Biological Hazard Possibly Produced by Aquaculture and Its Control

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Abstract

Blooms of *Heterocapsa circularisquama,* a novel dinoflagellate, have been causing mass mortality of both wild and cultured shellfish in embayments at the western part of Japan since 1988. Physiological and epidemiological studies suggest that the alga has been partly dispersed with the movement of shellfish in aquaculture activities.

A recent outbreak of an epizootic iridovirus in red sea bream (*Pagrus major*) has caused extensive damage to marine fish culture in Japan. A research group at the National Research Institute of Aquaculture (NRIA), collaborating with prefectural fisheries research laboratories and an R&D company, clarified the etiology and developed a diagnostic method and a commercial vaccine.

Penaeid acute viremia (PAV), a synonym of white spot syndrome, caused catastrophic loses in kuruma shrimp (*Penaeus japonicus*) culture in Japan. An epidemiological study of the research group at NRIA and the prefectural fisheries research laboratories strongly suggests that the causative virus was newly introduced to Japan from imported shrimp seeds for aquaculture. The group clarified the etiology and established diagnostic methods. Based on their studies, NRIA proposed a protocol to check the virus during larval culture and before seedlings are shipped.

Introduction

Aquaculture in Japan has encountered various severe biological and environmental damages such as red tide, diseases, and environmental pollution over the last decade. In the present article, three events in fisheries (red tide from a novel algae and new diseases), which were possibly produced or enhanced by aquaculture activities, were examined to estimate the biological impacts of aquaculture. The paper also presents how and what measures were developed to control them.

Red Tide of *Heterocapsa circularisquama*

Three types of harmful and toxic algae, which are important to fisheries in Japan, are known. The first type is especially harmful to fish. *Gymnodinium mikimotoi, Heterosigma akashiwo*, and *Chattonella antiqua* belong to this type. Red tides caused by these algae often result in mass mortality among cultured fish stocks and has been a major concern from the point of fisheries. The second type of algae does not kill shellfish species but are generally toxic to other aquatic species. The potent toxins produced are accumulated in shellfishes, which find their way through the food chain, ending in humans to cause a variety of food poisoning symptoms such as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), and neurotoxic shellfish poisoning etc. In Japan, *Allexandrium*

tamarense and *A. catenella* cause PSP and *Dinophysis fortii* causes DSP. Recently, a novel dinoflagellate, *Heterocapsa circularisquama,* which was first found in 1995 in Japan (Horiguchi, 1995), has been causing mass mortality in bivalves and has been demonstrated to be harmful also to gastropods. However, its harmful effects in fish and crustaceans, and in human public health has never been reported (Matsuyama, 1999).

A red tide bloom of *H. circularisquama* first occurred in Shikoku Island in 1988 and since then the occurrence of the red tide has expanded to all over the western part of Japan for the last decade (Fig. 1). The blooms have caused destructive mass mortality of both wild and cultured bivalves such as short-necked clam, Pacific oysters, and pearl oysters. In those places where the red tide of *H. circularisquama* once occurred, a series of blooms of the same alga often followed thereafter, suggesting that the alga had settled down in these areas. These places are semi-closed bays or rather calm areas, and the places are generally utilized for some kind of aquaculture including pearl oyster culture.

Honjo et al. (1998) examined the potential for accidental transfer of *H. circularisquama* via consignment of pearl oyster for aquaculture purpose. They experimentally exposed pearl oysters to *H. circularisquama* and then placed each oyster in a dry beaker and checked the state of algae thereafter. They found that the motile algae decreased with time but that some cells remained motile both inside and on the surface of the shell even 24 hr after the transfer to the dry beaker (Fig. 2). Moreover, they found that the most immotile cells became motile again in several days of culture in fresh culture medium. From these results, the authors suggested the possibility of transport of the algae with pearl oyster from an alga abundant area to new places by aquaculture activities.

Red Sea Bream Iridovirus (RSIV)

A new epizootic, which caused high mortalities of 20 to about 60% among cultured red sea

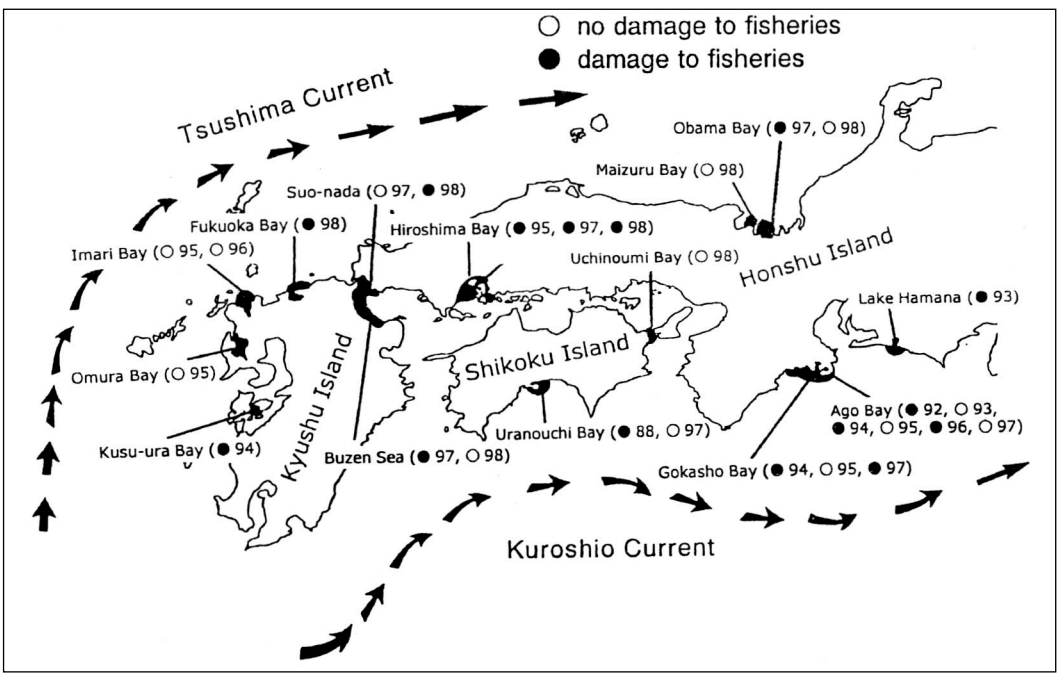

Figure 1. **Occurrence of red tide from *Heterocapsa circularisquama* blooms in Japan (Matsuyama, 1999)**

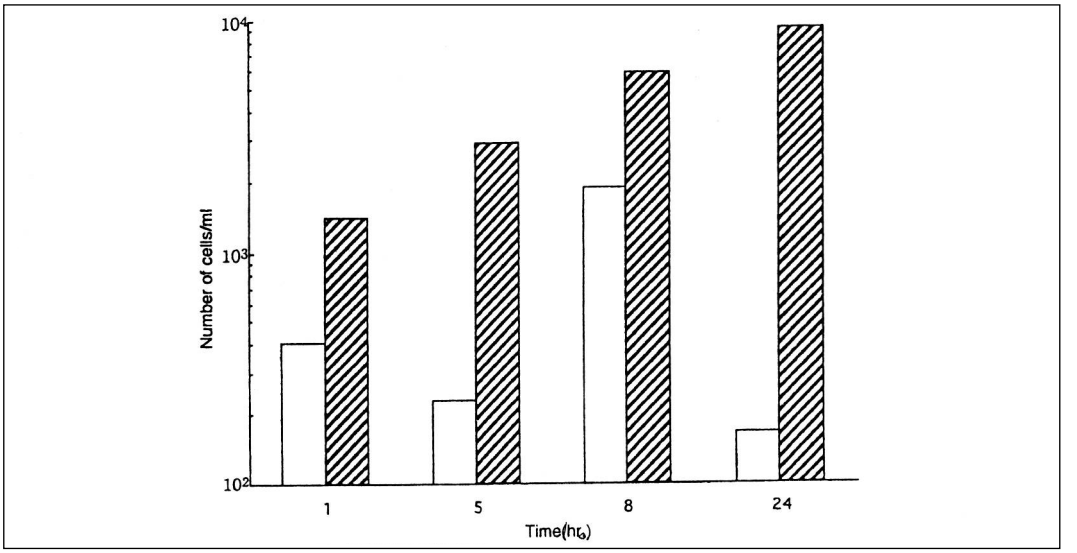

Figure 2. **Changes in the number of motile and immotile *H. circularisquama* in drip water from pearl oyster after the transfer of oysters, which were experimentally exposed to the algae, to individual dry beaker. Open bars denote motile cell number and hatched bars immotile cell number (Honjo *et al.*, 1998)**

bream *Pagrus major* in Shikoku Island, Japan in 1990, has been reported. The diseased fish became sluggish and showed severe anemia and petechiae of the gill. Histology revealed typically enlarged cells with basophillic stainability in the spleen, heart, kidney, liver, and gills. From localization and morphology of the cells, these enlarged basophilic cells were considered leucocytes (Inouye *et al.*, 1992). Electron micrograph showed a number of hexagonal virions in the cytoplasm of these enlarged cells. The virion measured 200~240nm in diameter and with a dense core and electron translucent zone. A cytopathic effect was produced on various fish cell lines by inoculation of the filtrate (450nm) of spleen homogenate of the diseased fish. Intraperitoneal inoculation of the filtrate (450nm) of both the spleen of the infected fish and the replicated virus on cell culture induced pathological changes similar to those of spontaneously diseased fish. These results indicate that the virus caused the disease. The morphology and physico-chemical properties of the virus as well as pathogenesis to various fish species indicate that the virus is new a one belonging to iridoviridae (Inouye *et al.*, 1992; Nakajima and Sorimachi, 1994).

Table 1. **Occurrence of red sea bream iridovirus disease in various prefectures and number of infected fish species in Japan (Matsuoka *et al.*, 1996)**

Year	Number of prefectures	Number of infected fish species
1990	1	1
1991	12	7
1992	15	8
1993	10	7
1994	14	15
1995	17	17
Total	17	20

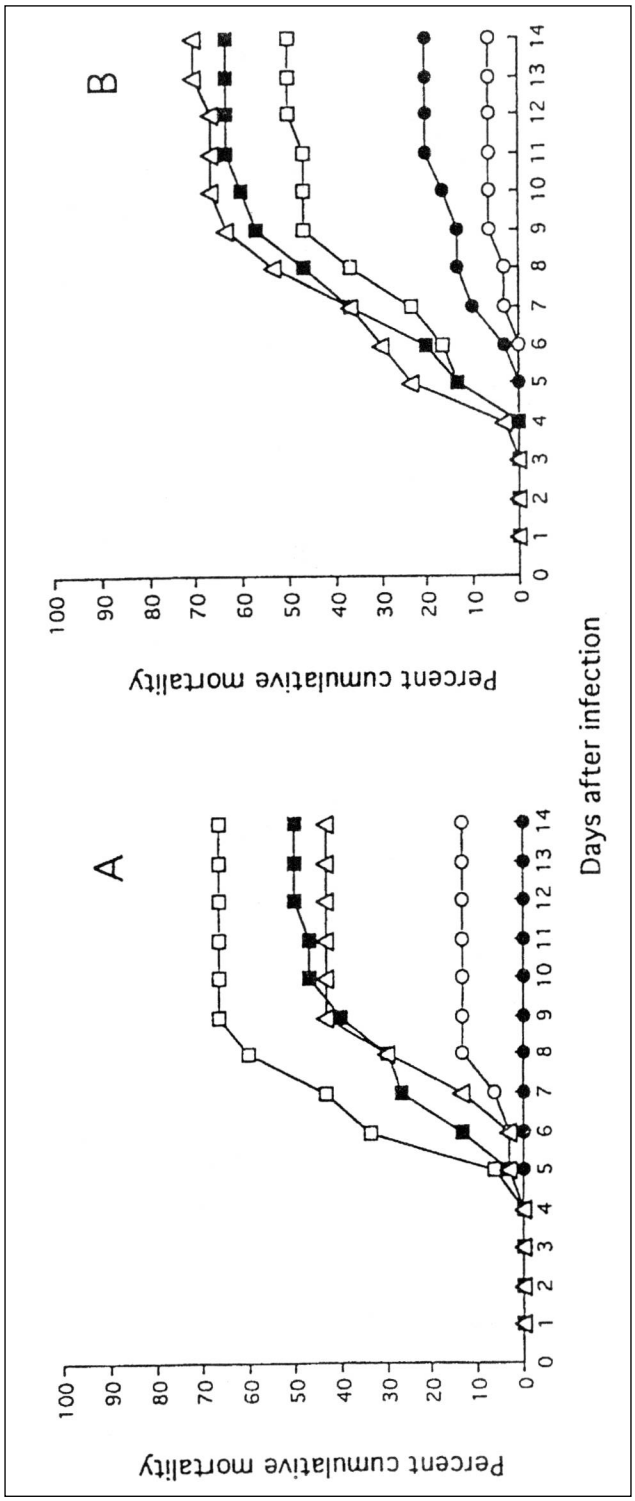

Figure 3. **Mortality of vaccinated juvenile red sea bream challenged with different doses of RSIV. Experimental fish were vaccinated by intraperitoneal injection of either formalin inactivated RSIV-infected GF cells (•), formalin inactivated cell culture supernatant of RSIV-infected GF cells (•œ), formalin inactivated non-infected GF cells (•) or formalin inactivated cell culture supernatant of non-infected GF cell (•¡). One group of fish (•¢) received no injection. Ten days after vaccination all the fish were challenged with RSIV at doses of $10^{3.5}$ $TCID_{50}$/0.1ml (A) or $10^{4.5}$ $TCID_{50}$/0.1ml (B) (Nakajima *et al.*, 1997)**

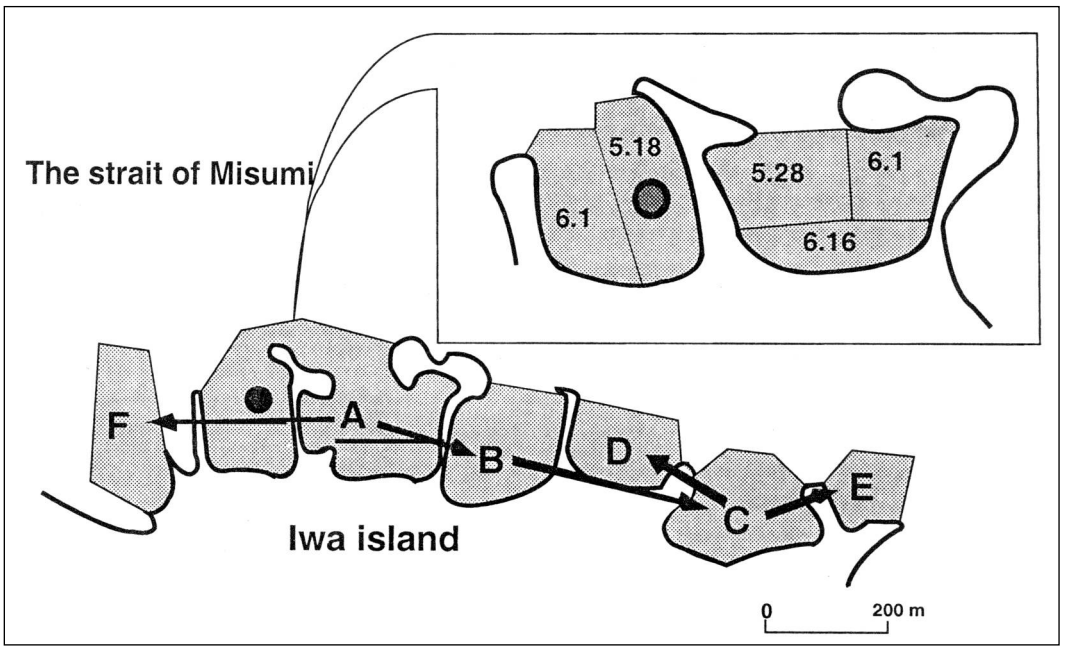

Figure 4. **Spread of mass mortality of kuruma shrimp due to PAV at Iwa Island in Kumamoto prefecture. The pond marked by the circle contained seedling shrimps from China introduced one month before the occurrence of the mass mortality. The arrows indicate the possible transmission route of the disease (Nakano *et al.*, 1994)**

The disease spread rapidly over farmed areas and among cultured fish species (Table 1). Disease occurrence during the early period was associated with red sea bream fingerlings produced by a specific hatchery, suggesting that the aquaculture activity may have spread the disease. The transfer of the disease from red sea bream to other species was also possibly a result of horizontal transmission in the aquaculture area. NRIA therefore organized a research group and collaborated with prefectural fisheries research laboratories. First, the group developed a presumptive diagnostic method using Giemsa staining on an imprint of the spleen (Inouye *et al.*, 1992). Then, they developed a monoclonal antibody against this virus (Nakajima and Sorimachi, 1995), and utilized this antibody to develop a rapid and confirmative diagnostic method using an immuno-fluorescence test (Nakajima *et al.*, 1995). The diagnostic method was then transferred to prefectural fisheries research laboratories through training programs. The research group of NRIA also developed a vaccine against the disease (Nakajima *et al.*, 1997). They found that vaccination of both formalin-inactivated RSIV infected GF cells and formalin-inactivated cell culture supernatant of RSIV-infected GF cells effectively increased the survival rate of the artificially infected fish (Fig. 3). In collaboration with an research and development company, the vaccine utilizing inactivated cell culture medium became commercially available since 1999.

Penaeid Acute Viremia

In 1993, a new epizootic disease causing high mortalities of more than 80% occurred among cultured kuruma shrimp in several prefectures in Japan. White spot on the surface skeleton and abnormal red coloration and discoloration were characteristic signs of the disease (Nakano *et al.*, 1994). Again, NRIA organized a research group that collaborated with prefectural fisheries research laboratories. Histology showed degenerated cells characterized by hypertrophied nuclei, which were

Table 2. **Occurrence of mass mortalities of cultured kuruma shrimp in Japan in 1993**

Prefecture	Total number of farms	Introduction of foreign kuruma shrimp			Mass morality				Dead shrimp	
		Number of farms	Exporting country	Month	Occurrence	Number of farms	Mortality (%)	Month	Number (10^6)	Body weight (gm)
Hiroshima	1	1	China	April	Yes	1	100	April	4.2	0.02-13.0
Yamaguchi	11	2	China	March-April	Yes	4	100	April-September	16.1	0.3-8.0
Ohita	5	1	China	April	Yes	1	86.8-100	April-July	1.8	5.5-11.0
Kumamoto	68	10	China	April-May	Yes	50	50-100	March-October	38.7	0.01-22.5
Kagoshima	15	1	China	March	Yes	1	90-100	May-June	3.4	0.8-4.0
Okinawa	19	1	China	April	Yes	1	100	April-June	10.2	2.0-20.0
Others[1]	31	0	-	-	No	-	-	-	-	-

[1] Niigata, Ishikawa, Kyoto, Wakayama, Hyogo, Ehime, Kagawa, Tokushima, Saga, Nagasaki, and Miyazaki prefectures (Nakano *et al*., 1994).

homogeneously stained with hematoxilin, in various tissues such as cuticular epidermis, connective tissue, lymphoid organ, antennal gland, hematopoietic tissue and nervous tissue (Momoyama *et al*., 1994). Electron microscopy revealed a number of rod-shaped, enveloped, non-occluded viruses in nuclei in these cells. The nucleocapsid of the virus measured 84±6x226±29nm (Inouye *et al*., 1994; 1996). The research group succeeded in inducing experimental infection similar to those of spontaneous infection by inoculating the filtrate (450nm) of the homogenate of the diseased shrimp (Nakano *et al*., 1994). From morphology, some physico-chemical properties, and host range of the virus, it was judged to be a new virus named penaeid rod shaped DNA virus and the disease penaeid acute viremia (PAV) since the virus was always found in the hemolymph of the infected shrimp (Inouye *et al*., 1996).

Fig. 4 typically shows how the disease spreads in an area. The first occurrence of mass mortality from the disease in Iwa Island was May 18 in a pond A area. Then, the disease spread all over A area in one month. In the next month, the disease then spread all over the shrimp pond of Iwa Island. The pond where the disease first occurred contained shrimp seedlings introduced from China about a month before the disease was detected. An epidemiological survey showed that all the places where seedlings were introduced from China in spring of 1993 had the disease during the spring to autumn in the same year. In contrast, the disease did not occur in other places where Chinese seedlings were not introduced in the same year (Table 2). In addition, the research group found typically degenerated cells with hypertrophied nuclei in several tissues of a shrimp seedling in a consignment from China, before the shrimp was transferred in Japanese waters (Momoyama *et al*., 1994). These results strongly suggest that the disease was newly introduced to Japan from imported shrimp seedlings for aquaculture.

The research group developed three types of diagnostic method: a rapid method using dark-field microscopy (Momoyama *et al*., 1995), a confirmative method using electron microscopy (Momoyama *et al*., 1995), and a rapid and confirmative method using PCR (Kimura *et al*., 1996). For the dark-field method and electron microscopy method, samples were taken either from the stomach or from the blood. For the dark field observation, samples were directly put on a slide and observed with a microscope. In the dark field, affected hypertrophied nuclei were seen as white fine particles. The dark-field method is simple and rapid. However, the method can not diagnose the early stage of the disease or virus carrier shrimp (Momoyama *et al*., 1995). For electron microscopy, stomach tissue from small shrimps was used and hemolymph from large shrimps. Virions or capsids were observed

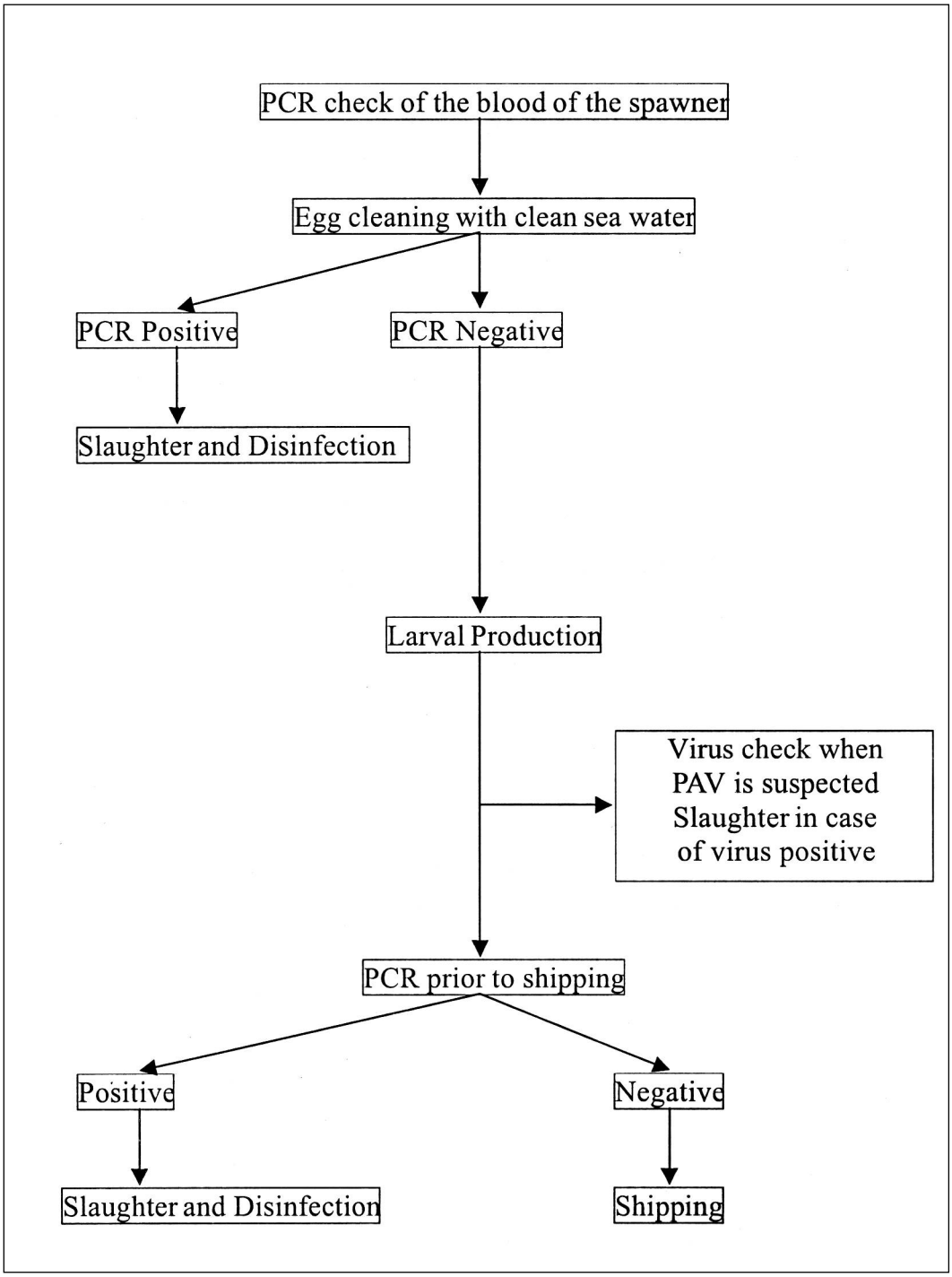

Figure 5. **Protocol for checking PAV during larval shrimp culture and before shipment of seedlings in the hatchery**

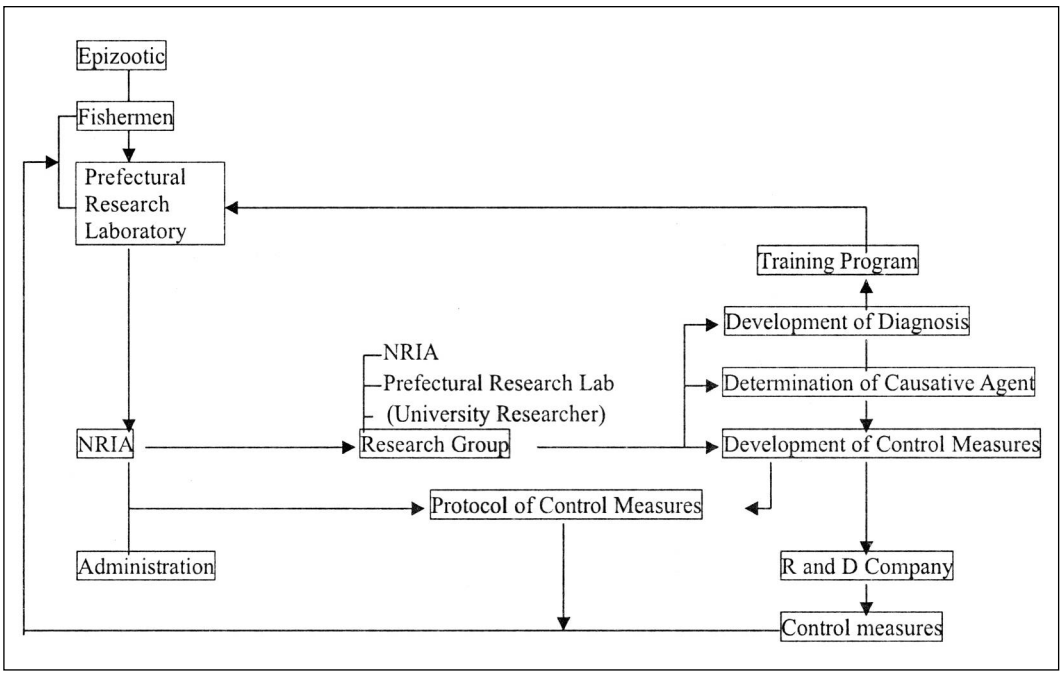

Figure 6. **NRIA's scheme for the development of control measures to a new epizootic**

on negatively stained preparations (Momoyama *et al.*, 1995). The research group purified the virus by gradient centrifugation, sequenced DNA fragment of the virus, and composed two pairs of PCR primers. Utilizing these primers, they established both one-step and nested PCR for the detection of the virus. The nested PCR can detect the virus during the early stage of the infection as well as from the excretion of experimentally infected kuruma shrimp (Kimura *et al.*, 1996). These diagnostic methods were transferred to prefectural fisheries research laboratories and public hatcheries through training programs. Based on the study and development of diagnostic method, NRIA proposed a protocol of checking PAV in the hatchery during larval culture and before the shipment of seedlings (Fig. 5). This hatchery protocol and some counter-measures in shrimp culture proposed by prefectural fisheries research laboratories (e.g., disinfection of the pond and reduction of the rearing density of farmed shrimp) appeared to have effectively reduced the occurrence of the disease.

NRIA's Scheme to Control a Novel Disease

Fig. 6 shows the scheme of NRIA to control new epizootics.

1. When a new disease occurs, the fisherman asks the diagnosis and control measures from prefectural fisheries research laboratory.
2. When the prefectural fisheries research laboratory finds the disease to be a new one or difficult to control, it informs NRIA.
3. NRIA usually makes some preliminary study and organizes a research group to collaborate with prefectural fisheries research laboratories and university researchers, if needed.
4. When diagnostic methods are developed, they are transferred to prefectural fisheries research laboratories through training programs.
5. If common control measures are needed, NRIA discusses with the administration office and

recommends control measures.

6. Research results are used to develop practical control measures in collaboration with a research and development company, if needed.

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