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Vibriosis and Furunculosis in Marine Cultured Salmon in Puget Sound, Washington

ANTHONY J. NOVOTNY

ABSTRACT—Infections in marine cultured Pacific salmon (genus Oncorhynchus*) and trout (genus* Salmo*) can include those caused by two bacterial pathogens,* Vibrio anguillarum *(vibriosis) and* Aeromonas salmonicida *(furunculosis). In the Puget Sound area, two distinct serotypes of* V. anguillarum *have caused extensive mortalities in net-pen culture. Although furunculosis is probably carried by fish from fresh water, it can be transmitted in seawater, and in the close confines of net-pen culture can reach epizootic proportions. In large-scale experiments, epizootics of vibriosis and furunculosis reduced the population of two sea cages (300,000) of chinook salmon (*O. tshawytscha*) by 80 percent during approximately 5 months of marine culture. Laboratory tests of the bacterial pathogen isolated from moribund fish indicated that the furunculosis organism was resistant to oxytetracycline and sulfa drugs, but was sensitive to furazolidone. Multiple infections of both diseases proved difficult to treat. The results of these experiments indicate the need for better management of furunculosis during the freshwater culture stages of salmon.*

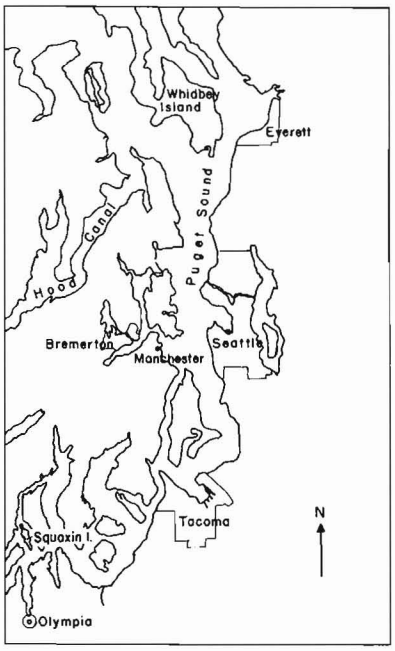

Figure 1.—Puget Sound, Washington. This inland arm of the northeastern Pacific Ocean is the site of major activities for the marine culture of Pacific salmon.

Since 1969, the Northwest and Alaska Fisheries Center of the National Marine Fisheries Service has been conducting research on the marine culture of salmonids (*Oncorhynchus* sp. and *Salmo* sp.) at its Aquaculture Experiment Station near Manchester, Wash. (Fig. 1). The major research effort at this station in central Puget Sound is focused on the culture of coho, *O. kisutch,* and chinook, *O. tshawytscha,* salmon in floating net pens.

The principle of this type of culture is the same as for an agriculture feedlot: the fish are concentrated to minimize space, materials, and labor, and are fed commercial pelleted rations. Tidal currents provide an almost continuous exchange of water through the knotless nylon net pens, insuring adequate supply of dissolved oxygen to the fish and the dilution and removal of excretory waste products.

The normal procedure is to transport unacclimated juvenile chinook or coho salmon from a freshwater hatchery and place them directly into seawater pens. The fish are then cultured for 6 months to a year prior to harvesting. The physiological stresses caused by direct transfer from fresh water to seawater, high population densities, repeated handling, and increasing water temperatures during the summer are conducive to diseases (Wedemeyer, 1970; Wedemeyer and Wood, 1974).

During an experiment to determine the effects of rearing densities on the growth and survival of chinook salmon in seawater pens, repeated epizootics of vibriosis and furunculosis were encountered. The objectives of this paper are to: 1) present a history of these diseases during the course of the experiment; 2) demonstrate drug resistance in furunculosis; 3) demonstrate the infectious nature of furunculosis in seawater; and 4) show that the presence of multiple infectious agents can be a serious threat to marine cultured salmonids.

DESCRIPTION OF VIBRIOSIS AND FURUNCULOSIS

The two most common diseases occurring in salmon cultured in seawater in Puget Sound are vibriosis and furunculosis. Both of these bacterial diseases can be either epizootic or chronic; they can also occur in marine fish other than salmon (Evelyn, 1971a, b; Kennedy, 1974; Egidius and Andersen, 1975; Novotny, 1975). The typical symptoms of both diseases are almost identical: a general hemorrhagic septicemia, external lesions, hemorrhaging of the fins, and bloody discharges from the vent.

Vibriosis in fish is an infection caused by the marine bacterium *Vibrio anguillarum* (Bergman, 1909), a motile, gram-negative rod which has been thoroughly described by Evelyn (1971b). At least two pathogenic serotypes have been identified (Novotny, 1975; Harrell et al., 1976).

Furunculosis in fish is an infection caused by the nonmotile, gram-negative rod, *Aeromonas salmonicida* (Lehmann and Neumann, 1896). This disease has been described (McCraw, 1952; Scott, 1968), but is generally considered to be a serious problem in freshwater environments only. Unlike

Anthony J. Novotny is with the Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Blvd. East, Seattle, WA 98112.

the vibrios, *A. salmonicida* is not considered to be a marine organism. In salmonids, the disease is undoubtedly first contracted during the freshwater culture stages. At low temperatures, the disease is latent and in a carrier state in infected fish (Snieszko, 1969). When the fish are transferred to seawater, the combined effect of osmotic stress and other environmental factors weakens the hosts, and the disease become infectious. Transmission in seawater can undoubtedly occur by direct contact (Scott, 1968) and probably by contact with fecal casts.

DISEASE OBSERVATIONS DURING 1972-73

Severe outbreaks of vibriosis and furunculosis among chinook salmon during an experiment to determine the effect of different fish densities on fish growth and survival in pens allowed extensive observation of the diseases. Observations on diagnosis, drug sensitivity tests, treatment, and mortality assessment are mentioned below.

In June 1972, juvenile chinook salmon were transferred from trucks to floating net pens by gravity flow through a large pipe. The fish went directly from the fresh water in the transport trucks to 30‰ seawater. The floating hexagon-shaped pens, made from knotless nylon webbing, measured 4.9 m on a side, were 3.8 m deep, and had an approximate volume of 220 m^3.

One hundred thousand salmon, weighing an average of 5.4 g each were placed in Pen I and 200,00 in Pen II. The temperature of the water in the transport truck was 13.9°C for Pen I fish and 17.9°C for Pen II fish. The temperature of the seawater was 11.2°C. The fish were to be reared in the pens for approximately 1 year, during which time they were to be fed a daily ration of Oregon Moist Pellets (OMP), based on a percentage of body weight.

Diagnostic procedures

Sick or freshly dead fish were routinely examined for disease. Samples of kidney tissue were aseptically streaked onto Petri dishes containing Trypticase Soy Agar (TSA)[1] with 1.5 percent NaCl or Tryptose Blood Agar (TBA). Sterile discs impregnated with the vibriostatic agent 2,4-diamino-6,7-di-iso-propyl pteridine phosphate (0/129) were placed on freshly streaked TSA plates. All plates were incubated at 24°C for 24 to 72 hours. A diagnosis of vibriosis was based on: 1) growth of smooth, opaque colonies in 24 to 48 hours on TSA, 2) motility, and 3) a zone of inhibition to the 0/129 disc of 8 mm or more. Furunculosis was considered to be the causative agent of disease when: 1) Pin-point colonies appeared within 72 hours on TBA, 2) the organisms were nonmotile, and 3) the TBA plates would be pigmented a light to chocolate brown.

Because of the large quantities of diseased fish that were processed, it was impossible to confirm all cultures by biochemical reactions. However, culture samples were periodically analyzed by the Department of Microbiology, University of Washington, for confirmation. In general, the accuracy of the field diagnosis was high.

Drug Sensitivity Tests

Drug sensitivity tests were conducted by using discs containing 30 μg of oxytetracycline or 100 μg of furazolidone placed on freshly streaked TSA or TBA plates. Triple sulfa (SSS) was tested by placing discs containing 250 μg SSS on freshly streaked plates containing Mueller-Hinton's agar. All plates were incubated at 24°C until sufficient growth occurred to indicate zones of inhibition. A zone of inhibition of less than 10 mm indicated a questionable effective value.

Therapeutic Treatment Procedures

All of the fish were placed on OMP-TM-50D (OMP-TM-50D is a commercial pellet containing 4 percent TM-50D; eleven percent of TM-50D is active oxytetracycline) medicated diets for the first 5 days after transfer as a precautionary measure to assist in combating any stress infection induced by transfer shock. Routinely thereafter, whenever the mortalities reached 0.1 percent/day and vibriosis or furunculosis was determined to be the cause, OMP-TM-50D diets were fed the fish for at least 5 days, or until the mortalities subsided to less than 0.1 percent/day. When it became evident later in the study that the furunculosis organism was resistant to oxytetracycline, standard OMP diets were prepared daily with commercial agriculture grade Furox 50. The Furox 50 (which is 50 percent active furazolidone) was mixed with herring oil and sprayed over the pellets at a rate of 2 percent oil by weight. The amount of Furox 50 added to the oil was adjusted to provide 0.8 g of Furox 50/kg fish per day.

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Mortality Assessments

Dead fish were removed from the pens each day and counted, except when mortalities were excessively high. In this case, total weights were taken, and an estimate of mortality was obtained from subsample weights and counts. Subsamples of the live fish population were obtained periodically to measure growth rates and to adjust feeding levels.

RESULTS AND DISCUSSION

The initial 10-day mortality in the high density pen (Pen II) was 0.5 percent compared to 0.1 percent in the low density pen (Pen I). This was believed to be due to the greater temperature shock during the transfer of Pen II fish.

Within 3 weeks of transfer, the nets became heavily fouled with sessile filamentous algae, reducing water flow through the pens. Dissolved oxygen concentrations in the center of the pens occasionally dropped to 3.5 ppm, creating an oxygen stress condition. This condition was somewhat alleviated by forcing large volumes of compressed air through perforated pipes beneath the pens. The rising air bubbles brought in new water, creating a partial exchange. This problem was solved in late July, when larger mesh net pens were installed.

Water temperatures reached 12°C early in July (Fig. 2). At this time, the first serious epizootic of vibriosis occurred. At times, the mortalities were

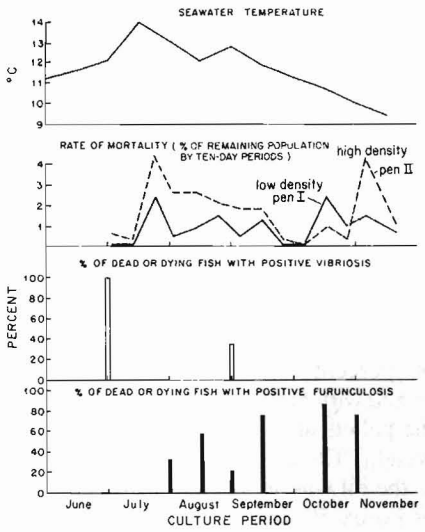

Figure 2.—Incidence of vibriosis and furunculosis in addition to estimated rates of mortality in the high and low density pens in relation to season and seawater temperature. Note that the declining seawater temperature in the fall did not inhibit an epizootic of furunculosis.

Table 1.—Salinity tolerance and anitbiotic sensitivity of *Aeromonas salmonicida*[1].

Sample no.	I	II	III	T_{30}	SSS	F_{100}
1	+ D	+ M	− N	0	11	25
2	+ D	+ M	+ N	0	8	12
3	+ D	+ L	− N	0	7	15
4	+ D	+ D	− N	Not conducted		
5	+ D	+ M	− N	0	11	22
6	+ D	+ L	− N	Not conducted		
7	+ D	+ M	− N	0	5	12
8	+ D	+ M	− N	0	0	10
9	+ D	+ M	− N	0	5	20
10	+ D	+ M	+ N	0	0	9
11	+ D	+ D	− N	0	0	15
12	+ D	+ M	+ N	0	0	15
13	+ D	+ M	+ L	0	0	15
14	+ D	+ M	− N	0	0	12

[1]Tests were performed with direct isolates from kidney smears on Tryptose Blood Agar and Mueller-Hinton's Agar. Samples collected 1-2 November 1972. I = 1.5 percent NaCl; II = 2.0 percent NaCl; III = 3.0 percent NaCl. + = good colony growth; − = no growth. Agar pigmentation: D = dark; M = medium; L = light; N = none. Antibiotic sensitivity discs (distance is the radius in mm): T_{30} = 30 μg oxytetracycline; SSS = 250 μg triple sulfa; F_{100} = 100 μg furizolidone.

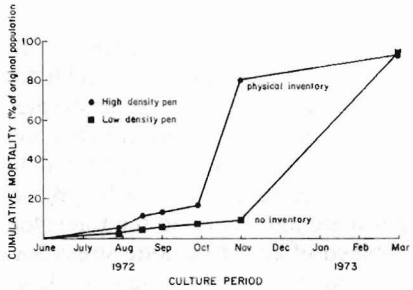

Figure 3.—Estimated cumulative losses of chinook salmon in the high and low density pens. The large disparity between the two pens in November is an estimating error. The high density pen was physically inventoried, whereas the low density pen was not inventoried until the following March.

so numerous that it was difficult to estimate mortality rates or standing populations. Deterioration of dead fish was so rapid that many were never recovered. Therefore, 10-day mortality rates in Figure 2 are based on estimates of the standing population. Mortality rates were generally in excess of 1 percent (≥0.1 percent/day), and the rate of mortality in Pen II was always higher than in Pen I, with the exception of a period in October (Fig. 2).

Our procedure for treatment, based on past experience, was to use OMP-TM-50D exclusively to combat vibriosis. Normally, an OMP-TM-50D diet was only necessary for a period of 5-7 days to control the disease. Rarely had oral medication been used more than three times during a growing season (summer-fall). However in this study, the mortalities were so numerous that we doubted we could control the disease with medicated feeds.

Figure 2 clearly indicates that the dominant problem was furunculosis. When the first epizootics of furunculosis appeared in July, we began testing cultures immediately. We found that the furunculosis organism was: 1) Resistant to oxytetracycline (whereas the vibrio was not) and triple sulfa compounds, but sensitive to furazolidone, and 2) it would grow on media containing between 2 and 3 percent NaCl (Table 1). This second finding indicated that this strain of furunculosis might be transmittable in seawater. Later, we isolated this organism from adult coho salmon cultured in pens within a 15-mile radius of Pens I and II. In addition, we isolated the organism from marine fish that accidentally entered the pens with the infected chinook salmon (Novotny, 1975).

Furox 50 was tried as a treatment for furunculosis. The first Furox 50 treatment period was extended to approximately 12 days in an attempt to reduce the numbers of low-level infectious or carrier fish as much as possible. We assumed that once the temperatures started dropping in early fall, our problems of recurring epizootics would be over. The rate of observed mortality declined through August, and a second treatment in August reduced the mortality rate to an even lower level in September (Fig. 2). However, by mid-October, the rate of mortality again began to climb in spite of decreasing water temperatures. *Aeromonas salmonicida* was isolated from almost all subsamples of dead or dying fish.

In late October, we inventoried the high density pen by passing all of the fish in the pen through a photocell fish counter. In spite of Furox 50 feedings before and after inventory, over 7,000 mortalities were recorded within 10 days. The inventory indicated a cumulative loss of 80 percent of the fish in Pen II (Fig. 3). Although the data in Figure 3 indicate a better survival in Pen I in November, this is an unreliable estimate, and the cumulative mortality was more likely between 70 and 80 percent. We continued the Furox 50 treatment in both pens periodically through the winter.

By February 1973, the surviving fish in both pens appeared to be in good enough condition to inventory. The fish were weighed and samples collected to determine average weights. We estimated only 6,000 fish surviving in Pen I and 14,000 in Pen II, for a total survival of 6 and 7 percent, respectively. At no time during the course of the experiment did we ever reach a planned high loading density of 32 kg/m³ (Fig. 4).

This total mortality of 93.5 percent is contrasted with a loss attributed to vibriosis of 3.2 percent during the 1971-72 rearing period at Manchester and a 1972-73 loss of 15.7 percent (directly attributable to vibriosis) of a different stock of chinook salmon (Moring, 1973). These lots represented stocks of chinook salmon from other hatcheries, no incidence of furunculosis was found, and all epizootics of vibriosis were easily controlled with OMP-TM-50D.

When the growth of the fish in the

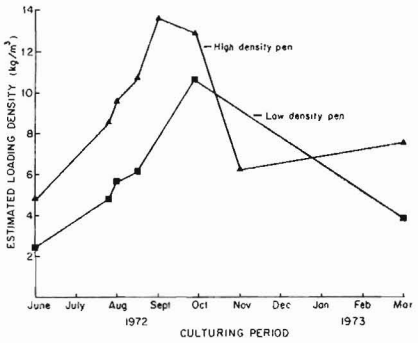

Figure 4.—Estimated loading densities of chinook salmon in the two pens during the spring-winter culturing periods. No physical inventory of the low density pen was taken in November, and the fall estimate of this pen is probably in error.

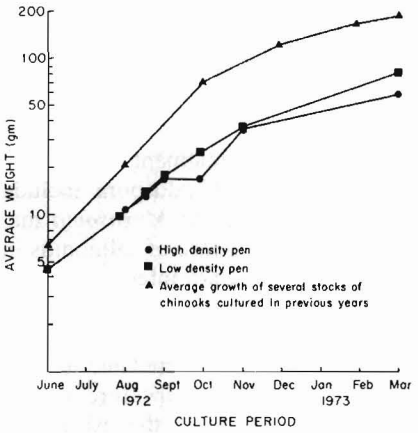

Figure 5.—Growth of chinook salmon in the density tests. A comparison is made with average growth rates for previous years.

loading density study was compared with the average growth of several stocks of chinook salmon cultured in previous years (Fig. 5), there is a depression in the rate of growth in the density of fish. Moring's (1973) average fish weights were 145 g in February 1972 and 92 g in February 1973. By contrast, the average weights of the Pen I and Pen II chinook salmon in February 1973 were 80 g and 59 g, respectively.

We felt that we had been unsuccessful in combating the infectious diseases for three primary reasons: 1) Environmental stress caused by low dissolved oxygen, 2) a carrier state of furunculosis, and 3) high loading densities. Secondary factors were handling stresses and the fact that the average size of the juvenile chinook salmon that were transferred to the net pens was considered to be too small for successful adaptation to saltwater. (Juvenile chinook salmon should average at least 8 g when transferred to seawater.) Osmotic stress is an important factor in predisposing fish to disease (Wedemeyer, 1970; Wedemeyer and Wood, 1974).

Approximately 20 percent of the fish were tagged, and the entire population was released between May and July 1973. The fish that were released were apparently healthy survivors. On the basis of actual tag recoveries, Moring (1973) estimated a 0.1 percent contribution to the sport fishery, and contributions from the tag recoveries of the released density-study fish were 0.3 percent. However, neither of these contributions is high. Moring (1973) notes that chinook salmon reared for 9 months in pens at Manchester and released in April 1971 had a 12 percent recovery rate in the sport fishery—a recovery 40 times greater than that of the fish used in the density study.

CONCLUSIONS

1) Juvenile chinook salmon are highly susceptible to vibriosis and furunculosis when subjected to excessive physical, environmental, and/or osmotic stress following transfer to seawater culture systems. Seawater temperatures in excess of 12°C contribute to the severity of epizootics of vibriosis and furunculosis. Epizootics of furunculosis recurred in the fall, in spite of declining seawater temperatures.

2) Furunculosis can be carried by the host fish in a latent state from fresh to saltwater. Furthermore, the growth of furunculosis on culture media containing 20-30‰ salt and other evidence suggests that the organism may be transmitted in saltwater.

3) Drug sensitivity tests demonstrated that the strain of *A. salmonicida* isolated in this study is resistant to oxytetracycline and sulfa compounds but sensitive to furazolidone. Severe combined epizootics of vibriosis and furunculosis could not be effectively controlled by feeding medicated diets containing oxytetracycline.

4) These observations suggest that a serious effort must be put forth to control furunculosis in fresh water.

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