

## Hygiene management is important to prevent red sea bream iridovirus transmission between net pens: Insights from a case study assessing cross-contamination in a fish farm

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**Extended Abstract:** Red sea bream iridovirus (RSIV) infection, which is currently listed as a notifiable disease by the World Organization for Animal Health (WOAH), has caused significant economic damage in Japanese mariculture since the 1990s. Although formalin-inactivated vaccines are commercially available to control RSIV outbreaks, fish farmers do not use the vaccine when the vaccination cost is not acceptable compared to the market value of a given cultured fish. Therefore, basic biosecurity management such as hygiene procedures could be important to control RSIV outbreak. Nevertheless, in the case of mariculture using net pens or cages which is defined as semi-open system aquaculture by WOAH, the hygiene procedures have been considered less effective than those applied to land-based aquaculture because there is no physical barrier to prevent pathogens from moving via environmental water between aquaculture units. Our latest study suggested that RSIV transmission via seawater is highly associated with the distance between net pens and that the environmental water could function as a potential barrier to prevent viral transmission. Hence, we hypothesized that the biosecurity management could effectively reduce the risk of RSIV transmission even in semi-open system aquaculture where environmental water can move freely.

For implementing aquaculture biosecurity, the significance of fomite transmission in fish farms has been described, especially in salmonid aquaculture. However, the studies for aquaculture biosecurity are based on epidemiological data and questionnaire results for fish farms. To the best of our knowledge, there is no previous study that directly assessed the intensity of contamination in each aquaculture equipment associated with the fomite transmission. In the present study, cross-contamination of RSIV in aquaculture equipment and facilities in a fish farm where RSIV outbreak occurred was investigated by surface swabbing tests and an environmental DNA technique. Based on the results in this case study, we assessed the risk factors for transmission of RSIV between net pens to identify an effective hygiene procedure in the semi-open system aquaculture.

The investigation was performed in a fish farm where the RSIV outbreak occurred between September and October in 2022. The outbreak was initiated from juvenile red sea bream *Pagrus major* and transmitted to juvenile Pacific bluefin tuna *Thunnus orientalis*. Our investigation during the RSIV outbreak in the fish farm demonstrated that landing nets and gloves associated with collecting dead fish (carcasses) were highly contaminated with RSIV. The viral load of the contaminated equipment was  $> 10^8$  copies of the RSIV genome. Because the equipment for collecting fish carcasses were used at all net pens without disinfection, the risk of fomite transmission was

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considered to be higher than the transmission via environmental seawater if the distances among net pens are appropriately secured. On the other hand, we could not request fish farmers to undertake a strict hygiene procedure that is often implemented in land-based aquaculture because disinfection on boats is generally difficult owing to limited space of boats and the direct influences of weather. Therefore, we proposed mitigation measures against RSIV transmission between net pens and the following actions were initiated in the fish farm. A daily operation for collecting dead fish started from the net pen where the disease had not occurred and moved to the net pen where RSIV outbreak occurred to ensure that RSIV was not transmitted to the clean net pens by cross-contamination. In addition, the landing nets used for collecting fish carcasses were disinfected at the end of each day to avoid carryover of the virus to the following day. As a result, RSIV was not transmitted to the clean net pens in the fish farm for more than 30 days. However, once the RSIV outbreak occurred in the net pen upstream in the operation for collecting dead fish, RSIV was transmitted to all net pens in one week, suggesting that the transmission was caused by cross-contamination.

This study indicated that appropriate hygiene management is important to reduce the risk of RSIV transmission between net pens, even in semi-open system aquaculture. However, careful attention to the sequence of operation could not be a sufficient strategy when the RSIV outbreak suddenly occurred in the upstream of operation as shown in the present study. For the future study, we need to seek disinfection procedures targeting the high-risk equipment such as landing nets and gloves for collecting dead fish, which can be undertaken on the boat considering cost-effective and labor-efficient method. We believe that the present study represents the first step for implementing appropriate biosecurity management in semi-open system aquaculture, which could be expanded for other viral or bacterial infections in aquaculture.

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**Key words:** red sea bream iridovirus, RSIV, cross-contamination, aquaculture biosecurity, hygiene management

#### Annotated Bibliography of Key Works

(1) Kawato Y, Ito T, Kamaishi T, Fujiwara A, Ototake M, Nakai T, Nakajima K (2016) Development of red sea bream iridovirus concentration method in seawater by iron flocculation. *Aquaculture*, **450**, 308–312.

It is the first report that the iron flocculation technique was applied to concentrate a virus causing fish disease. Since environmental DNA (eDNA) was directly extracted from the iron flocculation-trapped filter without an elution step, the procedure until real-time PCR assay became simpler and time effective.

(2) Kawato Y, Mekata T, Inada M, Ito T (2021) Application of environmental DNA for monitoring red sea bream iridovirus at a fish farm. *Microbiol. Spectr.*, **9**, e0079621.

Environmental DNA (eDNA) could be applied in monitoring waterborne viruses of aquatic animals. However, there are few data for practical application of eDNA in fish farms to control disease outbreaks. The results of their field research over three years targeting eDNA in a red sea bream

(*Pagrus major*) fish farm implied that red sea bream iridovirus (RSIV) outbreaks in juveniles originated from the virus shedding from asymptotically virus-infected broodstocks. Their work identified the infection source of RSIV in a fish farm by eDNA monitoring, and it could be applied as a tool in aquaculture to control fish diseases.

(3) Kawato Y, Takada Y, Mizuno K, Harakawa S, Yoshihara Y, Nakagawa Y, Kurobe T, Kawakami H, Ito T (2023) Assessing the transmission risk of red sea bream iridovirus (RSIV) in environmental water: insights from fish farms and experimental settings. *Microbiol. Spectr.*, **11**, e0156723.

This study aimed to understand the actual transmission risk of RSIV through environmental water among fish farms. The results indicated that the viral loads in the seawater were low, except for the net pens where RSIV outbreaks occurred. Furthermore, their experimental infection model indicated that the infection risk of RSIV-contained seawater with less than  $10^3$  copies/L was extremely low. These results suggest that the transmission of RSIV among fish farms via seawater is highly associated with the distance between the net pens, and the

environmental water is not always an infection source for the transmission of RSIV between fish farms.

(4) Kawato Y, Mizuno K, Harakawa S, Takada Y, Yoshihara Y, Kawakami H, Ito T (2024) Risk assessment of wild fish as environmental sources of red sea bream iridovirus (RSIV) outbreaks in aquaculture. *Dis. Aquat. Organ.*, **158**, 65–74.

RSIV in wild fish near aquaculture installations was surveyed to evaluate the risk of wild fish being an infection source for RSIV outbreaks in cultured fish. In total, 1102 wild

fish, consisting of 44 species, were captured from 2 aquaculture areas in western Japan between 2019 and 2022. Eleven fish from 7 species were confirmed to harbor the RSIV genome using a real-time PCR assay. Based on the diagnostic records of RSIV in the sampled area of wild fish, the RSIV-infected wild fish appeared during or after the RSIV outbreak in cultured fish, suggesting that RSIV detected in the wild fish was derived from the RSIV outbreak in cultured fish. Therefore, wild fish populations near aquaculture installations may not be a significant risk factor for RSIV outbreaks in cultured fish.