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CONTROL STRATEGY FOR VIRAL DISEASES OF SALMONIDS AND FLOUNDER

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ABSTRACT

Salmonid fish and flounders are economically important fish cultured in northern Japan. Viral diseases remain one of the limitations to the successful propagation in aquaculture. The incidence of disease, especially viral disease, has been increasing. Methods currently used to control viral disease in hatcheries are: (a) hygiene and sanitation, (b) disinfection of water supplies and wastewater, (c) selection of pathogen-free broodstock, (d) health monitoring of hatched fry, (e) control of normal intestinal flora, (f) vaccination, and (g) temperature control.

INTRODUCTION

Salmonid fish, such as chum salmon (*Oncorhynchus keta*), pink salmon (*O. gorbuscha*), and masu salmon (*O. masou*), and flounders, such as Japanese flounder (*Paralichthys olivaceus*) and barfin flounder (*Verasper moseri*), are economically important fish cultured in northern Japan. Salmonid fish are important species for hatchery reared and released fish. Flounders are also important fish species for seed production and sea-farming in northern Japan, because of their rapid growth at low temperatures and relatively high market prices. The incidence of disease, however, especially viral diseases, has been increasing. Viral diseases found in salmonid fish are infectious hematopoietic necrosis (IHN), *O. masou* virus disease (OMVD), erythrocytic inclusion body syndrome (EIBS), chum salmon virus disease, and viral erythrocytic necrosis. In cultured Japanese flounder and barfin flounder, lymphocystis disease (LCD), viral ascites, hirame rhabdovirus (HIRRV) disease, viral epithelial hyperplasia, reovirus disease, viral nervous necrosis (VNN), and viral hemorrhagic septicemia (VHS) are found.

Methods currently used to control viral diseases in hatcheries are: (a) hygiene and sanitation, (b) disinfection of water supplies and wastewater using ultraviolet (UV) irradiation, ozonization, and electron decomposition of seawater, (c) selection of pathogen-free broodstock using antibody detection and virus-specific gene detection by polymerase chain reaction (PCR), (d) health monitoring of hatched fry by immunological methods and PCR, (e) control of normal intestinal flora (e.g., by feeding an antiviral intestinal bacterium), (f) vaccination (e.g., against IHN and OMVD in masu salmon and rainbow trout, *O. mykiss*, and against LCD in Japanese flounder) using a formalin-inactivated virus or recombinant G protein expressed via yeast, and (g) temperature control.

HYGIENE AND SANITATION

General sanitation measures are standard practice in hatchery and seed producing facilities. Special care must be taken to avoid the movement of equipment from one tank to another and all articles should be disinfected after use. Methods adopted to sanitize a rearing unit have to be chosen carefully, due to factors such as chemical toxicity for fish and effects of temperature and repeats use. It should be remembered that workers themselves might serve as efficient vectors for pathogens, and proper disinfection of hands and boots are required to prevent dissemination of viruses. Although it may be difficult to sanitize a rearing unit during use, tanks and raceways should be disinfected with a chlorine-based disinfectant before and after use (Ahne et al. 1989; Kimura and Yoshimizu 1991). Equipment, nets, and brushes are disinfected with ozonated or electrolyzed seawater containing 0.5 mg/L of total residual oxidants (TROs) or chlorine for 30 min and are used separately for individual tanks (Watanabe and Yoshimizu 1998; 2001).

Some viruses are transmitted vertically from adult to progeny via infected eggs or sperm. Disinfection of the surface of fertilized eggs has proven to be effective in breaking the infection cycle for several viruses, such as rhabdovirus, herpesvirus, and nodavirus (Yoshimizu et al. 1989, Yoshimizu 1998, Watanabe et al. 2000). For IPN, however, surface disinfection may not always be successful, as the virus may be carried internally in salmonid eggs. The salmonid eggs are disinfected with iodine (50 ppm for 20 min) immediately after fertilization and at the eyed stage (Yoshimizu and Nomura 1989). Flounder eggs are disinfected with ozonated seawater (0.5 mg/L of TROs for 10 min) at the morula stage (Watanabe and Yoshimizu 2000).

DISINFECTION OF WATER SUPPLIES AND WASTEWATER

Water systems provide an efficient means for the introduction and spread of infectious diseases. Pathogen-free water sources are often essential for success in aquaculture. The surface waters from domestic bay commonly used in aquaculture contain fish pathogens or animal pathogens. Such open water supplies should not be used without prior treatment to kill the fish pathogens. Treatment systems usually make use of high efficiency sand filters to clarify the water before treating it with UV light or ozonization (Kasai et al. 2002).

Fish viruses are divided into two groups, based on their sensitivity to UV and TROs (Yoshimizu et al. 1986a; 1995; Kasai et al. 2002). Sensitive types are infectious hematopoietic necrosis virus (IHNV), *O. masou* virus (OMV), lymphocystis disease virus (LCDV), and Japanese flounder rhabdovirus (HIRRV). These viruses are inactivated by the treatment of 10^4 iW sec/cm² (UV dose) or 0.1 mg/ml (TROs) for 1 min. Resistant types are infectious pancreatic necrosis virus (IPNV), marine birnavirus, reovirus, and fish nodavirus, and are inactivated at a dose of 10^5 iW sec/cm² or 0.5 mg/ml (TROs) for 1 min (Ito et al. 1996a; Kasai and Yoshimizu 2001). Since ozonated seawater that contains TROs shows toxicity to fish, the TROs should be removed by charcoal (Ito et al. 1996b). Disinfection by electrolysis of seawater for aquaculture and waste seawater from the hatchery is very effective for treating the large volumes of seawater that are used (Kasai et al. 2000; 2001a; 2001b).

PATHOGEN-FREE BROODSTOCK

Management and monitoring of health conditions are very important for seed production in aquaculture (Yoshimizu 1998). Health inspections of broodstock are conducted to insure that fish are free from certain important diseases. Specialized diagnostic techniques are required to make specific pathogen-free broodstock for routine inspections (Yoshimizu and Nomura 1989). The tests have been made easier and more rapid by the development of enzyme-linked immunosorbent assay (ELISA) (Yoshimizu et al. 1997; 2001; Watanabe et al. 2000).

For salmonid fish, ovarian fluid is collected by the methods of Yoshimizu et al. (1985) and inspected routinely. Fertilized eggs were disinfected with iodofore 50 ppm for 20 min. Eyed eggs indicate that inside the egg membrane is pathogen-free (Yoshimizu et al. 1989). Since viruses and bacteria, including IHNV, OMV, *Aeromonas salmonicida*, and *Renibacterium salmoninarum*, may infect and thrive in the embryo, it is very important to disinfect the surface of eyed eggs with iodofore again.

In flounder hatcheries, tags are applied for identification of individual fish. For example, to control barfin flounder and Japanese flounder nervous necrosis virus (BFNNV and JFNNV, respectively), a standard sandwich ELISA uses an expressed protein of partial BFNNV coat protein for an antigen to capture the specific antibodies, and reverse transcription PCR (RT-PCR) to detect striped jack (*Caranx vinctus*) nervous necrosis virus-specific gene sequences for broodstock selection. ELISA is done three mo before spawning, and the negative fish by ELISA are reared for the broodstock. Eggs and sperm are tested by RT-PCR, and specimens are inoculated to fish cell line SSN-1 cells at the same time. The eggs or sperm that are positive by RT-PCR are removed (Watanabe et al. 1998; 2000).

HEALTH MONITORING OF HATCHED FRY

Except for Pacific salmon *Oncorhynchus*, fry from each spawner should be cultured in separate tanks. When the fry show abnormal swimming or signs of disease, the fish should be removed and brought to the laboratory for diagnosis as soon as possible. Moreover, health monitoring should be routinely done, using methods for viral detection such as cell culture, fluorescent antibody techniques (FAT), immuno-peroxidase stain (IPT), antigen detecting ELISA, and PCR tests. Reverse transcription PCR (RT-PCR) is suitable for detecting the fish nodavirus and flounder ascites virus, while FAT is commonly used to diagnose viral epithelial hyperplasia, lymphocystis disease, HIRRV disease, and reovirus disease (Yoshimizu 1995).

CONTROL OF NORMAL BACTERIAL FLORA

Generally, normal bacterial floras play an important role in inhibiting the growth of pathogenic bacteria in the intestine or on the skin, and in stimulating the immune response of the host animals. Sometimes, the bacterial flora of larvae cultured in disinfected water is not normal. It is important to establish the normal bacterial flora of the fish before they are released to the river or ocean.

Many bacterial strains that produce anti-viral substances against fish viruses have been reported (Yoshimizu et al. 1986b; Yoshimizu and Ezura 1999). Rainbow trout and masu salmon that are fed with the bacteria that have been isolated from the normal intestinal flora and which have an anti-IHNV activity have showed more resistance to artificial infection with IHNV (Yoshimizu et al. 1992). *Vibrio* spp. with anti-viral activity against IHNV, OMV and BFNNV have been isolated from the normal intestinal flora of barfin flounder and added to *Artemia* that had been disinfected at the egg stage and hatched out in disinfected water. Anti-IHNV, -OMV, and -BFNNV activities have been observed in homogenates of intestines of fish fed with the *Artemia* (Yoshimizu and Ezura 1999). Barfin flounder fed with *Artemia* that contained *Vibrio* sp. have showed more resistance to natural infection by BFNNV (Yoshimizu and Ezura 1999).

VACCINATION

Immunization represents the most effective method to control those diseases for which avoidance is not possible. Commercial vaccines have become available to protect the fish against important pathogens.

Formalin-inactivated OMV and recombinant IHNV-glycoprotein (IHNV-G protein) expressed by yeast have also been tested. Injecting the OMV vaccine into mature rainbow trout lowered the incidence of OMV in the ovarian fluid, and an immersion vaccine of IHNV-G protein expressed yeast has prevented infection by IHNV. Apparently healthy Japanese flounder with low ELISA antibody titers against Japanese flounder lymphocystis disease virus (JF-LCDV), have been injected with three types of inactivated JF-LCDV vaccine, including a formalin-killed Japanese flounder lymphocystis (JF-LC) cell suspension, a heat-killed JF-LC cell suspension, and a formalin-killed purified JF-LCDV. Three mo post-injection, ELISA antibody titers had increased to 1:40. In similar fish that had not been injected, the ELISA antibody titers remained low (1:5 or 1:10). Outbreaks of LCD had been observed at that time. It was reported that lymphocystis (LC) cells did not grow, but gradually disappeared from among the immunized fish that showed high ELISA titers. In contrast, it was reported that at 6 mo post-vaccination, LCD was observed among 53 % of the fish that had not been injected (Yoshimizu 2001; Iwamoto et al. 2001).

TEMPERATURE CONTROL

It is well known that many diseases of aquatic animals are temperature dependent. In the case of HIRRV, natural outbreaks of disease have disappeared when the water temperature increases to 15 C. Cumulative mortality of artificially infected Japanese flounder (IP $10^{5.3}$ TCID₅₀/fish) reared at 5, 10, 15, and 20 C, were reported at 40%, 60%, 10%, and 0%, respectively, with the highest virus infectivity from the fish cultured at 5 C, followed by the 10 C (Oseko et al. 1988). It has therefore been strongly recommended that Japanese flounder should be cultured at water temperatures between 15 C and 18 C. No outbreaks of HIRRV disease have been reported since 1988. Now, temperature control treatment is done against IHN.

LITERATURE CITED

- Ahne, W., J. R. Winton, and T. Kimura. 1989. Prevention of infectious diseases in aquaculture. *Journal of Veterinary Medicine B/Zentralblatt fuer Veterinaermedizin* Reihe 36:561-567.
- Ito, S., M. Yoshimizu, and Y. Ezura. 1996a. Disinfectant effects of low level of total residual oxidants in artificial seawater on fish pathogenic microorganisms. *Nippon Suisan Gakkaishi* 63:97-102.
- Ito, S., M. Yoshimizu, M.-J. Oh, S. Hyuga, K. Watanabe, Y. Hayakawa, and Y. Ezura. 1996b. Effect of ozonized seawater on bacterial population and survival of cultured flounders (*Paralichthys olivaceus* and *Verasper moseri*). *Suisanzoshoku* 44:457-463.
- Iwamoto, R., O. Hasegawa, S. LaPatra, and M. Yoshimizu. 2002. Isolation and characterization of the Japanese flounder (*Paralichthys olivaceus*) lymphocystis disease virus. *Journal of Aquatic Animal Health* 14: in press.
- Kasai, H. and M. Yoshimizu. 2001. Disinfection of water supply for aquaculture by ozonization. *Bulletin of Japanese Research Association for the Medical and Hygienic Use of Ozone* 8(3): 2-6.
- Kasai, H., M. Ishikawa, Y. Hori, K. Watanabe, and M. Yoshimizu. 2000. Disinfectant effects of electrolyzed salt water on fish pathogenic bacteria and viruses. *Nippon Suisan Gakkaishi* 66:1020-1025.
- Kasai, H., K. Watanabe, and M. Yoshimizu. 2001a. Bactericidal effect of continuous flow electrolyzer on hatchery waste-seawater. *Nippon Suisan Gakkaishi* 67:222-225.
- Kasai, H., K. Watanabe, and M. Yoshimizu. 2001b. Disinfectant effects of hypochlorite produced by batch electrolytic system on fish pathogenic bacteria and viruses. *Suisanzoshoku* 49:237-241.
- Kasai, H., M. Yoshimizu, and Y. Ezura. 2002. Disinfection of water for aquaculture. *Proceedings of the 70th Anniversary of The Japanese Society of Fisheries Science (JSFS) International Commemorative Symposium*. Yokohama, Japan, 1-5 October 2001. Japanese Society of Fisheries Science, Yokohama, Japan, in press.
- Kimura, T. and M. Yoshimizu. 1991. Disinfection methods of aquaculture unit. Pages 220-226 in M. Takano and R. Yokoyama, editors. *New Handbook of Sterilization Technology*. Science-Forum, Tokyo, Japan.

- Oseko, N., M. Yoshimizu, and T. Kimura. 1988. Effect of water temperature on artificial infection of *Rhabdovirus olivaceus* (hirame rhabdovirus: HRV) to hirame (Japanese flounder, *Paralichthys olivaceus*). *Fish Pathology* 23:125-132.
- Watanabe, K. and M. Yoshimizu. 1998. Disinfection of equipment for aquaculture and fertilized eggs by ozonated sea water. *Fish Pathology* 33:145-146.
- Watanabe, K. and M. Yoshimizu. 2000. Disinfection of viral nervous necrosis virus contaminated fertilized barfin flounder eggs by ozonated sea water. *Nippon Suisan Gakkaishi* 66:1066-1067.
- Watanabe, K. and M. Yoshimizu. 2001. Disinfection of equipment for aquaculture by electrolyzed seawater. *Nippon Suisan Gakkaishi* 67:304-305.
- Watanabe, K., S. Suzuki, T. Nishizawa, K. Suzuki, M. Yoshimizu, and Y. Ezura. 1998. Control strategy for viral nervous necrosis of barfin flounder. *Fish Pathology* 33:445-446.
- Watanabe, K., T. Nishizawa, and M. Yoshimizu. 2000. Selection of brood stock candidates of barfin flounder using an ELISA system with recombinant protein of barfin flounder nervous necrosis virus. *Diseases of Aquatic Organisms* 41:219-223.
- Yoshimizu, M. 1995. Virological inspection. Pages 144-148 in K. Muroga and S. Egusa, editors. *General Fish Pathology*. Koseisha Koseikaku, Tokyo, Japan.
- Yoshimizu, M. 1998. Health management of brood stock and its methods. Pages 1-9 in Japan Sea Farming Association, editors. *Prevention Methods for Diseases of Larval Stages*, No. 9. Fisheries Agency, Ministry of Agriculture, Forest, and Fisheries, Tokyo, Japan.
- Yoshimizu, M. 2001. Effective vaccination against lymphocystis disease in hirame (Japanese flounder *Paralichthys olivaceus*). *International Federation of Consulting Engineers (FIDIC)*, in press.
- Yoshimizu, M. and Y. Ezura. 1999. Biological control of fish viral diseases by anti-viral substance producing bacteria. *Microbes and Environment* 14:269-275.
- Yoshimizu, M. and T. Nomura. 1989. An improved method for isolation of fish pathogenic bacteria and viruses from mature salmonids. *Technical Reports of Hokkaido Salmon Hatchery; Fish and Egg* 158:49-59.
- Yoshimizu, M., T. Kimura, and J. R. Winton. 1985. An improved technique for collecting reproductive fluid samples from salmonid fishes. *Progressive Fish-Culturist* 47:199-200.

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- Yoshimizu, M., H. Takizawa, and T. Kimura. 1986a. U.V. susceptibility of some fish pathogenic virus. *Fish Pathology* 21:47- 52.
- Yoshimizu, M., H. Takizawa, Y. Kamei, and T. Kimura. 1986b. Interaction between fish pathogenic viruses and microorganisms in fish rearing water: Survival and inactivation of infectious pancreatic necrosis virus, infectious pancreatic necrosis virus and *Oncorhynchus masou* virus in rearing water. *Fish Pathology* 21: 223-231.
- Yoshimizu, M., M. Sami, and T. Kimura. 1989. Survivability of infectious haematopoietic necrosis virus in fertilized eggs of masu salmon and chum salmon. *Journal of Aquatic Animal Health* 1:1-17.
- Yoshimizu, M., Y. Fushimi, K. Kouno, C. Shinada, Y. Ezura, and T. Kimura. 1992. Biological control of infectious hematopoietic necrosis by antiviral substance producing bacteria. Pages 301-307 in T. Kimura, editor. *Salmonid Diseases*. Hokkaido University Press, Sapporo, Japan.
- Yoshimizu, M., S. Hyuga, M.-J. Oh, S. Ito, Y. Ezura, and G. Minura. 1995. Disinfectant effect of oxidant produced by ozonation of sea water on fish pathogenic viruses, bacteria, and ciliata. Pages 203-209 in M. Shariff, J. R. Arthur, and R. Subasighe, editors. *Diseases in Asian Aquaculture II. Fish Health Section*. Asian Fisheries Society, Manila, Philippines.
- Yoshimizu, M., K. Suzuki, T. Nishizawa, J. R. Winton, and Y. Ezura. 1997. Antibody screening for the identification of nervous necrosis virus carriers in a flounder brood stock. Pages 124-130 in *New Approaches to Viral Diseases of Aquatic Animals*, National Research Institute of Aquaculture (NRIA) International Workshop, National Research Institute of Aquaculture, Mie, Japan.
- Yoshimizu, M., Y. Hori, T. Yoshinaka, T. Kimura, and J. A. Leong. 2001. Evaluation of methods used to detect the prevalence of infectious haematopoietic necrosis (IHN) virus in the surveillance and monitoring of fish health for risk assessment. Pages 276-281 in C. J. Rodgers, editor. *Proceedings of the Office International des Epizooties (OIE) International Conference on Risk Analysis in Aquatic Animal Health*. Paris, France, 8-10 February 2000. Office International des Epizooties, Paris, France.