Important Diseases and Practical Control Measures in Shrimp Culture in Japan

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Abstract

The gross product from sea culture in Japan was about USD 3.4 billion in 2013 with kuruma shrimp *Marsupenaeus japonicus* constituting 2% of the total production. In recent years, annual shrimp production has reached about 1,600 metric tons (MT) and 99% of the species produced comprised of *M. japonicus*. Kuruma shrimp is highly traded at market price of USD 40-60 per kg. At present, 65% of cultured kuruma shrimp are produced in Okinawa and Kagoshima prefectures. To increase or maintain kuruma shrimp fishery in the natural environment, 200 million fry have been annually released into the sea along the coast of Japan. In addition, whiteleg shrimp *Penaeus vannamei* has also been produced in a private farm since 2007 with an annual production of approximately 40 MT. Recently, the number of farms that ventured into whiteleg shrimp culture has increased.

The most serious obstacle faced by the kuruma shrimp industry in Japan in the 1990s was the outbreaks of white spot disease (WSD) caused by white spot syndrome virus (WSSV). The seed production of kuruma shrimp has been dependent on captured wild broodstocks. However, broodstocks obtained from the wild could be carriers of WSSV that may vertically transmit the virus to fertilized eggs. To prevent the spread of WSD, measures for disinfecting WSSV-infected fertilized eggs of shrimp and detection of the causative virus by polymerase chain reaction (PCR) have been developed. Lately, with the application of an improved technology for broodstock rearing, production losses ascribed to WSD have significantly decreased because majority of the hatcheries have been using specific WSSV-free shrimps.

In the last decade, in kuruma shrimp hatcheries and grow-out ponds, vibriosis due to *Vibrio penaeicida* has been frequently encountered. Fusariosis caused by *Fusarium solani* is also common. The first cases of vibriosis and fusariosis in Japan occurred in 1973 and 1972, respectively. Taking the case of Okinawa prefecture as example, production losses in 2005 due to vibriosis and fusariosis were 66% and 34%, respectively. Cleaning of the pond bottom prior to the commencement of grow-out culture has been recommended to prevent vibriosis. Some antibiotics have also been orally administered to reduce mortality of shrimps affected by vibriosis. However, farmers have been properly cautioned regarding their use as emergence of antibiotic-resistant bacteria could consequently arise. Because no practical treatment for fusariosis is currently available, farmers either dispose or harvest infected shrimps followed by disinfection of ponds with 10 ppm chlorine.

Acute hepatopancreatic necrosis disease (AHPND) has not yet been detected in Japan. As kuruma shrimp is also susceptible to the disease, the Government of Japan plans to designate AHPND as *Specific Disease*. Under Japanese law, enlistment of AHPND as a quarantinable disease will also be instituted to prevent the introduction of this disease into Japanese hatcheries and grow-out facilities. However, in case an AHPND outbreak inadvertently occurs, Prefectural Fisheries Experimental Stations (PES) could immediately conduct preliminary diagnosis using the AP4 polymerase chain reaction (PCR) method. In addition, confirmatory diagnosis using AP4 nested PCR method and sequencing could be conducted at the National Research Institute of Aquaculture (NRIA), a central laboratory for aquatic animal health. NRIA's task is not only to perform confirmatory diagnosis but likewise disseminate diagnostic techniques to the staff of the National Quarantine and PES.

Introduction

The origin of shrimp farming could be dated back to the 1930s when the kuruma shrimp spawned in captivity and was cultured for the first time in Japan (Hudinaga, 1942). A small shrimp farming industry emerged in Japan in the 1960s. Later that year, commercial shrimp farming proliferated (Hudinaga and Kittaka, 1975). In recent years, annual production of shrimp has reached approximately 1,600 metric tons (MT) with 99% of the species produced comprised of kuruma shrimp (FAO, 2012). However, the occurrence of infectious diseases attributed to vibriosis, fusariosis and white spot disease (WSD) has, over the past years, deterred the sustainability of the shrimp industry in Japan (Satoh, 2012).

The introduction of WSD caused by white spot syndrome virus (WSSV) through shrimp larvae from overseas in 1993 had caused serious economic losses in Japan's shrimp industry (Inouye *et al.*, 1994; Nakano *et al.*, 1994). Because the primary infection route of the causative virus was identified to be via vertical transmission, i.e. from spawners to larvae (Lo and Kou, 1998), eggs obtained from broodstocks after spawning were screened by polymerase chain reaction (PCR). In addition, fertilized eggs were washed with disinfectant prior to hatchery rearing (Mushiake *et al.*, 1999). In spite of these interventions, in some cases, occurrence of WSD was encountered during culture due to latent virus infection because viral loads in shrimps with inapparent infection could be extremely low and very difficult to detect even by sensitive methods such as real-time PCR.

WSSV-free-cultured shrimps have been recently available as broodstocks for captive breeding in several shrimp hatcheries chiefly due to improvements in the rearing techniques (Sugiyama, 2007). As a result, production losses resulting from WSD have markedly decreased over the past several years. Okinawa prefecture is the largest shrimp producer in Japan wherein approximately 70% of larvae are obtained from mature captive broodstock (Matsumoto, 2015). However, some hatcheries are still dependent on wild caught broodstock. Aside from WSD, vibriosis and fusariosis could infect penaeid shrimps cultivated in grow-out ponds.

Acute hepatopancreatic necrosis disease (AHPND) caused by unique strains of *V. parahaemolyticus* has not yet been detected in Japan. However, the domestic shrimp price in Japan soared to USD 15.5 from USD 10.5, when the import volume from Thailand and Malaysia decreased due to AHPND outbreaks. Notably, importation of shrimps from Viet Nam and India momentarily increased (Editorial office, 2015). As kuruma shrimp has been demonstrated to be susceptible to the disease via experimental infection, the Government of Japan plans to designate AHPND as *Specific Disease*. Under Japanese law, enlistment of AHPND as a quarantinable disease will also be instituted to prevent the introduction of this disease into Japanese hatcheries and grow-out facilities. However, in the event that an AHPND outbreak inadvertently occurs in Japan, Prefectural Experimental Stations (PES) could immediately conduct the preliminary diagnosis using the AP4 polymerase chain reaction (PCR) method (Dangtip *et al.*, 2015). In addition, confirmatory diagnosis using AP4 nested PCR method coupled with sequencing could be conducted at the National Research Institute of Aquaculture (NRIA), a central laboratory for aquatic animal health.

Major diseases of kuruma shrimp and their diagnostic methods

Three major infectious diseases, i.e. vibriosis, fusariosis and WSD of penaeid shrimps have been mainly documented among cultivated kuruma shrimps in Japan. While considerable economic losses caused by infectious diseases were encountered in the 1990's, these diseases occur sporadically at present. From a total production valued at about USD 79.8 million in 2008, production losses were estimated at USD 8.8 million with vibriosis constituting 53% of the losses, followed by WSD (31%), and fusariosis (16%). However, economic losses due to fusariosis has gradually increased in the last decade.

Almost all life stages of kuruma shrimp are susceptible to WSD, i.e. from juveniles to mature adults. WSD has not only caused serious economic losses to the shrimp aquaculture industry, but also huge troubles in stock enhancement of kuruma shrimp for decades. Kuruma shrimps infected with WSD exhibit embedded white spots within the exoskeleton, rapid reduction of food consumption, and lethargic behavior. Nested PCR or real-time PCR methods have been employed to diagnose the disease.

Vibriosis has been observed in shrimps weighing 1- 45 g with the most sensitive stage noted in shrimps with body weights ranging from 15-25 g. *Vibrio penaeicida* infection in cultured kuruma shrimp was first observed in 1973 (Ueda, 1973) and has been the primary cause of shrimp losses until the emergence of WSD in Japan in 1993. Affected shrimps exhibit small black spots in the gill filaments. In addition, lymphoid organs become enlarged with brown spots. Diagnostic methods include bacterial isolation on marine agar or thiosulfate-citrate-bile salts-citrate (TCBS) agar followed by the identification of isolated bacteria using anti-serum slide aggregation test or PCR.

Only kuruma shrimp is so far sensitive to *Fusarium* spp. Fusariosis has been documented in cultivated shrimps both in hatcheries and grow-out ponds. In addition, mortality in younger stages, i.e. from nauplius to postlarvae 10 has been noted to be higher compared to those cultivated in grow-out ponds during the latter stages of culture. Fusariosis first occurred in 1972 (Egusa and Ueda, 1972) and has been persistently observed up to the present. The gills of shrimps infected with fusariosis turn blackish in color when viewed under the microscope due to the presence of fungal hyphae. *Fusarium* spp. can be isolated from the infected gills using Mycosel agar or artificial seawater yeast extract peptone glucose (PYGS) agar. Additionally, *Fusarium* spp. can be identified microscopically based on the morphological characteristics of the hyphae and conidia.

Disease prevention and control

The primary infection route of WSD in hatcheries has been ascribed to the vertical transmission of WSSV from spawners to eggs and larvae. This is supported by the fact that wild shrimps used for induced spawning have

Table 1. Prevalence of white spot syndrome virus (WSSV) in wild mature kuruma shrimp from 1996 to 1998.

Sex	Organs	Prevalence	Number of WSSV positive/total
Female	Gonad	10.1%	96/955
	Stomach	7.3%	70/955
	Hemolymph	5.8%	52/892
Male	Gonad	4.8%	15/314
	Stomach	6.7%	21/314
	Hemolymph	0.0%	0/314

Table 2. Frequency of white spot disease (WSD) occurrences in the seed production of kuruma shrimp at National Research Institute of Aquaculture from 1996 to 1999.

Year	Selection of spawner by PCR		Disinfection treatment of eggs	Number of cases with WSD/Total (%)
	Target Organ	Timing of sampling		
1996	Ovary	not done	not done	8/14 (57.1%)
1997	Ovary	before spawning	with iodine	2/23 (8.7%)
1998	Receptaculum seminis	after spawning	with iodine	0/11 (0%)
1999	Receptaculum seminis	after spawning	with iodine	0/16 (0%)

been often infected by WSSV (Table 1). Thus, to inhibit the vertical transmission of WSSV, eggs are selected based on WSSV detection in broodstocks by PCR after spawning. The target organ for PCR is the receptaculum seminis. In addition, the fertilized eggs are disinfected with povidone iodine (5 mg/l for 5 min). Frequency of WSD occurrences in the seed production of kuruma shrimp at NRIA from 1996 to 1999 is shown in Table 2. Our established protocol involving a combination of WSD negative spawner selection by PCR that detects the virus from the receptaculum seminis and disinfection of fertilized eggs with iodine could successfully prevent larvae and post-larvae from being contaminated with WSSV. In addition, to prevent the horizontal transmission of WSSV, seawater is treated by UV irradiation before being pumped into the rearing tanks of shrimp larvae or juveniles. Stable production of specific-pathogen-free shrimp has been accomplished by these countermeasures against WSSV transmission. Control measures aimed at preventing WSD in hatcheries are shown in Figure 1. Additionally, horizontal transmission of WSSV among pond cultivated shrimps has been primarily identified to be via cannibalism and the waterborne route. Vaccines (subunit and DNA) targeting major envelope proteins such as VP28 of WSSV in penaeid shrimp have been shown to elicit good protection against WSD (Satoh *et al.*, 2008). Unfortunately, field trial has not yet been conducted due to patent limitation and high costs.

Vibriosis often occurs when the sand in the pond bottom contains excessive organic matter. Thus, to prevent this condition, the pond bottom should be turned over after harvesting shrimp or the sand should be renewed, if possible. While oxytetracycline hydrochloride or oxolinic acid have been used to treat vibriosis in kuruma shrimp, no approved antibiotics for shrimp vibriosis is currently available in Japan. Immunostimulants such as beta-1,3-glucan and peptidoglycan have been proven effective in reducing shrimp mortality due to vibriosis (Itami *et al.*, 1989; Takahashi *et al.*, 1995), but its application is seemingly not economically practical. No practical treatment for fusariosis is currently available; hence, farmers either dispose or harvest infected shrimps followed by disinfection of pond water with 10 ppm chlorine.

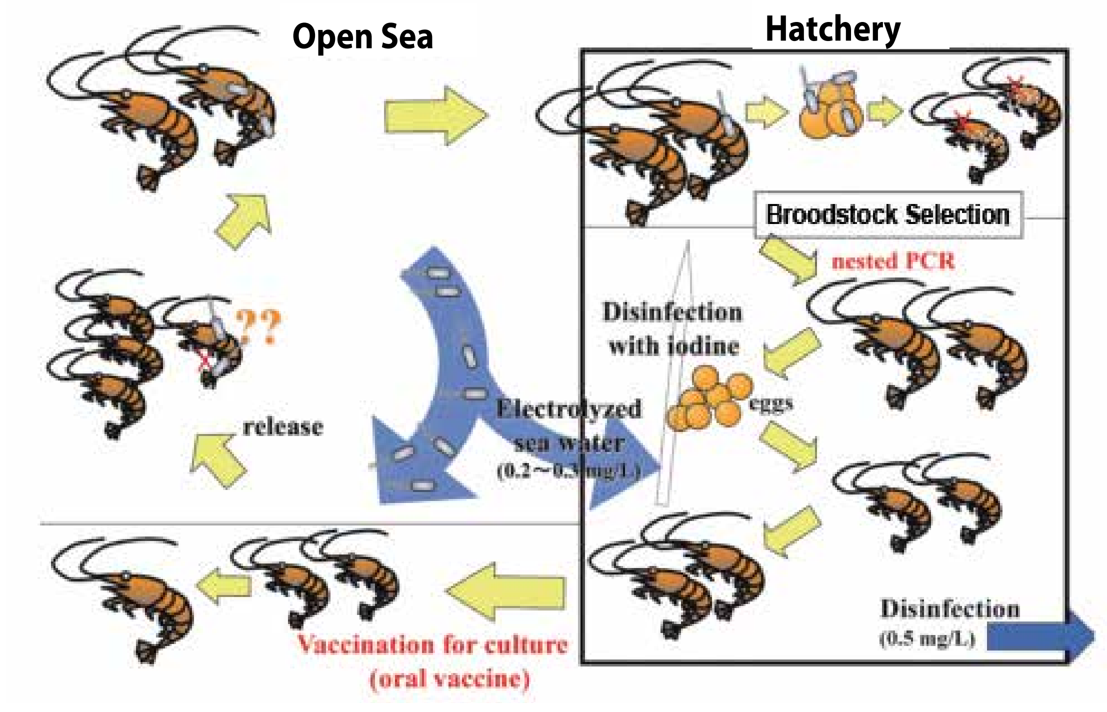

Figure 1. Control measures to prevent shrimp from viral contamination (extracted from Bull. Fish. Res. Agen. No. 36, 57-106, 2012).

Preventive measures against the spreading of important diseases of aquacultured organisms in Japan have been implemented according to two Japanese laws, i.e. *Sustainable Aquaculture Production Assurance Act* (Law No.51 of 1999, amended in Law No.69 of 2014) and *Act on the Protection of Fisheries Resources* (Law No.313 of 1951, amended in Low No. 70 of 2015). The former law clearly stipulates that necessary measures should be aimed at preventing the spread of specified infectious diseases among farms in order to maintain the stable supply of aquatic products. On the other hand, the latter stipulates that quarantine procedures for imported aquatic animals should be strictly followed. The laws designated 11 diseases as *Specified Diseases*, including 5 shrimp diseases. At the moment, the Ministry of Agriculture, Forestry and Fisheries (MAFF) is planning to increase the number of the *Specified Diseases* to a total of 24 diseases including AHPND and 9 other shrimp diseases (Table 3).

The Animal Quarantine Service (AQS) of the MAFF performs import quarantine inspection at 30 ports located around Japan. The staff of AQS inspect documents for imports including heath certificate and confirm the number/condition of imported animals. When necessary, the animals are kept at a designated facility during the control period specified for each disease. If animals show clinical signs or mortality during the period, the staff of AQS visit the facility for on-site inspection and sampling to detect the *Specified Diseases* by PCR. When the samples turn out to be positive for one of the *Specified Diseases*, all animals are destroyed and the facility is thoroughly disinfected.

On the other hand, according to the Sustainable Aquaculture Production Assurance Act, the manager and employee engaging in aquaculture have to notify the prefectural government in case they recognize or suspect that cultured animals are infected with any of the *Specified Diseases*. The Prefectural Government's Experimental Station that receives the notification conducts preliminary diagnoses for the *Specified Diseases* according to the diagnostic manual issued by MAFF. If the result of the diagnosis is positive, the prefectural government requests a confirmatory diagnosis for the disease to the

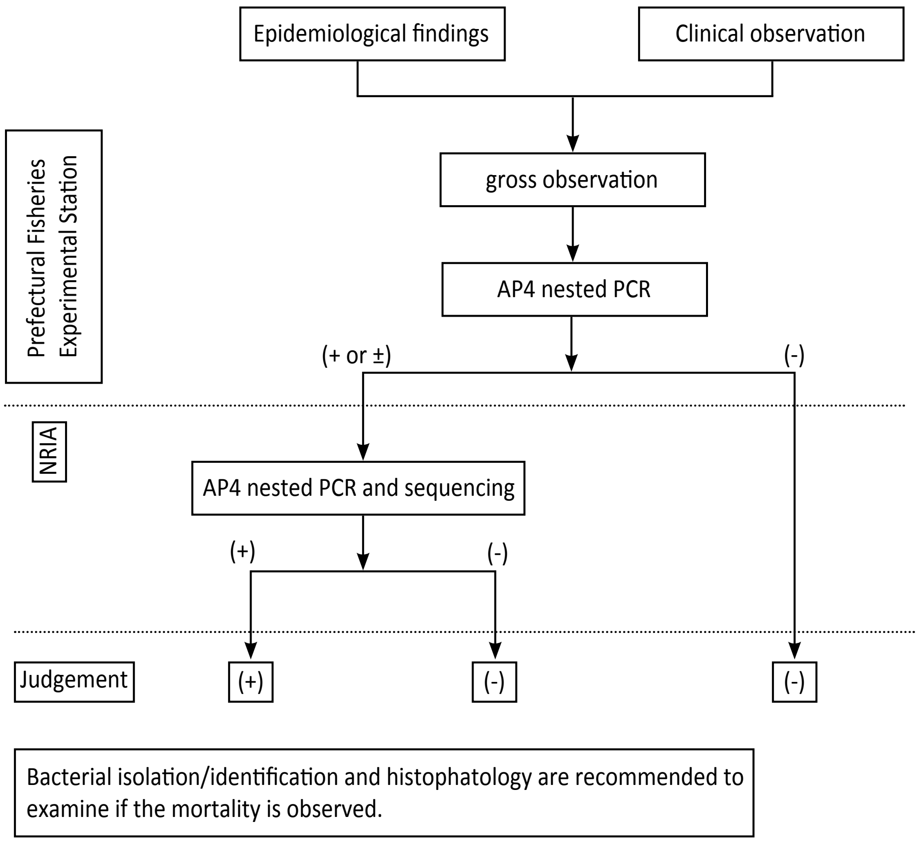

Figure 2. Draft of inspection chart for acute hepatopancreatic necrosis disease (AHPND).

National Research Institute of Aquaculture. Under the instruction of the MAFF, the NRIA should promptly address the request using PCR that is specified in the manual. The PCR adopted for the confirmatory diagnosis is often distinct from the PCR used in the preliminary diagnosis. If the result by the NRIA is also positive for the disease, the prefectural governor can direct the manager or owner of the aquaculture farm to stop transferring the animals from the facility and destroy the animals that are possibly infected with the disease (Figures 2 and 3). In case of outbreaks in the wild environment, the governor can ban fishermen from transferring the susceptible animals from a particular watershed to other waters.

By and large, because the preventive measures highlighted below have been stringently and strictly implemented throughout Japan, occurrence of exotic shrimp diseases including the *Specified Diseases* and those listed in the OIE in Japanese shrimp culture facilities and natural waters has not been so far encountered.

Table 3. List of *Specified Diseases* of shrimp and corresponding diagnostic methods.

Specified Disease	Diagnostic Methods	
	Preliminary test*	Confirmatory test**
Yellow head disease (YHD)	RT-PCR	Nested-PCR
Taura syndrome (TS)	RT-PCR	RT-PCR
Infectious hypodermal and haematopoietic necrosis (IHHN)	PCR	PCR
Tetrahedral baculovirosis	PCR	PCR
Spherical baculovirosis	PCR	PCR
Acute hepatopancreatic necrosis disease (AHPND)	nested-PCR	Nested PCR+sequencing
Necrotising hepatopancreatitis (NHP)	PCR	Real-time PCR
Infectious myonecrosis (IMN)	RT-PCR	Nested-PCR
Covert mortality disease of shrimp (CMD)	RT-PCR	Nested-PCR
Gill-associated virus disease	RT-PCR	Nested-PCR

*Preliminary test is performed at the prefectural experimental stations
**Confirmatory test is performed at the National Research Institute of Aquaculture

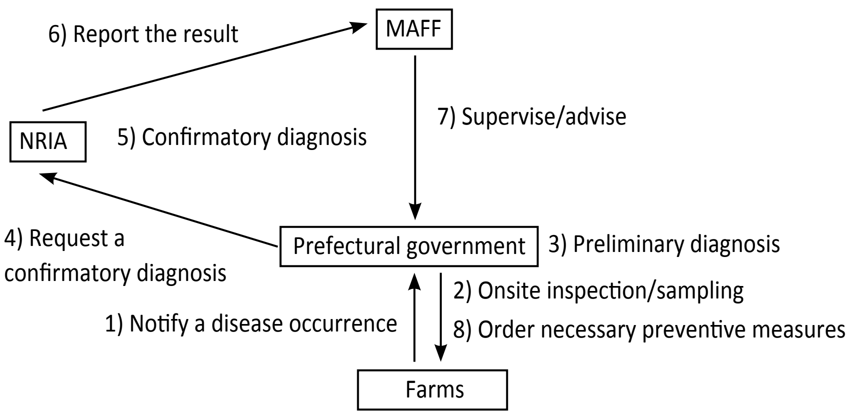

Figure 3. Preventive measures when a *Specified Disease* occurs.

Summary and way forward

Outbreaks of WSD in 1993 due to imported shrimp caused economic losses to the shrimp industry of Japan. Recently, the frequency of WSD occurrence has significantly decreased as a result of WSSV-free broodstock selection, disinfection of fertilized eggs, and UV-treatment of rearing water in juvenile production facilities. On the basis of the lessons learned from cases of exotic infectious disease outbreaks in the past, the government has strengthened regulations to prevent the entry of exotic diseases in the country. Importantly, through stringent and strict implementation of the regulations, Japan has so far been spared from AHPND and other OIE-listed diseases, except WSD. It is therefore evident that the best way to control disease problems in cultivated shrimps and other aquatic animals is to continue embarking on the conduct of scientific researches geared at establishing effective and practical epidemic prevention system for the importation/ exportation and domestic movement of live aquatic organisms.

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