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# Control Strategy for Viral Nervous Necrosis of Barfin Flounder

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Barfin flounder (*Verasper moseri*) is one of the most important fish species for seed production and sea-farming in northern Japan, because of its rapid growth at low temperatures and relatively high market prices. Akkeshi Station of Japan Sea-Farming Association (JASFA) has established the rearing method of this species in 1986. Subsequently, the production of barfin flounder in Akkeshi Station of JASFA had been multiplied every year. In 1990, rearing juveniles of barfin flounder with abnormal swimming and hemorrhaging in eyes and brain were observed but no major pathogen was found at that time. However, in 1993, mass mortalities occurred frequently in seed production of barfin flounder in the station, and the affected fish showed abnormal swimming and vacuolation in the retinal and brain tissue. Unenveloped and round-shaped viral particles approximately 25 to 28 nm in diameter were observed in the cytoplasm of the retinal and brain cells around the vacuolation. Additionally, these affected juveniles showed positive reaction by PCR test with primers, which were designed based on the nucleotide sequence of striped jack nervous necrosis virus (SJNNV) for detection of fish nodaviruses[1]. Therefore, these juveniles were considered to be affected with viral nervous necrosis (VNN)[2]. In a case of striped jack (*Psuedocaranx dentex*), the causative agent of VNN was vertically transmitted from spawners to their offsprings. The incidence of VNN in striped jack larvae has been reduced in hatcheries by elimination of virus-carrying fish from spawner candidates based on the PCR test[3]. However, this PCR-elimination of spawners was not complete enough to prevent the occurrence of VNN with vertical transmission[4]. It was deduced that vertical transmission also occurred in the VNN of barfin flounder, based on the fact that the causative agent was detected by PCR from egg fluid and the positive number of egg fluid samples increased with repeated artificial fertilizations. In the present study, ELISA system to determine the quantity of specific antibodies against the causative agent, barfin flounder nervous necrosis virus (BFNNV), was applied for selection of brood stocks for spawner candidates, and occurrence of VNN was monitored in their offsprings.

A standard sandwich ELISA was used in the present study. Briefly, an expressed protein of partial BFNNV coat protein was used for the ELISA as an antigen to capture the specific antibodies in barfin flounder serum. The expressed protein was obtained from *Escherichia coli* cells transformed with the pET25b(+) expression vector (Novagen) having T2 region of BFNNV coat protein gene. Estimated size of the expressed protein was 31,808 Da. A rabbit antiserum against barfin flounder IgM and peroxidase conjugated swine antibodies to rabbit IgG were used as the 2nd and 3rd antibodies respectively for detection of barfin flounder IgM. *O*-phenylenediamine was used as a substance, and an absorbance at 492 nm in wave length was measured after 15 min incubation at room temperature with microtiter plate reader (Corona). An end point of maximum dilution of fish sera showing values higher than 0.01 of absorbance, which was defined as the ELISA antibody titer[5], was determined for quantitative analysis of the specific antibodies to BFNNV. The PCR detection of BFNNV from sexual materials and offsprings was performed with the method described by Nishizawa *et al.*[1]

For seed production of barfin flounder in 1995, 37 PCR-negative brood stocks were submitted to the ELISA antibody titer test 3 months before their spawning, and 35 having ELISA antibody titer of 1:40 or less were selected. Just before the artificial fertilization, 7 pairs of fish were selected as spawner candidates by the ELISA and PCR tests. All of the selected spawners had not antibody titers higher than 1:20 and BFNNV was not detected from their sexual materials by PCR test. As the result of rearing of their offsprings in separated tanks, VNN occurred in only one group of larvae obtained from a pair of spawners. In 1996, 20 brood stocks with ELISA antibody titers lower than 1:20 and negative result in PCR were selected 3 months before spawning. And 5 pairs of spawners with low antibody titers ($\leqq$1:10) and negative PCR were selected just before the artificial fertilization. Eventually, no occurrence of VNN was observed in their offsprings and larvae. From these results, it was considered that the selection of brood stocks for spawner candidates by the ELISA antibody titer and PCR test was efficacious to reduce the incidence of VNN in seed production of barfin flounder.

Base on the results of these experiments, the following strategy (Fig.1) has been enforced to control VNN in seed production of barfin flounder in Akkeshi Station of JASFA. Brood stocks with lower ELISA antibody titers ($\leqq$1:10) are selected and reared at individual tanks. Eggs and sperms are inspected of the presence of BFNNV by PCR in spawning season. Only the brood stocks whose sexual materials are negative by PCR test are submitted to artificial fertilization. The fertilized eggs at the morula stage are disinfected with ozonated sea water [0.5 mg/*l* of total residual oxidants (TROs) for 5 min], and the

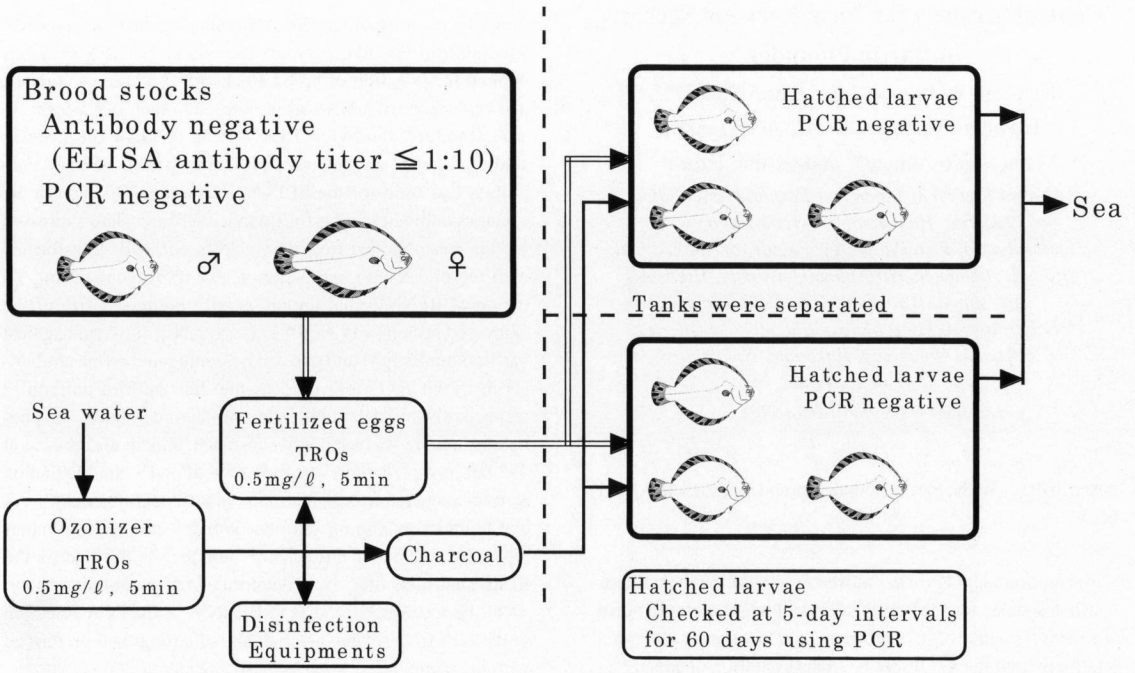


**Fig. 1.** Control strategy for viral nervous necrosis of barfin flounder.

hatched larvae are submitted to PCR detection of BFNNV at 5-day intervals for 60 days. All rearing equipments are disinfected with ozonated seawater with 0.5 mg/*l* of TROs for 1 h and are separately used for individual tanks. Eggs and larvae are reared in separated tanks with charcoal-treated ozonated sea water (0.5 mg/*l* of TROs for 5 min) and then removed[6].

## References

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