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The Fungistatic Effect of NaCl on Rainbow trout Egg Saprolegniasis

Nilubol Kitancharoen*[1], Atsushi Ono*[2], Atsushi Yamamoto*[3]
and Kishio Hatai*[1]

*[1]*Division of Fish Diseases, Nippon Veterinary and Animal Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180, Japan*
*[2]*Tokyo Metropolitan Fisheries Experimental Station Okutama Branch, 720, Okutama-machi, Nishi-Tama-gun, Tokyo 198-01, Japan*
*[3]*Oshino Trout Hatchery, Yamanashi Prefectural Fisheries Technology Center, Oshino, Yamanashi 401-05, Japan*

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With 1 hour exposure time, the maximum concentration of NaCl which was not toxic to rainbow trout eggs was 25 ppt at 13°C. The present study showed that interval treatment with 25 ppt NaCl for 1 h, twice a week, decreased fungal infection and increased the hatching rate of rainbow trout eggs. Continuous treatments with NaCl at concentrations of 3, 5, 7 ppt apparently increased the hatching rate to almost the same level. Furthermore, in the group treated with 7 ppt NaCl, no fungal infection was detected.

Key words: fungal infection, hatching rate, NaCl, rainbow trout, saprolegniasis

To control water molds (Order Saprolegniales), which are notorious for causing serious disease problems in fish culture systems, has been one of the big problems to be solved in aquaculture. In Japan, salmonid hatcheries have long faced the problem of fungal infection of incubating eggs. To combat this problem, malachite green and formalin have been introduced for treatment. The use of formalin to control fungal infection of fish eggs is limited in Japan, but malachite green is widely used for this purpose in varied dosages, typically at 2-5 μg/m*l* for 1 h, twice a week.

Although malachite green is effective in the control of fungal infection on fish and fish eggs, it is prohibited by the FDA to use as a fungicide in fish culture in the U.S. (Marking *et al.*, 1994; Fitzpatrick *et al.*, 1995). Also, concerning about its teratogenic properties (Meyer and Jorgenson, 1983) and its metabolites which are still obscured, its use should be avoided. The residue of malachite green is considered to remain in market-sized fish hatched from eggs exposed to the chemical (Meinertz *et al.*, 1995). Formalin is also quite harmful in terms of users, safety and residue remaining in the environment. According to the FDA registration, the formalin use is limited to eggs of salmonids and esocids (Fitzpatrick *et al.*, 1995).

Salt (NaCl) was brought to our attention as a safe, common substance, which leaves no hazardous residue. Several reports mentioned the effects of salt on fungal control and improving of the hatching rate (Canfield, 1947; Taylor and Bailey, 1979; Phelps and Walser, 1993). The use of NaCl in hatchery has not hitherto been reported in Japan. Also several reports about dosages of NaCl, which was effective for fungal control, were varied. This study focused on the effect of NaCl as a fungistatic agent and its effect on the hatching rate of incubated eggs of rainbow trout. Interval and continuous NaCl exposures for treatments were performed, with the purpose of application for use in both open and closed systems.

Materials and Methods

Toxicity of NaCl to rainbow trout eggs

The minimum concentration of NaCl which affects the survival of rainbow trout eggs was verified to determine the dosages for the next experiment. Eyed eggs of rainbow trout were prepared in groups of 50 eggs. The eggs were bathed in various concentrations of NaCl for 1 h (Table 1) in 500 m*l* plastic cups containing 400 m*l* of NaCl solution at water temperature of 13°C. Aeration was provided during dipping. The eggs were subsequently transferred to plastic mesh baskets (about 10 cm in diameter) in 20 *l* plastic tanks with running water at the temperature around 13°C. The survival

rate was determined after 72 h.

Interval treatment with NaCl in open system

Rainbow trout eggs were collected from four 2-year-old females. The eggs were pooled together for insemination. About 1 h after insemination, the eggs were randomly separated into groups of 300 eggs. Eggs from each group were held in a 10 × 10 × 3 cm stainless mesh basket. The experimental groups were treated with various concentrations of NaCl which had previously been determined to have no toxicity to rainbow trout eggs (Table 2) in 2 *l* water with aeration for 1 h, twice a week until they hatched. The control group was manipulated in the same manner, but without NaCl. After each interval treatment, the eggs were separately held in 20 *l* plastic tanks in running water. The water supply was drawn from the main line and then sent to each tank. The water-flow rate was about 1.8 *l*/min. The eggs were kept in the baskets through the experiment without screening off the infected eggs. The number of eggs with fungal infection and the hatching rate were determined after treatment for 37 days. The water temperature during this experiment was 8-12°C. The number of zoospores in the inflow and outflow water was ascertained during the experiment by spreading 10 m*l* samples on GY agar plates, and expressed as the average of 10 counts ± standard deviations.

Table 1. Mortality of rainbow trout eggs in the eyed stage after treatment with various concentrations of NaCl for 1 h

NaCl concentration (ppt)	Mortality (%)
15	0
20	0
25	0
30	4
35	30
40	84
45	100
50	100
Control (without NaCl)	0

Continuous treatment with NaCl in closed system

Rainbow trout eggs used in this experiment were collected from two 4-year-old females and inseminated as described above. At 1 h post-insemination, eggs were separated into groups of 400 eggs and kept in the plastic mesh baskets. These trays were placed in 20 *l* tanks containing NaCl of various concentrations (Table 3) in 10 *l* of well water with aeration. The water source used in this test was previously confirmed to be continuously contaminated with fungi when flowing through the incubating eggs without any chemical treatment. Concentrations of NaCl below 0.9% (9 ppt)

Table 2. Effects of NaCl treatment with twice a week intervals on the hatching rate and fungal control in rainbow trout eggs

NaCl (ppt)	Number of eggs infected (%) after 37 days incubation	Hatching rate (%)	Number of zoospores/ml water (means±SD)					
			Inflow			Outflow		
			0 day	20 days	37 days	0 day	20 days	37 days
15	65.8	25.7	0.60±0.55	0.60±0.55	0.60±0.55	0.60±0.55	0.80±0.45	8.17±2.48
20	18.7	46.0	ND*	0.60±0.55	0.80±0.45	0.60±0.55	0.80±0.45	12.83±3.60
25	2.3	78.7	0.80±0.45	0.60±0.55	0.80±0.45	0.60±0.55	0.60±0.55	8.80±2.59
Control	90.3	5.7	0.60±0.55	1.00±0.00	0.80±0.45	0.60±0.55	1.67±0.82	14.50±3.62

ND*: not detected

Table 3. Effects of continuous treatment with NaCl on hatching rate and fungal control in rainbow trout eggs

NaCl (ppt)	Number of eggs colonized (%) after 34 days incubation	Hatching rate (%)
3.0	8.0	90.5
5.0	7.5	91.0
7.0	0	89.5
Control	91.5	8.5

were selected to avoid osmoregulation failure and embryonic developmental interference. Water was changed once or twice a week depending on the water conditions, while the initial NaCl concentrations were maintained. The water temperature during the experiment was 12-13°C.

Results

Toxicity of NaCl to rainbow trout eggs

Treatment with NaCl for 1 h at concentrations from 15 ppt to 25 ppt was not toxic to rainbow trout eggs (Table 1). However, NaCl above 30 ppt appeared to be toxic to the eggs. Mortality of the eggs increased simultaneously with the NaCl concentration, and reached 100% in the groups exposed to 45 and 50 ppt.

The rate of deformity in hatched larvae or any abnormalities upon hatching was very low (less than 3%) in all experimental groups.

Interval treatment with NaCl in open system

From the results in Table 2, treatment with the 3 selected concentrations of NaCl for 1 h exhibited various degrees of effectiveness on the fungal control and hatching rate. Twenty-five ppt of NaCl offered the highest hatching rate, with a very low fungal infection. Fungal infection in the control group was extremely high, at 90.3%, which resulted in the low hatching rate of 5.7%. Zoospore numbers in the inflow water were consistently low in all experimental groups during the experiment, whereas those in the outflow water tended to increase as the incubating time was extended.

Continuous treatment with NaCl in closed system

The results shown in Table 3 revealed that the fungal infection decreased as the NaCl concentration increased, whereas hatching rates appeared to be only slightly affected at the concentrations tested. There was no significant difference in the hatching rate among the groups treated (Duncan's New Multiple Range Test; $P>0.05$). Eggs incubated in well water containing 7 ppt of NaCl showed no fungal infection. A serious fungal infection in the control group resulted in a very low hatching rate.

Discussion

The present study concluded that interval treatment with 25 ppt NaCl for 1 h, twice a week, was the most effective procedure to control fungal infection of rainbow trout eggs. With regard to fungal infection on eggs, *Saprolegnia diclina* was the dominant species found on infected eggs. From the observation of the zoospore number in the water, it was inferred that incubated eggs were continuously exposed to a low number of zoospores which were carried in the water supply. Zoospores attached to the eggs, especially dead ones which supported fungal growth as an enriched substrate, then germinated and developed to the hyphal stage. From this stage, large amounts of zoospores were reproduced and liberated into the water, resulting in the increase in the risk of fungal infection of the incubated eggs. Thus, in practice, the hatching rate can be increased by routinely discarding dead eggs. Also, the sole usage of malachite green or formalin, without screening dead eggs from the batch, could not completely inhibit fungal infection of incubated eggs (unpublished data). The exposure time of 1 h was chosen as a standard throughout this study because hatchery procedures in Japan traditionally involve 1 h exposure for prophylactic treatment. In the case of continuous treatment, the results indicated that concentration of NaCl as low as 3-7 ppt was effective in controlling fungal infection and also improving the hatching rate. In comparison, the hatching rate of channel catfish eggs showed improvement at 0-5 ppt, salinity lower than that of rainbow trout eggs (Phelps and Walser, 1993). Naturally, the continuous treatment procedure may be applied in hatcheries of other fish species, or where management is performed by recirculatory system.

There are several reports on the use of salt or NaCl to control fungal infections of fish and fish eggs. Canfield (1947) suggested using the dip method with 0.5% salt for fungal control on fish and fish eggs. Taylor and Bailey (1979) reported that sea water was effective for the control of *Saprolegnia diclina* on the eggs of pink salmon, *Oncorhynchus gorbuscha*. Marking *et al.* (1994) reported that 30 ppt of salt solution effectively inhibited fungal infection and increased the hatching rate. Edgell *et al.* (1993) recommended 20 ppt of NaCl-$CaCl_2$ mixture for fungal control on salmonids eggs. The present study found that 25 ppt NaCl was best for fungal control without affecting the egg condition. Egg conditions, fish species and water parameters may call for varying concentrations of NaCl.

Even though the use of NaCl did not completely eliminate fungal infection, it may reduce the frequency of the use of malachite green which is known for its hazard. However, to introduce NaCl as a fungistatic agent in hatcheries.

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