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AN EPIDEMIOLOGICAL STUDY OF FURUNCULOSIS IN SALMON PROPAGATION

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INTRODUCTION

Furunculosis of salmonid fishes, caused by *Aeromonas salmonicida*, was first reported in 1890's by Emmerich and Weibel (1890a,b). Since these first reports, furunculosis has been reported in virtually all parts of the world where wild or cultured salmonids occur (Herman 1968, Smith 1960, Snieszko 1972, Austin and Austin 1987).

Furunculosis is not a serious problem in rainbow trout (*Oncorhynchus mykiss*) culture in Japan because this species is more resistant to the causative agent of the disease. However, serious mortality has been documented in juvenile amago (*O. rhodurus*) and masu salmon (*O. masou*) in accordance with increased production of these fish.

In Hokkaido, outbreaks of furunculosis have been reported in chum salmon (*O. keta*) by Nishino (1967), in masu salmon and pink salmon (*O. gorbuscha*) by Kimura (1970) during maturation in the holding ponds. Nomura (1983), Nomura and Kimura (1981), Nomura *et al.* (1983) and Nomura *et al.* (1991a) reported isolating *A. salmonicida* from the kidneys of the mature chum, pink and masu salmon that showed no apparent clinical signs of furunculosis.

Recently, there have been no systematic epidemiological studies from which quarantine and disease control policy could be based to establish control measures for fish furunculosis in salmonids. In this paper, we report a recent epidemiological study of *A. salmonicida* which was carried out for the purpose of establishing control measures for furunculosis.

ISOLATION OF *A. SALMONICIDA* FROM COELOMIC FLUID

In previous papers (Nomura *et al.* 1991a, 1991b), we reported the distribution and prevalence of *A. salmonicida* in the kidney of mature chum, pink and masu salmon. Also, we speculated from the results that the agent infects the salmon after they enter the rivers (Nomura *et al.* 1991b). For the purpose of revealing the route of infection of *A. salmonicida*, isolation of the agent from coelomic fluid of mature salmonids was conducted. In 1989 in Hokkaido, the coelomic fluid was collected from the chum, pink and masu salmon, according to the method

of Yoshimizu *et al.* (1985). The samples were cultured on nutrient agar at 20C for 7 days, and bacterial colonies that produced brown pigment in the medium and showed the characteristics indicated by Popoff (1984) were considered as *A. salmonicida*. Incidence of the agent from coelomic fluids is shown in Table 1.

Table 1. Isolation of *A. salmonicida* from the coelomic fluid(CF) and kidney of mature chum, pink and masu salmon.

Species	Rivers	Number of fish			
		Examined	Isolated from		
			CF	CF and Kidney	Kidney
Chum	Ishikari	120	0	0	0
	Ichani	60	0	1	10
	Tohoro	60	3	3	18
	Tokachi	120	15	7	23
Pink	Shibetsu	60	1	0	4
	Nishibetsu	60	0	0	1
Masu	Shiribetsu	60	0	1	1
	Shibetsu	60	0	0	3

A. salmonicida was isolated from the coelomic fluid of fish showing no apparent clinical signs of furunculosis. For example, *A. salmonicida* was isolated from the coelomic fluid of 22 fish of the 120 examined from the Tokachi River. The populations in which *A. salmonicida* existed in the coelomic fluid, also showed high incidence of the agent in the kidney (Nomura *et al.* 1991a). In populations of fish from the Shibetsu and Teshio rivers, the number of *A. salmonicida* in the coelomic fluid ranged from 10^5 to 10^7 cfu/ml. The coelomic fluid containing *A. salmonicida*, is expelled from the fish at the time of egg stripping or during the process of maturation in the pond. Consequently, *A. salmonicida*, together with the coelomic fluid, could contaminate the river water because the sewage of the egg stripping facility and the holding pond is not disinfected in Hokkaido. The bacteria from these source could be a source of infection for other anadromous salmon that ascend the river for spawning.

PRESENCE OF *A. SALMONICIDA* ON EGGS

The presence of the bacterium in the coelomic fluid suggests that the surface of eggs taken from the fish may also be contaminated by *A. salmonicida*. Contaminated eggs could play a role in the dissemination of the agent to places where the eggs are transplanted. We studied the existence of *A. salmonicida* on the surface of the eggs by artificially contaminating eggs with *A.*

salmonicida.

Table 2. Changes in the number of *A. salmonicida* (cfu/egg) on surface of artificially contaminated chum salmon egg after fertilization.

Time after fertilization (Hour)	Experiment No.			
	1	2	3	4
0	4.3x10 ⁶ *	5.8x10 ⁵	1.6x10 ⁴	1.1x10 ⁴
1	68	11.3	6.4	15
24	<4.5**	<4.5	<4.5	<4.5
48	<4.5	<4.5	<4.5	<4.5

*1 Colony forming unit/ egg.

*2 Not detected.

Chum salmon egg surfaces were bathed in 1.1x10⁴ to 4.3x10⁶ cfu/ml of *A. salmonicida*. The number of *A. salmonicida* decreased to 68 to 6.4 cfu/ml an hour after fertilization and *A. salmonicida* could not be isolated after 24 hours (Table 2). McCarthy (1980) also studied the changes of the number of *A. salmonicida* on the surface of the egg by artificially contaminating eggs. He reported that the number of bacteria was 10⁶ initially, and decreased gradually, and could not be isolated after 5 days. We attempted to isolate *A. salmonicida* from the eggs in the incubation box at the hatchery. These eggs were taken from brood fish in which the prevalence of *A. salmonicida* was high, but fortunately, *A. salmonicida* was not isolated from any of the eggs.

SURVIVAL OF *A. SALMONICIDA* IN WATER

A. salmonicida is considered to be an obligate pathogen (Popoff 1984). Its ability to survive and remain infectious in the external environment may be a major determinant in the spread of furunculosis. The agent is not easily isolated from non-sterile water. McCarthy (1980) studied the survival of the agent in water using an antibiotic resistance strain of *A. salmonicida*, and found the agent could survive for 8 days in water. We studied the viability of *A. salmonicida* in non-sterile water, filter sterilized water, and autoclaved water. In sterilized fresh water, *A. salmonicida* survived for sixty days and in non-sterile water, only four days. The survival of *A. salmonicida* in sea water was shorter than in fresh water. The results revealed that *A. salmonicida* survived long enough to infect other fish in the water.

THE DISTRIBUTION OF *A. SALMONICIDA*

Fig. 1. explains the distribution of *A. salmonicida* in salmon propagation according to

our results. The prevalence of *A. salmonicida* in the mature fish in the pond was high. *A. salmonicida* increased in both the kidney and the coelomic fluid. Consequently, *A. salmonicida*, together with the coelomic fluid, is expelled into the river water because the sewage of the egg stripping place and the holding pond is not disinfected in Hokkaido. The bacteria would become a source of infection for other anadromous salmon that ascend the river for spawning.

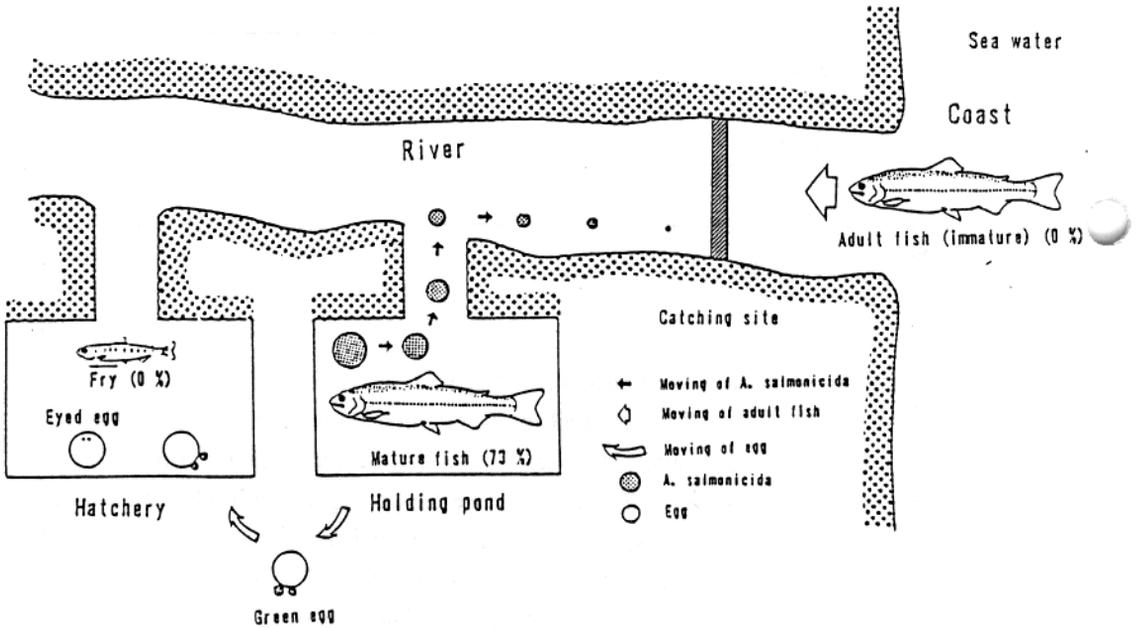


Fig. 1. Distribution of *Aeromonas salmonicida* in salmon propagation.

VARIATION OF AGGLUTINATION TITERS AGAINST *A. SALMONICIDA* IN THE SERA

A survey of agglutination titers against *A. salmonicida* in sera of chum, pink and masu salmon showed great variability among species. Few of the chum salmon contained antibodies, but a wide range of variation was observed in pink and masu salmon. The diversity in prevalence of agglutination titers in each of the three species indicates a continuous, widespread interaction among individuals of the host population and *A. salmonicida*. The difference in the agglutination titer is proportionate to the period of *A. salmonicida* infection. In general, the percentage of serologically reactive salmon increased as their length of freshwater residence increased. It was suggested that the fish were infected with *A. salmonicida* shortly before their eggs were stripped.

Kimura (1970) reported that the immunological method of preventing of furunculosis was

useful in adult masu salmon during the holding period because the salmon stay in freshwater for a period long enough to produce antibodies after antigen inoculation. In chum salmon, however, the freshwater residence is short, so this method would not be useful.

ISOLATION OF BACTERIOPHAGE OF *A. SALMONICIDA* FROM WATER

There is no sensitive medium for selecting *A. salmonicida*. Therefore when the number of *A. salmonicida* in water is low, the isolation of *A. salmonicida* from the water is difficult, because *A. salmonicida* cannot grow on the culture medium with competition of other naturally bacterial populations. We attempted to isolate bacteriophage of *A. salmonicida* to ascertain the presence of *A. salmonicida* in the water. The phage of *A. salmonicida* was isolated from the water of several rivers. We believed that this indicated that *A. salmonicida* can survive in the river water after leaving the fish and that its presence may be a source of infection of salmonid fish.

McCraw (1952) stated that the presence of the phage of *A. salmonicida* indicates the existence of the bacterium. He suggested that phage could be useful in studying the existence of the bacterium in water.

PATHOGENICITY OF THE ISOLATED *A. SALMONICIDA*

The isolated strains were identified as *A. salmonicida* subsp. *salmonicida* by their biological, biochemical, and immunological characteristics. All of the isolated *A. salmonicida* strains auto-agglutinated and produced protease, so we also expected them to be pathogenic. In order to examine the pathogenicity of the isolated strains, we injected them into chum and masu salmon fry and into adult chum salmon. All examined fish showed typical signs of furunculosis 3 to 4 days after injection. The number of *A. salmonicida* in the kidneys of moribund fish was approximately 10^8 cfu/g kidney, the same number reported by Morikawa and Tashiro (1981) in the kidney of moribund amago salmon. On the basis of these results, we conclude the isolates to be a pathogenic strains.

CONTROL OF *A. SALMONICIDA* ON THE SURFACE OF THE EGG

To establish a method of controlling *A. salmonicida* on the eggs, the bactericidal effect of popidon-iodine (Isodine) and its toxicity to chum salmon eggs were studied. *A. salmonicida* was completely killed by treatment with 25 ppm Isodine for 5 minute and this solution showed no toxic effect to chum salmon eggs within 1 hour treatment. We confirmed that Isodine solution has a sanitary effect on the bacterium, and that it does not have adverse effects on chum salmon eggs.

METHODS FOR DECREASING THE PREVALENCE OF *A. SALMONICIDA* IN CHUM SALMON

From the results of our epidemiological study, we suspected that the incidence of *A.*

salmonicida was affected by the density of fish in the holding ponds during the maturation period; as the density of the brood fish in the ponds decreases, incidence of *A. salmonicida* in examined fish also decreased. Therefore, we examined the relationship between the stocking density of fish in the pond and the prevalence of *A. salmonicida* in the fish.

Chum salmon in the Ishikari river were randomly assigned to experimental holding ponds and held under low (4.9 fish/m³) and high density (14.7 fish/m³) condition. As we expected, we found that 12.4 % of the fish examined harbored *A. salmonicida* when they were stocked at a higher density, but no fish stocked at a lower density contained the agent (Fig. 2). The prevalence of *A. salmonicida* in the fish that were held under low dissolved oxygen conditions, was significantly higher than in the fish held under high dissolved oxygen condition. These results indicate that high stocking density of the fish and low dissolved oxygen in the water of the holding pond has an effect on the prevalence of the agent in the fish. We concluded that fish maturation in low density conditions and disinfection of the eggs is necessary to prevent fish furunculosis in the artificial propagation of salmon.

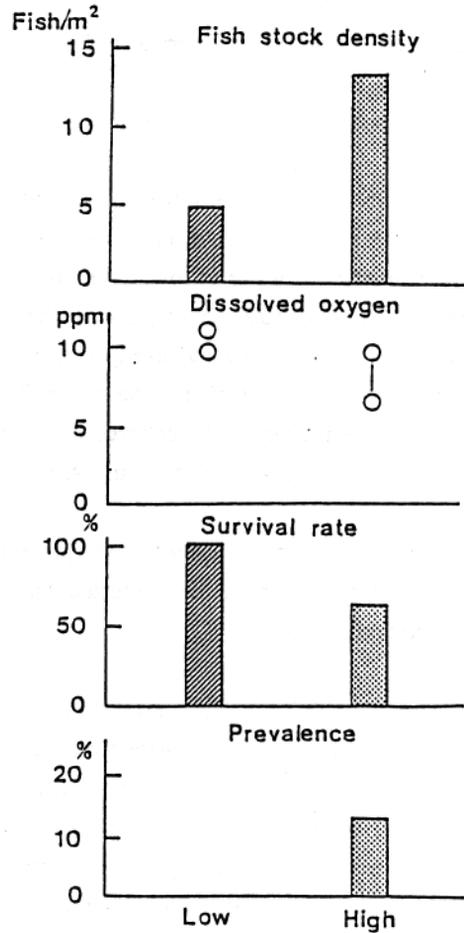


Fig. 2 The relationship between the density of fish stock in holding ponds, the concentration of dissolved oxygen in the water, survival rate and the prevalence of *Aeromonas salmonicida*.

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