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MFR PAPER 1330

R. A. Robohm and C. Brown are with the Milford Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Milford, CN 06460.

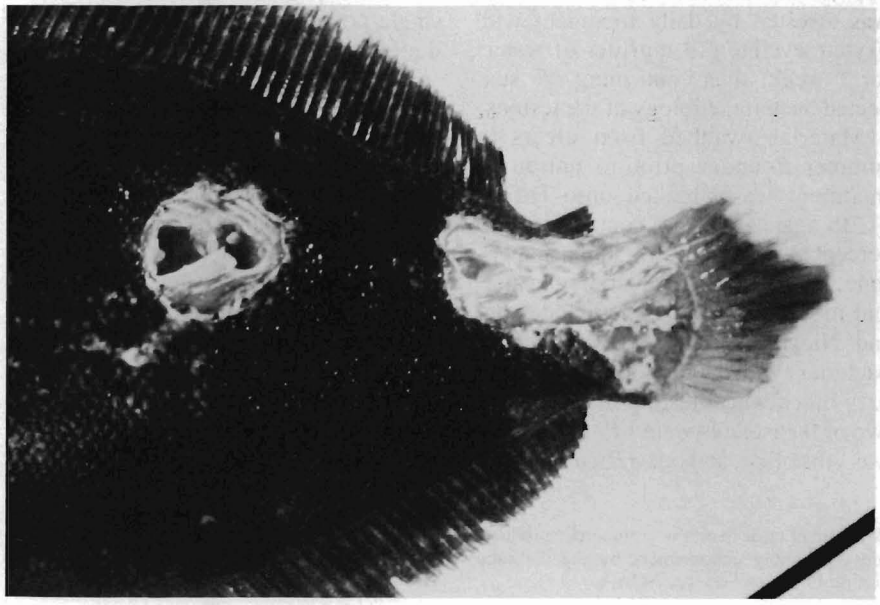
A New Bacterium (Presumptive *Vibrio* Species) Causing Ulcers in Flatfish

R. A. ROBOHM and C. BROWN

Summer flounder, *Paralichthys dentatus,* captured in the wild and held for 10 days in a tank supplied with flowing seawater (about 25 ppt salinity) from Milford harbor developed ulcerative lesions of the tail and dorsal muscle. Lesions began as a white patch at the flexure point of the tail (where the caudal fin meets the body) and sometimes were accompanied by hemorrhagic necrosis at the tip of the caudal fin. Frank ulceration extending into the skeletal muscle could be seen within a few days when the epithelium sloughed off under the patches (Fig. 1). Frequently, bony rays of the entire caudal fin were exposed by progression of the ulcerative process either anteriorly from the tip of the fin or from the initial lesion caudally (Fig. 2).

First appearance of the ulcers in two summer flounder was followed within 1 week and progressively for 4 weeks by ulcers in other summer flounder in the same tank, as well as in winter flounder, *Pseudopleuronectes americanus,* and hogchokers, *Trinectes maculatus,* in an adjacent tank. Although the lesions resemble those caused by *Vibrio*

Figure 1.—Posterior portion of a summer flounder infected with the new *Vibrio* species. Ulcers are seen to extend deep within the dorsal muscle.



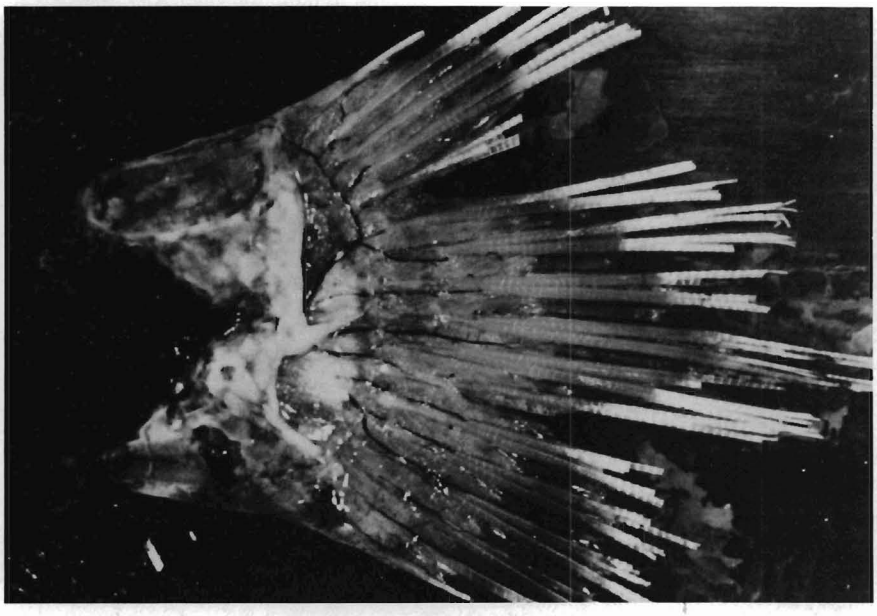


Figure 2.—Caudal fin of a summer flounder infected with the new *Vibrio* species. Epithelium is sloughed off to expose the underlying bony rays.

anguillarum infections (Haastein and Holt, 1972; Levin et al., 1972; ZoBell and Wells, 1934), other bacteria are known to cause similar ulcers in fish. These include lesions due to *Aeromonas hydrophila* (Amlacher, 1961), *A. salmonicida* (Herman, 1968), *Hemophilus piscium* (Snieszko et al., 1950), and *Cytophaga psychrophila* (Borg, 1960; Rucker et al., 1954). The disease in summer flounder was arrested by daily treatment with oxytetracycline (13 mg/liter of water) for 1 week, thus confirming the suspected bacterial etiology of the lesions.

Material swabbed from ulcers in summer flounder prior to antibiotic treatment was streaked onto Difco[1] TCBS agar, a medium containing 0.05 percent yeast extract, 0.05 percent peptone, 1.5 percent agar and seawater, and modified Twedt agar (Vanderzant and Nickelson, 1972). Four distinct bacterial types were isolated. Preliminary biochemical testing indicated that two of the isolates were *Vibrio* sp., one was vibriolike, and one a *Pseudomonas* sp. Sera drawn 3 weeks after the start of symptoms, from five antibiotic-treated summer flounder, contained antibodies against one of the *Vibrio* isolates at reciprocal agglutination titers of 64 to 256. Since the five sera had low antibody titers (less than 16) against the three other bacterial isolates, the first isolate was strongly implicated as the ulcer-disease bacterium. This organism was a short, gram-negative rod with a single polar flagellum when grown on liquid or solid medium.

Subcutaneous inoculation of varying numbers of the bacterium into 36 winter flounder produced small ulcers at the inoculation site in 31 fish; ulcers were accompanied by subcutaneous hemorrhages along the base of nearby fins and petechia on the nonpigmented side. Fish with these symptoms usually died within 2 days of inoculation. The organism was recovered repeatedly from the spleens of dead or dying fish—usually in pure culture. The subcutaneous LD_{50} calculated by the Reed and Muench (1938) method was 15,985 bacteria, but some fish died at a dose of 7,000 bacteria. Fish inoculated with 700 bacteria routinely survived. Because subcutaneous inoculation produced a disease different in form from that seen naturally in the holding tanks, another method of disease induction was tried. After first scraping away scales from a small patch at the tail-flexure point with a scalpel blade, winter flounder were placed in 76-liter tanks containing recirculating, charcoal-filtered seawater. Ulcerative lesions resembling those of the natural disease could be induced in the fish within 3-7 days by inoculating the water on alternate days with 360 bacteria/ml. Within 24 hours, bacterial counts were 10/ml; within 48 hours, counts could no longer be made because of competing microflora. The diseased fish died within 10 days; the organism was recovered from the lesions and from the spleens of dead fish and confirmed biochemically. An inoculum of 36 bacteria/ml into tank water on alternate days failed to induce disease.

Eight characteristics of the ulcer-disease organism are recognized by the Subcommittee on Vibrios of the International Committee on Systematic Bacteriology as necessary to place an organism in the genus *Vibrio* (Hugh and Sakazaki, 1972; Subcommittee on Taxonomy of Vibrios, 1972). These are: gram reaction −, motility +, single polar flagellum +, oxidase +, catalase +, glucose fermentation +, gas from glucose −, and acid from glucose +. A ninth characteristic which should be included is a 40-50 percent guanine plus cytosine (G-C) content in the bacterial DNA. Repeat analyses of the G-C content has given us conflicting results. Final analysis is yet to be completed. However, a total of 53 biochemical and physical tests indicate that the ulcer-disease organism is sufficiently dissimilar to currently known vibrios and closely related organisms to merit provisional designation as a new species.

We examined the sera of a variety of wild fish species for antibodies against the ulcer-disease organism. Sera of healthy blackfish, *Tautoga onitis*, and cunners, *Tautogolabrus adspersus*, from Long Island Sound near Milford had antibody levels to the ulcer-disease bacterium which rivaled levels in in-

[1]Mention of trade names or commercial products does not imply endorsement by the National Marine Fisheries Service, NOAA.

fected summer flounder. The antibody in cunners appeared to be protective against the disease; 50 percent of the fish had significant antibody levels and 50 percent survived intraperitoneal injections of bacteria. Most of the sera from 13 windowpane flounder, *Scophthalmus aquosus,* 15 weakfish, *Cynoscion regalis,* 54 summer flounder, and 101 winter flounder from Long Island Sound and New York Bight had low antibody titers to the organism. These results suggest that the high susceptibility of flounder species to the disease may be due to low natural exposure to the organism; whereas, immunity in two fish species near Milford may be due to a high degree of exposure to the organism; this could arise from a food source or from proliferation of the organism locally in nutrient-rich waters.

As a measure of the ability of the organism to persist and be disseminated in seawater, we inoculated a washed broth culture of the bacterium into filtered, sterilized seawater (25 ppt salinity) at a level of 10^6 cells/ml. Viable numbers decreased over a 5-month period at two temperatures; however, by 155 days, 89 and 141 bacterial/ml remained at 25°C and 15°C, respectively. This appears to be a relatively long survival when compared with that of *V. anguillarum;* Evelyn (1971) reported that 10^7 *V. anguillarum* cells were reduced to 10^1 in 14 days at 0°-3°C. These results suggest that a requirement for direct fish to fish contact may be more important in maintaining infection with *V. anguillarum* than with the ulcer-disease organism.

From the results of this work, we find that a previously undescribed bacterium 1) is pathogenic for flatfish, 2) is probably a new *Vibrio* species which survives for extended periods of time in seawater, and 3) has a capacity to cause ulcers which may be modulated by route of entry and/or the presence of competing microflora.

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