



HOKKAIDO UNIVERSITY

Title	The Epidemiological Study of Furunculosis in Salmon Propagation
Author(s)	Nomura, Tetsuichi; Yoshimizu, Mamoru; Kimura, Takahisa
Description	Nineteenth U.S.-Japan Meeting on Aquaculture. 29-30 October and 2 November 1990. Ise, Japan.
Relation	Edited by Ralph S. Svrjcek. (NOAA Technical Report NMFS ; 111)
Citation	Control of disease in aquaculture : proceedings of the nineteenth U.S.-Japan meeting on aquaculture, Ise, Mie Prefecture, Japan 29-30 October, 1990, satellite symposium: 2 November, 101-108
Issue Date	1992-10
Doc URL	https://hdl.handle.net/2115/39004
Type	conference paper
File Information	yoshimizu-103.pdf



The Epidemiological Study of Furunculosis in Salmon Propagation

TETSUICHI NOMURA*, MAMORU YOSHIMIZU**, and TAKAHISA KIMURA**

*Hokkaido Salmon Hatchery, Fisheries Agency
Nakanoshima 2-2, Toyohiraku
Sapporo, Japan.

** Faculty of Fisheries, Hokkaido University
Minatomachi 3-1
Hakodate, Japan

ABSTRACT

The authors attempted to determine the distribution and prevalence of *Aeromonas salmonicida* in mature chum (*Oncorhynchus keta*), pink (*O. gorbuscha*), and masu salmon (*O. masou*) in Hokkaido that showed no apparent clinical signs of furunculosis. From September 1979 to November 1989, a total of 12,891 chum, pink, and masu salmon were collected from 30 rivers. The changing pattern of the annual prevalence of *A. salmonicida* in salmon was closely related to changes in fish density in the holding ponds: the prevalence of *A. salmonicida* increased in proportion to the increase in the number of fish in the ponds. We concluded from the results of histological and bacteriological examinations that fish with *A. salmonicida* in the kidney were not diseased but were carriers of *A. salmonicida*. The agent could not be isolated from the immature fish examined. *A. salmonicida* was also isolated from the ovarian fluid of fish showing no apparent clinical sign of furunculosis. Few *A. salmonicida* were found on the surface of the eggs one hour after fertilization. A survey of agglutination titers against *A. salmonicida* in sera of chum, pink, and masu salmon showed great variability within the species. The isolated strains were identified as *A. salmonicida* subsp. *salmonicida* and were pathogenic to salmonids. We concluded that the *A. salmonicida* carrier state in fish poses a serious problem in the prevention of furunculosis and its reduction plays a key role in salmon propagation. Both maturation of fish under conditions of low density in ponds, and disinfection of their eggs, are necessary to prevent fish furunculosis during artificial propagation of salmon.

Introduction

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

Furunculosis is not a serious problem in rainbow trout (*Oncorhynchus mykiss*) culture in Japan because this species is resistant to the causative agent of the disease. However, serious mortality has been

documented in juvenile amago salmon (*O. rhodurus*) and masu salmon (*O. masou*) in accordance with increased production of these fish. In Hokkaido, outbreaks of furunculosis have been reported to occur in chum salmon (*O. keta*) by Nishino (1967), and in masu and pink salmon (*O. gorbuscha*) by Kimura (1970) during the maturation of these species in holding ponds. Nomura and Kimura (1981), Nomura (1983), and Nomura et al. (1983, 1991, a and b) reported isolating *A. salmonicida* from the kidneys of mature chum, pink, and masu salmon that showed no apparent clinical signs of furunculosis.

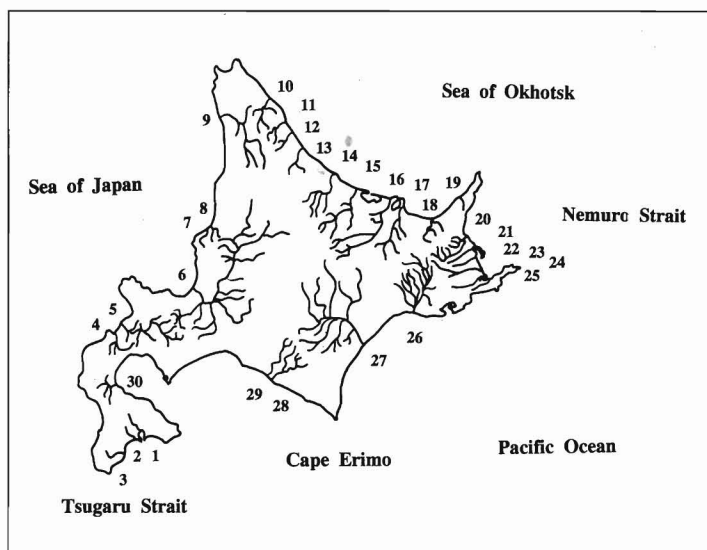


Figure 1

Rivers where chum, pink, and masu salmon were collected (after Nomura et al. 1991a).

1 Moheji	2 Hekirichi	3 Shiriuchi	4 Shubuto
5 Shiribetsu	6 Ishikari	7 Shokanbetsu	8 Nobusha
9 Teshio	10 Tonbetsu	11 Hrobetsu	12 Tokushibetsu
13 Horonai	14 Shokotsu	15 Yuubetsu	16 Tokoro
17 Abashiri	18 Shari	19 Iwaobetsu	20 Ichani
21 Shibetsu	22 Tohoru	23 Nishibetsu	24 Furen
25 Bettouga	26 Kushiro	27 Tokachi	28 Shizunai
29 Niikappu	30 Yurappu		

Recently, no systematic epidemiological studies have been done to establish control measures for furunculosis in salmonids from which quarantine and disease control policies could be based.

In this paper, we report the recent epidemiological study of *A. salmonicida* which was carried out for the purpose of establishing control measures for the disease.

Distribution of *A. salmonicida* in Salmonids in Hokkaido

We attempted to determine the distribution and prevalence of *A. salmonicida* in mature chum, pink, and masu salmon populations in Hokkaido that showed no apparent clinical signs of furunculosis (Nomura et al. 1991a).

From September 1979 to November 1989, a total of 12,891 chum, pink, and masu salmon were collected from 30 rivers (Fig. 1). At each sampling, a total of 60 fish of each species were randomly selected from the rivers' salmonid populations in accordance with Amos (1985). The fish were separated by species and river and held in individual ponds at each river for about 1 month until maturity. After spawning, they were processed for examination. Kidney materials were streaked onto nutrient agar plates (Eiken Co., Tokyo, Japan) and cultured at 20°C for 7 days. No clinical signs of furunculosis were observed in the examined fish. Bacterial colonies that produced a soluble brown pigment and showed the following characteristics were classified as *A. salmonicida*: Gram-negative staining, lack of motility, failure to grow at 37°C, tested positive for cytochrome oxidase, and had the ability to ferment on oxidative fermentative basal medium.

We isolated *A. salmonicida* from chum salmon in 11 of the 22 rivers examined; the percent occurrence of the bacterium in this species of fish ranged from 0.6 to 49.2%. Populations of pink salmon, from 13 rivers were tested and *A. salmonicida* was isolated from 6 of these rivers with percent occurrence ranging from 0.2 to 13.3%. In masu salmon the bacterium was isolated from 5 of 10 rivers examined and the percent occurrence ranged from 1.0 to 5.6%. Hence, *A. salmonicida* was determined to be distributed widely in the salmonid populations of Hokkaido, except those of rivers located between Tsugaru Strait and Cape Erimo (Fig. 2).

In the Ishikari, Shari, Iwaobetsu, Shibetsu, and Tokachi rivers, the prevalence of *A. salmonicida* was found to vary yearly. In the Ishikari river, the prevalence of *A. salmonicida* in chum salmon was high from 1979 to 1984 but has gradually been decreasing since 1985 (Fig. 3). In the chum salmon of the Tokachi River and in all three species in the Shibetsu River, the prevalence of the bacterium remained high throughout the examination period. In the Shari river from 1979 to 1988, *A. salmonicida* was not isolated from any of the fish examined; however, it was isolated from 4 of 60 fish examined in 1989.

From 1979 to 1984, changes in the monthly prevalence of the agent could be observed in fishes in the Ishikari river. The incidence of *A. salmonicida* increased until the middle of October and then decreased thereafter. The pattern of change was closely related with changes in fish density in the holding pond; the prevalence of the bacterium appeared to increase proportionately as density of fish in the pond increased (Fig. 4).

The number of *A. salmonicida* bacteria found in kidney tissues ranged from 10^1 to 10^5 colony forming units per gram (cfu/g) (Nomura et al. 1991a).

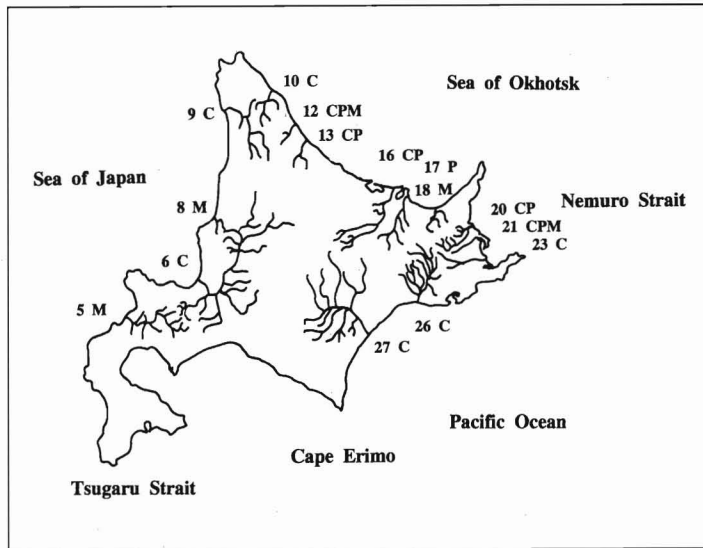


Figure 2

Rivers where *Aeromonas salmonicida* was isolated from chum, pink, and masu salmon from 1979 to 1989. C: isolated from chum salmon; P: isolated from pink salmon; M: isolated from masu salmon (after Nomura et al. 1991a).

5 Shiribetsu	6 Ishikari	8 Nobusha
9 Teshio	10 Tonbetsu	12 Tokushibetsu
13 Horonai	16 Tokoro	17 Abashiri
18 Shari	20 Ichani	21 Shibetsu
23 Nishibetsu	26 Kushiro	27 Tokachi

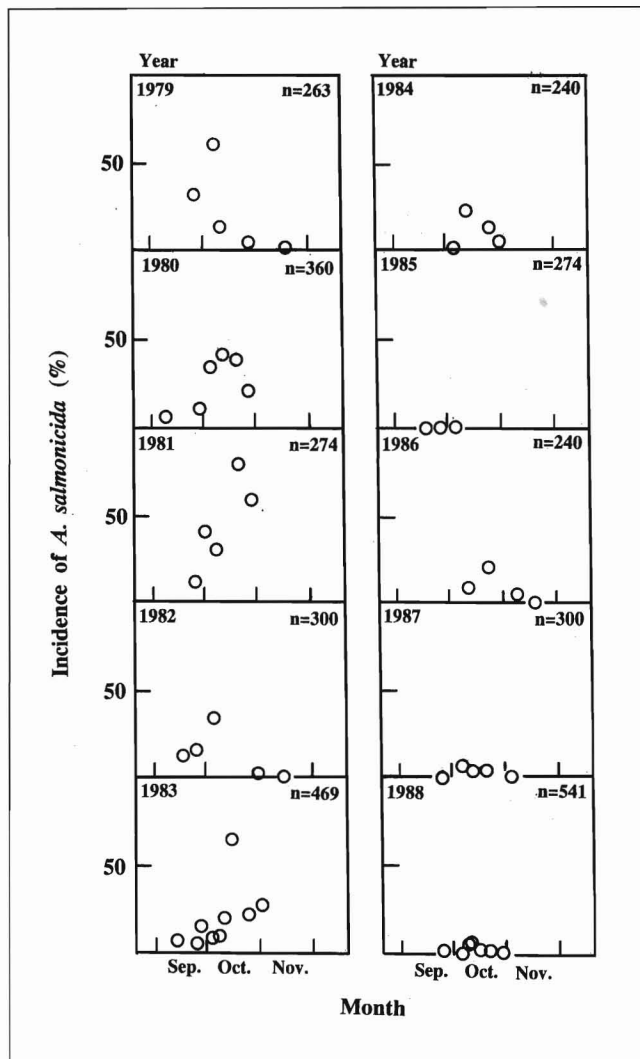


Figure 3

Changes in the monthly incidence of *Aeromonas salmonicida* in chum salmon collected from the Ishikari river, and held for maturation periods during the period September to November, 1979–88 (Modified from Nomura et al. 1991a).

The kidney materials of chum salmon in which *A. salmonicida* was isolated were fixed with Bouin's solution for histopathological examination. The kidney organs were dehydrated and embedded in Paraplast, and sections of the samples were made and stained with HE and Gimsa stain.

Histopathological examination of the infected fish did not, however, reveal colonies of *A. salmonicida* typically observed in fishes with furunculosis. Also, no outbreaks of furunculosis were recorded in the examined populations during the research period (Nomura et al. 1991a).

There are few reports examining the prevalence of *A. salmonicida* in the organs of apparently normal mature fish. In fact, as far as we know, there is only one report by Daly and Stevenson (1985). They reported that *A. salmonicida* was detected in 31 of 286 brown trout (*Salmo trutta*) sampled from spawning runs in the Ganaraska River, Ontario, Canada, over a period of two years. Our results showed that the incidence of this agent in apparently normal chum salmon was higher compared to that of Daly and Stevenson's (1985) estimated for brown trout, and that *A. salmonicida* is distributed widely in the river populations of salmonids in Hokkaido.

Morikawa et al. (1981) reported that the number of *A. salmonicida* in the kidneys of moribund amago salmon was 10^8 to 10^9 cfu/g. The reason why diseased fish were not found in the population we examined, even though they had *A. salmonicida* in their kidneys, was that the degree of infection was not high enough. From the results of histological and bacteriological examinations, we conclude that fish with *A. salmonicida* in the kidney are not diseased fish but carriers of *A. salmonicida*.

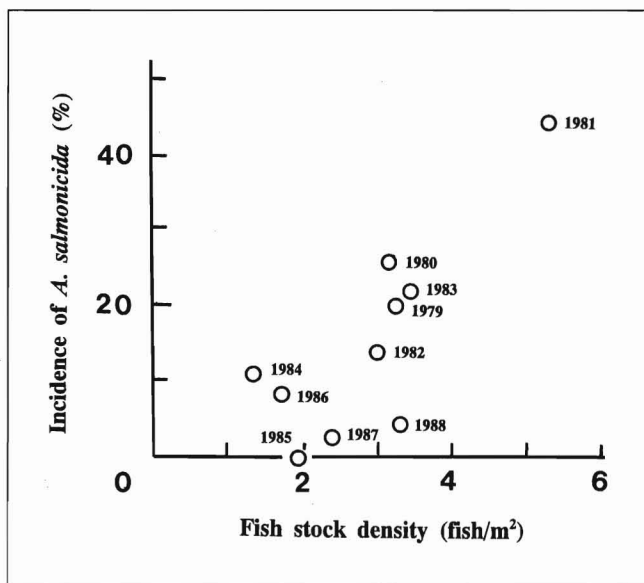


Figure 4

The relationship between average density of the fish in holding ponds and the incidence (% occurrence) of *Aeromonas salmonicida* in fish taken from the Ishikari River. Numbers in the figure indicate the year of examination (unpubl. data).

A. salmonicida in Immature Fish

We attempted to isolate *A. salmonicida* from immature chum and masu salmon (Nomura et al. 1991b). A total of 680 fish were collected in four coastal set nets off Hokkaido, and a total of 1,200 juvenile masu salmon and chum salmon fry were collected from 11 hatcheries of the Hokkaido Salmon Hatchery system.

The bacterium was not isolated from any of the examined fish.

A. salmonicida in Ovarian Fluid

In 1989 in Hokkaido, we attempted to isolate *A. salmonicida* from the ovarian fluids of mature chum, pink, and masu salmon.

Ovarian fluids were collected according to the method of Yoshimizu et al. (1985). The ovarian fluids were streaked onto nutrient agar plates (Eiken Co., Tokyo, Japan) and the plates were cultured at 20°C for 7 days. Number of viable counts of *A. salmonicida* in ovarian fluid and kidney were measured in accordance with the method of Nomura et al. (1991a).

A. salmonicida was isolated from the ovarian fluid of fish showing no apparent clinical signs of furunculosis. For example, *A. salmonicida* was isolated from the ovarian fluid of 22 of 120 fish examined from the Tokachi River. The number of *A. salmonicida* bacteria

in ovarian fluids ranged from 10^1 to 10^7 cfu/mL in the populations from the Shibetsu and Teshio rivers.

Ovarian fluid containing *A. salmonicida* flows out of the fish at the time eggs are stripped or during the process of maturation in the pond. Consequently, infected water and infected ovarian fluid are expelled into the river because the sewage from the egg stripping areas and the holding ponds is not disinfected in Hokkaido. We suspect from these results that the agent drained from the fish may be a source of infection for other anadromous salmon that ascend the river for spawning.

Horne and Maj (1928), McCraw (1952), and Hastein and Lindstad (1991) stated that the most important source of *A. salmonicida* in the spread of furunculosis is the existence of fish carrying this agent. Fish carrying the bacterium pose a serious problem to the prevention of furunculosis, and their reduction in fish plays a key role in salmon propagation.

A. salmonicida on Egg Surfaces

The existence of the bacterium in the ovarian fluid suggests that the surface of eggs taken from the fish will also be contaminated. Contaminated eggs may spread the agent to areas where the eggs will be transplanted. We studied the existence of *A. salmonicida* on the surface of eggs by artificially contaminating chum salmon eggs with *A. salmonicida*.

The *A. salmonicida* 20-1, strain, which was isolated from the kidneys of chum salmon in the Tokachi River, was used as inoculum. The strain was cultured and harvested, then was suspended in phosphate buffer saline (PBS). The chum salmon eggs were bathed in PBS containing the agent for an hour. The eggs were incubated in well water at 8°C in the laboratory. At one hour and at 24 hours after fertilization, we took 20 eggs from the incubator and put them into sterilized water. The flask was shaken strongly for 5 minutes, we then measured the viable number of *A. salmonicida* in the water according to the method of Nomura et al. (1991b).

Egg surfaces were initially bathed with 1.1×10^4 to 4.3×10^6 cfu/egg of *A. salmonicida*. The number of *A. salmonicida* present on the egg surfaces decreased from 68 to 4.6 cfu/egg an hour after fertilization and *A. salmonicida* could not be isolated from the eggs cultured on plates 24 hours after the initial bath treatment.

We also attempted to isolate *A. salmonicida* from eggs in the incubation boxes at the Satsunai, Nakagawa, Nemuro, and Tokachi hatcheries. These eggs were taken from brood fish in which the preva-

lence of *A. salmonicida* was high (Nomura et al. 1991a). Fortunately, *A. salmonicida* was not isolated from any of the 15 hatcheries' eggs (Nomura et al. 1991b).

In Hokkaido, fertilized eggs are transported to a hatchery from the egg collection location 1 hour after fertilization. From the results of our experiment, it appears that *A. salmonicida* is able to exist on the surface of an egg. This makes us concerned that we may be transporting the bacteria to the hatchery with the fertilized egg. We believe that it is necessary to prevent the transfer of *A. salmonicida* via eggs in order to control furunculosis.

Survival of *A. salmonicida* in Water

By definition, *A. salmonicida* is considered to be an obligate pathogen (Popoff 1984) and is never found in surface water. Its ability to survive and remain infectious in the external environment may be a major determinant in the spread of furunculosis. We studied the viability of *A. salmonicida* in nonsterile, sterile filtered, and autoclaved fresh water and in salt water.

A. salmonicida strain 20-1 isolated from chum salmon in the Tokchi River was used in this experiment. The strain was cultured at 20°C and harvested, then suspended in fresh water or in sea water. The suspended cells were inoculated into 200 mL of nonsterile, sterile filtered, and autoclaved fresh water and saltwater and were incubated at 10°C.

In sterilized fresh water, *A. salmonicida* survived for 60 days and in nonsterile water, only 4 days. The survival of *A. salmonicida* in sterile salt water was 8 days; this was a shorter survival period than that in sterile fresh water.

It is believed that *A. salmonicida* is not able to exist for long time in water without fish, but McCarthy (1980) studied the survival of the agent in water using an antibiotic-resistant strain of *A. salmonicida* and found the agent could survive for 8 days in water.

The results of McCarthy (1980) and this study indicate that *A. salmonicida* survives long enough to infect other fish in the water.

Variation of Agglutination Titer Against *A. salmonicida* in the Serum

A serological survey of adult salmon was made from blood samples collected in 1988 in Hokkaido, from mature chum, pink, and masu salmon. Blood was aseptically extracted from the dorsal artery with 10 mL of Vacteinor (Terumo Co., Tokyo, Japan). The resulting serum was separated from the blood-cell clot by centrifugation and was stored at -90°C until assayed. The serum was tested for agglutinating antibody titers individually, by test-tube methods with *A. salmonicida* ATCC14174.

Agglutinin titers against *A. salmonicida* in the serum of mature chum, pink, and masu salmon in Hokkaido in 1988 are shown in Table 1.

Of a total of 75 serum samples taken from mature chum salmon, 73.3% did not have the agglutinin, and the range of titers was 8 to 32. In pink salmon, 10% of the sample did not have the agglutinin, and the range was 4 to 32. In masu salmon, 16.6% of the examined serum did not have the agglutinin, and the range was 4 to 128.

The diversity in the incidence of agglutination titer within each of the three species indicates a continuous, widespread interaction between individuals of

Table 1
Prevalence and ranges of agglutinin titers against *A. salmonicida* in the serum of mature chum, pink, and masu salmon ascending various rivers in Hokkaido.

Species	River	No. of fish examined	Agglutinin titers		
			Negative (%)	Modes	Range
Chum salmon	Ichani	15	80.0	32	16- 32
	Tokachi	30	76.6	8	8- 32
	Shizunai	30	66.6	16	8- 32
Pink salmon	Shokotsu	30	16.6	16	4- 32
	Iwaobetsu	30	6.7	16	4- 32
	Nishibetsu	30	6.7	16	4- 32
Masu salmon	Shiribetsu	39	15.4	8	4-128
	Shari	18	0	16	4-128
	Nishibetsu	27	29.6	8	4- 32

the host populations and *A. salmonicida*. The difference in the amount of 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 residency increased. Weber and Zwicker (1979) reported that of a total of 43 serum sampled from Atlantic salmon (*Salmo salar*) in the Miramichi or Margareen rivers in Canada, none had *A. salmonicida* agglutinin, but of 27 Restigouche River salmon, four had a titer of 10, five had a titer of 20, and one had a titer of 640. They confirmed that Atlantic salmon have previously contacted *A. salmonicida* in the Restigouche River.

In our study, agglutinate titers in the serums were low. 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 furunculosis was useful in adult masu salmon during the holding period because these salmon stay in fresh water for a long enough period to allow them to produce antibodies after antigen inoculation. In chum salmon, however, the freshwater residency period is short, so this method of prevention would not be practical.

Isolation of the Bacteriophage of *A. salmonicida* from Water

There is no sensitive medium for selecting *A. salmonicida*. This means that when the number of *A. salmonicida* in water is low, the isolation of *A. salmonicida* from the water will be difficult. This is because *A. salmonicida* cannot grow on the culture medium under competitive conditions with other natural bacteria populations. We attempted to isolate the bacteriophage of *A. salmonicida* to ascertain the existence of *A. salmonicida* in the water.

Water samples, from 11 hatcheries and 4 rivers were examined. Nutrient agar (Eiken Co., Tokyo, Japan) was employed for the routine culture, dilution, and enumeration of *A. salmonicida* and its phage strain. One hundred mL of sample was added to 500 mL of cultured *A. salmonicida* Ar-32, Ar-43, Ar-71, and H-70 strains in the logarithmic phase. Detection and enumeration of phage were achieved using the medium and double agar layer technique (Paterson et al. 1969). The results are shown in Table 2.

McCraw (1952) stated that when the bacteriophage of *A. salmonicida* exists, its presence may indicate the existence of the bacterium. The bacteriophage was isolated from two samples of river water and five samples from hatchery water. From this result, it was

suspected that *A. salmonicida* can survive for a long time in the river water in Hokkaido after leaving the fish and that its existence may be a source of infection to salmonid fish. The results suggest that such bacteriophage could be very useful for studying the existence of the agent in water.

Table 2

Isolation of *Aeromonas salmonicida* bacteriophage from the samples of river water and hatchery water.

Sample	Samples	
	Numbers examined	Isolated containing phage
River water	4	2
Hatchery water	9	2
Sewage of hatchery	13	3

Pathogenicity of the Isolated *A. salmonicida*

The isolated strains were identified as *A. salmonicida*, subspecies *salmonicida*, by their biological, biochemical, and immunological characteristics. All of the isolated *A. salmonicida* strains showed auto-agglutination and produced protease in the medium, so we also expected them to be pathogenic.

In order to examine the pathogenicity of the isolated strain, we injected it into chum and masu salmon fry and adult chum salmon.

A. salmonicida 20-1 was cultured for 48 hours at 20°C. The cells were washed three times in PBS and were suspended in PBS. The strain was injected into chum salmon fry, yearling masu salmon, and chum salmon brood fish at concentrations of 1.7×10^2 , 1.8×10^3 and 6.0×10^5 cfu/fish, respectively.

All of the 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 around 10^8 cfu/g kidney tissue, the same number reported by Morikawa et al. (1981) in the kidneys of moribund amago salmon. On the basis of these results, we suspect the isolate is a pathogenic strain.

Control of *A. salmonicida* on the Surface of Egg

To establish a method of controlling *A. salmonicida* on the eggs, the bactericidal effect of popidon-iodine (Isodine), and the toxicity of this agent to the chum salmon egg were studied.

The bactericidal effects of popidon-iodine to *A. salmonicida* were determined in accordance with the method of Amend and Fryer (1972).

A. salmonicida was completely killed by treatment with 25 ppm isodine for five minutes and this solution was not toxic to the chum salmon eggs for treatments lasting up to one hour. Thus, the authors confirmed that isodine solution has a sanitizing effect on the agent, and that it does not have adverse effects on chum salmon eggs.

Method 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 during their maturation period in the holding ponds; as the average density of brood fish stocked in ponds decreased, the incidence of *A. salmonicida* in examined fish also decreased (Fig. 4). Therefore, we examined the relationship between the stocking density of fish in the pond and the prevalence of *A. salmonicida* in the fish.

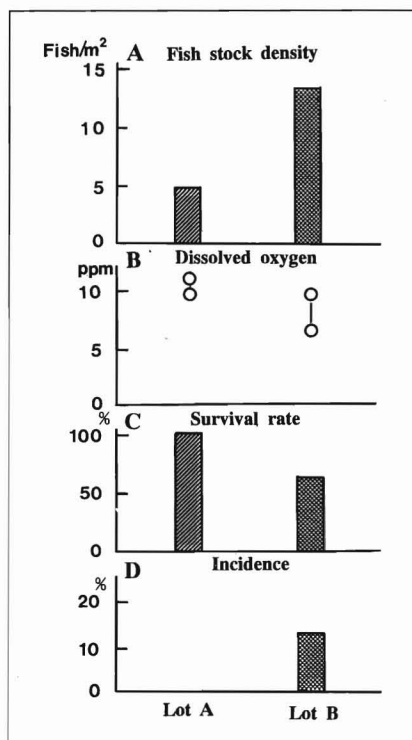


Figure 5

The relationship between the density of fish stock in holding ponds, the concentration of dissolved oxygen in the water, survival rate, and the incidence (% occurrence) of *Aeromonas salmonicida* (unpubl. data).

Chum salmon in the Ishikari River were randomly assigned to experimental holding ponds and held under low (4.9 fish/m²) and high density (14.7 fish/m²) conditions until maturation. The kidney tissues of all the fish used in experiment were cultured on nutrient agar in accordance with the method of Nomura et al. (1991).

As we expected, we found that 12.4% of the fish examined harbored *A. salmonicida* when they were stocked at a high density, but no examined fish contained the agent when they were stocked at a low density (Fig. 5A). The incidence of the bacterium in fish that were held under low dissolved oxygen conditions was higher than that of fish held under high dissolved oxygen levels (Fig. 5D). These results clearly indicate that high stocking densities and low dissolved oxygen levels in holding ponds have a marked effect on the prevalence of the agent in the fish. We concluded that fish maturation in the pond under low density conditions and disinfection of the eggs, are necessary to prevent fish furunculosis in the artificial propagation of salmon.

Citation

- Amend, D.F., and J.L. Fryer.
1972. Virucidal activity of two iodo-phors to salmonid virus. J. Fish. Res. Board Can. 29:61-65.
- Amos, K.H., ed.
1985. Fish health blue book-procedures for the detection of certain fish pathogens. Fish health section, Am. Fish. Soc., 114 p.
- Austin, B., and D.A. Austin.
1987. Bacterial fish pathogens: disease in farmed and wild fish. Ellis Horwood Ltd., Chichester, West Sussex, England, 195 p.
- Daly, J.G., and R.M.W. Stevenson.
1985. Importance of culturing several organs to detect *Aeromonas salmonicida* in salmonid fish. Trans. Am. Fish. Soc. 114:909-910.
- Emmerich, R., and C. Weibel.
1890a. Über eine durch Bakterien verursachte Infektionskrankheit der Forellen. Allg. Fish. Zgt. 15:73-77.
1890b. Über eine durch Bakterien verursachte Infektionskrankheit der Forellen. Allg. Fish. Zgt. 15:85-92.
- Hastein, T., and T. Lindstad.
1991. Disease in wild and cultured salmon: possible interaction. Aquaculture. 98:277-288.
- Herman, R.L.
1968. Fish furunculosis 1952-1966. Trans. Am. Fish. Soc. 97:221-230.
- Horne, J.H., and I.M.S. Maj.
1928. Furunculosis in trout and the importance of carriers in the spread of the disease. J. Hyg. 28:67-78.
- Kimura, T.
1970. Studies on a bacterial disease occurred in the adult "Sakuramasu" (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) rearing for maturity. Sci. Rep. Hokkaido Salmon Hatchery. 24:9-100. (In Japanese; English abstr.)

- McCraw, B.M.
1952. Furunculosis of fish. U.S. Fish and Wildl. Serv. Spec. Sci. Rep. 84:87 p.
- McCarthy, D.H.
1980. Some ecological aspects of the bacterial fish pathogen—*Aeromonas salmonicida*. In Aquatic microbiology: symposium of the Society of Applied Bacteriology 6:299–324.
- Morikawa, S., S. Miki, and F. Tashiro.
1981. Changes in hemato logical properties and viable cell number of bacteria in amago salmon artificially infected with *Aeromonas salmonicida*. Fish Pathol. 16:43–49. (In Japanese; English abstr.)
- Nishino, K.
1967. On a bacterial disease of adult "sake" *Oncorhynchus keta*, in captivity. Fish Pathol. 2(1):73–74. (In Japanese.)
- Nomura, T.
1983. Incidence of *Aeromonas salmonicida* among anadromous salmonids, 1980–1982. Sci. Rep. Hokkai- do Salmon Hatchery. 37:63–65. (In Japanese; English abstr.)
- Nomura, T., T. Kimura.
1981. Incidence of *Aeromonas salmonicida* among anadromous salmonids. Fish Pathol. 16:69–74. (In Japanese; English abstr.)
- Nomura, T., T. Kimura, I. Shimizu, and K. Nara.
1983. Incidence of *Aeromonas salmonicida* among chum salmon, *Oncorhynchus keta*, in Chitose River. Sci. Rep. Hokkaido Salmon Hatchery. 37:53–61. (In Japanese; English abstr.)
- Nomura, T., M. Yoshimizu, and T. Kimura.
1991a. Prevalence of *Aeromonas salmonicida* in the chum salmon (*Oncorhynchus keta*), pink salmon (*O. gorbuscha*) and masu salmon (*O. masou*) returning to rivers in Hokkaido. Fish Pathol. 28, 139–147. (In Japanese; English abstr.)
- Nomura, T., M. Yoshimizu and T. Kimura.
1991b. Prevalence of *Aeromonas salmonicida* in the kidney of chum salmon (*Oncorhynchus keta*) and masu salmon (*O. masou*) at various life stages. Fish Pathol., 26:149–153. (In Japanese; English abstr.)
- Paterson, W.D., R.J. Douglas, I. Grinyer, and L.A. McDermott.
1969. Isolation and preliminary characterization of some *Aeromonas salmonicida* bacteriophages. J. Fish. Res. Board Can. 26:629–632.
- Popoff, M.
1984. Family II. Vibrionaceae. In Bergey's manual of systematic bacteriology, Vol. 1 (Noel R. Krieg and John G. Holt eds.), p. 545–550. Williams and Wilkins, Baltimore.
- Smith, I.W.
1960. Furunculosis in salmon kelts. Nature 186:733–734.
- Snieszko, S.F.
1972. Panel review on furunculosis of salmonidae. In Report of the symposium on the major communicable fish diseases in Europe and their control; 20–22 April 1972, Amsterdam (William A. Dill, ed.): p 157–163. European Inland Fisheries Advisory Committee EIFA Technical paper 17 (suppl. 2).
- Yoshimizu, M., T. Kimura, and J.R. Winton.
1985. An improved technique for collecting reproductive fluid samples from salmonid fish. Prog. Fish-Cult. 47:199–200.
- Weber, J.M. and B.M. Zwicker.
1979. *Aeromonas salmonicida* in Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 36:1102–1107.