

NOTES

ISOLATION AND DESCRIPTION OF TWO VIBRIOS PATHOGENIC TO PACIFIC SALMON IN PUGET SOUND, WASHINGTON

Vibrio anguillarum (Bergman 1909) is recognized worldwide as a saltwater pathogen in fish (Anderson and Conroy 1970). Most epizootics caused by marine bacteria have been attributed to this organism (Rucker 1959; Sindermann 1966). This note describes recent mortalities resulting from vibriosis of Pacific salmon in the marine waters of Puget Sound, Wash., and heterogeneity observed in vibrios isolated from diseased fish.

The National Marine Fisheries Service (NMFS) is engaged in the experimental culture of Pacific salmon in salt water at the NMFS Aquaculture Experiment Station near Manchester, Wash. Epizootics caused by marine vibrios have occurred regularly in cultured salmon during the spring and summer months; the organisms were also isolated from diseased fish on a minor scale in every month during fall and winter (Novotny 1975). Vibrios originally isolated from diseased fish at Manchester were typical of *Vibrio anguillarum* (Evelyn 1971); strain 775 was representative.

In November 1973, a commercial salmon farm in the Manchester area suffered a high mortality of pen-reared, 0-age, 250-g coho salmon, *Oncorhynchus kisutch*. Past experience with vibriosis in the area indicated that the first serious outbreaks usually began in April when water temperatures exceeded 9°C and continued until water temperatures dropped below 12°C in early October (Novotny 1975). Water temperatures in November 1973 were 10° to 11°C; therefore, problems from vibriosis were not anticipated.

Mortalities also began to occur at about the same time, although not on an epizootic scale, in coho salmon held at the NMFS facility at Manchester. These fish had been vaccinated in late spring by injecting a heat-killed bacterin prepared from *V. anguillarum* 775. Oral antibiotics were administered, but the period required to bring the disease under control appeared to be almost twice that usually required for *V. anguillarum*.

Diseased fish sampled from the NMFS pens and

the commercial farm exhibited the common signs of vibriosis, most notably a hemorrhagic septicemia. Bacteria characterized as vibrios were consistently isolated from dead or dying fish, but the growth rate of the isolated bacteria was markedly different from that of the typical *V. anguillarum*. Also, this bacterium was not agglutinated by rabbit anti-*V. anguillarum* 775 serum in rapid slide agglutination tests.

The new isolates were confirmed as pathogens by injecting pure cultures of them into salmon. All the injected fish died and the organism was routinely re-isolated from kidneys. We designated this bacterium as *Vibrio* sp. 1669.

In June 1974, NMFS conducted cooperative vaccination tests with a second commercial salmon farm in the Manchester area. Approximately 280,000 coho salmon smolts were injected with a heat-killed bacterin of *V. anguillarum* 775 at least 2 wk prior to their transfer to saltwater pens. Mortalities were exceptionally low until late August (less than 6% from all causes and less than 2% from vibriosis). At that time the rate of mortality began to increase and *Vibrio* sp. 1669 was isolated.

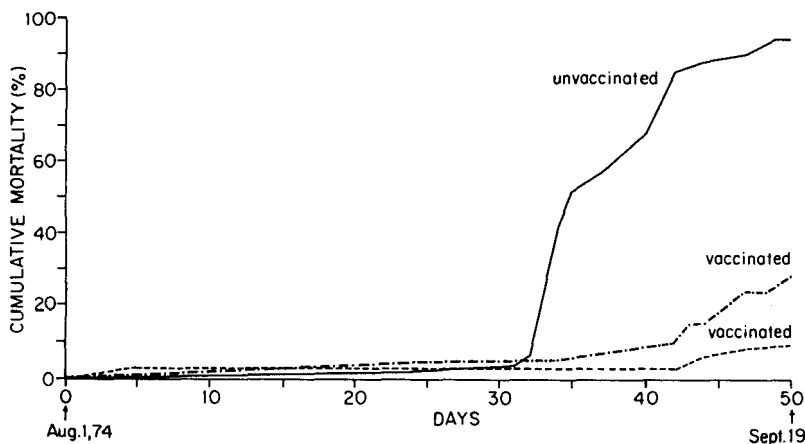
Further tests were made in early August 1974, when 450 0-age sockeye salmon, *O. nerka*, smolts were transferred to NMFS saltwater pens. One pen contained 150 unvaccinated control fish, and two pens contained 150 fish each that had been vaccinated in fresh water with a heat-killed *V. anguillarum* 775 bacterin. After 50 days in the saltwater pens, 95% of the unvaccinated fish had died. During the same period the mortalities in the vaccinated lots were 9% and 27% (Figure 1). Vibrios isolated from the vaccinated fish were only of the 1669 type, based on results of slide agglutination tests.

Materials and Methods

Samples of kidney, eye, or spleen from freshly dead or moribund fish were streaked on trypticase soy agar (TSA) (Difco)¹ with 1% salt added, or on 50% seawater cytophaga agar (Pacha and

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

FIGURE 1.—Comparative rate of mortality of three lots of 0-age sockeye salmon raised in net pens at Manchester, Wash. Two of the lots were vaccinated with an intraperitoneal injection of a heat-killed bacterin prepared from *Vibrio anguillarum* 775.



Ordal 1967). Plates were incubated aerobically at 23°C. Presumptive identifications of the bacteria were based on the following tests: gram stain, motility and morphology characteristics under phase contrast microscopy, oxidase test (Kovacs), fermentation or oxidation of glucose, and sensitivity to the vibriostatic compound 0/129 (2,4-diamino-6,7-diisopropyl pteridine phosphate).

Further biochemical characterization included tests in Moeller's media for an alkaline reaction with arginine and for lysine decarboxylase, the production of indole, the production of acetyl-methylcarbinol (Voges-Proskauer test), and the ability to ferment arabinose, glycerol, mannitol, sucrose, and galactose. These tests were selected because they were found to be variable among marine vibrio groups established by deoxyribonucleic acid homology characteristics (E. J. Ordal, University of Washington School of Medicine, Seattle, pers. commun.). In all of these tests additional NaCl (1%) was added.

TABLE 1.—Selected properties of *Vibrio anguillarum* 775 and *Vibrio* sp. 1669.

| Property | <i>V. anguillarum</i> 775 | <i>Vibrio</i> sp. 1669 |
|----------------------------|---------------------------|------------------------|
| Gram reaction | - | - |
| Motility | + | + |
| Oxidase (Kovacs) test | + | + |
| Fermentative (glucose) | + | + |
| Gas from glucose | - | - |
| Moeller's media: | | |
| Arginine-alkaline reaction | + | - |
| Lysine decarboxylase test | - | - |
| Indole production | - | - |
| Voges-Proskauer reaction | + | - |
| Acid from: | | |
| Arabinose | + | - |
| Glycerol | + | - |
| Mannitol | + | + |
| Sucrose | + | + |
| Galactose | + | - |

Antisera for serological comparisons were prepared in both rabbits and coho salmon with heat-killed bacterins of *V. anguillarum* 775 and *Vibrio* sp. 1669 in Freund's complete adjuvant. Rapid slide agglutination tests with the specific antisera were used for initial differentiation. The microtiter system (Cooke Engineering Co.) was used later to determine agglutinin titers, and immunodiffusion techniques were used to further compare antigenic structure and relatedness. Tests were run with unabsorbed antisera and with anti-*Vibrio* sp. 1669 sera absorbed with *V. anguillarum* 775.

Results and Discussion

Vibrio sp. 1669 was typical of the marine vibrio group: it was characterized as a gram negative, motile, curved, asporogenous rod that was oxidase positive, an anaerogenic fermenter, and sensitive to the vibriostatic compound 0/129. A slower rate of growth of *Vibrio* sp. 1669, in comparison to *V. anguillarum* 775, was observed on TSA, as well as variations in certain culture reactions (Table 1).

Coho salmon anti-*V. anguillarum* 775 serum with an agglutinin titer of 512 against the homologous bacterium had a titer of 8 against *Vibrio* sp. 1669. Immunodiffusion also revealed differences between the two vibrios. In Figure 2, the inner precipitin lines demonstrate antigenic cross-reactivity (reaction of identity). An additional antigen unique to *V. anguillarum* 775 is demonstrated by the outer precipitin line which is not present in reactions with *Vibrio* sp. 1669.

After all detectable agglutinin activity against

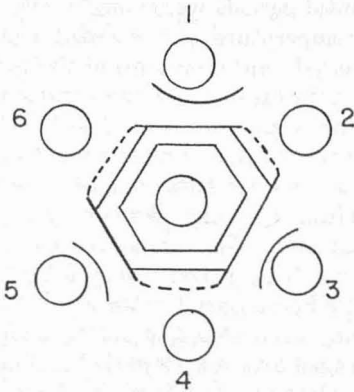
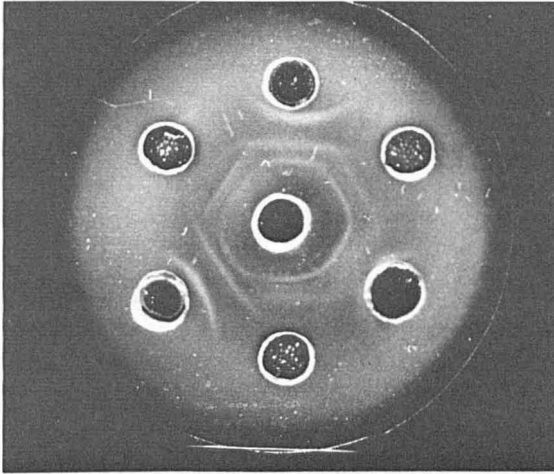


FIGURE 2.—Immunodiffusion comparison of *Vibrio anguillarum* 775 and *Vibrio* sp. 1669. Wells 1, 3, and 5 contain *V. anguillarum* 775 sonicate and wells 2, 4, and 6 contain *Vibrio* sp. 1669 sonicate. The center well contains rabbit anti-*V. anguillarum* 775 serum.

V. anguillarum 775 in rabbit anti-*Vibrio* sp. 1669 serum was removed by absorption, a titer of 16 against 1669 remained (Table 2), indicating that *Vibrio* sp. 1669 also contains antigenic determinants not present on *V. anguillarum* 775.

Whether a vaccine containing antigens from both vibrios would be more protective than vaccines containing antigens from only one of the

TABLE 2.—Agglutinin titers of rabbit anti-*Vibrio* sp. 1669 serum unabsorbed and absorbed with *V. anguillarum* 775 antigen.

| Condition | Titer | |
|-----------------------------------|-------|------|
| | 775 | 1669 |
| Unabsorbed anti-1669 serum | 8 | 32 |
| Anti-1669 serum absorbed with 775 | 0 | 16 |

vibrios is not known. This possibility is currently being investigated. Deoxyribonucleic acid homology experiments are also in progress to better clarify the taxonomic relation of the two vibrios.

Literature Cited

- ANDERSON, J. I. W., AND D. A. CONROY.
1970. *Vibrio* disease in marine fishes. In S. F. Snieszko (editor), *A Symposium on Diseases of Fishes and Shellfishes*, p. 266-272. Am. Fish. Soc., Spec. Publ. 5.
- EVELYN, T. P. T.
1971. First records of vibriosis in Pacific salmon cultured in Canada, and taxonomic status of the responsible bacterium, *Vibrio anguillarum*. *J. Fish. Res. Board Can.* 28:517-525.
- NOVOTNY, A. J.
1975. Net-pen culture of Pacific salmon in marine waters. *Mar. Fish. Rev.* 37(1):36-47.
- PACHA, R. E., AND E. J. ORDAL.
1967. Histopathology of experimental columnaris disease in young salmon. *J. Comp. Pathol.* 77:419-423.
- RUCKER, R. R.
1959. *Vibrio* infections among marine and fresh-water fish. *Prog. Fish-Cult.* 21:22-25.
- SINDERMANN, C. J.
1966. Diseases of marine fishes. *Adv. Mar. Biol.* 4:1-89.

LEE W. HARRELL
ANTHONY J. NOVOTNY
MICHAEL H. SCHIEWE
HAROLD O. HODGINS

Northwest Fisheries Center
National Marine Fisheries Service, NOAA
2725 Montlake Boulevard East
Seattle, WA 98112

RELATION OF FISH CATCHES IN GILL NETS TO FRONTAL PERIODS

A study was conducted in 1972 relating gill net catches of fishes to webbing material, time of day, and water depth in St. Andrew Bay, Fla. (Pristas and Trent¹). While conducting the study, Pristas and Trent observed that catches in the nets appeared to be greater when atmospheric fronts moved through the area in the autumn. We decided to test the hypothesis that catches of fishes in gill nets increase during frontal periods. Experimental data were collected in September-December 1973, and the results of the analysis are presented in this paper.

¹Pristas, P. J., and L. Trent. 1974. Comparisons of catches of fishes in gill nets in relation to webbing material, time of day, and water depth in St. Andrew Bay, Florida. Unpubl. manuscr.