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Survivability of Fish Pathogenic Viruses in Environmental Water, and Inactivation of Fish Viruses

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Abstract

Survival of three salmonid viruses and two marine fish viruses in fish rearing water or coastal sea water were observed at 0, 5, 10 and 15 ℃ for 7 or 14 days. Interaction between viruses and microorganisms present in the rearing water was observed. Infectious pancreatic necrosis virus (IPNV) and fish nodavirus (BF-NNV) were stable in waters used at every temperature tested for 14 days, but it was observed that, for infectious hematopoietic necrosis virus (IHNV), Oncorhynchus masou virus (OMV), and hirame rhabdovirus (HIRRV), as the temperature increased, the loss of infectivity also increased. When IHNV and OMV were suspended in filtrated and autoclaved rearing water, infectivity was reduced in comparison with the untreated water. Subsequently, adsorption of IHNV to mud or small particles was studied. IHNV adsorbed to several clays (kaolin, bentonite, Japanese acid clay) and diatomaceous earth in sterilized water with a wide range of pH (5-11) at concentrations of 1, 10, and 100 mg/mL. Except for bentonite, infectivity of clay-adsorbed IHNV persisted for at least 9 weeks. The clay-adsorbed IHNV also persisted in infectivity to rainbow trout Oncorhynchus mykiss, causing cumulative mortality rates of more than 73 %. Then, inactivation effects of UV irradiation, ozonization, and electrolyzation of water were studied against six fish rhabdoviruses, three fish herpesviruses, one fish birnaviruses, one fish iridovirus, and one fish nodavirus. Six rhabdoviruses, three herpesviruses, and lymphocystis disease virus were found to be sensitive to UV irradiation, ozonization, and electrolyzation. Susceptibility of IPNV, chum salmon virus (CSV), and BFNNV to UV was found to be low. IPNV and CSV were low sensitive to ozonization and electrolyzation. Virucidal effects of six kinds of disinfectants were examined against OMV, IPNV, IHNV, and HIRRV at 15 and 20℃ for 30 sec and 20 min. At 15℃ for 20 min, minimum concentrations showing 100 % plaque reduction of viruses tested by iodophore, sodium hypochlorite solution, benzalconium chloride solution, saponated cresole solution, formaldehyde solution, and potassium permanganate solution were 40, 50, 100, 100, 3500, and 16 ppm, respectively.

Key words: fish virus, survivability, environmental water, inactivation, disinfectant, UV, ozonization, electrolyzation

Introduction

The knowledge of survival of fish viruses in the fish rearing water and interaction of viruses and other microorganisms in aquatic environments are critical for establishing the control methods of fish viral diseases. Several previous reports have described the inactivation of viruses in natural water and sludge which at certain times seem to be related to microorganisms inherent in these environments (Kamei *et al.*, 1987 a,b, Kamei *et al.*, 1988 a,b, Kimura *et al.*, 1988). Disinfection of the water for aquaculture is also critical for preventing the introduction and spread of infectious disease. A pathogen free water source is essential for success in aquaculture. Surface waters commonly used in aquaculture come from coastal waters or rivers and may contain some fish pathogens and such open water supplies should not be used without treatment. Disinfection of wastewater before discharging is necessary to avoid the pathogen contamination in the

environment (Kasai *et al.*, 2002, Yoshimizu and Kasai 2002). In this paper, we introduce the survival of fish viruses in the fish rearing water and virucidal effect of ultraviolet (UV) irradiation, ozonization, and electrolyzation of water. Additionally, virucidal effects of disinfectant against fish viruses are introduced.

Materials and Methods

Survivability of fish viruses in environmental water

Survival of three salmonids viruses; infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV) and *Oncorhynchus masou* virus (OMV) in fish rearing water, de-chlorinated city water, double-distilled water, and Hanks' balanced water (HBSS) (Yoshimizu *et al.*, 1986), two marine fish viruses; hirame rhabdovirus (HIRRV) and fish nodavirus (barfin flounder nervous necrosis virus; BF-NNV) in coastal sea water, filtrate sea water, and autoclaved sea water, were observed at 0, 5, 10 and 15℃ for 7 or 14 days. Interaction between viruses and microorganisms present in the rearing water was observed.

Adsorption of IHNV to mud

Adsorption of IHNV to sea sand, Japanese acid clay, diatomaceous earth, kaolin, bentonite, quarts sand, chitin, cellulose powder, ion exchange hydrophobic Toyopearl and Cellulofine, alundum, active carbon, silica gel glass, plastic, and bacterial cells was studied (Yoshinaka *et al.*, 2000). Infectivity of clay-adsorbed IHNV was observed using a cell line and rainbow trout *Oncorhynchus mykiss*.

Inactivate effects of UV irradiation, ozonization, and electrolyzation

Inactivation effects of UV irradiation, ozonization, and electrolyzation of water were studied against six fish rhabdoviruses; IHNV, HIRRV, pike fry rhabdovirus (PFRV), spring viremia of carp virus (SVCV), eel virus from America (EVA), and eel virus from Europe X (EVEX), three fish herpesviruses; OMV, *Herpesvirus salmonis* (*H. salmonis*), and channel cat fish herpesvirus (CCHV), fish birnaviruses; IPNV, fish reovirus; chum salmon virus (CSV), fish iridovirus; Japanese flounder lymphocystis disease virus (JF-LCDV), and fish nodavirus (BF-NNV) (Kasai *et al.*, 2002).

Virucidal effects of disinfectants

Virucidal effects of six kinds of disinfectants; iodophore, sodium hypochlorite solution, benzalconium chloride solution, saponated cresole solution, formaldehyde solution, and potassium permanganate solution were examined against OMV, IPNV, IHNV, and HIRRV at 15 and 20℃ for 30 sec and 20 min (Hatori *et al.*, 2003). Virucidal effects were measured by plaque reduction methods (Kamei *et al.*, 1987c).

Result and Discussion

Survivability of fish viruses in environmental water

IPNV and BFNV were stable in all kinds of waters used at every temperature tested for 14 days but it was observed that for IHNV and HIRRV, as the temperature increased, (Figs. 1 and 2).

OMV was labile even in HBSS and inactivated rapidly as the temperature was raised. It was not survive at 5 to 7 days later in the fish rearing water. When IHNV and OMV were suspended in filtrated and autoclaved rearing water, infectivity was reduced in comparison with the untreated water (Fig. 3) (Yoshimizu *et al.*, 1986).

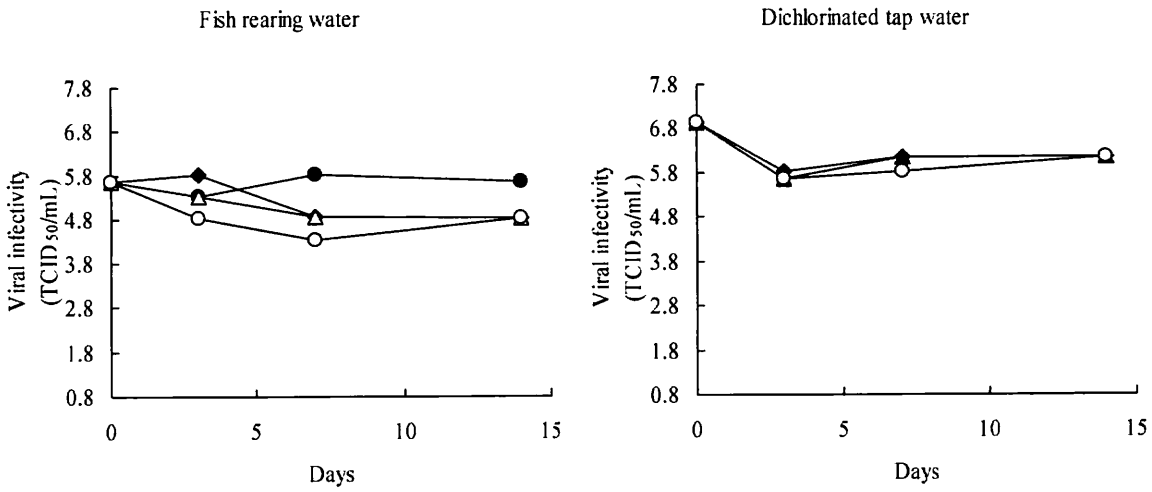

Fig. 1. Survival of IPNV in fish rearing water at 0, 5, 10 and 15 ℃.

—●— ; 0℃, —◆—; 5℃, —▲— ; 10℃, —○—; 15℃.

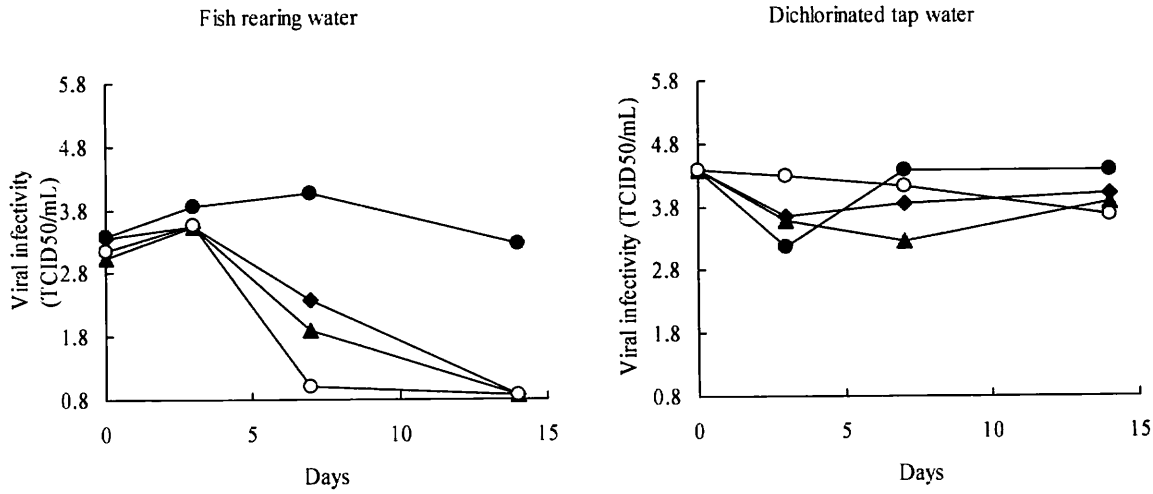

Fig. 2. Survival of IHNV in fish rearing water at 0, 5, 10 and 15 ℃.

—●— ; 0℃, —◆—; 5℃, —▲— ; 10℃, —○—; 15℃.

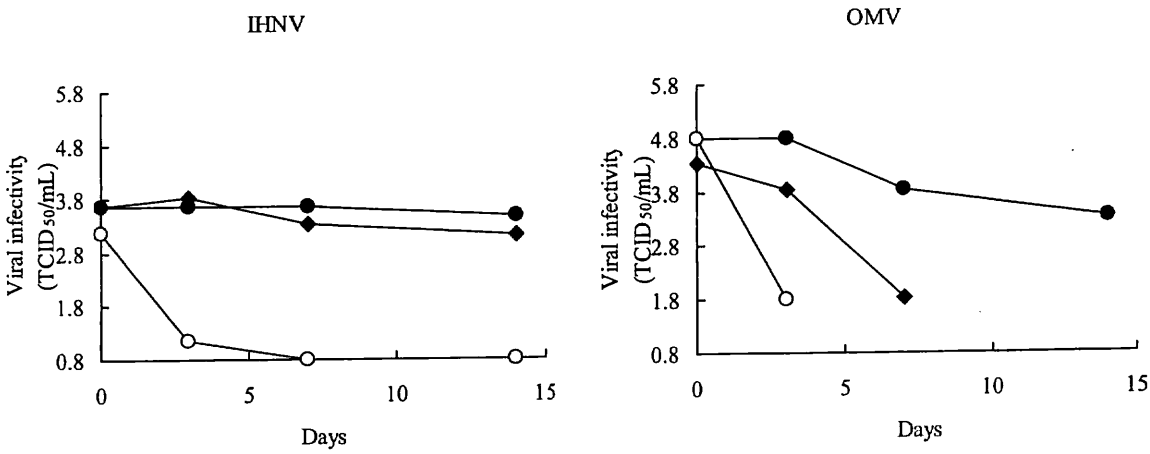

Fig. 3. Survival of IHNV in fish rearing water at 0, 5, 10 and 15 ℃.
●— ; Autoclaved, ◆— ; Filtered (0.22 μm), —○— ; Non-treated,
▼ ; Under the detection limit.

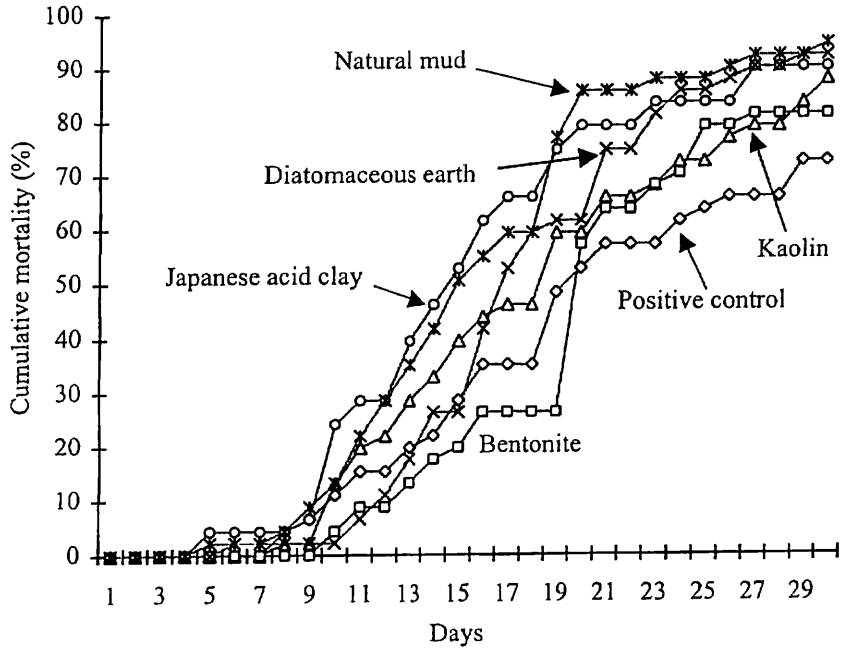

Fig. 4. Cumulative mortality of rainbow trout with bath challenge of IHNV absorbed to solid.

Representative isolates *Pseudomonas fluorescence* strain 46NW-04 has been proven to have the ability to produce anti IHNV substance.

Adsorption of IHNV to mud

IHNV adsorbed to several clays (kaolin, bentonite, Japanese acid clay) and diatomaceous earth in sterilized water with a wide range of pH (5-11) at concentrations of 1, 10, and 100 mg/mL (Table 1). Except for bentonite, infectivity of clay-adsorbed IHNV persisted for at least 9 weeks. The clay-adsorbed IHNV also persisted in infectivity to rainbow trout Oncorhynchus mykiss, causing cumulative mortality rates of more than 73 % (Fig. 4). The results suggest that IHNV adsorbed to naturally occurring substances in various aquatic environments may provide a source of infection for susceptible fish inhabiting these environments (Yoshinaka *et al.*, 2000).

Table 1. IHNV adsorption onto suspended solids.

Samples	Virus adsorbed*		Virus adsorbed
bentonite	>99.9	polystyrene	44.8
diatomaceous earth	>99.9	polyethylene	83.2
kaolin	>99.9	polyvinyl chloride	83.2
Japanese acid clay	>99.9	ion exchange hydrobic	44.8
active carbon	99.8	(Cellfine)	
quartz sand	0	ion exchange hydrobic	44.8
seasand	0	(Toyopearl)	
glass beads	0	chitin	83.2
craft glass pink	69.4	cellulose powder	44.8
craft glass yellow	69.4	bacteria IC-4	90.0
craft glass black	90.0	bacteria IE-1	90.0
craft glass orange	83.2	bacteria IE-2	99.0
craft glass green	69.4	bacteria IG-1	98.2
alundum	44.8	bacteria 2FI6	99.4
polypropylene	44.8	bacteria 46NW04	98.2

*: (1-supernatant titer / original IHNV titer) x 100.

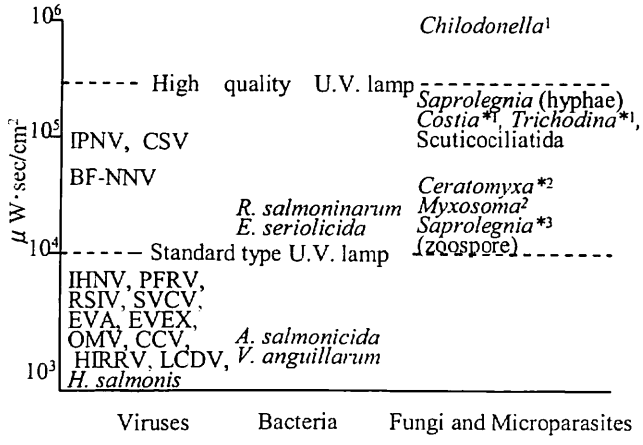

Fig. 5. U.V. susceptibility of fish pathogens.
*1: Vlasenko, M.I. (1969), *2: Hoffman, G.L. (1974), *3: Normandeau, D.A. (1968).

Inactivate effects of UV irradiation, ozonization, and electrolyzation

Six rhabdoviruses, three herpesviruses, and JF-LCDV were found to be sensitive to UV irradiation (99 % infectivity reduction dose ID_{99} = 10^3 μ W·sec/cm^2), ozonization ($ID_{99.9}$ = total residual oxidants; TROs, 0.5 mg/L for 15 sec), and electrolyzation ($ID_{99.9}$ = Hypochlorite, 0.3 mg/L for 1 min). Susceptibility of IPNV, CSV, and BFNNV to UV were found to be low, and ID_{99} was measured 10^5 μ W·sec/cm^2 (Fig. 5). IPNV and CSV have low sensitivity to ozonization and electrolyzation, and $ID_{99.9}$ was measured 0.5 mg/L TROs for 30 sec and 0.5 mg/L (Tables 2 and 3), hypochlorite for 1 min, respectively (Kasai *et al.*, 2002).

Table 2. Effects of total residual oxidants (TROs) concentrations produced by ozonization of seawater on fish pathogens.

Pathogens	TRO concentration (mg/L)	Treatment time (sec)	Reduction (%)	Initial number (Log. TCID50/mL)
IPNV	0.5	60	>99	4
CSV	0.5	60	>99	4
YTAV	0.5	60	>99	4.3
IHNV	0.5	15	>99	4
HIRRV	0.5	15	>99	5.5
OMV	0.5	15	>99	3
V. anguillarum	0.5	15	>99.9	5.6
E. serioricida	0.5	15	>99.9	5.8
A. salmonicida	0.5	15	>99.9	5.1
A. hydrophila	0.5	15	>99.9	4.6
E. coli	0.5	15	>99.9	6.5
Scuticociliatida	0.8	30	>99.9	5.3

Table 3. Chlorine concentration required to reduce 99.9% of viable bacteria or virus infectivity in 1 minute treatment.

Pathogens		Chlorine concentration (mg/L)
V. anguillarum	NCMB 6	0.07
A. salmonicida	ATCC 14174	0.06
E. coli	O-26	0.14
YTAV	Y-1	0.45
HIRRV	8401H	0.34

Table 4. Minimum concentration of disinfectants to show the 100% plaque reduction of OMV, IHNV, HIRRV and IPNV.

Disinfectant	Time	Concentration (ppm) OMV		Concentration (ppm) IHNV		Concentration (ppm) HIRRV		Concentration (ppm) IPNV	
		0℃	15℃	0℃	15℃	0℃	15℃	0℃	15℃
Iodophor	30 S	40	40	100	100	100	100	200	100
	20 min	40	40	100	100	100	100	100	100
Sodium hypochloride	30 S	50	50	50	50	50	50	100	100
	20 min	1	1	50	10	50	10	100	100
Benzalkonium chloride	30 S	100	100	200	200	200	1000	ND*	ND
	20 min	100	100	200	100	100	100	ND	ND
Saponated cresol	30 S	100	100	500	100	1000	500	5000	5000
	20 min	100	100	100	50	500	500	ND	ND
Formaldehyde	30 S	700	700	3500	3500	3500	3500	3500	3500
	20 min	700	350	3500	3500	3500	700	3500	700
Potassium permanganate solution	30 S	16	16	158	158	158	158	32	32
	20 min	16	16	32	16	16	16	16	16

Virucidal effects of disinfectants

At 15℃ for 20 min, minimum concentrations showing 100 % plaque reduction of viruses tested by iodophore, sodium hypochlorite solution, benzalconium chloride solution, saponated cresole solution, formaldehyde solution, and potassium permanganate solution were 40, 50, 100, 100, 3500, and 16 ppm, respectively (Table 4) (Hatori *et al.*, 2003). Koi herpesvirus, the causative agent of mass mortalities of koi and common carp, is a newly isolated virus in Israel, United States, Europe and Asian countries including Japan. Wild resource of carp is also damaged by KHV, thus it's important to monitor the survivability of KHV in the lake and river to control the KHV disease. Although survivability of KHV in the ambient water has not been studied, KHV is considered to be difficult to keep its infectivity in natural environment because this virus belongs to the Herpesviridae same as OMV. With the results of our recent study, KHV was inactivated in the waters and/or mud collected from Lake Kasumigaura during 3 days. While KHV is unstable in the environmental conditions, it shows strong pathogenicity to koi and common carp. Therefore it's necessary to establish an effective control method.

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