cultured Gymnodinium sp. Type' 84K, Chattonella antiqua, C. marina and Cylindrotheca closterium were exposed to acrinol at concentrations between 0-30 ppm to examine the coagulation / sinking effect.
➢ Cultured C. marina and Cylindrotheca closterium were added into an acrinol / seawater mixture to examine the growth inhibition effect of acrinol.
➢ To examine the effect of C. marina and acrinol mixture on fish survival, three Japanese amberjacks (700-1,000 g) were released into a mixture of C. marina (4,000 cells/ml) and acrinol in a 500 L tank. Tests were conducted at four different acrinol concentrations (3-30 ppm) and one with no acrinol (control).

*Acrinol: an anti-infective agent</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ At acrinol concentrations above 3 ppm, coagulation / sinking and cell lysis of C. marina and Chattonella antique were observed. Growth inhibition was also observed at acrinol concentrations between 1-3 ppm.
➢ Coagulation / sinking and cell lysis were not observed for Gymnodinium sp. Type' 84K at all concentrations. All Cylindrotheca closterium cells died when acrinol concentrations were above 5 ppm.
➢ According to the growth inhibition test of C. marina and Cylindrotheca closterium with acrinol / seawater mixture, the growth of C. marina was inhibited with increasing acrinol concentration. A similar trend was observed for Cylindrotheca closterium but was not as distinct as C. marina.
➢ Japanese amberjack in the control tank showed erratic movements after 35 minutes, and all died after 102 minutes. The cell concentration of C. marina did not change during the experiment. At acrinol concentration of 3 and 5 ppm, half of the C. marina cells ceased swimming and two out of three Japanese amberjack died. At acrinol concentration of 10 and 30 ppm, most of the C. marina cells were destroyed, while all three Japanese amberjack survived.</td></tr>
</table>

10) Impact on the environment / ecosystem	(1) Impact on fish ➢ Two fish species (minnow and yellowtail) were exposed to acrinol to examine fish toxicity. The 24-hr LC50 value for minnow ranged between 15-20 ppm. No deaths of yellowtail were recorded within 48 hrs at acrinol concentrations of 8 and 40 ppm. (2) Impact on environment ➢ Under natural light conditions, most of the acrinol decomposed after 2 hrs. ➢ Montmorillonite and agar culture did not show any adsorption of acrinol. ➢ The distribution / diffusion pattern of acrinol in seawater was surveyed by spraying 64 L of 1% acrinol solution. The acrinol solution mainly diffused along the surface layer, and mostly did not reach to 1 m depth.
11) Others	No description
12) References	➢ Kagoshima Prefectural Fisheries Experimental Station (1987): Report on the development of red-tide countermeasures Year 1986, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1988): Report on the development of red-tide countermeasures Year 1987, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1989): Report on the development of red-tide countermeasures Year 1988, Fisheries Agency. ➢ Muhammad, S.H., Nozawa, K., Onoue, Y., Matsumoto, S. & Aramaki, T. (1991), Control of Red-Tide Organisms, Especially the Genus *Chattonella* by Chemical Acrinol, Aquaculture Science, Vol.39 (2), 141-145.

Biological secretion:

No. : J-C-10

1) Title	Algicidal Effect of Autolysate of Jellyfish *Aurelia aurita* on New Type Red Tide Flagellate *Heterocapsa circularisquama*	
2) Category	Chemical control	
3) Implementing organization	Shinya Handa, Juro Hiromi, and Naoyuki Uchida (Nihon University, Japan)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Heterocapsa circularisquama*
5) Implemented period	1998	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ Cultured *Heterocapsa circularisquama* was exposed to autolysate of jellyfish (*Aurelia aurita*), to examine its algicidal effect. ➢ The autolysate was extracted from three *Aurelia aurita* individuals (average wet-weight: 300g/individual) by allowing it to autolyse at 20 °C or 23 °C for 24 hrs. The autolysate was then sterilized through either autoclaving or filter sterilization. ➢ The autolysate was then added to a culture medium to produce 5% (v/v) culture mediums. *Heterocapsa circularisquama* was then inoculated into the autolysate culture medium, at initial cell concentrations of 300, 1500 and 3,000 cells/ml. The cell concentration of each sample was counted 24, 48 and 72 hrs after inoculation. ➢ The tests were conducted at water temperature 23±1 °C, illumination 96-119 μE/m^2/sec and 12h light 12h dark photo-cycle. ➢ Additional tests were conducted to examine the effect of *Aurelia aurita* autolysate on pearl oyster and short-neck clam.	
9) Results	➢ Algicidal effects of *Aurelia aurita* autolysate on *Heterocapsa circularisquama* were observed for both autoclave and filter sterilized medium. However, the algicidal effect of the filter-sterilized medium was twice as effective compared to the autoclaved medium (Fig. 1).	
10) Impact on the environment / ecosystem	(1) Impact on shellfish ➢ No significant impact of *Aurelia aurita* autolysate (concentration: 5% v/v) was observed on pearl oyster and short-necked clam. (2) Impact on environment ➢ No description	
11) Others	➢ The impact of *Aurelia aurita* autolysate on other organisms and algae species should be investigated.	
12) References	➢ Shinya Handa, Juro Hiromi, and Naoyuki Uchida (1998): Algicidal effect of Autolysate of Jellyfish *Aurelia aurita* on New Type Red Tide Flagellate *Heterocapsa circularisquama*, Nippon Suisan Gakkaishi, Vol.64(1), 123-124.(in Japanese)	

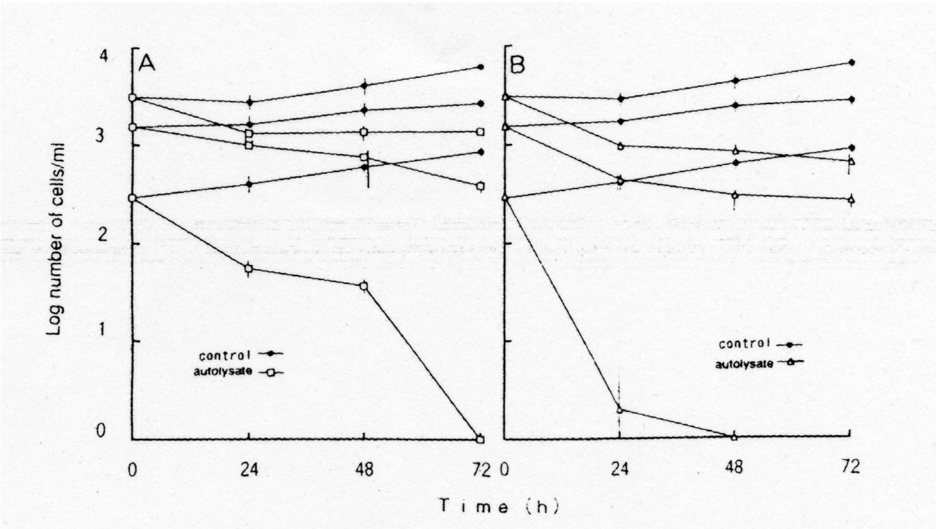


Figure-1　Algicidal effect of autolysate (concentration = 5% v/v) of jellyfish, *Aurelia aurita*, on the growth of *Heterocapsa circularisquama*. (A) Autolysate sterilized with autoclave, (B) Autolysate sterilized with Millex-GS filter. Vertical bars mean the maximum and minimum values among the triplicates.

Source：　Handa et al (1998)

No. : J-C-11

<table>
<tr><td>1) Title</td><td colspan="2">Algicidal effect of phlorotannins from the brown alga Ecklonia kurome on red tide microalgae</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Koki Nagayama, Toshiyuki Shibata, Ken Fujimoto, Tuneo Honjo, and Takashi Nakamura (Kumamoto Prefectual Fisheries Research Center, Japan etc.)</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, Cochlodinium polykrikoides</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella antiqua</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2003</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Algicidal effects of phlorotannins* from the brown alga Ecklonia kurome on three species of red tide microalgae (Karenia mikimotoi, Cochlodinium polykrikoides and Chattonella antiqua) were examined.
➢ Red tide microalgae used in the experiment were collected from the surface of the Ariake Sea and Shiranui Sea. They were cultured in modified SWM-3 medium.
➢ The crude phlorotannin used in the experiment was extracted from E. kurome, collected from Tuuji Island in Kumamoto Prefecture.
➢ Crude phlorotannins was dissolved in 70% methanol, and aliquots in the 25µL solutions were added to 20mL of microalgal suspensions in test tubes. The cells were then cultured. Cell numbers were counted with a microscope after 0.1, 0.5, 1, 2, 3, and 24 hrs.

*: Phlorotannin is a type of polyphenol contained in seaweeds.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Swimming cell density of K. mikimotoi and C. polykrikoides decreased to less than 2% of the initial density within 30 min in medium containing 150mg/L crude phlorotannins.
➢ After losing their motility, almost all of the cells became round. They then expanded and burst. Once cells had become round, none of them recovered to normal vegetative cells within 24 h even if they were transplanted to normal medium.
➢ No change was observed on C. antiqua in the 500mg/L medium within 3 h after inoculation. However, by 24 h, over 99% of the cells were destroyed even in the 100mg/L medium.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ Acute toxicity of 200mg/L phlorotannins on red sea bream (ca. 13g), tiger puffer (ca. 102g) and blue crab (ca. 2mm) were investigated. No mortality was observed among them.
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ Among the five phlorotannins isolated from E. kurome, phlorofucofuroeckol A, a pentamer of phloroglucinol, had the strongest algicidal activity, which was comparable to that of epogallocatechin gallatet.</td></tr>
</table>

12) References	➢ Koki Nagayama, Toshiyuki Shibata, Ken Fujimoto, Tuneo Honjo and Takashi Nakamura (2003): Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae, Aquaculture, Vol. 218, 601-611.

No. : J-C-12

<table>
<tr><td>1) Title</td><td colspan="2">The effectiveness of Ulva fasciata and U. pertusa (Ulvales, Chlorophyta) as algicidal substances on harmful algal bloom species</td></tr>
<tr><td>2) Category</td><td colspan="2">Chemical control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Mochammad Amin Alamsjah, Fumito Ishimashi, Hitoshi Kitamura, and Yuji Fujita (Nagasaki Univ., 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>Alexandrium catenella, Karenia mikimotoi</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella marina, Fibrocapsa japonica, Heterosigma akashiwo</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2006</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ The algicidal activity of fresh tissue, dry powder and methanol extracts of Ulva fasciata and U. pertusa were evaluated against HABs species (Alexandrium catenella, Karenia mikimotoi, Chattonella marina, Fibrocapsa japonica, Heterosigma akashiwo).
➢ HABs species were obtained from the National Institute for Environmental Studies, Japan.
➢ Ulva fasciata and U. pertusa were collected from the coastal area of Nagasaki City, Japan.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ The sporophyte of fresh tissue from U. fasciata and U. pertusa induced the growth inhibition and lethal effects on H. akashiwo and A. catenella higher than their gametophyte strains.
➢ The dry powder of sporophyte of U. fasciata and U. pertusa induced significantly high rate of reduced growth and cell death than gametophyte strains on H. akashiwo species. On the contrary. The dry powder of Ulva spp. had low effect on A. catenella species.
➢ The methanol extracts of sporophyte of U. fasciata and U. pertusa showed higher algicidal effects than their gametophyte strains on HABs. These assays were most effective against the cells of C. marina, H. akashiwo, and were moderately effective against F. japonica and K. mikimotoi cells.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ No description</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Mochammad Amin Alamsjah, Fumito Ishimashi, Hitoshi Kitamura, and Yuji Fujita (2006): The effectiveness of Ulva fasciata and U. pertusa (Ulvales, Chlorophyta) as algicidal substances on harmful algal bloom species, Aquaculture Science, Vol. 54(3), 325-334.</td></tr>
</table>

Other chemicals:

No. : J-C-13

1) Title	Effects of fatty acids on *Chattonella marina*	
2) Category	Chemical control	
3) Implementing organization	Kagoshima Prefectural Fisheries Experimental Station, Japan(now Kagoshima Prefectural Fisheries Technology and Development Center)	
4) Target species	Class	Genus and Species
	Raphidophyceae	*Chattonella marina*
5) Implemented period	1986-1989 (published year: 1987-1989)	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ Cultured *Chattonella marina* was exposed to two types of fatty acid solutions (S-100 and S-200)* for 10 minutes, to examine their coagulation effects. The solutions are mainly composed of arachidonic acid and eicosapetaenoic acid (EPA). The exposure concentrations were set at 10 levels between 0.1-250 ppm. ➢ Cultured *C. marina* was exposed to 2 types of saturated fatty acid solutions and 2 types of polyunsaturated fatty acid (PUFA) solutions at concentrations between 0.25-25 ppm, to examine their coagulation effects. The cell concentrations were counted 1 and 30 minutes after exposure. Saturated fatty acid type: palmitic acid (16:0, 93.6% purity) and EPA (90% purity) Polyunsaturated fatty acid (PUFA) type: PUFA methyl ester (PUFA purity 92.7%) and sardine oil mixed fatty acid (PUFA purity 29.5%) ➢ Cultured *C. marina* was exposed to 3 types of fatty acid containing solutions (10%FL, 50%EC and NK ekoro)*, to examine their coagulation and cell lysis effects. The cell concentration of *C. marina* was measured 10 minutes after exposure. The exposure concentrations were set at 7 levels between 0.5-32 ppm. *: product of MIYOSHI OIL & FAT CO., LTD. （http://www.miyoshi-yushi.co.jp/）	
9) Results	➢ The rate of swimming cessation and cell lysis increased with increasing concentration of fatty acid S-100. Swimming cessation was mainly observed between 10-100 ppm. The cell lysis rate became high above 150 ppm, and at 250 ppm all cells showed cell lysis. ➢ Similar to S-100, the rate of swimming cessation and cell lysis increased with increasing concentration of fatty acid S-200. However, cell lysis of all cells was observed at a higher concentration of 500 ppm. ➢ Neither swimming cessation or cell lysis was observed with palmitic acid (16:0) and PUFA methyl ester. High rate of swimming cessation was observed when sardine oil mixed fatty acid concentrations were 2.5 ppm and 25 ppm. Both swimming cessation and cell lysis were observed when EPA concentrations were 2.5 ppm and 25 ppm. Cell lysis of all cells was observed after 30 minutes at 25 ppm. ➢ The rate of swimming cessation and cell lysis of *C. marina* increased with increasing concentration of 10%FL, 50%EC and NK ekoro solutions. The rate of cell lysis at 32 ppm was below 5% for 10%FL and 82% for 50%EC, and no cell lysis occured with NK ekoro.	

10) Impact on the environment / ecosystem	(1) Impact on fish ➢ The 50% lethal concentration (24h LC50) of fatty acid S-200 was estimated for Japanese amberjack (length 35.7-38.8 cm) and red seabream (length 29.0-31.8 cm). The LC50 for Japanese amberjack was 60 ppm, and 320 ppm for red seabream. ➢ The LC50 of 10%FL, 50%EC and NK ekoro for Japanese amberjack (wet weight 62.5-137.3 g) were approximately 10.5 ppm (20h), 5.8 ppm (6h) and 19.0 ppm (9h), respectively. (2) Impact on the environment ➢ S-100 and S-200 were decomposed by aeration, and the decomposition rates were measured. Although the fat composition was reduced to approximately 50% after 24 hours, the decomposition rate slowed down from then onwards and 30% remained even after 96 hours.
11) Others	➢ S-200 was highly toxic to fish, which was assumed to be caused by the emulsifier in S-200. Therefore, S-200 was considered to be impractical for red-tide removal. ➢ In respect to PUFA, singular free fatty acids (EPA) were more effective towards *C. marina* than mixed free fatty acids (sardine oil mixed fatty acid). No effects on *C. marina* were observed with saturated fatty acid (palmitic acid).
12) References	➢ Kagoshima Prefectural Fisheries Experimental Station (1987): Report on the development of red-tide countermeasures Year 1986, Fisheries Agency. ➢ Kagoshima Prefectural Fisheries Experimental Station (1988): Report on the development of red-tide countermeasures Year 1987, Fisheries Agency. ➢ Murata H., Sakai T., Endo M., Kuroki A., Kimura M. & Kumanda K. (1989): Screening of Removal Agents of a Red Tide Plankton *Chattonella marina*-with Special Reference to the Ability of the Free Radicals Derived from the Hydrogen Peroxide and Polyunsaturated Fatty Acids, Bulletin of the Japanese Society of Scientific Fisheries, 55(6), 1075-1082.

Biological control:
Algicida bacteria:

No. : J-B-1

1) Title	Isolation and Properties of a Bacterium Inhibiting the Growth of *Gymnodinium nagasakiense*	
2) Category	Biological control	
3) Implementing organization	Kimio Fukami, Atsushi Yuzawa, Toshitaka Nishijima and Yoshihiko Hata (Kochi University, Japan)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Gymnodinium nagasakiense* (=*Karenia mikimotoi*)
5) Implemented period	1992	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ A bacterium (5N-3) possessing a remarkable inhibitory effect on the growth of *Gymnodinium nagasakiense* was isolated from Uranouchi Inlet, Kochi in October 1989. ➢ The algicidal effects of this bacterium were examined by inoculation of the bacterium into *G. nagasakiense* cultures and three other red tide phytoplankton species cultures (*Heterosigma akashiwo*, *Chattonella antiqua*, and *Skeletonema costatum*). The initial density of the bacteria was 1 x 10^6 cells/mL. ➢ It was also checked whether the filtrate of the bacterium culture had an inhibitory effect on *G. nagasakiense* or not. The initial density of *G. nagasakiense* was 5 x 10^2 cells/mL.	
9) Results	➢ A bacterium 5N-3 was tentatively identified as *Flavobacterium* sp. by the taxonomical characteristics. ➢ The growth inhibiting effects of 5N-3 on *G. nagasakiense* was drastic in particular when the alga was in logarithmic growth phase, and cell density decreased to less than1% of the initial concentration with in 4 days after inoculating 5N-3 (Fig. 1, Fig. 2). ➢ The inhibitory effect of 5N-3 on the growth of *G. nagasakiense* was detected in the filtrate of the bacterium. Especially, the growth of *G. nagasakiense* was completely suppressed by 30mL or more of bacterial culture fluid (Fig. 3). ➢ On the other hand, the algicidal effect of 5N-3 was only observed on *G. nagasakiense* but not on other red tide phytoplankton species (Fig. 4).	
10) Impact on the environment / ecosystem	➢ No description	

11) Others	➢ These results suggest that the effect of 5N-3 was *G. nagasakiense* specific, and the effective algicidal activity of 5N-3 was obtained when its cell density was more than 10^6 cells/mL. ➢ The growth-inhibiting effect of 5N-3 could be due to some chemical materials released from the bacterial cells. ➢ 5N-3 grew very rapidly in the mixed culture with any phytoplankton of four species. These results suggest that it is possible to expect that 5N-3 grows and increases cell density to a significant level in the field by using natural organic carbon from phytoplankton.
12) References	➢ Fukami, K., Yuzawa, A., Nishijima, T. and Hara, Y. (1992): Isolation and Properties of a Bacterium Inhibiting the Growth of *Gymnodinium nagasakiense*, Nippon Suisan Gakkaishi, Vol.58 (6), 1073-1077.

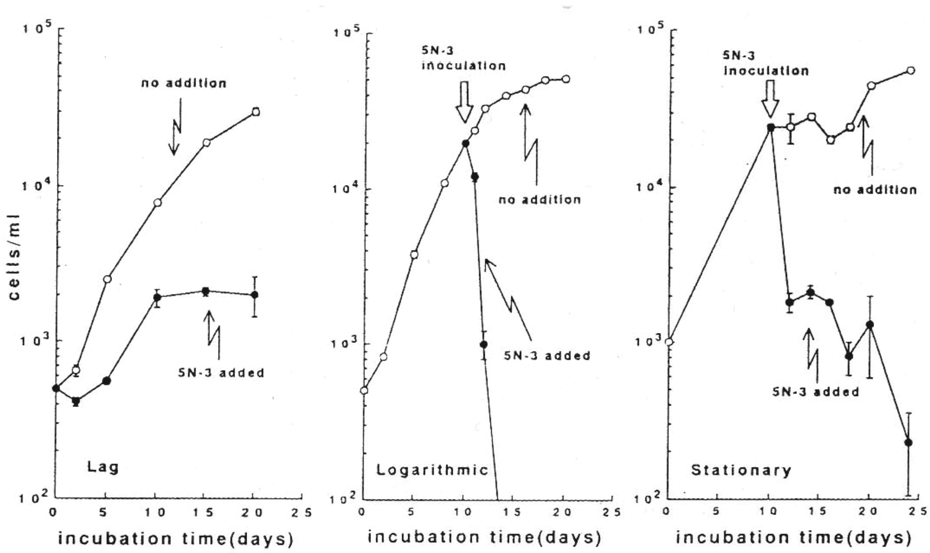


Figure-1 Effects of bacterium 5N-3 on the growth of *Gymnodinium nagasakiense* indifferent growth stages. Time of bacterial inoculation is indicated by the open arrow.

Source：Fukami et al (1992)

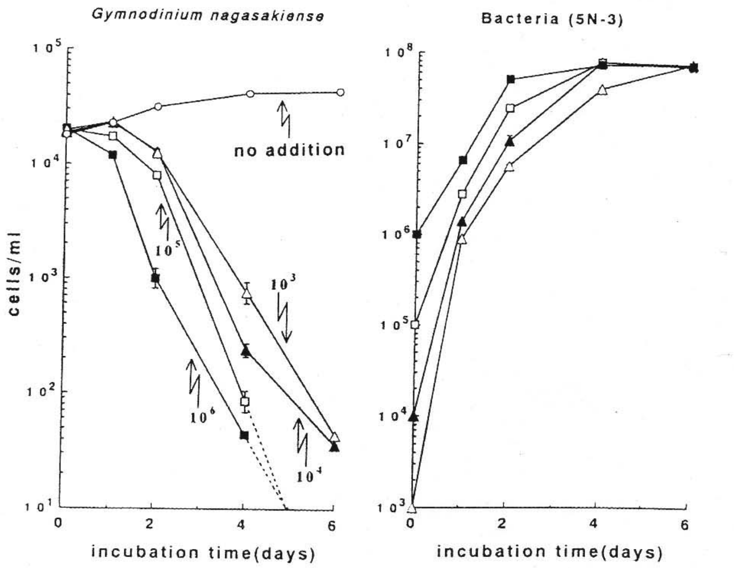


Figure-2　Changes in cell densities of *G. nagasakiense* and bacterium 5N-3 after inoculating of 5N-3 with different initial densities.

Source：Fukami et al (1992)

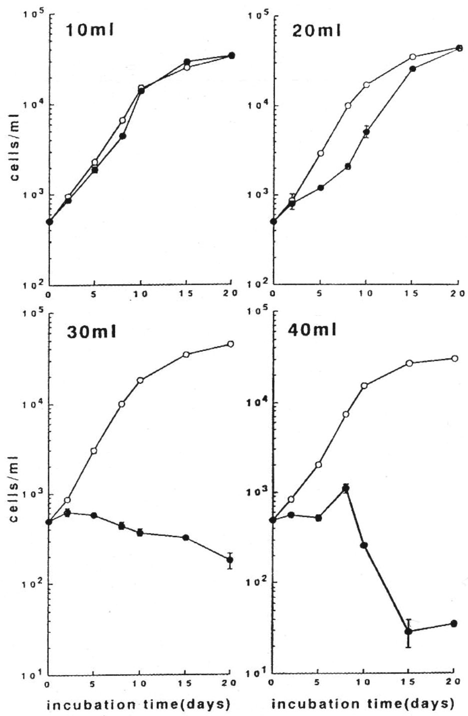


Figure-3　Effects of the culture filtrate of bacterium 5N-3 at different volumes in 80mL of incubation system on the growth of *G. nagasakiense*. ○:No addition; ●:culture filtrate added.

Source：Fukami et al (1992)

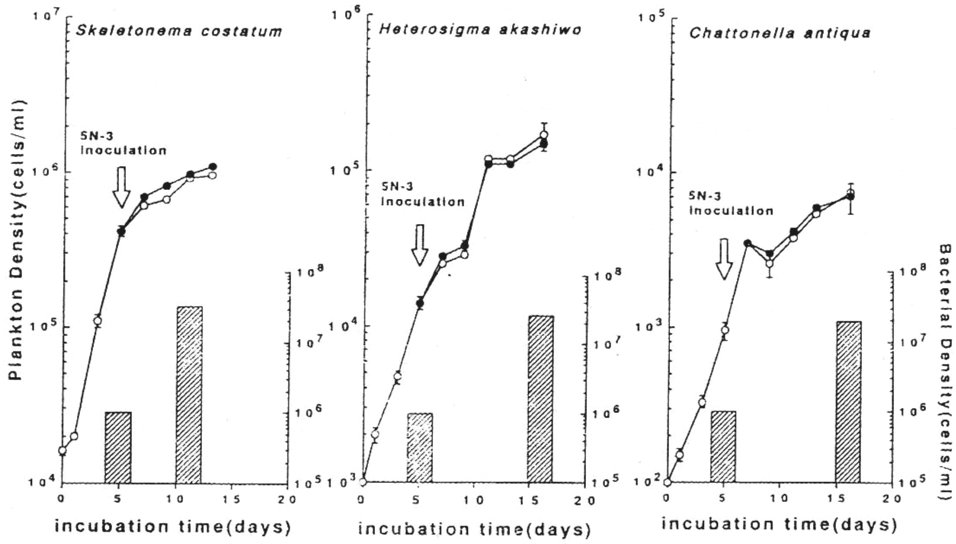


Figure-4　Effects of the bacterium 5N-3 on the growth of three phytoplankton species. The 5N-3 densities at the initiation and the end of experiments are also shown by shallow columns. ○:No addition; ●:culture filtrate added.

Source：Fukami et al (1992)

No. : J-B-2

<table>
<tr><td>1) Title</td><td colspan="2">The algicidal effects of *Alteromonas* sp. (6/6-46 strain) on *Gymnodinium mikimotoi*</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Mie Prefectural Fisheries Technology Center, Japan</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>*Gymnodinium mikimotoi* (=*Karenia mikimotoi*)</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1994</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ The algicidal effect of *Alteromonas* sp. (6/6-46 strain) on *Gymnodinium mikimotoi* was examined, when co-occurring with other bacteria species (*Pseudomonas, Moraxella,* and *Vibrio*).
➢ The *Alteromonas* sp. (6/6-46 strain) was isolated from Gokashyo Bay of Mie Prefecture.
➢ *Alteromonas* sp. (6/6-46 strain) was incubated with each bacteria species (*Pseudomonas, Moraxella,* and *Vibrio*), and *G. mikimotoi* was added on the forth day of incubation. During the test, the number of *G. mikimotoi* cells and bacteria were counted regularly.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ When the initial density of *Alteromonas* sp. (6/6-46 strain) and the other bacteria were 10^{3} cfu/mL, the growth of *G. mikimotoi* was not inhibited (Figure-1).
➢ However, when the initial density of *Alteromonas* sp. (6/6-46 strain) was set at 10^{5}～10^{6} cfu/mL, and the other bacteria at 10^{3} cfu/mL, the growth of *G. mikimotoi* was inhibited (Figure-1).</td></tr>
<tr><td>10) Impact on environment / ecosystem</td><td colspan="2">➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ In order for *Alteromonas* sp. (6/6-46 strain) to inhibit the growth of *G. mikimotoi*, the initial density must be between 10^{6}～10^{7} cfu/mL.
➢ When the initial density of *Alteromonas* sp. (6/6-46 strain) and the other bacteria were incubated at similar concentration, the growth of *Alteromonas* sp. (6/6-46 strain) appeared to be inhibited.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Mie Prefectural Fisheries Technology Center (1994): Development of Red Tide Countermeasures by Marine Biotechnology, Report of Mie Prefectural Fisheries Technology Center Year 1993, 95-99.</td></tr>
</table>

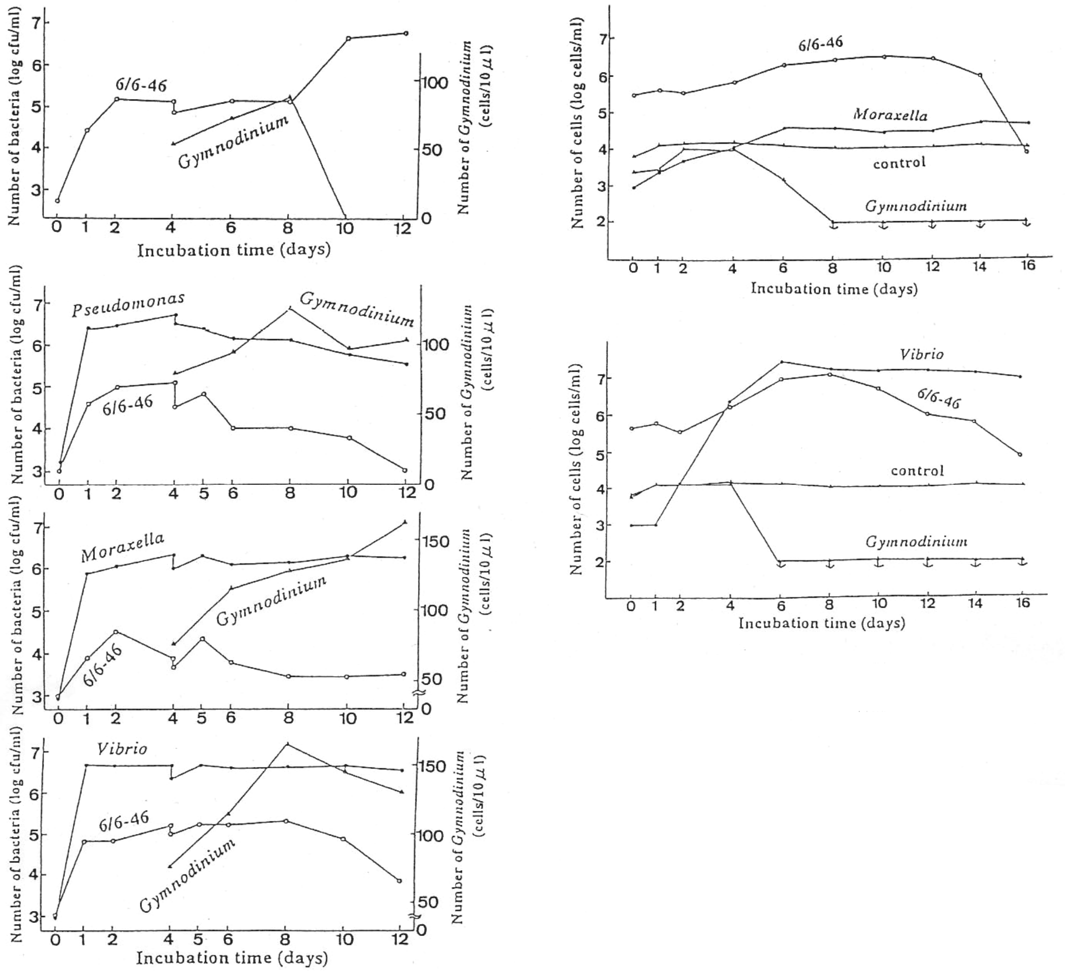


Figure-1 The algicidal effect of 6/6-46 strain on *G. mikimotoi*, when incubated with other bacteria species

Source：Mie Prefectural Fisheries Technology Center (1994)

No. : J-B-3

<table>
<tr><td>1) Title</td><td colspan="2">Analysis of Algicidal Ranges of the Bacteria killing the Marine Dinoflagellate Gymnodinium mikimotoi Isolated from Tanabe Bay, Wakayama pref., Japan</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Ikuo Yoshinaga (Kyoto University, Japan)</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Gymnodinium mikimotoi (=Karenia mikimotoi),
Alexandrium catenella</td></tr>
<tr><td>Raphidophyceae</td><td>Heterosigma akashiwo</td></tr>
<tr><td>Bacillariophyceae</td><td>Skeletonema costatum, Ditylum brightwellii,
Thalassiosira sp.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1997</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ 28 strains of the marine bacteria that killed Gymnodinium mikimotoi were isolated in Tanabe Bay, Japan in 1990.
➢ To study the algicidal ranges of Gm-GIB (Gymnodinium mikimotoi's-growth inhibiting bacteria), Gm-GIB were cultured with 6 species of marine phytoplankton (Gymnodinium mikimotoi, Alexandrium catenella, Heterosigma akashiwo, Skeletonema costatum, Ditylum brightwellii and Thalassiosira sp.). Each strain of Gm-GIB was inoculated at initial cell density of ca 10^3 cells/mL into the long-phase culture.
➢ The algal-bacterial co-cultures were incubated at 20 °C with the L:D cycle of 14:10 under 8000 lux.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Among the 28 strains, 22 strains belonged to the genus Vivrio, three to Flavocacterium, two to Acinetobacter and one to Pseudomonas-Alteromonas.
➢ Most of the killing bacteria did not affect the growth of three marine diatoms, S. costatum, D. brightwellii and Thalassiosira sp., and some of them did not affect the growth of the marine dinoflagellate, A. catenella (Figure-1).</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ These findings strongly suggest that the killing bacteria influence the change of microalgal community in a marine environment.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Ikuo Yoshinaga et al (1997): Analysis of Algicidal Ranges of the Bacteria killing the Marine Dinoflagellate Gymnodinium mikimotoi Isolated from Tanabe Bay, Wakayama pref., Japan, Fisheries Science, Vol. 63(1), 94-98.</td></tr>
</table>

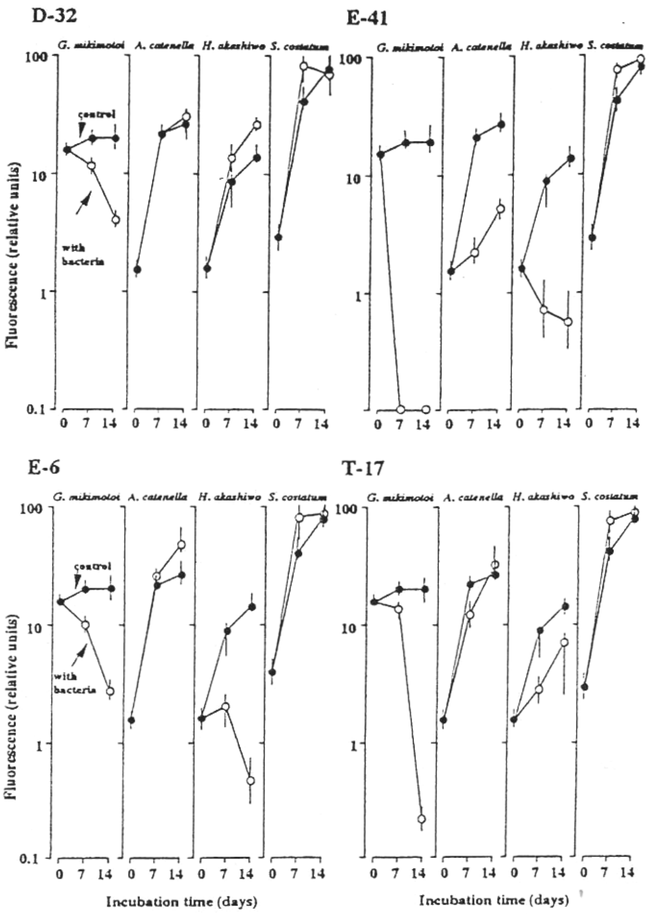


Figure-1 Effects of Gm-GIB(D32, E41, E6, and T17) against marine microalgae, *G. mikimotoi*, *A. catenella*, *H. akashowo* and *S. costatum*. Control: no addition of bacteria.

Notes: Each algal growth was expressed by autofluorescence of chlorophyll a. Error bars are shown.

Source: Yoshinaga et al (1997)

Table1 Algicidal ranges of Gm-GIB isolated in Tanabe Bay in 1990

Table 2. Algicidal ranges of Gm-GIB isolated in Tanabe Bay in 1990

Strain	Prey Microalgae				
	Alexandrium catenella	*Thalassiosira* sp.	*Ditylum brightwellii*	*Skeletonema costatum*	*Heterosigma akashiwo*
A47	+	+	−	−	++
B42	+	++	−	−	+
B46	+	+	−	−	+
C1	+	−	−	−	++
C4	+	++	−	−	++
C42	+	−	−	−	++
C49	+	−	−	−	++
T10	−	−	−	−	−
T16	−	−	−	−	−
T17	−	−	ND	−	+
T26	+	−	−	−	+
T27	+	−	−	−	++
D6	+	−	−	−	++
D26	−	−	−	−	+
D32	−	−	−	−	−
D35	−	−	−	−	−
E6	−	−	−	−	++
E26	−	−	−	−	++
E27	−	−	−	−	−
E40	−	−	−	−	++
E41	+	−	−	−	+
E45	+	−	−	−	ND
E46	−	−	−	−	++
F36	+	−	−	−	++
F37	−	−	−	−	++
G42	+	−	−	−	++
G62	++	−	−	−	++
G63	−	−	ND	ND	−

++: kill
+: inhibit
−: no effect
ND: not detected bacterial growth

Source: Yoshinaga et al (1997)

No. : J-B-4

1) Title	Distribution and Fluctuation of Algicidal Bacterium in the Decay Process of *Karenia mikimoti* in Cylindrical Culture Instrument	
2) Category	Biological control	
3) Implementing organization	Yuzo Iwata, Isao Sugahara, Hiroto Maeda, Toshio Kimura, Kentaro Noritake, and Hiroe Kowa (Mie university, Japan)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Karenia mikimotoi*
5) Implemented period	2006	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ Algicidal bacteria of *Karenia mikimotoi* were inoculated into a *K. mikimotoi* culture in a 1.5 L cylindrical culture instrument (Figure-1). The distribution and fluctuation of the algicidal bacteria were investigated for vertically and horizontally placed cylindrical culture. ➢ *Alteromonas* sp. and *Flavobacterium* sp. were used as the algicidal bacteria of *K. mikimotoi*, which were isolated from the coastal area of Mie Prefecture. ➢ The algicidal bacteria were inoculated into the *K. mikimotoi* culture at a concentration of approximately 10^8 cfu/mL, and cultured under the following conditions. Water temperature: 20 °C Illuminance: 45.3 - 74.4 μE/m^2/s Photoperiod: 14 hr light, 10 hr dark ➢ The culture medium was sampled regularly and the cell number and bacteria were counted.	
9) Results	➢ Both *Alteromonas* sp. and *Flavobacterium* sp. increased evenly throughout the cylindrical culture after inoculation and killed *K. mikimotoi*. ➢ After the inoculation of the algicidal bacteria, the cell concentration of *K. mikimotoi* was reduced to 1 % of the initial level after 18-108 hours. ➢ In the horizontal cylindrical culture with distinguished light and dark area, *K. mikimotoi* was mainly distributed in the light area. After inoculation of the algicidal bacteria in the light area, the bacteria diffused and increased throughout the cylindrical culture and killed *K. mikimotoi* in the process.	
10) Impact on environment / ecosystem	➢ No description	
11) Others	➢ Further experiments should be conducted to investigate the effectiveness of algicidal bacteria on large-scale red tides. ➢ Since algicidal bacteria rapidly diffuse, it is necessary to develop anti-diffusion methods for field application. ➢ It is necessary to investigate the safety aspects of the algicidal bacteria.	
12) References	➢ Iwata, Y. et al. (2006): Distribution and Fluctuation of Algicidal Bacterium in the Decay Process of *Karenia mikimoti* in Cylindrical Culture Instrument, Aquaculture Science, 54(1), 55-59.	

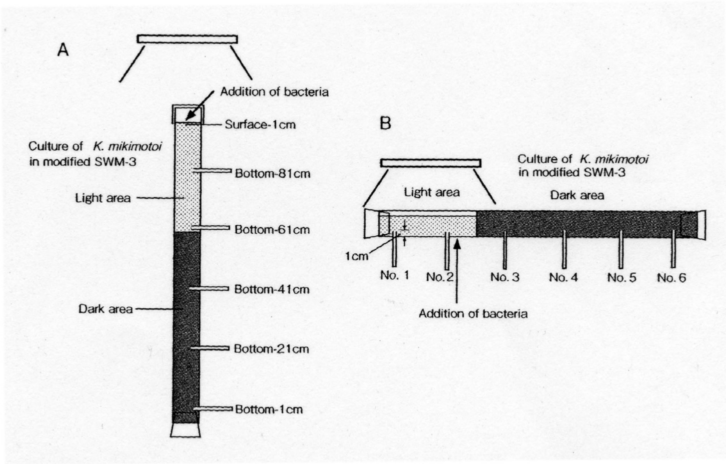


Fig.1　Schematic diagram of vertical cylindrical culture (A) and horizontal cylindrical culture (B) used in this experiment.

Source：Iwata et al (2006)

No. : J-B-5

<table>
<tr><td>1) Title</td><td colspan="2">Development of red-tide removal technologies using algicidal bacteria fixed carriers</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Marino-Forum 21</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Heterocapsa circularisquama, Gymnodinium mikimotoi(= Karenia mikimotoi)</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2003</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Algicidal bacterium was fixed onto different carrier materials, and their algicidal effects were examined.
➢ Algicidal bacteria EHK-1 strain fixed carriers were added into Heterocapsa circularisquama and Gymnodinium mikimotoi culture medium, to examine their algicidal effects.
➢ Three materials were selected as carriers of algicidal bacteria EHK-1 strain: calcium alginate, poly-vinyl alcohol and ceramic. The cell density of the fixed EHK-1 strain was 10^8 cells/g wet weight for calcium alginate and poly-vinyl alcohol, and approximately 7×10^8 cells/carrier for ceramic.
➢ Calcium alginate (1 g wet weight), poly-vinyl alcohol (1 g wet weight) and ceramic (0.6 g) carriers were added into a test tube containing 10 mL (cell density: 1000 cells/mL) of H. circularisquama and G. mikimotoi culture medium. Then the algicidal effects were monitored every 24 hours.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Within the selected carriers, algicidal effects on H. circularisquama and G. mikimotoi were confirmed with the ceramic carrier. The algicidal rate was calculated as 1000 cells/carrier/day.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on the ecosystem
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ The algicidal range in field application was calculated when using 40 L volume spraying devices containing ceramic carrier with 70 ml/carrier treatment capacity. The effective algicidal range for complete extermination was 6 m^2, and 600-6000 m^2 to achieve 20% reduction.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Marino-Forum 21 (2003): Report on the Development of Red-tide Countermeasures and Practical Application Experiments Year 2002, Fisheries Agency.</td></tr>
</table>

No. : J-B-6

<table>
<tr><td>1) Title</td><td colspan="2">Detection and isolation of micro-organisms that inhibit the growth of noxious red-tide dinoflagellate Heterocapsa circularisquama</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Imai, I., et al. (Kyoto University, Japan)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Heterocapsa circularisquama</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1996</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Algicidal bacteria of Heterocapsa circularisquama were collected from Ago Bay, Mie Prefecture. The algicidal bacteria were isolated using the Most Probable Number method (MPN method).
➢ The isolated algicidal bacteria (AA8-2 strain) were incubated with H. circularisquama to investigate its algicidal effects.
➢ The initial concentration of H. circularisquama and the algicidal bacteria were 2.2×10^4 cells/mL and $3.8 \sim 4.5 \times 10^3$ cells/mL, respectively.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ The algicidal bacteria (AA8-2 strain) inhibited the growth of H. circularisquama (Figure-1). However, when the cell density of H. circularisquama was low, the algicidal effect tended to be restricted.</td></tr>
<tr><td>10) Impact on environment / ecosystem</td><td colspan="2">➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ No description</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Imai, I., et al. (1996): Detection and isolation of micro-organisms that inhibit the growth of noxious red-tide dinoflagellate Heterocapsa circularisquama, Research Report on Bloom Mechanism and Prediction Methods of Dinoflagellate / Raphidophyceae Year 1995, 36-41, Fisheries Agency.</td></tr>
</table>

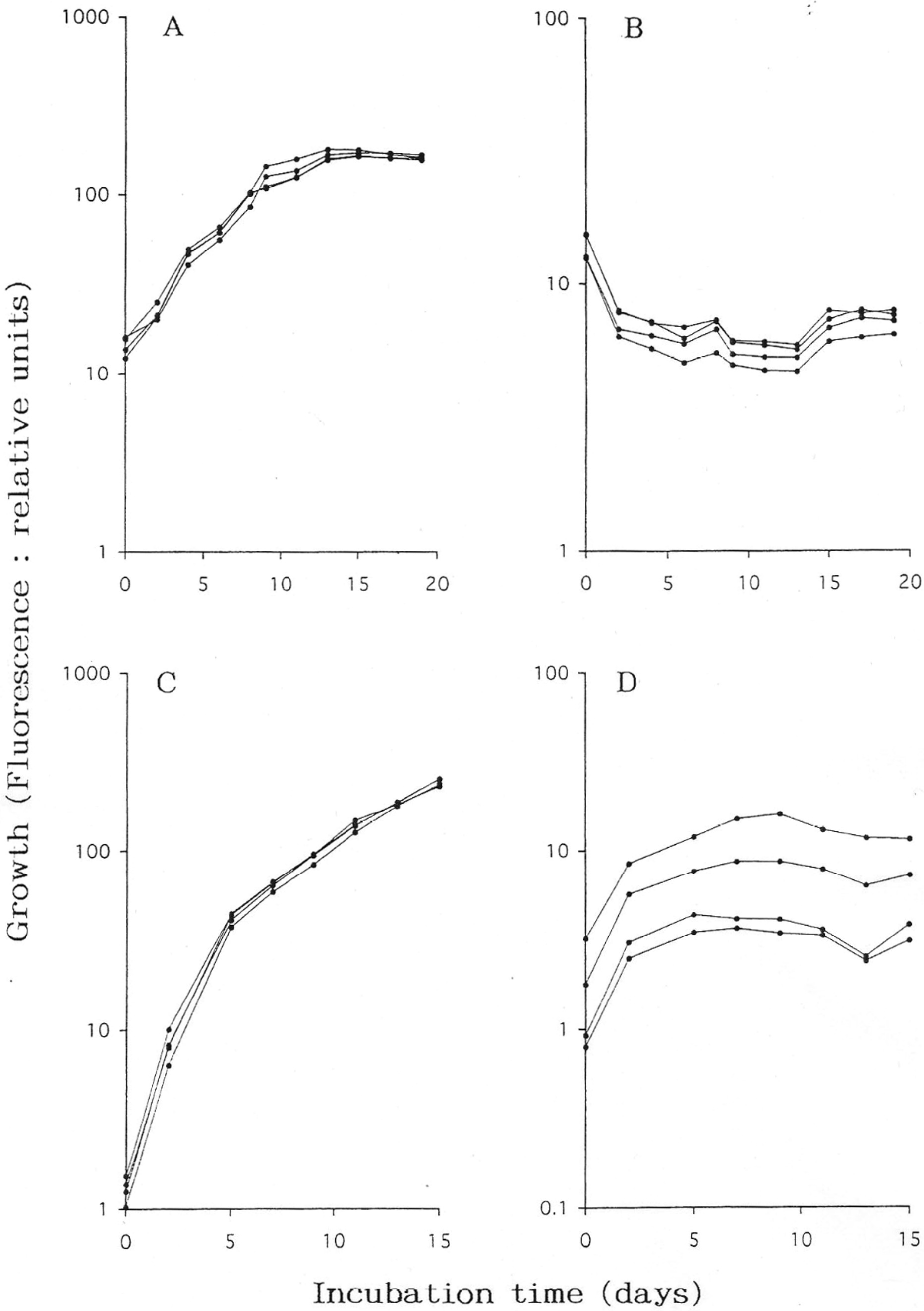


Figure-1 Fluctuation of *H. circularisquama* growth rate when cultured with algicidal bacteria (AA8-2 strain) of *H. circularisquama*

Note: A and C are control (no addition of algicidal bacteria (AA8-2 strain)). For B and D, algicidal bacteria (AA8-2 strain) were added at a concentration of 4.5×10^3 cells/mL and 3.8×10^3 cells/mL, respectively. The initial cell density of *H. circularisquama* was 2.2×10^4 cells/mL for A and B, which was higher than C and D (concentrations of C and D are unknown).

Source：Imai et al (1996)

No. : J-B-7

1) Title	Algicidal activity of a killer bacterium against the Harmful red tide dinoflagellate *Heterocapsa circularisquama* isolated from Ago Bay,Japan	
2) Category	Biological control	
3) Implementing organization	Keizo Nagasaki, Mineo Yamaguchi, and Ichiro Imai (National Research Institute of Fisheries and Environment of Inland Sea, Kyoto University, Japan)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Heterocapsa circularisquama*
5) Implemented period	2000	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ Algicidal activity of a bacterium strain *Cytophaga* sp. AA8-2 against the harmful red tide causing alga *Heterocapsa circularisquama* was investigated. ➢ The algicidal effects of *Cytophaga* sp. AA8-2 were examined against the following factors: the physiological conditions of the host cell, incubation temperature, presence of ambient organic substrate and co-existing bacteria. ➢ Seven different *H. circularisquama* strains were used for the experiment, with some strains containing intracellular bacteria.	
9) Results	➢ Bacterial lysis of *H. circularisquama* was more rapid at higher incubation temperature (20-30°C). ➢ Growth of 6 among 7 *H. circularisquama* strains tested was inhibited by *Cytophaga* sp. AA8-2, the levels of which were varied. ➢ Some *H. circularisquama* cells in a culture formed temporary cysts to survive the bacterial attack. The envelope of the temporary cyst of *H. circularisquama* was composed of a markedly thicker layered structure (209±72 nm) than that of the vegetative cell (40±15 nm).	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ Prior to practical application of algicidal bacteria for red-tide control, methods for containing the bacterial numbers and the impact on the environment must be considered.	
12) References	➢ Keizo Nagasaki, Mineo Yamaguchi, and Ichiro Imai (2000): Algicidal activity of a killer bacterium against the Harmful red tide dinoflagellate *Heterocapsa circularisquama* isolated from Ago Bay, Japan, Nippon Suisan Gakkaishi, Vol. 66(4), 666-673.	

No. : J-B-8

1) Title	Isolation of a marine gliding bacterium that kills *Chattonella antiqua*	
2) Category	Biological control	
3) Implementing organization	Ichiro Imai, Yuzaburo Ishida, Shigeki Sawayama, and Yoshihiko Hata (Kyoto University, Japan etc.)	
4) Target species	Class	Genus and Species
	Raphidophyceae	*Chattonella antiqua*
5) Implemented period	1991	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ Marine bacterium that kills *Chattonella antiqua* was isolated from northern Hiroshima Bay, the Seto Inland Sea, Japan in 1990. One strain (J18/M01) was selected and its algicidal characteristics were examined.	
9) Results	➢ The isolated algicidal bacterium was tentatively identified as *Cytophaga* sp. ➢ When 0.1 mL bacterial culture (ca. 10^8 cells/mL) in the liquid medium was added to 25 mL of *Chattonella antiqua* culture, the algal cells settled to the bottom of flask and were completely killed within 2-3 days. *Chattonella* cells were deformed, and then burst.	
10) Impact on the environment / ecosystem	➢ No description	
11) Others	➢ The present finding suggests that marine algicidal gliding bacteria are potentially significant agents controlling red tide occurrences.	
12) References	➢ Ichiro Imai, Yuzaburo Ishida, Shigeki Sawayama, and Yoshihiko Hata (1991): Isolation of a marine gliding bacterium that kills *Chattonella antiqua* (Rhaphidophyceae), Nippon Suisan Gakkaishi, Vol. 57(7), 1409.	

No. : J-B-9

<table>
<tr><td>1) Title</td><td colspan="2">Algicidal Marine Bacteria Isolated from Northern Hiroshima Bay, Japan</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Ichiro Imai, Yuzaburo Ishida, Keiichi Sakaguchi, and Yoshihiko Hata (Kyoto University, Japan)</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Gymnodinium mikimotoi (=Karenia mikimotoi)</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella antiqua, C. marina, Heterosigma akashiwo</td></tr>
<tr><td>Bacillariophyceae</td><td>Chaetoceros didymum, Ditylum brightwellii</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1995</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Marine bacteria that kill Chattonella antiqua were screened and isolated from northern Hiroshima Bay, the Seto Inland Sea, Japan in 1991. Four strains (S, K, D, R) were selected and examined on characteristics of algicidal activities.
➢ The algicidal ranges of the 4 strains of algicidal bacteria were examined by co-culture experiment with 6 species of marine phytoplankton (Chattonella antiqua, C. marina, Heterosigma akashiwo, Gymnodinium mikimotoi, Chaetoceros didymum and Ditylum brightwellii). The bacterial cultures were inoculated at final concentrations of about 10^3 cells/mL. Incubations were made at 22 °C and a light intensity of about 130-160 $\mu mol/m^2/sec$ with a 14h light: 10h dark photo-cycle.
➢ The effects of the culture filtrates in which C. antiqua was completely killed by the 4 strains of algicidal bacteria in medium on C. antiqua growth (or death) were examined. Each culture filtrate (0.1 μm pore filter) was added at concentrations of 50-99.9%.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Strains S and R showed wide algicidal range, killing all cells of the 6 species of marine phytoplankton. Algicidal activities of the strains K and D depended on prey phytoplankton species (Figure-1, 2).
➢ Bacterial culture filtrate experiment showed that the bacterial strains K and D give lethal effects on C. antiqua by means of extracellular products, and the strains S and R not by such substances but by predation (Figure-3).
➢ If one or two bacterial cells were inoculated into C. antiqua culture, all of the host cells were killed by the 4 strains of algicidal bacteria within 7 days.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">➢ No description</td></tr>
</table>

11) Others	➢ 4 strains of isolated algicidal bacteria were tentatively identified as *Alteromonas* spp. by their toxonomical characteristics. ➢ These results suggest that the algicidal activity by bacteria may be a significant factor influencing the population dynamics of phytoplankton, and potentially might account for rapid termination of red tides in the coastal sea.
12) References	➢ Ichiro Imai, Yuzaburo Ishida, Keiichi Sakaguchi, and Yoshihiko Hata (1995): Algicidal Marine Bacteria Isolated from Northern Hiroshima Bay, Japan, Fisheries Science, Vol. 61(4), 628-636.

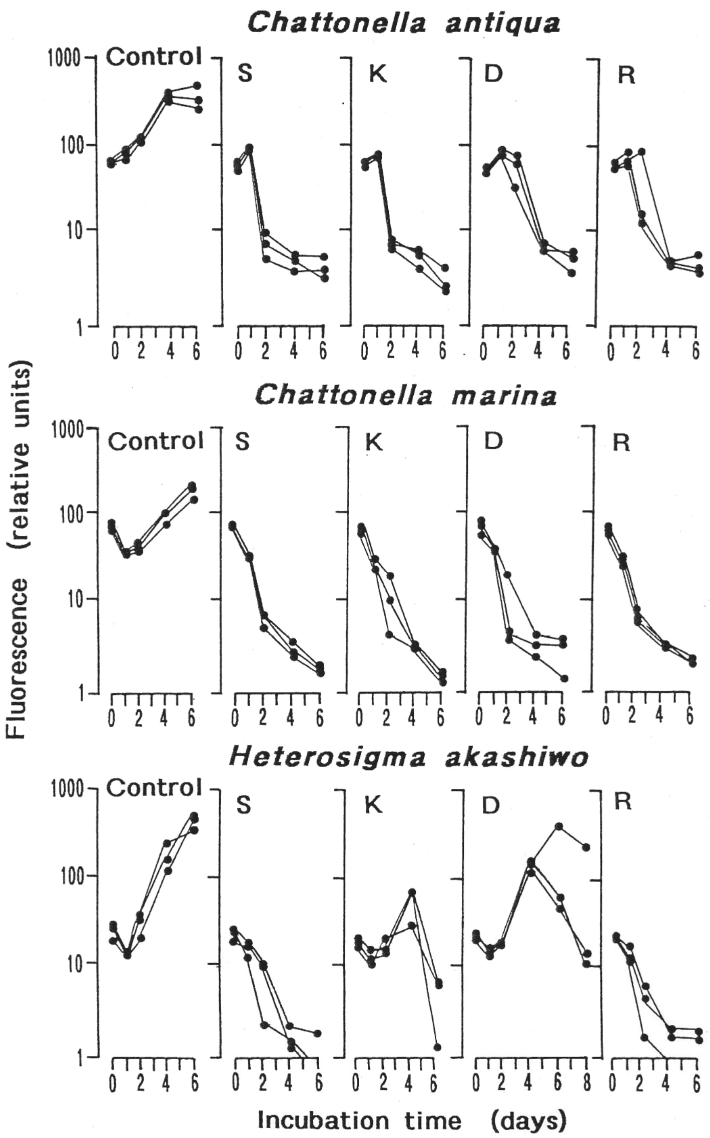


Figure-1 Effects of bacterial strains (S, K, D, R) on the growth or survival of *C. antiqua, C. marina*, and *H. akashowo*. Control: no addition of bacteria.

Source：Imai et al (1995)

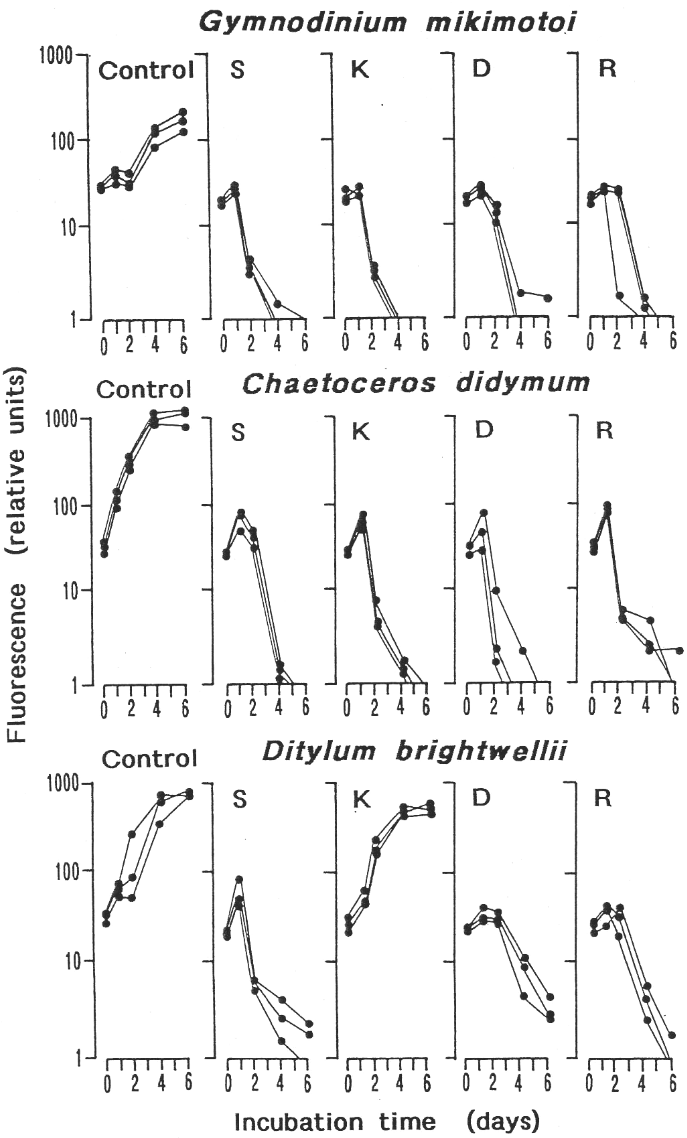


Figure-2 Effects of bacterial strains (S, K, D, R) on the growth or survival of *G. mikimotoi, C. didymum*, and *D. brightwellii*. Control: no addition of bacteria.

Source：Imai et al (1995)

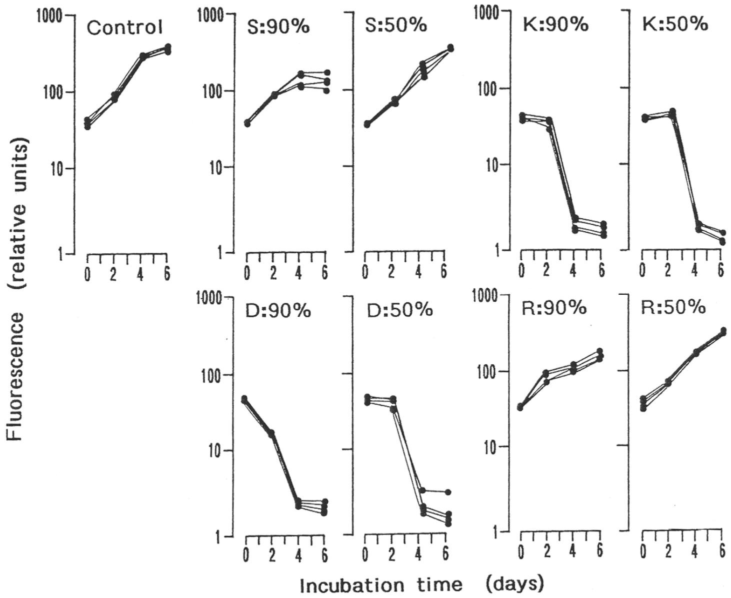


Figure-3 Effects of mixed culture filtrates on the growth or survival of *Chattonella antiqua*. Culture filtrates in which the algal cells were completely killed by the 4 strains of bacteria in SWM-3 medium were used. Numerals with % show ratios of the filtrates added to the algal culture. Control: naddition of culture filtrate.

Source：Imai et al (1995)

No. : J-B-10

1) Title	Algicidal Ranges in Killer Bacteria of Direct Attack Type for Marine Phytoplankton	
2) Category	Biological control	
3) Implementing organization	Imai, I. (Kyoto University, Japan)	
4) Target species	**Class**	**Genus and Species**
	Dinophyceae	*Alexandrium tamarense, Heterocapsa circularisquama*
	Raphidophyceae	*Chattonella ovata, C. verruculosa*
	Others	*Eutreptiella gymnastica, Oltmannsiellopsis viridis*
5) Implemented period	1997	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ The algicidal activities of the direct attack killer bacteria *Alteromonas* sp. strains S, R and *Cytophaga* sp. J18/M01 were examined against six phytoplankton species listed above. ➢ The phytoplankton species and algicidal bacteria (approximately 10^3 cells/mL) were cultured together in a test tube under the following conditions. Water temperature: 22 °C Illuminance: 130-160 μmol/m^2/s Photo-cycle: 14 hr light, 10 hr dark ➢ The growth of phytoplankton was monitored measuring invo fluorescence with a fluoroceter.	
9) Results	➢ *Chattonella ovata, C. verruculosa* and *Eutreptiella gymnastica* were killed effectively by all the algicidal bacteria. *Oltmannsiellopsis viridis* was also killed by all the algicidal bacteria, but the rate of decline was different depending on the type of algicidal bacteria (Figure-1). ➢ Although the growth rate of *A. tamarense* and *H. circularisquama* were restricted by *Cytophaga* sp. and *Alteromonas* sp. (S strain), no significant decline was observed in their abundance (Figure-1). ➢ *Alteromonas* sp.(R strain) did not show any algicidal effect on *A. tamarense* and *H. circularisquama* (Figure-1).	
10) Impact on environment / ecosystem	➢ No description	
11) Others	➢ Algicidal bacteria kill phytoplankton by two means: direct attack or production of killer substances. The direct attack type appears to be effective in killing naked flagellates and non-motile diatoms (Figure-2).	
12) References	➢ Ichiro Imai (1997): Algicidal ranges in killer bacteria of direct attack type for marine phytoplankton, Bulletin of Plankton Society of Japan, Vol.44, 3-9. (in Japanese)	

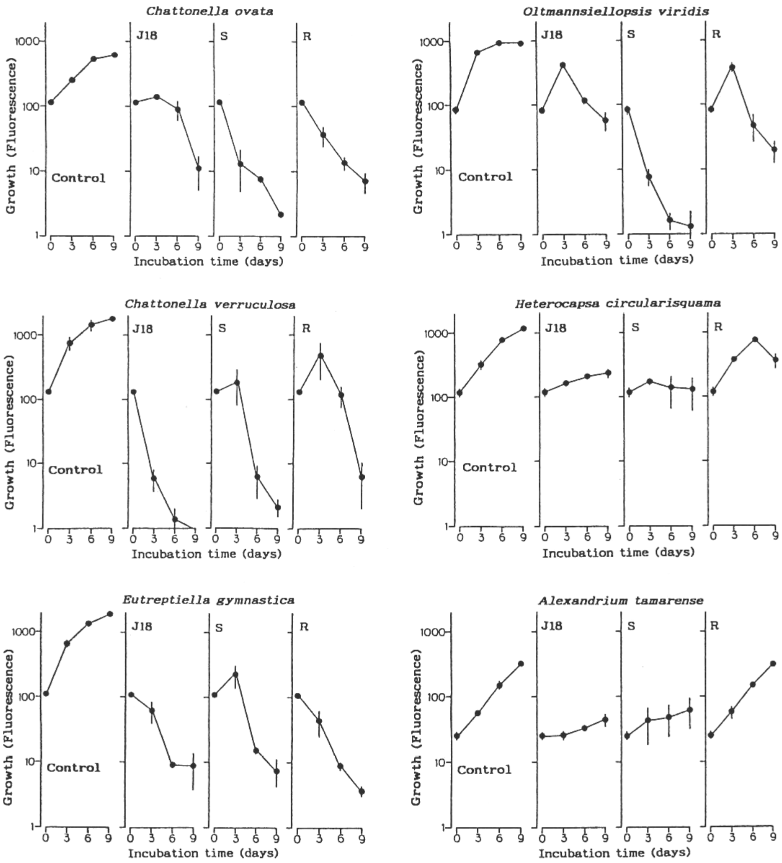


Figure-1 Effects of the 3 killer bacteria of direct attack type on the growth or survival of 6 species of marine phytoplankton

Note: Bacterial concentrations initially added were about 10^3 cells/mL. The growth or survival of phytoplankton was monitored measuring in vivo fluorescence with a fluorometer. (Control) no addition of killer bacterium; (J18) *Cytophaga* sp. J18/M01; (S) *Alteromonas* sp.S; (R) *Alteromonas* sp. R

Source：Imai (1997)

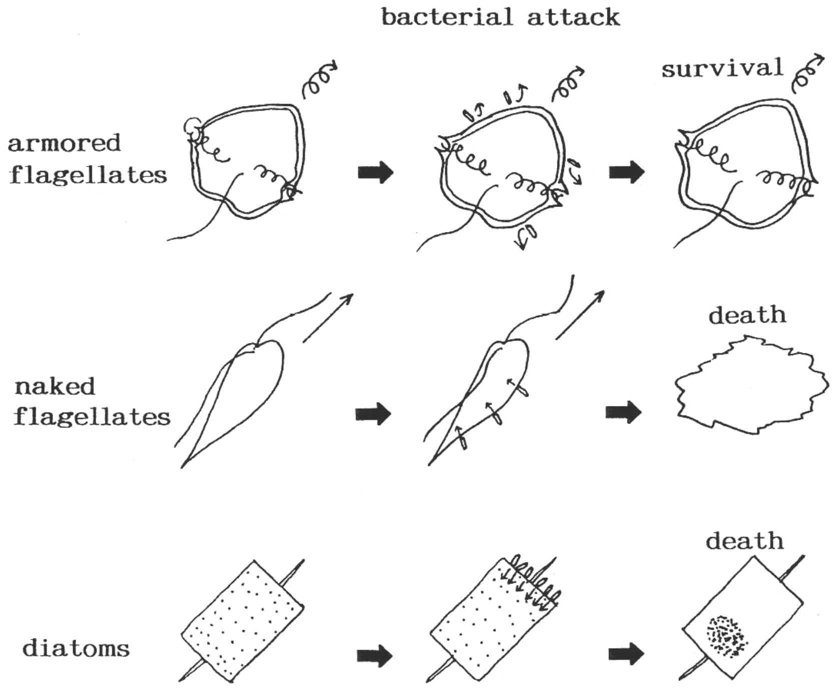


Figure-2 Schematic representation of patterns of algicidal activity in the killer bacteria of direct attack type.

Note: Small arrows represent attacks of killer bacteria such as direct injection of toxic substances to phytoplankton cells.

Source：Imai (1997)

No. : J-B-11

<table>
<tr><td>1) Title</td><td colspan="2">Lysis of Skeletonema costatum by Cytophaga sp. Isolated from the coastal water of the Ariake Sea</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Atsushi Mitsutani, Kaoru Takesue, Masanori Kirita, and Yuzaburo Ishida (Shimonoseki University of Fisheries, Japan etc.)</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Gymnodinium nagasakiense (= Karenia mikimotoi)</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella antiqua</td></tr>
<tr><td>Bacillariophyceae</td><td>Skeletonema costatum, Ditylum brightwellii, Chaetoceros didymum, Thalassiosira sp.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1992</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ A bacterium strain of Cytophaga sp., which has strong algal-lytic activity against the diatom S. costatum was isolated from the coastal water of the Ariake Sea, Japan.
➢ In order to determine the host range, lytic bacterium was inoculated into the algal cultures. Host algae were 6 species (Skeletonema costatum, Ditylum brightwellii, Chaetoceros didymum, Thalassiosira sp. Chattonella antiqua, and Gymnodinium nagasakiense). The initial concentration of the bacterium was about 1×10^4 cells/mL. After one or two weeks of incubation, lysis of algae was examined under a microscope.
➢ In order to investigate the mechanism of the lysis of algae, Cytophaga sp. was cocultured with Skeletonema costatum under various conditions.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Cytophaga sp. could lyse Skeletonema costatum, Ditylum brightwellii, Thalassiosira sp., Chattonella antiqua, but could not lyse Chaetoceros didymum or Gymnodinium nagasakiense.
➢ Several days after the bacterium was inoculated to the culture of Skeletonema costatum, the cell number of the bacterium increased rapidly without lysing algal cells, and after it reached the order of 10^6 cells/mL the lysis of algae began to be observed. The bacterial number increased again with the progress of lysis and reached the order of 10^7 cells/mL.
➢ A significant increase in the number of protoplasts of diatom was microscopically observed in the process of lysis of the algae, following which these protoplasts were thoroughly lysed and disappeared in the last stage of lysis.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ Microscopic examination indicated that the bacterial cells were attached to the living cells or protoplasts of diatom by one long rod.</td></tr>
</table>

12) References	➢ Atsushi Mitsutani, Kaoru Takesue, Masanori Kirita, and Yuzaburo Ishida (1992): Lysis of *Skeletonema costatum* by *Cytophaga* sp. Isolated from the coastal water of the Ariake Sea, Nippon Suisan Gakkaishi, Vol. 58(11), 2159-2169.

No. : J-B-12

1) Title	Growth inhibition of diatoms with algicidal bacteria	
2) Category	Biological control	
3) Implementing organization	Sakata T. (Kagoshima University, Japan)	
4) Target species	**Class**	**Genus and Species**
	Bacillariophyceae	*Chaetoceros ceraposporum*
5) Implemented period	1990-1994 (published year: 1991-1995)	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ Algicidal bacteria were isolated from seawater samples collected from the coast and tiger prawn farm in Kagoshima Bay. ➢ The isolated bacteria belonged to the genus *Saprospira*, *Vitreoscilla*, *Amoeba* and *Labyrinthula*. ➢ Algicidal bacteria *Saprospira* (SS91-40 strain) and its culture supernatant fluid were added to a diatom culture medium to examine the algicidal effects.	
9) Results	➢ The growth of diatoms was inhibited by the algicidal bacteria *Saprospira* (SS91-40 strain). ➢ The growth of diatoms was also inhibited by the *Saprospira* (SS91-40 strain) culture supernatant fluid. The growth inhibition effect of the supernatant fluid was not lost even after heat treatment.	
10) Impact on the environment / ecosystem	(1) Impact on the ecosystem ➢ No description (2) Impact on the environment ➢ No description	
11) Others	No description	

12) References	➢ Kagoshima University, Faculty of Fisheries (Sakata, T.) (1991): Report on the development of red-tide countermeasures Year 1990, Fisheries Agency. ➢ Kagoshima University, Faculty of Fisheries (Sakata, T.) (1992): Report on the development of red-tide countermeasures Year 1991, Fisheries Agency. ➢ Kagoshima University, Faculty of Fisheries (Sakata, T.) (1993): Report on the development of red-tide countermeasures Year 1992, Fisheries Agency. ➢ Kagoshima University, Faculty of Fisheries (Sakata, T.) (1994): Report on the development of red-tide countermeasures Year 1993, Fisheries Agency. ➢ Kagoshima University, Faculty of Fisheries (Sakata, T.) (1995): Report on the development of red-tide countermeasures Year 1994, Fisheries Agency. ➢ Taizo Sakata(1990): Occurrence of marine Saprospira sp. possessing algicidal activity for diatoms, Nippon Suisan Gakkaishi, 56(7), 1165. ➢ Taizo Sakata, Yoshiyuki Fujita, Hiroyuki Yasumoto (1991): Plaque formation by algicidal Saprospira sp. on a lawn of Chaetoceros ceratosporum, Nippon Suisan Gakkaishi, 57(6), 1147-1152. ➢ Taizo Sakata, Hiroyuki Yasumoto (1991): Colony formation by algicidal Saprospira sp. on marine agar plates, Nippon Suisan Gakkaishi, 57(11), 2139-2143 ➢ Taizo Sakata, Kozo Iwamoto (1991): Isolation of marine algicidal microorganisms on diatom double layer agar plates, Fisheries Science, 61(1) 173-174.

No. : J-B-13

<table>
<tr><td>1) Title</td><td colspan="2">Possibility for bio-control of harmful diatom blooms in Coscinodiscus wailesii by marine bacteria</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Satoshi Nagai and Ichiro Imai</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Bacillariophyceae</td><td>Coscinodiscus wailesii</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1999</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ A marine bacterium Alteromonas sp., lethal to Coscinodiscus wailesii, was isolated from eastern Seto Inland Sea, Japan.
➢ The algicidal effects of the isolated Alteromonas sp. (Strain K12) bacterium were examined against C. wailesii.
➢ The algicidal effects of the isolated Alteromonas sp. (Strain K12) bacterium were examined against 17 phytoplankton species.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Alteromonas sp. (Strain K12) showed algicidal effects on C. wailesii. The mortality rate of C. wailesii increased significantly when the concentration of Alteromonas sp. (Strain K12) reached close to 10^6 cells/mL.
➢ C. wailesii was also killed when incubated with a Alteromonas sp. (Strain K12) culture filtrate, which indicates that Alteromonas sp. (Strain K12) produces some toxic substances towards C. wailesii.
➢ Alteromonas sp. (Strain K12) also showed algicidal effects towards many other phytoplanktons.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ The toxin produced by Alteromonas sp. (Strain K12) was deduced to be a non-volatile peptide-like substance, which passed through ultrafiltration membrane with molecular weight cut off 5000, and became completely inactive after 60 minutes of 80°C heat treatment.
➢ Prior to applying microorganism for red-tide control, the following criteria should be considered:
  ➢ only kills the target red-tide species,
  ➢ exists in natural waters and can be cultured,
  ➢ is non-toxic to fish and other animals.
Hence the use of algicidal bacteria can have some problems, since these bacteria kill non-target red-tide species as well.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Nagai, S. and Imai, I. (1999): Possibility for bio-control of harmful diatom blooms in Coscinodiscus wailesii by marine bacteria., Microb. Environ, 14(4), 253-262.(in Japanese)</td></tr>
</table>

Algicida viruses:

No. : J-B-14

<table>
<tr><td>1) Title</td><td colspan="2">Isolation of a virus infecting the novel shellfish-killing dinoflagellate Heterocapsa circularisquama</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Kenji Tarutani, Keizo Nagasaki, Shigeru Itakura, Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea, Japan)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Heterocapsa circularisquama</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2001</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ A virus infecting the novel shellfish-killing dinoflagellate Heterocapsa circularisquama (H. circularisquama Virus: HcV) was isolated from Japanese coastal waters in August 1999 during a H. circularisquama bloom.
➢ General characteristics of a virus infecting and lysing H. circularisquama were observed using transmission electron microscopy and epifluorescence microscopy.
➢ The host range of the virus (HcV) was tested on 25 phytoplankton species, including 18 strains of H. circularisquama isolated from various embayments throughout central and western Japan.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ The virus was icosahedral, lacking a tail, ca. 180 to 210 nm (mean ± standard deviation = 197 ± 8 nm) in diameter and contained an electron-dense core. It was a double-stranded DNA virus, and the appearance of the virus particles was associated with a granular region (viroplasm) in the cytoplasm that did not appear within uninfected cells.
➢ The virus infected and lysed all H. circularisquama strains that were tested, but did not cause lysis in any of the other 24 phytoplankton species.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ This is the first report of a virus infecting dinoflagellates, which has been isolated and maintained in culture, and these results demonstrated that viruses which infect and cause lysis of dinoflagellates are a component of natural marine viral communities.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Kenji Tarutani, Keizo Nagasaki, Shigeru Itakura, Mineo Yamaguchi (2001): Isolation of a virus infecting the novel shellfish-killing dinoflagellate Heterocapsa circularisquama, Aquatic Microbial Ecology, Vol. 23, 103-111.</td></tr>
</table>

No. : J-B-15

1) Title	Dynamics of *Heterocapsa circularisquama* and its viruses in Ago Bay, Japan	
2) Category	Biological control	
3) Implementing organization	Keizo Nagasaki, Yuji Tamaru, Katsuya nakanishi, Naotsugu Hata, Noriaki Katanozaka. Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea, Japan etc.)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Heterocapsa circularisquama*
5) Implemented period	2004	
6) Experiment type	Field and lab experiment	
7) Application	No description	
8) Method / mechanism	➢ To examine the relationship between the bloom-forming dinoflagellate *Heterocapsa circularisquama* and its infectious viruses, field surveys were conducted once a week at the Tategami Station in Ago Bay, Japan from April through November 2001. Seawater samples were collected from several layers using Kitahara's water bottle, and sediment samples were collected using Ekman-Birge bottom sampler. ➢ Phytoplankton abundance was immediately assessed by direct counting with optical microscopy, and the titration of viruses infecting *H. circularisquama* was carried out within 24 h of collection. ➢ The abundance of viruses infecting *H. circularisquama* in the seawater samples (5m and B-1m) and the sediment samples was enumerated by means of the extinction dilution method.	
9) Results	➢ The abundance of viruses infectious to *H. circularisquama* was high from the peak of the bloom (mid July) and throughout the post-bloom period, but ceased by the end of August. At the peak of the bloom, 88% of the *H. circularisquama* cells in the population harbored small virus like particles. ➢ Based on transmission electron microscopic observation, morphological resemblance between these virus-like particles and the single-stranded RNA (ssRNA) virus infecting *H. circularisquama* isolated from the bloom was noticeable. ➢ The fluctuation patterns of the viruses indicated that at least 2 distinct types of virus with different host specificity spectra coexisted. A specific increase in viral abundance in the sediments was observed in the middle of the bloom, and these viruses were likely able to maintain their infectivity for at least 3 months.	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ This study provides further evidence of the possible viral impacts on the biomass and clonal composition of algal populations in the natural environment, and offers support for the hypothesis that sediments are a reservoir of algal viruses.	

12) References	➢ Keizo Nagasaki, Yuji Tamaru, Katsuya nakanishi, Naotsugu Hata, Noriaki Katanozaka, Mineo Yamaguchi (2004): Dynamics of *Heterocapsa circularisquama* (Dinophyceae) and its viruses in Ago Bay, Japan, Aquatic Microbial Ecology, Vol. 34, 219-226.

No. : J-B-16

<table>
<tr><td>1) Title</td><td colspan="2">Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga Heterocapsa circularisquama</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Yuji Tomaru, Noriaki Katanozaka, Kensho Nishida,Yoko Shirai, Kenji Tarutani, Mineo Yamaguchi, Keizo Nagasaki (National Research Institute of Fisheries and Environment of Inland Sea, Japan etc.)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Heterocapsa circularisquama</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2004</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ HcRNAV, a novel single-stranded RNA (ssRNA) virus specifically infecting the bivalve-killing dinoflagellate Heterocapsa circularisquama was isolated from the coastal waters of Japan in 2000 and 2001.
➢ To examine the intra-species host specificity of the pathogens, clonal pathogens were screened against putative hosts to test their infectivity. An aliquot of each lysate was inoculated independently into exponentially growing cultures of the 56 H. circularisquama strains. The inter-species host specificity of HcRNAV was also tested by adding aliquots of each suspension to cultures of the exponentially growing 36 phytoplankton strains, which included 4 H. circularisquama strains. The occurrence of algal lysis was monitored by optical microscopy.
➢ To examine the algicidal effect of HcRNAV, aliquots of the pathogen suspension were filtered through a 0.1 µm pore-size polycarbonate membrane filter and added to exponentially growing cultures of H. circularisquama strains. The titer of the pathogen was measured by means of the extinction-dilution method.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ 107 clonal pathogens to H. circularisquama were isolated from 9 coastal waters of western Japan.
➢ Through the intra-species host-range assay, the HcRNAV strains were divided into 2 types on the basis of their infection spectra: UA-type and CY-type. The infection spectra of UA-type and CY-type pathogens were complementary to each other. On the basis of these results, HcRNAV*34 and HcRNAV109 were selected as representatives of UA-type and CY-type pathogens, respectively.
➢ Both virus strains were icosahedral, ca. 30nm in diameter, and harbored a single molecule of ssRNA approximately 4.4 kb in size.
➢ Both virus strains were not lytic to all the tested phytoplankton strains, except the 4 H. circularisquama strains.

*HcRNAV (H. circularisquama RNA Virus: single-stranded RNA virus)</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
</table>

11) Others	➢ The findinig of RNA viruses infecting microalgae such as HaRNAV and HcRNAV emphasizes the diversity of algicidal viral pathogens.
12) References	➢ Yuji Tomaru, Noriaki Katanozaka, Kensho Nishida,Yoko Shirai, Kenji Tarutani, Mineo Yamaguchi, Keizo Nagasaki (2004): Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*, Aquatic Microbial Ecology, Vol. 34, 207-218.

No. : J-B-17

1) Title	Widespread occurrence of viruses lytic to the bivalve-killing dinoflagellate *Heterocapsa circularisquama* along the western coast of Japan
2) Category	Biological control
3) Implementing organization	Yuji Tomaru and Keizo Nagasaki (National Research Institute of Fisheries and Environment of Inland Sea, Japan)
4) Target species	**Class** / **Genus and Species** Dinophyceae / *Heterocapsa circularisquama*
5) Implemented period	2004
6) Experiment type	Field experiment and Lab experiment
7) Application	No description
8) Method / mechanism	➢ To clarify the ecological implications of viral infection on *Heterocapsa circularisquama*, the abundances of viruses infecting *H. circularisquama* were examined at six sites along the western coast of Japan in 2001. ➢ Water samples were collected from *H. circularisquama* blooms at 6 sampling site. The titer of infectious viruses in the filtrated water sample was measured by MPN method.
9) Results	➢ Viral agents lytic to *H. circularisquama* were detected from all water samples, and the maximum abundance was 2.08×10^5 infectious units mL^{-1}. ➢ Transmission electron microscopy revealed the coexistence of two distinct virus-like particles in a *H. circularisquama* bloom that occurred in Fukura Bay (Hyogo Pref.): large (210±17nm) and small (28±2nm) virus-like particles that were morphologically quite similar to HcV and HcRNAV, respectively. HcV (*H. circularisquama* Virus: double-stranded DNA virus) HcRNAV (*H. circularisquama* RNA Virus: single-stranded RNA virus)
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description
11) Others	➢ The results suggest a close relationship between *H. circularisquama* blooms and the lytic viruses in natural environments.
12) References	➢ Yuji Tomaru and Keizo Nagasaki (2004): Widespread occurrence of viruses lytic to the bivalve-killing dinoflagellate *Heterocapsa circularisquama* along the western coast of Japan, Plankton Biol. Ecol., Vol. 51(1), 1-6.

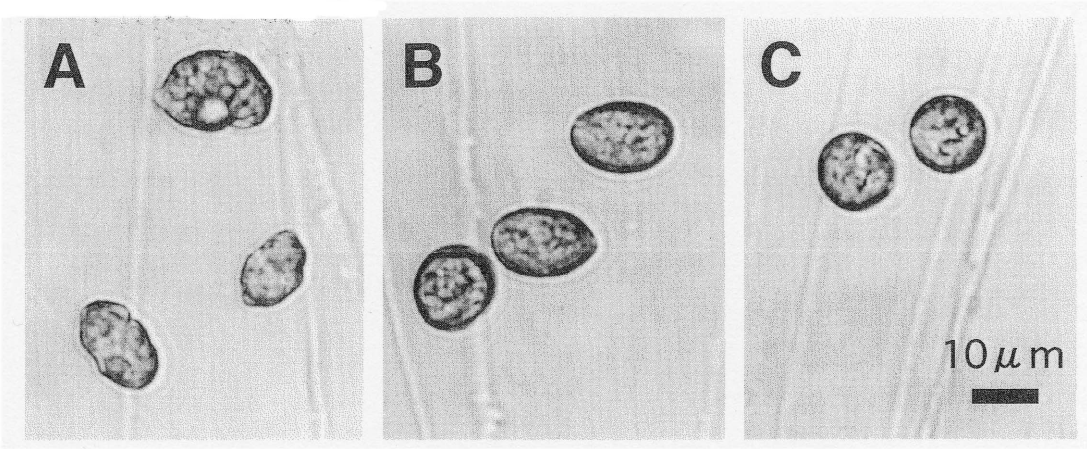
No. : J-B-18

1) Title	Effect of temperature on the algicidal activity and the stability of HaV (*Heterosigma akashiwo* virus)	
2) Category	Biological control	
3) Implementing organization	Keizo Nagasaki and Mineo Yamaguchi (Nansei National Fisheries Research Institute, now National Research Institute of Fisheries and Environment of Inland Sea, Japan)	
4) Target species	**Class**	**Genus and Species**
	Raphidophyceae	*Heterosigma akashiwo*
5) Implemented period	1998	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ The effect of temperature on the algicidal activity and stability of HaV (*Heterosigma akashiwo* Virus), which infects the harmful bloom causing alga, *H. akashiwo*, was determined by growing *H. akashiwo* culture inoculated with HaV under various conditions. ➢ Two strains of *H. akashiwo* were used, one was *H. akashiwo* H93616 isolated from northern part of Hiroshima Bay (Hiroshima Pref., Japan) in 1993, and the other was *H. akashiwo* NM96 isolated from Nomi Bay (Kochi Pref., Japan) in 1996. ➢ Two HaV clones isolated seawater samples taken from a *H. akashiwo* red tide were used; one was HaV01 from Unoshima Fishing Port (Fukuoka Pref., Japan) and the other was HaV08 from Nomi Bay (Kochi Pref., Japan) in 1996.	
9) Results	➢ Temperature and growth stage of the host culture are considered to be important factors determining the algicidal activity of HaV. The optimum temperature for the algicidal activity of HaV ranged from 20 to 25 °C. ➢ Comparing the viral susceptibility of *H. akashiwo* strains and the algicidal activity of the HaV clones at different temperatures, both were suggested to be phenotypically diverse. ➢ In regards to the effect of temperature on the HaV stability, HaV showed a relatively rapid decrease in infectious titer even when preserved at 5 °C in the dark.	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ No description	
12) References	➢ Keizo Nagasaki and Mineo Yamaguchi (1997): Isolation of a virus infectious to the harmful bloom causing microalga, *Heterosigma akashiwo* (Raphidophyceae), Aquatic Microbial Ecology, Vol.13, 135-140. ➢ Keizo Nagasaki and Mineo Yamaguchi (1998): Effect of temperature on the algicidal activity and the stability of HaV (*Heterosigma akashiwo* virus), Aquatic Microbial Ecology, Vol.15, 211-216.	

No. : J-B-19

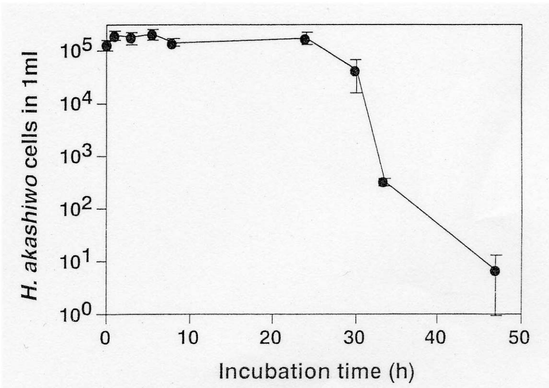
<table>
<tr><td>1) Title</td><td colspan="2">Growth characteristics of Heterosigma akashiwo virus and its possible use as a microbiological agent for red tide control</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Nansei National Fisheries Institute, Japan (now National Research Institute of Fisheries and Environment of inland Sea, Fisheries Research Institute, Japan)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Raphidophyceae</td><td>Heterosigma akashiwo</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1998 – 1999</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ 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</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ 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).</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ 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.</td></tr>
</table>

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.



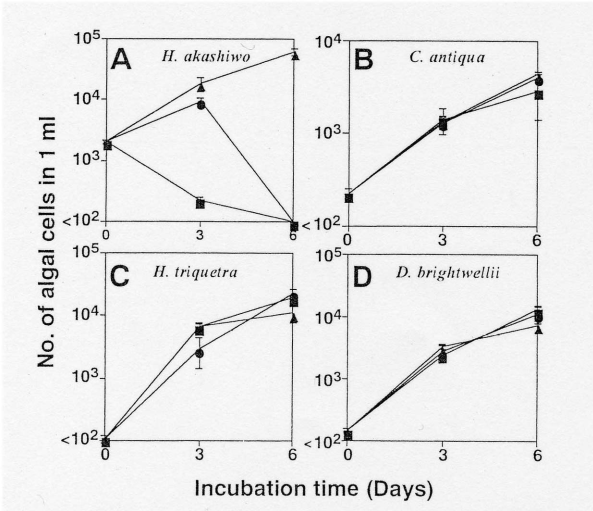
Source: Nagasaki et al (1999)

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



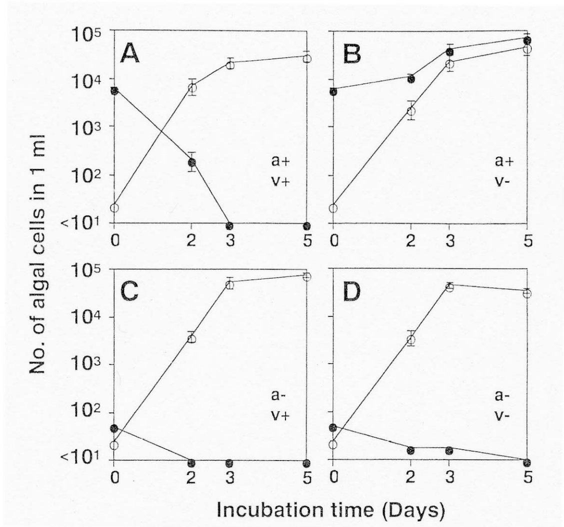
Source: Nagasaki et al (1999)

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.



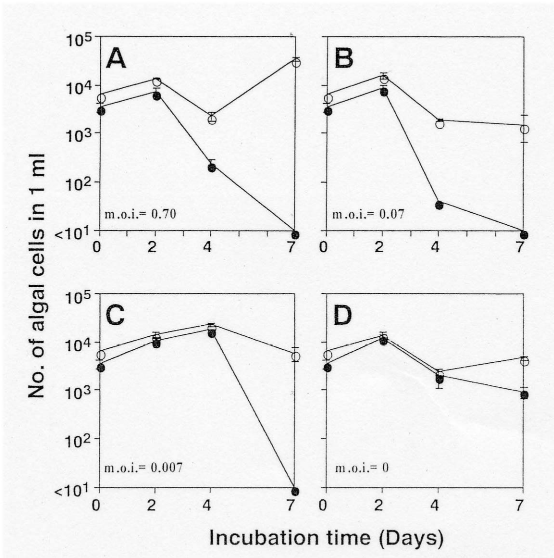
Source: Nagasaki et al (1999)

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.

No. : J-B-20

1) Title	Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo*	
2) Category	Biological control	
3) Implementing organization	Kenji Tarutani, Keizo Nagasaki, Mineo Yamaguchi (National Research Institute of Fisheries and Environment of Inland Sea, Japan)	
4) Target species	Class	Genus and Species
	Raphidophyceae	*Heterosigma akashiwo*
5) Implemented period	2000	
6) Experiment type	Field and lab experiment	
7) Application	No description	
8) Method / mechanism	➢ The population dynamics of the harmful bloom-forming phytoplankton *Heterosigma akashiwo* and the infectious *H. akashiwo* viruses (HaV) were monitored in Hiroshima Bay Japan, from May to July 1998. Concurrently, a number of *H. akashiwo* and HaV clones were isolated, and their virus susceptibilities and host ranges were determined through laboratory cross-reactivity tests. ➢ Cell counts and taxonomic identification of *H. akashiwo* and other phytoplankton species were carried out with a Sedgewick-Rafter chamber under optical microscopy on the sampling day without fixation of the sample water. The abundance of *H. akashiwo* in seawater was estimated by the most probable number (MPN) technique. ➢ The virus susceptibilities of *H. akashiwo* isolates were examined by using a range of HaV clonal isolates.	
9) Results	➢ A sudden decrease in cell density of *H. akashiwo* was accompanied by a drastic increase in the abundance of HaV, suggesting that viruses contributed greatly to the disintegration of the *H. akashiwo* bloom as mortality agents. ➢ Despite the large quantity of infectious HaV, however, a significant proportion of *H. akashiwo* cells survived after the bloom disintegration. ➢ The viral susceptibility of *H. akashiwo* isolates demonstrated that the majority of these surviving cells were resistant to most of the HaV clones, whereas resistant cells were a minor component during the bloom period. Moreover, these resistant cells were displaced by susceptible cells, presumably due to viral infection.	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	

11) Others	➢ These results demonstrated that the properties of dominant cells within the *H. akashiwo* population change during the period when a bloom is terminated by viral infection, suggesting that viruses also play an important role in determining the clonal composition and maintaining the clonal diversity of *H. akashiwo* populations. Therefore, data indicate that viral infection influences the total abundance and the clonal composition of one host algal species, suggesting that viruses are an important component in quantitatively and qualitatively controlling phytoplankton populations in natural marine environments.
12) References	➢ Kenji Tarutani, Keizo Nagasaki, Mineo Yamaguchi (2000): Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo*, Applied and Environmental Microbiology, Vol. 66(11), 4916-4920.

No. : J-B-21

1) Title	Quantitative and qualitative impacts of viral infection on a *Heterosigma akashiwo* bloom in Hiroshima Bay, Japan	
2) Category	Biological control	
3) Implementing organization	Yuji Tamaru, Kenji Tarutani, Mineo Yamaguchi, Keizo Nagasaki (National Research Institute of Fisheries and Environment of Inland Sea, Japan)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Heterosigma akashiwo*
5) Implemented period	2004	
6) Experiment type	Field and lab experiment	
7) Application	No description	
8) Method / mechanism	➢ To clarify the relationship between *Heterosigma akashiwo* and its infectious viruses (HaV), both algal and viral dynamics were monitored in Hiroshima Bay, Japan from May through July 2000. Water samples were collected 1 to 3 times weekly from May through July 2000 at Itsukaichi Fishing Port. ➢ Cell number of *H. akashiwo* was immediately assessed by direct counting with optical microscopy without fixation of the sample waters. The abundance of viruses lytic to *H. akashiwo* in seawater samples was estimated by MPN technique. The abundance of lytic viruses was calculated with a BASIC program from the number of wells in which lysis occurred. ➢ To examine intraspecies host specificity of the virus strains, 90 *H. akashiwo* clones and 65 HaV clones were obtained during the survey. ➢ An aliquot of each lysate was inoculated independently into exponentially growing cultures of the 94 *H. akashiwo* strains (4 strains used for previous studies and 90 strains isolated during the survey), and the occurrence of algal lysis was monitored by optical microscopy. For comparison, growth of host cultures without pathogen inoculation was also monitored.	
9) Results	➢ The abundance of viruses lytic to *H. akashiwo* showed its own dynamics pattern, but the viruses shared similar trends with each other, exhibiting a marked increase accompanied by a sudden decrease in host abundance. ➢ Based on the results of laboratory cross-reactivity tests between 90 *H. akashiwo* clones and 65 HaV clones, they were divided into 6 and 3 groups, respectively, showing their high diversity with regard to their virus sensitivity and host specificity.	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ The viral infection was one of the most important factors determining quantity (biomass) and quality (clonal composition) of the *H. akashiwo* population.	

12) References	➢ Yuji Tamaru, Kenji Tarutani, Mineo Yamaguchi, Keizo Nagasaki (2004): Quantitative and qualitative impacts of viral infection on a *Heterosigma akashiwo* bloom in Hiroshima Bay, Japan, Aquatic Microbial Ecology, Vol. 34, 227-238.

Plankton grazers:

No. : J-B-22

1) Title	Experiment on *Gymnodinium mikimotoi* prey-predation relationship	
2) Category	Biological control	
3) Implementing organization	Kagawa Prefecture Fisheries Research Institute / Red Tide Research Institute （Yoshimatsu, S. & Tatsumitsu, N.）	
4) Target species	Class	Genus and Species
	Dinophyceae	*Gymnodinium mikimotoi* (= *Karenia mikimotoi*)
5) Implemented period	1992	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ *Gymnodinium mikimotoi* and its possible predator *Gyrodinium fissum*, were incubated together for 5 days to examine their prey-predator relationship. ➢ Five *Gym. mikimotoi* and *Gyr. fissum* mixtures were prepared at different cell concentrations, and incubated under the following condition: Water temperature: 25.5 C° Iluminance: 2,000 Lux Photoperiod: 14 hr light, 10 hr dark Cell numbers of both species were counted once per day with a microscope.	
9) Results	➢ The cell number of *Gym. mikimotoi* showed rapid decrease when *Gyr. fissum* was present, and disappeared completely after 1-3 days (Table-1).	
10) Impact on the environment / ecosystem	➢ No description	
11) Others	➢ During the experiment, the cell number of *Gyr. fissum* showed a maximum of 8-fold increase in one day. ➢ The results of the experiment clearly shows that *Gyr. fissum* is a predator of *Gym. mikimotoi*. Similar experiments should be further conducted to examine the relationship between predator predation rate and red-tide blooms.	
12) References	➢ Kagawa Prefecture Fisheries Research Institute / Red tide Research Institute (Yoshimatsu, S. & Tatsumitsu, N.) (1992): Report on the development of red-tide countermeasures Year 1991, Fisheries Agency.	

Table-1　The change in cell numbers in the predation experiment of *Gyr. fissum* and *Gym. Mikimotoi*

Unit: cells/mL

		Number of days					
		0	1	2	3	4	5
Lot 1	Gym.m.	430	536	664	750	706	1.6
	Gyr.f	0	0	0	2.0	1.0	0
Lot 2	Gym.m.	382.0	412.0	117.2	0	0	0
	Gyr.f	4.0	18.4	148.4	258.6	77.4	21.6
Lot 3	Gym.m.	336.0	35.2	0	0	0	0
	Gyr.f	18.6	122.4	157.4	122.8	56.4	0.4
Lot 4	Gym.m.	332.0	0	0	0	0	-
	Gyr.f	52.2	324.0	207.0	12.6	0	-
Lot 5	Gym.m.	0	0	0	0	0	-
	Gyr.f	520.0	686.0	528.0	20.0	0	-

Source：Yoshimatsu and Tatsumitsu (1992)

Note: The cell number of Lot 4 and 5 were not counted in the fifth day, since all cells had disappeared in the forth day

No. : J-B-23

1) Title	Studies on the effects of grazing pressure on red-tide development
2) Category	Biological control
3) Implementing organization	Nagasaki University (Shoji Iizuka)
4) Target species	**Class**: Dinophyceae **Genus and Species**: *Gymnodinium* sp.65' type (= *Karenia mikimotoi*)
5) Implemented period	1980-1983 (published year: 1981-1984)
6) Experiment type	Lab experiment, Field experiment (Omura Bay, Nagasaki Prefecture)
7) Application	No description
8) Method / mechanism	➢ The grazing effects of zooplanktons on red-tide species *Gymnodinium* sp. (65' type) were investigated. ➢ *Gymnodinium* and 2 copepod species (*Paracalanus crassirostris* and *Oithona brevi-cornis*) were cultured together, to examine the grazing rate on *Gymnodinium*. The cell density of *Gymnodinium* and the copepods were measured after 3 and 6 hours. ➢ Two types of seawater samples were prepared: one containing both zooplankton and *Gymnodinium*, and one containing only *Gymnodinium*. Both samples were enclosed in a cellulose dialysis membrane tube, and then installed in a natural sea area for 1-7 days. The *Gymnodinium* cell concentrations were monitored during the experiment.
9) Results	➢ When *Gymnodinium* and *P. crassirostris* were cultured together, the *Gymnodinium* cell density was reduced on average by 31% of the initial level. For *Gymnodinium* and *O. brevi-cornis*, the *Gymnodinium* cell density was reduced on average by 56% of the initial level. ➢ No zooplankton grazing effects on *Gymnodinium* were observed with the cellulose dialysis membrane tube field experiment.
10) Impact on the environment / ecosystem	(1) Impact on the ecosystem ➢ No description (2) Impact on the environment ➢ When *Gymnodinium* and *O. brevi-cornis* were cultured together, both species showed high mortality. This result suggested that *Gymnodinium* might have some toxic effect on *O. brevi-cornis*.
11) Others	➢ Germination test were conducted with cysts collected from the bottom sediments of Omura Bay. Although 11 dinoflagellates species were identified, *Gymnodinium* was not present.
12) References	➢ Nagasaki University (Shoji Iizuka) (1981): Report on the development of red-tide countermeasures Year 1980, Fisheries Agency. ➢ Nagasaki University (Shoji Iizuka) (1982): Report on the development of red-tide countermeasures Year 1981, Fisheries Agency. ➢ Nagasaki University (Shoji Iizuka) (1983): Report on the development of red-tide countermeasures Year 1982, Fisheries Agency. ➢ Nagasaki University (Shoji Iizuka) (1984): Report on the development of red-tide countermeasures Year 1983, Fisheries Agency.

No. : J-B-24

1) Title	Investigation and identification of zooplanktons that graze on red-tide species
2) Category	Biological control
3) Implementing organization	Shin-Nippon Meteorological & Oceanographical Consultant Co., Ltd. (now IDEA Consultants, Inc.)
4) Target species	Class: Raphidophyceae Genus and Species: *Chattonella angiqua, C. marina*
5) Implemented period	1985 – 1987 (published year: 1986-1988)
6) Experiment type	Lab experiment
7) Application	No description
8) Method / mechanism	➢ Zooplankton (copepods) generation rearing and grazing experiments (of red-tide species) were conducted. ➢ Cultured phytoplankton species were reared with copepods (*Acartia clausi, Pseudodiaptomus marinus, Calanus sinicus*) for 150 days. ➢ Grazing rates of *Acartia clausi* and *Pseudodiaptomus marinus* on *Chattonella antique* and *C. marina* were investigated. Twenty zooplankton individuals were tested with varying concentration of *C. antique* and *C. marina* (100-800 cells/mL), using a 200 mL flask. The cell density of *Chattonella* was measured after 6 hours, and based on the values the grazing rates were calculated.
9) Results	➢ Generation rearing was possible with *A. clausi* and *P. marinus*. Rearing of *P. marinus* was especially stable over the 150 days test period. On the other hand, rearing of *Calanus sinicus* was difficult. ➢ *A. clause* and *P. marinus* grazed actively on *C. antique* and *C. marina.* ➢ The grazing rate of *A. clausi* on *C. antiqua* was 10-18.5 cells/individual/hour, and 27-44 cells/individual/hour on *C. marina*. ➢ The grazing rate of *P. marinus* on *C. antiqua* was 13-24.5 cells/individual/hour, and 13-18 cells/individual/hour on *C. marina*.
10) Impact on the environment / ecosystem	(1) Impact on the ecosystem ➢ No description (2) Impact on the environment ➢ No description
11) Others	➢ The feasibility of using zooplankton (copepods) as a red-tide control option was considered under the following assumptions. Scale of the *Chattonella* red tide: area of red tide = 0.01 km^2, depth range of red tide = 0-1 m, cell density of *Chattonella* = 1,000 cells/mL Under the above assumptions, the number of copepods required for removing the *Chattonella* red tide was calculated as $3{,}300\times10^6$ individuals. Since the rearing limit of copepods is 100 individuals/L, the required rearing capacity (water volume) for the above case was calculated as 33×10^3 tons.

12) References	➢ Shin-Nippon Meteorological & Oceanographical Consultant Co., Ltd. (1986): Report on the development of red-tide countermeasures Year 1985, Fisheries Agency. ➢ Shin-Nippon Meteorological & Oceanographical Consultant Co., Ltd. (1987): Report on the development of red-tide countermeasures Year 1986, Fisheries Agency. ➢ Shin-Nippon Meteorological & Oceanographical Consultant Co., Ltd. (1988): Report on the development of red-tide countermeasures Year 1987, Fisheries Agency.

No. : J-B-25

<table>
<tr><td>1) Title</td><td colspan="2">Rearing technologies of zooplanktons for use as a red-tide control agent</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Akashiwo Research Institute of Kagawa Prefecture (Kagawa Pref., Japan)</td></tr>
<tr><td rowspan="3">4) Target species</td><th>Class</th><th>Genus and Species</th></tr>
<tr><td>Dinophyceae</td><td>Gyrodinium instriatum</td></tr>
<tr><td>Raphidophyceae</td><td>Chattonella angiqua, C. marina, Heterosigma akashiwo</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">1985 – 1987 (published year: 1986 – 1988)</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Lab experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Zooplankton grazing was considered as a possible red-tide control option.
➢ Tintinnid ciliates (Favella taraikaensis, F. ehrenbergii, Codonellopsis sp., Tintinopsis sp.) were collected from the sea, and were reared with phytoplanktons (Chattonella angiqua, C. marina, Heterosigma akashiwo, Gyrodinium instriatum etc.) in two types of container: 100-300 ml and 4,000 ml container. 20-60 zooplankton individuals were added per container. The phytoplankton concentration was set between 500-10,000 cells/mL.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Tintinnid ciliates F. taraikaensis, F. ehrenbergii and Codonellopsis sp. were possible to rear. Especially, rearing of F. taraikaensis and F. ehrenbergii were achieved successfully on a stable basis. On the other hand, rearing of Tintinopsis sp. was not possible.
➢ When F. taraikaensis was reared with 4 types of phytoplankton, the growth rate was highest when reared with Gyrodinium instriatum. Rearing of F. taraikaensis with 3 Raphidophyceae species (Chattonella angiqua, C. marina, Heterosigma akashiwo) was not successful, which suggests that Raphidophyceae species are not suitable food source for F. taraikaensis.
➢ The growth of F. taraikaensis was fastest when the cell density of Gyrodinium instriatum was set at 2,000 times (500-1,000 cells/mL) that of F. taraikaensis.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on the ecosystem
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ The feasibility of using zooplankton (tintinnid ciliates) as a red-tide control option was considered under the following assumptions.
Scale of the red tide: area of red tide = 1 km^2, depth range of red tide = 0-1 m, cell density of red tide = 4,000 cells/mL
Zooplankton density required for removing the red tide: 1/100 of red-tide density (40 individuals/mL)
Under the above assumptions, the required capacity (water volume) for rearing tintinnid ciliates was estimated at 10,000 tons, which was concluded as impractical for application.</td></tr>
</table>

12) References	➢ Akashiwo Research Institute of Kagawa Prefecture (1986): Report on the development of red-tide countermeasures Year 1985, Fisheries Agency. ➢ Akashiwo Research Institute of Kagawa Prefecture (1987): Report on the development of red-tide countermeasures Year 1986, Fisheries Agency. ➢ Akashiwo Research Institute of Kagawa Prefecture (1988): Report on the development of red-tide countermeasures Year 1987, Fisheries Agency.

No. : J-B-26

1) Title	The growth and grazing rate of tintinnid ciliates on the red-tide species *Heterocapsa circularisquama*	
2) Category	Biological control	
3) Implementing organization	Kamiyama, T. (Nansei National Fisheries Research Institute)	
4) Target species	**Class**	**Genus and Species**
	Dinophyceae	*Heterocapsa circularisquama* *Heterocapsa triquetra*
5) Implemented period	1996	
6) Experiment type	Lab experiment	
7) Application	No description	
8) Method / mechanism	➢ The growth and grazing rate of two tintinnid ciliate species *Favella azorica* and *F. taraikaensis* were examined, when mixed with red-tide species *Heterocapsa. circularisquama* and *H. triquetra*. The impact of tintinnid ciliate grazing on *H. circularisquama* bloom formation was then examined from the obtained results. ➢ Test samples of *H. circularisquama* were prepared at 5 different cell densities ($2.2 \times 10^2 \sim 1.6 \times 10^4$ cells/mL), and then *F. azorica* and *F. taraikaensis* were added into each samples at concentration of 1 individual / ml. After 24 hour incubation (water temperature: 20 °C, iluminance: 3 μE/m^2/s, photoperiod: 14 hr light, 10 hr dark, shaking: 1 rpm), the number of *F. azorica* and *F. taraikaensis* individuals were counted, and the cell density of *H. circularisquama* measured with a fluorometer.	
9) Results	➢ Both *F. azorica* and *F. taraikaensis* preyed on *H. circularisquam* or *H. triquetra*. ➢ When the initial *Heterocapsa* cell density was between 100-1000 cells/mL, the average doubling rate of *F. azorica* and *F. taraikaensis* were 2.13-2.15 and 1.92-1.97 doublings / day, respectively (Figure-1). ➢ When *H. circularisquam* cell density was above 10^4 cells/mL, all *F. taraikaensis* individuals died (Figure-1). ➢ The clearance rate of *F. azorica* and *F. taraikaensis* were 0.9-27.5 μL/ind/h and 0.3-22.1 μL/ind/h, respectively (Figure-2). ➢ The grazing rate of *F. azorica* and *F. taraikaensis* were 0.7-28.7 and 0.1-13.0 cells/ind/h, respectively (Figure-3).	
10) Impact on the environment / ecosystem	➢ No description	
11) Others	➢ At low *H. circularisquam* density, *F. azorica* and *F. taraikaensis* showed high level of feeding, which may imply that these species have a important role in controlling the initial phases of the *H. circularisquam* blooms. ➢ The relationship of *Favella* ingestion rate and *H. circularisquam* cell numbers were calculated (Figure-4). When the number of *Favella* individuals were set at 100-900 ind/L, and the cell density of *H. circularisquam* at 540 cells/mL, the clearance rate of *Favella* was calculated as 6-50% of the *H. circularisquam* cell number.	

12) References	➢ Kamiyama, T. (1996): Growth and grazing rate of tintinnid ciliates when *Heterocapsa circularisquama* was supplied as food, Report of Nansei National Fisheries Research Institute Year 1995, Fisheries Agency.

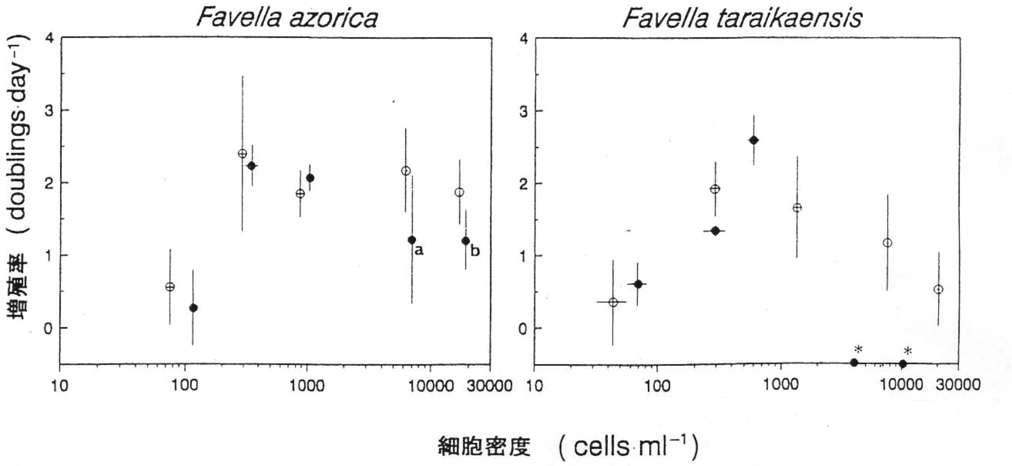


Fig.-1 The doubling rate of *F. azorica* and *F. taraikaensis*, when mixed with *H. circularisquama* and *H. triquetra*

Source：Kamiyama (1996)

Note: the asterisk shows that almost all *F. taraikaensis* individuals were dead after the experiment

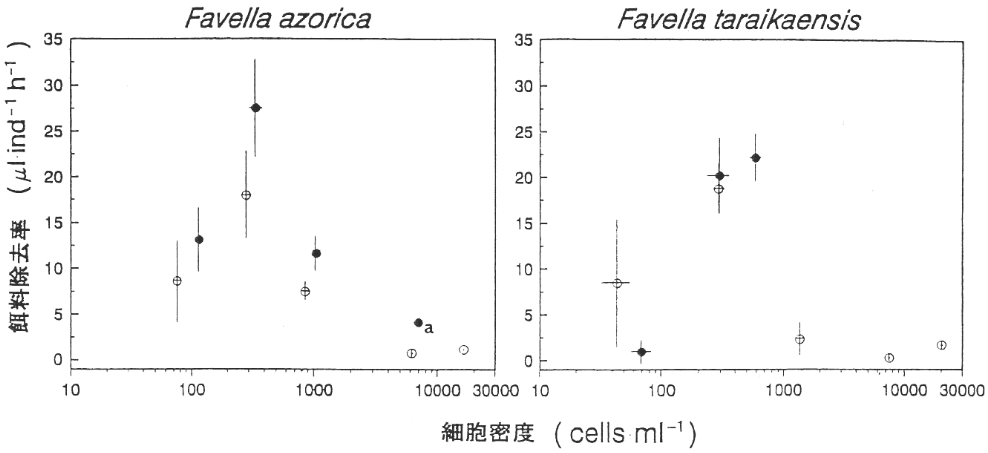


Fig.-2 The clearance rate of *F. azorica* and *F. taraikaensis*, when mixed with *H. circularisquama* and *H. triquetra*

Source：Kamiyama (1996)

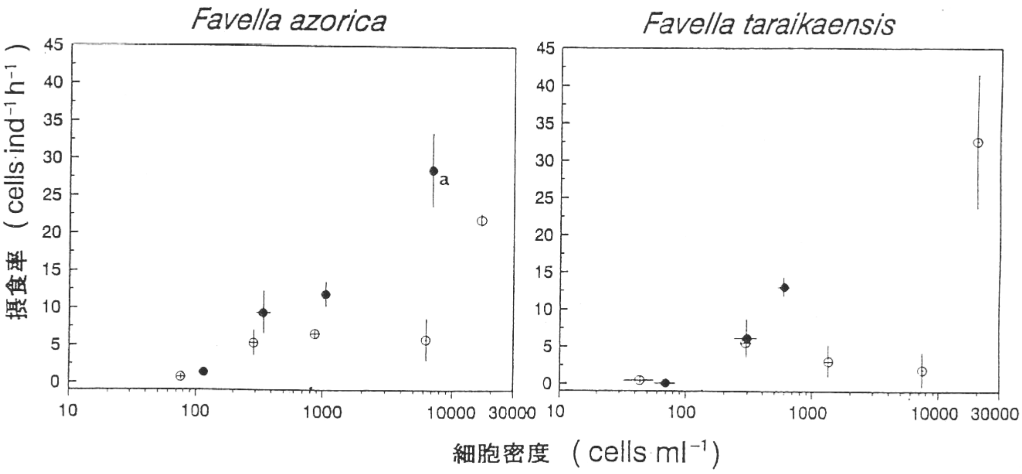


Fig.-3 The grazing rate of *F. azorica* and *F. taraikaensis*, when mixed with *H. circularisquama* and *H. triquetra*

Source：Kamiyama (1996)

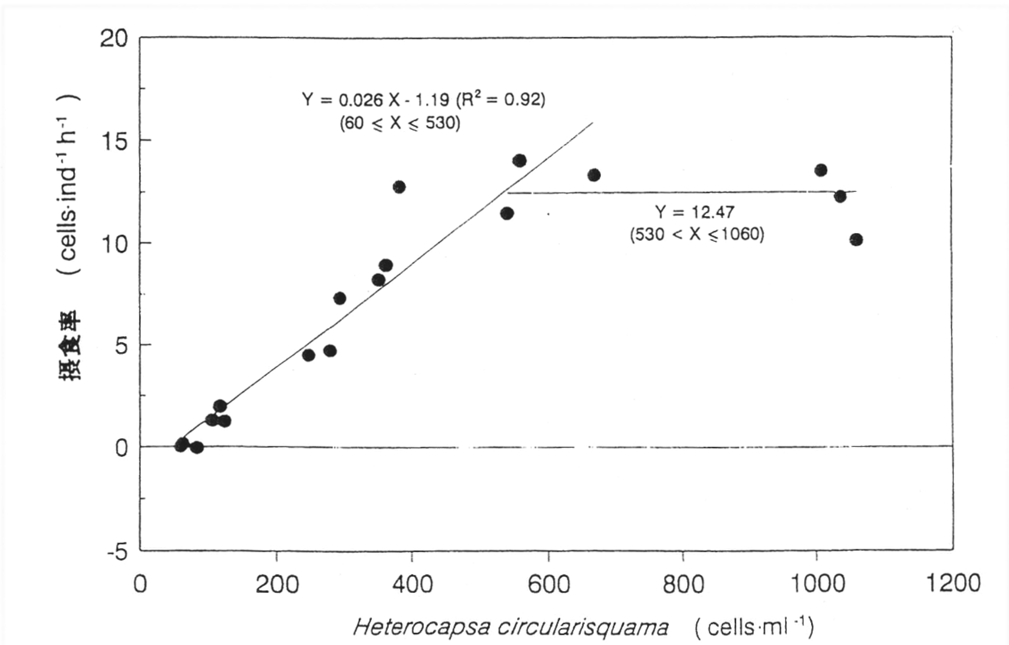


Fig.-4 The *Favella* grazing rate when the *H. circularisquam* cell numbers was in the order of 10^2 cells/mL

Source：Kamiyama (1996)

No. : J-B-27

1) Title	Grazing impact of the field ciliate assemblage on a bloom of the toxic dinoflagellate *Heterocapsa circularisquama*	
2) Category	Biological control	
3) Implementing organization	Takashi Kamiyama, Haruyoshi Takayama, Yoshinori Nishii, Takuji Uchida (National Research Institute of Fisheries and Environment of Inland Sea, Japan etc.)	
4) Target species	Class	Genus and Species
	Dinophyceae	*Heterocapsa circularisquama*
5) Implemented period	2001	
6) Experiment type	Field and lab experiment	
7) Application	No description	
8) Method / mechanism	➢ The ingestion rates of the toxic dinoflagellate *Heterocapsa circularisquama* by ciliate species were measured using the fluorescently labeled algae (FLA) method with the vital fluorescent dye CMFDA. ➢ Seawater samples were collected from the surface or 1-m layer at a coastal site in western Hiroshima Bay, the Seto Inland Sea, Japan. One liter of the seawater was poured into 1-liter polycarbonate bottles, and then the CMFDA-labeled *H. circularisquama* was added to the bottle to a final concentration of 6.4 to 7.8×10^2 cells/mL. After 10 and 30 min. of incubation, aliquot of the water was sampled from each incubated bottle and then fixed by 20% buffered formaldehyde. The fixed samples were settled in an Utermohl chamber and ciliates in the samples were observed with epifluorescence microscope. ➢ Ingestion rates for each ciliate species on *H. circularisquama* were calculated from the increase in the average number of ingested cells between 10 and 30 min of incubation. ➢ Field investigation was carried out when a bloom of *H. circularisquama* occurred in a part of the bay on 20 and 24 August 1998. Seawater samples were collected, and the abundance of *H. circularisquama,* ciliates and copepod nauplii were counted with a microscope. Then based on the species-specific ingestion rates and their abundances, the grazing impact of the ciliate assemblage on the *H. circularisquama* concentration was estimated.	
9) Results	➢ 16 species of tintinnid ciliates and 3 species of aloricate ciliates that can feed on *H. circularisquama* were recognized, and the mean ingestion rate of each species ranged from 0.2 to 14.5 cells/indv./h. ➢ The daily grazing loss by the ciliate assemblage ranged from 3 to 53% of the *H. circularisquama* population.	
10) Impact on the environment / ecosystem	(1) Impact on fish and shellfish ➢ No description (2) Impact on the environment ➢ No description	
11) Others	➢ This study indicated that information on grazer ciliates is important to improve the prediction model for the outbreak of *H. circularisquama* red tides.	

12) References	➢ Takashi Kamiyama, Haruyoshi Takayama, Yoshinori Nishii, Takuji Uchida (2001): Grazing impact of the field ciliate assemblage on a bloom of the toxic dinoflagellate *Heterocapsa circularisquama*, Plankton Biol. Ecol., Vol. 48(1), 10-18.

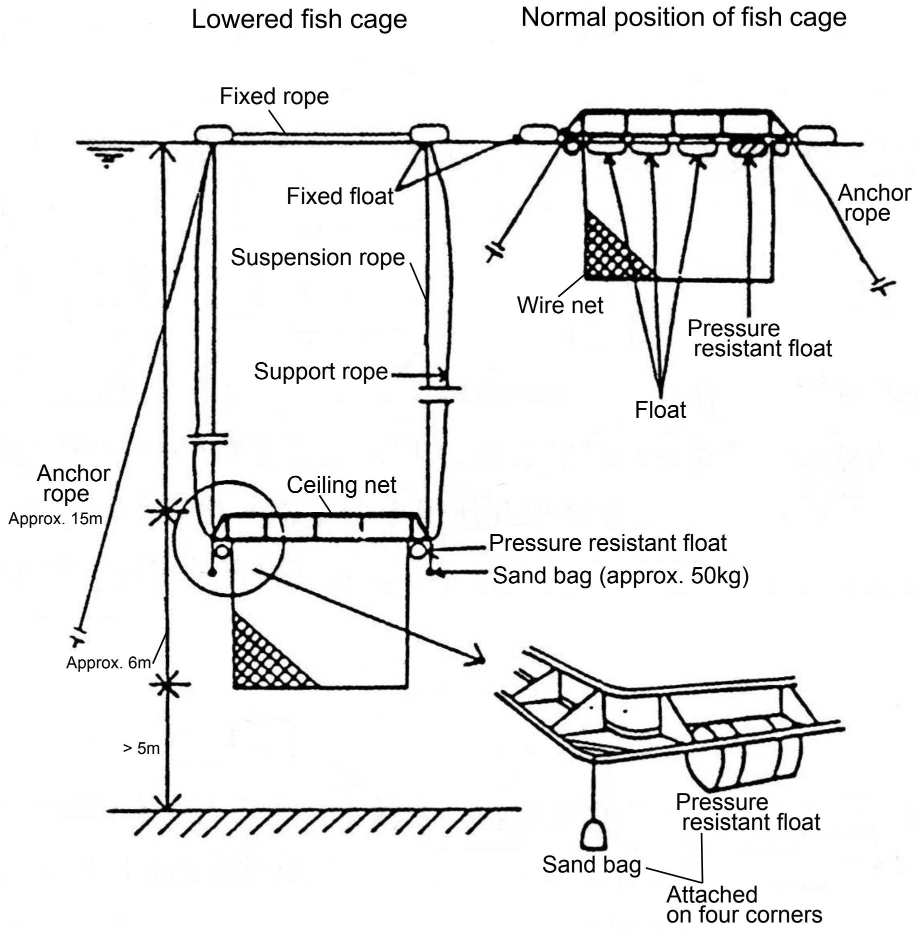
No. : J-B-28

<table>
<tr><td>1) Title</td><td colspan="2">Temporal changes in the ciliate assemblage and consecutive estimates of their grazing effect during the course of a Heterocapsa circularisquama bloom</td></tr>
<tr><td>2) Category</td><td colspan="2">Biological control</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Takashi Kamiyama and Yukihiko Matsuyama (Tohoku National Fisheries Research Institute, Japan etc.)</td></tr>
<tr><td rowspan="2">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td>Dinophyceae</td><td>Heterocapsa circularisquama</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">2005</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Field experiment</td></tr>
<tr><td>7) Application</td><td colspan="2">No description</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">➢ Temporal changes in ciliate assemblages during the course of a bloom the harmful microalga Heterocapsa circularisquama were investigated and consecutive estimates of species-specific maximum grazing losses were analyzed from August to September 1998 at a site in western Hiroshima Bay, the Seto Inland Sea of Japan.
➢ Seawater samplings were carried out at a fixed station in Hiroshima Bay from 22 August, 1998 to 20 September, 1998, during the course of a H. circularisquama bloom. The abundance of phytoplankton, ciliates and metazoans were counted with a Sedgwick-Rafter chamber under a microscope.
➢ The grazing loss of H. circularisquama by ciliates was estimated.</td></tr>
<tr><td>9) Results</td><td colspan="2">➢ Temporal increases of the H. circularisquama mean concentration in the water column were observed twice (25-29 August and 7-10 September) with the maximum concentration (ca. 4000 cells/mL) being recorded on 25 August. The main ciliate genera during the bloom were Favella, Tontonia, Eutintinnus, Tintinnopsis and Amphorellopsis. Increases of Favella and Tontonia were observed when the concentration of H. circularisquama ranged from 260 to 1170 cells /mL.
➢ The total maximum grazing loss ranged from 1 to 75% standing stock removed per day of the H. circularisquama concentration. High grazing losses mainly due to the genera Favella and Tontonia occurred during the period when the H. circularisquama concentration was decreasing.</td></tr>
<tr><td>10) Impact on the environment / ecosystem</td><td colspan="2">(1) Impact on fish and shellfish
➢ No description
(2) Impact on the environment
➢ No description</td></tr>
<tr><td>11) Others</td><td colspan="2">➢ The results suggest that grazing by ciliate assemblages can influence the population dynamics of H. circularisquama despite the potentially toxic nature of the phytoplankter.</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Takashi Kamiyama and Yukihiko Matsuyama (2005): Temporal changes in the ciliate assemblage and consective estimates of their grazing effect during the course of a Heterocapsa circularisquama bloom, Journal of Plankton Research, Vol. 27(4), 303-311.</td></tr>
</table>

Avoidance measure:
Submersion of fish cages:

No. : J-O-1

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

Conuntermeasures against HABs in Korea

List of Countermeasures against HABs in Korea

Study No.	Category	Methods	Title	Implementing organization (author)
K-P-1	Physical Control	Clay dispersal	Direct control of using residual clays	Local municipal authorities disperse the clays based on the red tide alert issued by NFRDI
K-P-2	Physical Control	Centrifugal separation	Centrifugal separation equipment	Korean Ocean Research and Development Institute (KORDI) responsible for this works, and fish farmers can installed in their fish culture farm especially land-based container
K-O-1	Avoidance measure	Perimeter skert or shield curtain	Perimeter skirt or shield curtain	Fish farmers can install in their fish culture farm
K-O-2	Others	Red tide removal system	Automated HAB warning and oxygen supplying system	Aquaculturists, and the government give subsidiary financial aids to the fish farmers who want to install this system in their fish culture farm
**	Indirect measure	**	The monitoring and prediction of HABs	National Fisheries Research & Development Institute (NFRDI) responsible for this works, and Regional Maritime Affairs and Fisheries Office (RMAFO) collect HABs data and information
**	Indirect measure	**	The bioassay monitoring for PSP, DSP, and ASP	National Fisheries Research & Development Institute (NFRDI)

Physical control:
Clays:

No.: K-P-1

1) Title	Direct control of using residual clays	
2) Category	The residual yellowish clays scavenge dinoflagellates from seawater and carry them to bottom sediments.	
3) Implementing organization	Local municipal authorities disperse the clays based on the red tide alert issued by NFRDI	
4) Target species	Class	Genus and Species
	Dinophyceae	*Cochlodinium polykirkoides*
5) Implemented period	When the density of *C. polykrikoides* exceeds 300 cells/mL i.e., from red tide alert to the warning lift on the early warning system in Korea	
6) Experiment type	Already taken laboratory test and field experiment to assess the removal rate and impacts on living animals and marine environment	
7) Application	Disperse clays directly over pen cages accommodating cultured fish using clay dispersing gun installed in HABs mitigation vessel and fishing vessels	
8) Method / mechanism	Clay minerals be powdered to a particle size of less than 50μm, and dispersed at concentrations of 100-400g/m^2 by mixing with seawater at mid-day (because *the C. polykrikoides* cells migrate to subsurface layers in mid-day). Taking into account the diffusion and sinking rate of clay minerals, the surface area of clay dispersion at fish cages would be about three times that of the area of the cages in order to protect fish staying at the bottom of the cage in mid-day. The interval for dispersion time is 30-40 minutes taking into account the sinking rate of clay and 10m depth of the fish pens. The clay is dispersed in the tidal currents so that it drifts in the direction of the fish farm. If HABs are already inside of the fish cages, clay suspensions are dispersed in a "merry go-round" fashion. Acknowledging that the higher the density of *C. polykrikoides*, the better the removal efficiency of the clay, the local government recommends dispersing the clay when the density exceeds 1,000cells/ml, the level of a "Red Tide Alert", taking into account the expenses and manpower for clay dispersion.	
9) Results	Clay is one of the promising agents for HAB mitigation and control especially in Korea where the culture fish is provided as raw fish, if its environmental effects are minimized.	
10) Impact on environment / ecosystem	Assessing the toxicity of yellow clay on fish and shellfish including abalone and flatfish, there were no significant impacts at a clay concentration of 20g/l within 24hours (NFRDI, 1999). A five-year survey of benthic fauna at the clay dispersal site near Tongyong, Korea, where clay has been distributed every year since 1996, showed no changes in the species composition, diversity and abundance of benthos (NFRDI, 1999).	
11) Others	The price of one set including application ship, seawater electrolyzing system, shooting gun capable of dispersing 5ton of clay waters per minute is about 210,000 US$	
12) References	➢ NFRDI, 2002. The impacts of red tide and its mitigation techniques (in Korean), 23pp. ➢ Kim, 2006. Mitigation and controls of HABs, 327-338. In :Ecology of Harmful Algae, Edna Granéli, J.T. Turner (Eds,). Springer.413pp. ➢ Kim et al., 1999. Management and mitigation techniques to minimize the impacts of HABs. 527pp.	

Centrifugal separation:

No.: K-P-2

<table>
<tr><td>1) Title</td><td colspan="2">Centrifugal separation equipment</td></tr>
<tr><td>2) Category</td><td colspan="2">Remove the dinoflagellates cells from the pumping seawater by centrifugal force.</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Korean Ocean Research and Development Institute (KORDI) responsible for this works, and fish farmers can installed in their fish culture farm especially land-based container</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td rowspan="3">Dinophyceae</td><td>Cochlodinium polykirkoides</td></tr>
<tr><td>Karenia mikimotoi</td></tr>
<tr><td>Gyrodinium sp.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">In C. polykrikoides bloom season generally from July to September</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Collect field observation data and information</td></tr>
<tr><td>7) Application</td><td colspan="2">Applied in the land-based tank for fish culture</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">Direct remove the dinoflagellates cells from the pumping seawater by centrifugal force and supply the treated seawater free of dinoflagellates to fish containers in the land-based tank</td></tr>
<tr><td>9) Results</td><td colspan="2">Can available at a small scale fish farm in the land</td></tr>
<tr><td>10) Impact on environment / ecosystem</td><td colspan="2">No impact if the treated supernatants were not input to the tank or coastal waters</td></tr>
<tr><td>11) Others</td><td colspan="2">The price is about 21,000US$ for a small scale aquaculture yard</td></tr>
<tr><td>12) References</td><td colspan="2"></td></tr>
</table>

Avoidance measure:
Perimeter skert or shield curtain:

No.: K-O-1

<table>
<tr><td>1) Title</td><td colspan="2">Perimeter skirt or shield curtain</td></tr>
<tr><td>2) Category</td><td colspan="2">Wrap up fish cages so as not to allow the fish killing dinoflagellates entering inside of the fish tank.</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">Fish farmers can install in their fish culture farm</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td rowspan="3">Dinophyceae</td><td>Cochlodinium polykirkoides</td></tr>
<tr><td>Karenia mikimotoi</td></tr>
<tr><td>Gyrodinium sp.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">In C. polykrikoides bloom season generally from July to September</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Wrap up fish cages accommodating culture fish inside, and asses how many days they can survive with or without air supply</td></tr>
<tr><td>7) Application</td><td colspan="2">The pilot experiment was done on the field fish cages</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">Enclosed the fish cages by perimeter skirt or shield curtain designed to protect the entrance of fish-killing dinoflagellates into the fish cages</td></tr>
<tr><td>9) Results</td><td colspan="2">Can available at a small scale fish cages for a short period</td></tr>
<tr><td>10) Impact on environment / ecosystem</td><td colspan="2">This system cause no impact on the culture animals and surrounding environment</td></tr>
<tr><td>11) Others</td><td colspan="2">The price of one perimeter skirt for one fish cage is about 8,500 US$</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Kim, 2006. Mitigation and controls of HABs, 327-338. In :Ecology of Harmful Algae, Edna Granéli, J.T. Turner (Eds,). Springer.413pp.
➢ Kim et al., 1999. Management and mitigation techniques to minimize the impacts of HABs. 527pp.</td></tr>
</table>

Others:
Red tide removal system:

No.: K-O-2

1) Title	Automated HAB warning and oxygen supplying system	
2) Category	Take warning the managers of approaching fish-killing dinoflagellates blooms, and let them to take emergent actions to protect culture animals	
3) Implementing organization	Aquaculturists, and the government give subsidiary financial aids to the fish farmers who want to install this system in their fish culture farm	
4) Target species	Class	Genus and Species
	Dinophyceae	*Cochlodinium polykirkoides*
		Karenia mikimotoi
		Gyrodinium sp.
5) Implemented period	Should be installed before the HABs season, and operate in *C. polykrikoides* bloom season generally from July to September	
6) Experiment type	Laboratory and then field experiment to check the sensitivity and safety	
7) Application	Available at the land-based fish culture	
8) Method / mechanism	Count the density of fish-killing dinoflagellates, and alarm the manager to take emergent actions such as stop pumping water and supply liquefied oxygen to fish container automatically in case of high density enough to kill fish. The alarm can be send to manager through cellular phone.	
9) Results	Can widely available at a small scale fish cages for a short period	
10) Impact on environment / ecosystem	This system cause no impact on the culture animals and surrounding environment	
11) Others	The price of the full set of this system is about 8,500 US$	
12) References	➢ NFRDI, 2002. The impacts of red tide and its mitigation techniques (in Korean), 23pp. ➢ Kim, 2006. Mitigation and controls of HABs, 327-338. In :Ecology of Harmful Algae, Edna Granéli, J.T. Turner (Eds,). Springer.413pp. ➢ Kim et al., 1999. Management and mitigation techniques to minimize the impacts of HABs. 527pp.	

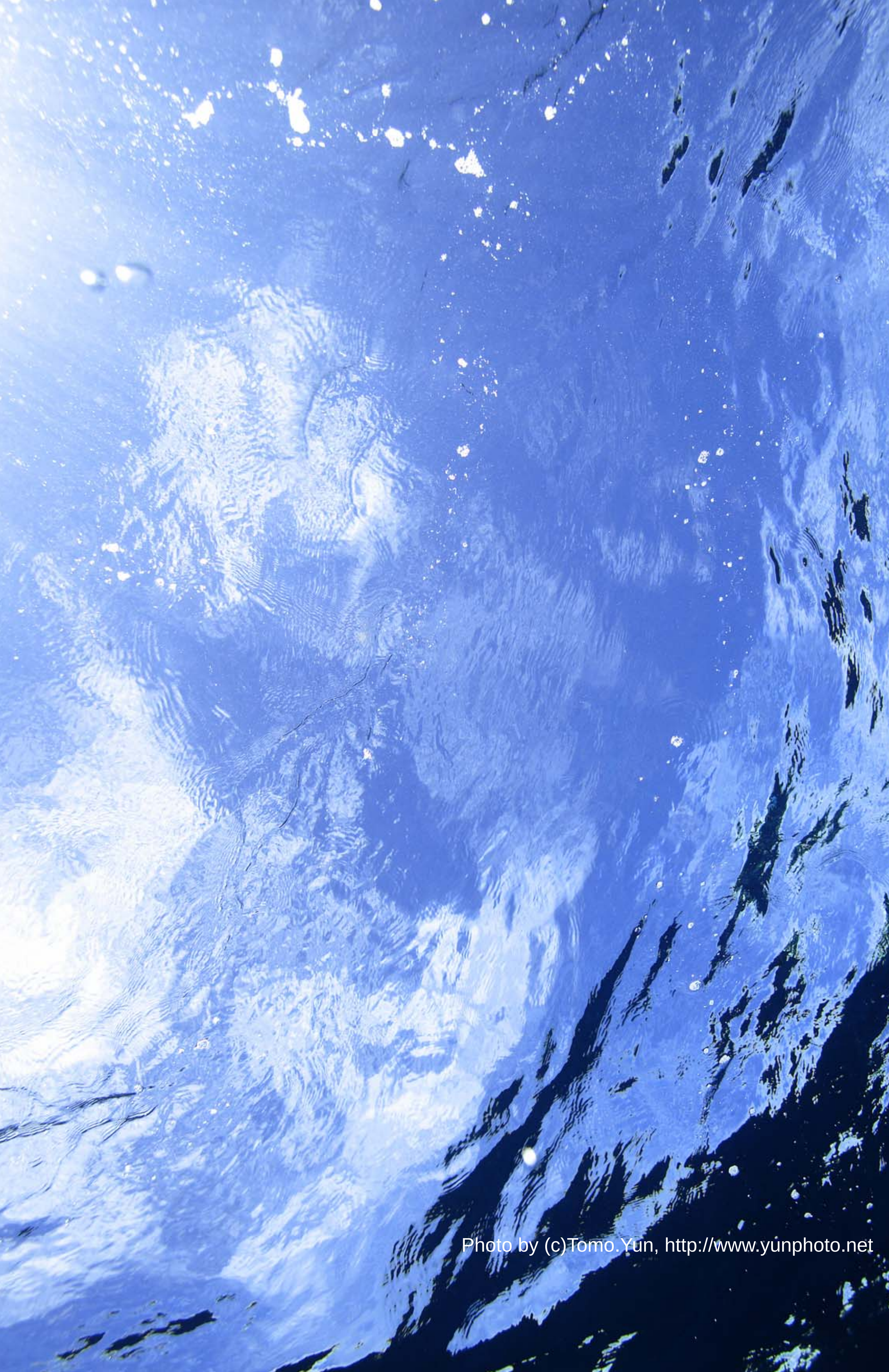
Indirect measure:

No.1:

<table>
<tr><td>1) Title</td><td colspan="2">The monitoring and prediction of HABs</td></tr>
<tr><td>2) Category</td><td colspan="2">The role of monitoring is to detect HABs and their associated toxins in algae or fish and shellfish. Prediction involves more scientific approaches based on the oceanography and ecology. Accurate forecasting of the timing and transport pathway of HABs can help fish farmers and other affected parties to take emergency actions.</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">National Fisheries Research & Development Institute (NFRDI) responsible for this works, and Regional Maritime Affairs and Fisheries Office (RMAFO) collect HABs data and information</td></tr>
<tr><td rowspan="4">4) Target species</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td rowspan="3">Dinophyceae</td><td>Cochlodinium polykirkoides</td></tr>
<tr><td>Karenia mikimotoi</td></tr>
<tr><td>Gyrodinium sp.</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">➢ Normal monitoring from March to December
➢ Special monitoring for C. polykirkoides bloom
· Initiative monitoring : June to detect C. polykirkoides
· Emergent monitoring : Red tide alert to the warning lift</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">Collect field observation data and information and announcement</td></tr>
<tr><td>7) Application</td><td colspan="2">Precautionary prevention and direct control of the blooms</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">The identification of target species, determination of toxins, understanding oceanographic properties underlying population dynamics, and analysis of environmental and meteorological changes to build integrated prediction models.</td></tr>
<tr><td>9) Results</td><td colspan="2">All stakeholders make use of them for mitigation and public health</td></tr>
<tr><td>10) Impact on environment / ecosystem</td><td colspan="2">No description</td></tr>
<tr><td>11) Others</td><td colspan="2">Satellite image of SST and chlorophyll are available for prediction of HABs</td></tr>
<tr><td>12) References</td><td colspan="2">➢ Park et al., 198. Manual of methods for research and monitoring of marine pollution and red tide. NFRDI.297pp.
➢ UNESCO/IOC, 2003. Manual on Harmful Marine Microalgae" - Monographs on Oceanographic Methodology in 2003, 793pp.</td></tr>
</table>

No.2:

<table>
<tr><td>1) Title</td><td colspan="2">The bioassay monitoring for PSP, DSP, and ASP</td></tr>
<tr><td>2) Category</td><td colspan="2">This monitoring is to detect paralytic, diarrhetic and amnesic algal toxins</td></tr>
<tr><td>3) Implementing organization</td><td colspan="2">National Fisheries Research & Development Institute (NFRDI)</td></tr>
<tr><td rowspan="6">4) Target species and toxins</td><td>Class</td><td>Genus and Species</td></tr>
<tr><td rowspan="3">Dinophyceae</td><td>Alexandrium tamarense</td></tr>
<tr><td>Gymnodinium catenatum</td></tr>
<tr><td>Dinophysis acuminata</td></tr>
<tr><td>Bacillariophyceae</td><td>Pseudonitzschia pungens</td></tr>
<tr><td>Toxins</td><td>PSP, DSP, ASP</td></tr>
<tr><td>5) Implemented period</td><td colspan="2">➢ PSP monitoring : March to May since 1980
➢ DSP & ASP monitoring : sporadic since 1995</td></tr>
<tr><td>6) Experiment type</td><td colspan="2">➢ Covering regions : the south and west coast of Korea
➢ Frequency of shellfish toxin
Once a month : All the year round
Every week : Toxic season (Usually Mar. to May)
➢ Monitoring target shellfish species
Blue mussel(Mytilus edulis), oyster (Crassostrea gigas), ark-shell (Scapharca broughtonii), short necked clam (Ruditapes philippinarum) and etc.</td></tr>
<tr><td>7) Application</td><td colspan="2">Aquaculture and wildlife animals in the suspected areas</td></tr>
<tr><td>8) Method / mechanism</td><td colspan="2">Regular shellfish monitoring PSP and DSP using mouse bioassay and HPLC, and ASP using HPLC.</td></tr>
<tr><td>9) Results</td><td colspan="2">The government bans the harvesting and marketing the suspected shellfish when the PSP level exceeds the standard 80 μ g/100g.</td></tr>
<tr><td>10) Impact on environment / ecosystem</td><td colspan="2">No description</td></tr>
<tr><td>11) Others</td><td colspan="2"></td></tr>
<tr><td>12) References</td><td colspan="2">➢ UNESCO/IOC, 2003. Manual on Harmful Marine Microalgae" - Monographs on Oceanographic Methodology in 2003, 793pp.</td></tr>
</table>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Booklet of Countermeasures against Harmful Algal Blooms in the NOWPAP Region

NOWPAP CEARAC