

SH 151 .N67 1973
24th annual Northwest Fish Culture
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THE SIZE OF COHO SALMON AND TIME OF ENTRY INTO
SEA WATER: PART 2 EFFECTS OF VIBRIO VACCINATION

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Our previous attempts to accurately define the effects of the size of zero-age coho salmon and the time of entry into sea water upon subsequent growth have been frustrated to a certain extent by the interactions of disease. Direct transfers of zero-age coho salmon from fresh water to 30 0/00 sea water are usually conducted (both experimentally and on a production scale) during the period from late May to early August in our Puget Sound region. This period also coincides with the peak incidence of vibriosis, especially in the Manchester area. Epidemics of this bacterial disease in sea water tanks and experimental floating net-pens in past years have created serious problems in maintaining statistical control, and in making repeated measurements of fish growth. We felt that these problems could be eliminated by the intra-peritoneal (IP) injection of a vaccine prepared from a local isolate of the infectious agent, Vibrio anguillarum. Research conducted in Oregon by J. Fryer and R. Garrison, indicated that a single injection of 300 to 1,000 ugm of killed, wet-packed cells in physiological saline would be sufficient to provide immunity against the pathogen.

Isolates of V. anguillarum from salmon cultured in net-pens in Clam Bay (Manchester), Washington were used to inoculate flasks of sterile trypticase soy broth containing 2 percent NaCl. The broth cultures were incubated for 24 hours at 24°C. The broth was then centrifuged, and washed three times with sterile physiological saline, centrifuging after each wash. The cells were concentrated in a single tube, and the tube was placed in a boiling water bath for two hours to produce the heat-killed antigen. Excess moisture was removed, and the wet-packed cells were weighed and suspended in sterile physiological saline. The final dilution contained approximately 2,240 ugm of wet-packed cells per cc. The vaccine was kept in refrigerated storage after adding 0.5 percent phenol as a preservative.

The time interval between the placement of the first experimental group of coho into full sea water and the last group was designed to be 60 days (see Part 1 of this presentation). We felt that an interval of 60 days between vaccination and natural exposure to the bacterium might be too long. Therefore, in order to provide equal treatments to all fish, all four groups were vaccinated once with 560 ugm of wet-packed cells on May 9, 1973, and again with 560 ugm on July 10, 1973.

The fish were anesthetized in MS222, and an automatic syringe was set to deliver $\frac{1}{4}$ cc of vaccine with each injection (it was interesting to note that with this technique, we could vaccinate fish at a rate of 720/hour/person).

The first group (I) was introduced to full sea water (30 0/00) within 24 hours post-injection. The second group (II) was introduced 19 days later, and the third group (III) 41 days post-injection. At 62 days post-injection the vaccination procedure was repeated on all groups (July 9) and the fourth group (IV) was introduced to sea water within 24 hours. Each of the four groups tested were represented by replicates of 100 fish each (800 total). The average size of the fish at the first injection was 10.3 gm. Both fresh and sea water were maintained at the temperature of the ambient sea water.

The mortalities of groups II and III in fresh water ranged from 0.023 percent per day to .0.21 percent per day. Group IV was in fresh water for the longest period, and mortalities ranged from 0.13 percent per day to 0.39 percent per day. There was a great deal of variation in mortality between tanks in fresh water. In almost all cases, the causative agent (isolated from the kidneys of moribund fish) was Aeromonas salmonicida (furunculosis). No medication was used in either fresh or sea water.

The average weights of the coho when introduced to 30 0/00 sea water were: Group I - 10.3 gm; Group II - 12.4 gm; Group III - 15.0 gm; and Group IV - 19.3 gm. At 120 days post-salt water entry, there was little difference in the cumulative mortality between Groups III and IV (Figure 1). Zero-age coho introduced to full sea water when the average weight is less than 15 gm appear to suffer from a higher stress mortality (Groups I and II). Fresh dead fish were examined as they appeared, and smears from the kidney were streaked onto tryptose blood agar and trypticase soy agar (2 percent NaCl) plates. Smaller fish were usually negative (no bacteria found), and the larger fish were frequently infected with furunculosis. No vibrios were found while the fish were in circular tanks in the hatchery.

In July, all groups were transferred to small experimental net-pens, arranged to maintain separate identities. Throughout the summer, we were only able to isolate vibrios from several moribund fish. The cumulative mortality in the Group IV fish was 10 percent (0.16%/day) through November 12th, a period of 64 days from the last injection. In contrast, the cumulative mortality of the Group I fish was 34 percent (0.5%/day) during the same period. When one considers that each fish was anesthetized, weighed and measured every three weeks, and received no medication throughout the experiment, the survival of the Groups III and IV fish is remarkable. If these fish were handled in the same way without vaccination or medication, the mortality would be in the order of 95 percent or higher, primarily from vibriosis.

Serum agglutination titers against the vibrio antigen were negative prior to vaccination. Dr. Robert Gunnels (College of Fisheries, University of Washington) conducted serum agglutination titers on sub-samples of these coho after the second vaccine injection, and the results are shown in Table 1.

Table 1. Serum agglutination titers of sub-sampled (vaccinated) coho against the vibrio antigen.

Date of Sub-sample	No. of days Post-2nd Injection	TITER									
		N	0	2	4	8	16	32	64	128	256
August 7	29	18	0	0	0	0	1	0	2	3	12
September 19	71	24	1	4	9	6	3	0	1	0	0

We expected that the natural, continuous challenge by the live vibrio organisms in the bay would stimulate antibody production. Apparently, this is not the case, as antibody production was dropping rapidly by mid September. Vibriosis outbreaks in September can be severe in Clam Bay, and indeed were in other non-vaccinated cultured salmon. Mortalities in vaccinated Groups III and IV were low throughout September, October and early November. However, in late November, at a temperature of 9.5 to 10.3°C., a serious epidemic of vibriosis occurred in the vaccinated groups.

The total mortality at this writing is not known, but prior to treatment with antibiotics, it approximated 5%/day. Blood samples were collected from dying fish, and the highest serum agglutination titer against our stock vibrio antigen was 1:2. Most were negative.

Large lots of other experimental coho that were routinely vaccinated during the summer with single injections ranging from 500 to 1,500 ugm of wet-packed cells were not affected by this late fall outbreak of vibriosis. However, these fish were not handled as frequently as the salinity test coho.

It appears to us (at this writing), that a single IP injection of vibrio vaccine will afford a great deal of protection to zero-age coho salmon throughout the summer and early fall, but probably cannot be extended beyond late fall without the use of an adjuvant. However, I think that we have clearly demonstrated the use of physical vaccination in clarifying the causes of mortalities in young coho, other than vibriosis.

Figure 1. Saltwater Mortality of Four Lots of Zero-Age Coho Salmon

