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NOWPAP CEARAC 2007: Booklet of countermeasures against Harmful Algal Blooms (HABs) in the NOWPAP Region.

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.

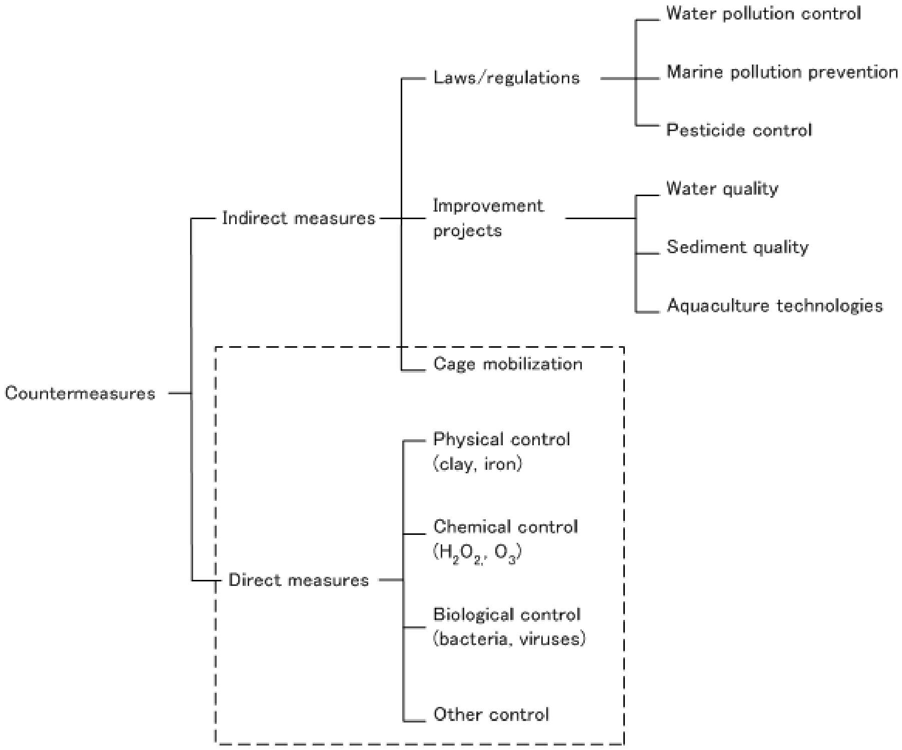

Figure 1 Classification of red-tide countermeasures

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).

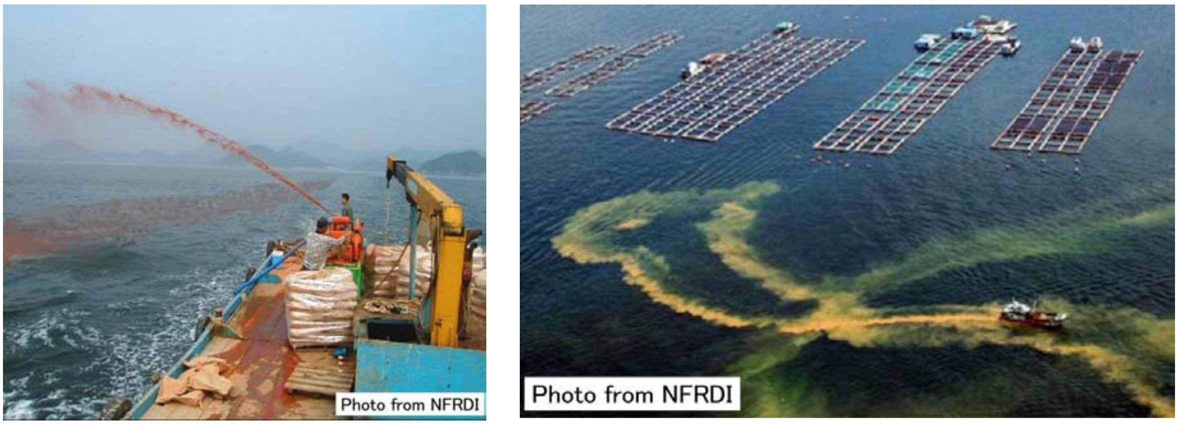

Photo from NFRDI

Photo from NFRDI

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.

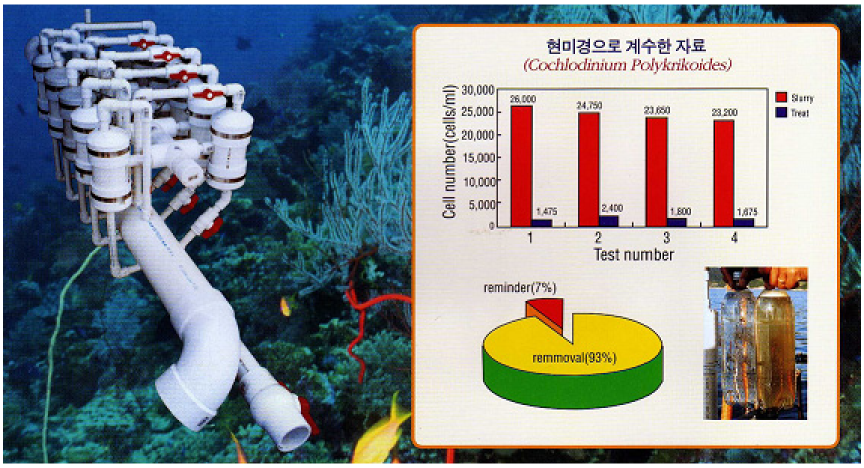

Figure 3 Centrifugal separation system

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 $\mu W/cm^2$ 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)

<u>Herbicides</u>

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-

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2.2.4 Avoidance measures

Submersion of fish cages

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.

Perimeter skirt or shield curtain

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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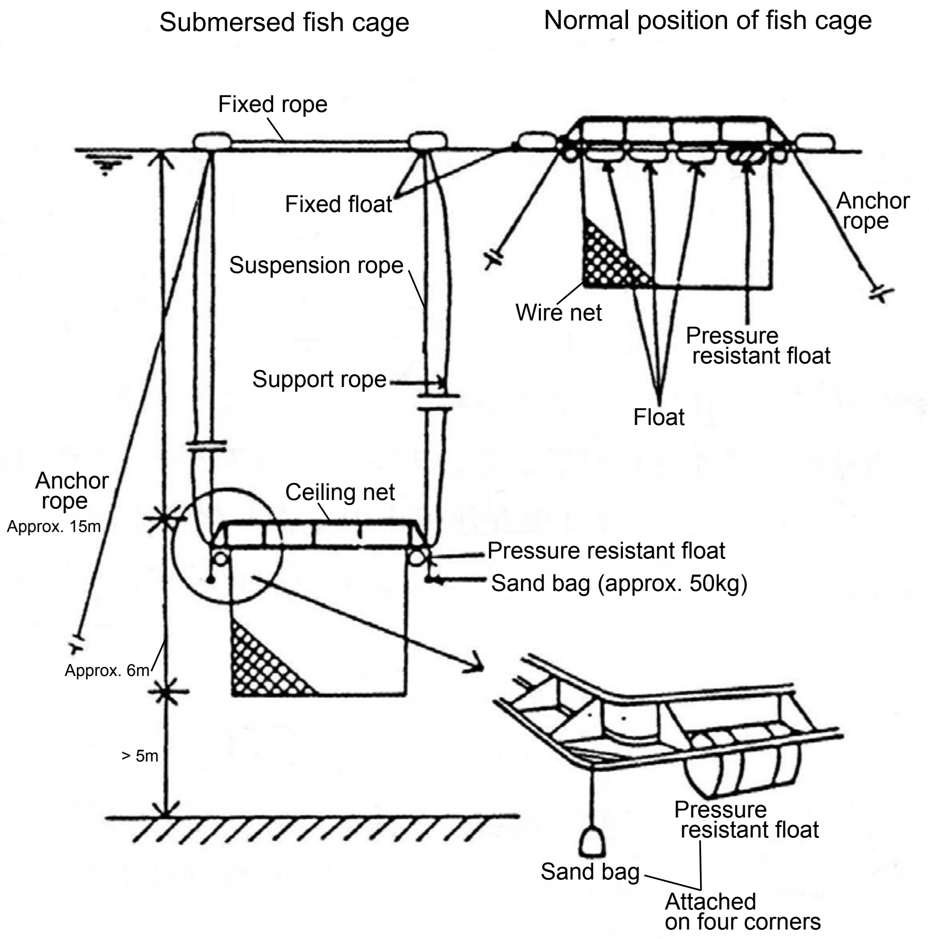

Figure 4 Schematic diagram of a fish-cage submersion system

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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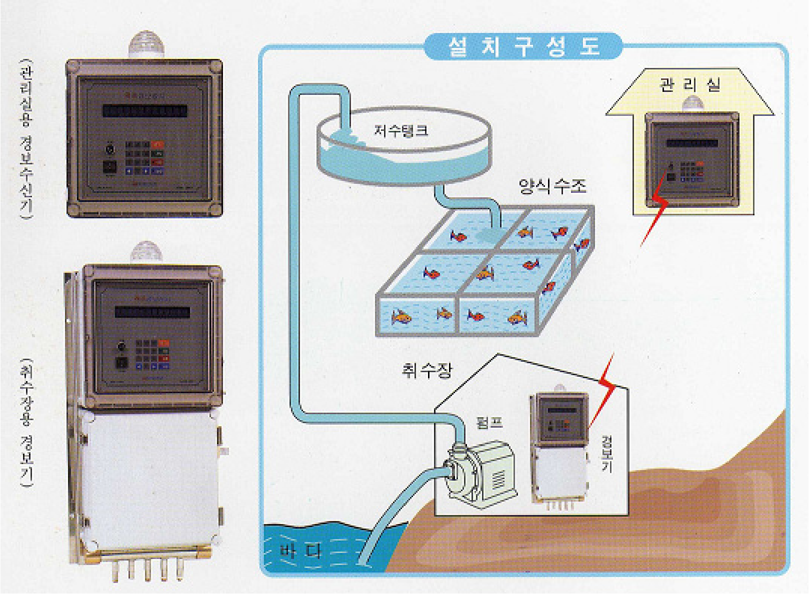

Figure 6 Automated HAB warning and oxygen supplying system for land-based fish tanks

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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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