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Distribution of *Aeromonas hydrophila* in Natural and Man-Made Thermal Effluents

TERRY C. HAZEN[1]* AND CARL B. FLIERMANS[2]

Biology Department, Wake Forest University, Winston-Salem, North Carolina 27109,[1] and Savannah River Laboratory, E. I. du Pont de Nemours & Co., Inc., Aiken, South Carolina 29801[2]

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Densities of *Aeromonas hydrophila* showed distinct thermal optima (25 to 35°C) and thermal maxima (45°C) when measured along thermal gradients created by geothermal and nuclear reactor effluents. Survival of *A. hydrophila* never exceeded 48 h at temperatures of >45°C. Thermophilic strains could not be isolated at any site.

Aeromonas hydrophila has long been recognized as a pathogen in poikilothermic and homeothermic animals, including humans (1, 2, 8, 11, 14). The cosmopolitan distribution and facultative pathogenicity of this bacterium is, at least in part, due to its ability to survive in a wide variety of aquatic systems; *A. hydrophila* has been found in lotic, lentic, oligotrophic, eutrophic, and marine systems (8).

Large epizootics of fish by red-sore disease, the etiological agent of which is *A. hydrophila*, have recently caused extensive losses to both commercial and sports fisheries in the southeastern United States (4, 6, 14); e.g., in one reservoir alone, 37,500 fish succumbed to the disease over a 13-day period in 1973 (11). Recent studies have shown that the incidence of red-sore disease is significantly higher among fish in thermally altered parts of a South Carolina reservoir that receives heated effluent from a nuclear production reactor (4). The positive correlation between red-sore disease and thermal effluent was related to thermal stress on potential fish hosts (3, 7). A more recent study demonstrated a significant positive correlation between densities of *A. hydrophila* in the water column and the incidence of red-sore disease in the fish population over a 3-year period (T. C. Hazen, Ph.D. thesis, Wake Forest University, Winston-Salem, N.C., 1978). Since the survival of *A. hydrophila* appears to be enhanced by reactor effluents in this reservoir (5), it was hypothesized that the higher incidence of infection in thermal areas could also be related to increased densities of *A. hydrophila* (3).

Because *A. hydrophila* is increasing in importance as a fish pathogen and because the natural distribution and abundance of this bacterium seem to be related to the incidence of fish disease and thermal effluents, the distribution and abundance of *A. hydrophila* were examined in thermal gradients created by nuclear reactors in South Carolina (man-made) and geothermal activity in Yellowstone National Park (natural) (YNP).

The effluent canals of three nuclear production reactors (C, K, and P) located on the Savannah River Plant near Aiken, S.C., served as one set of study sites. The temperature of the reactor effluent water never exceeded 80°C; C and K reactors receive their cooling waters entirely from the Savannah River, whereas P reactor receives most of its cooling water from Par Pond, with supplemental water from the Savannah River (2.84×10^4 liters min^{-1}). Water is pumped into C canal at 6.85×10^5 liters min^{-1}, into K canal at 7.0×10^5 liters min^{-1}, and into P canal at 6.81×10^5 liters min^{-1}. Sampling sites in YNP were along the Firehole River, Madison River, and Ojo Caliente Spring. Flow rates at these sites were reported by Zeikus and Brock (17). Heating of these waters is related to geothermal activity. Hot springs empty directly into rivers or riverlets which connect to the rivers at irregular intervals (17).

Samples were collected and analyzed using Rimler-Shotts medium (15) as previously described (8, 10, 12). Survival of *A. hydrophila* after reactor shutdown was determined by placing 48 precleaned, glass microscope slides (75 by 25 mm) in a rubber-coated test tube rack and suspending the rack in the center of the reactor canal at a depth of 1 m. At 12-h intervals, three slides were removed from the rack and stained immediately with fluorescent anitbody (9). Temperature and other water-quality parameters were measured in situ (8).

Zones within Firehole River (YNP) varied in pH from 6.3 to 9.8, in turbidity from 0 to 49 Jackson turbidity units (JTU), and in temperature from 15.0 to 65.5°C. The density of *A. hydrophila* ranged from 0 to 60.0 colony-forming units (CFU) ml^{-1}; the highest densities of *A. hydrophila* occurred at 23.3 and 21.0°C. The Madison River (YNP) varied in pH from 7.1 to 7.8, in turbidity from 8 to 44 JTU, and in temperature from 17.0 to 30.0°C. The density of *A. hydrophila* varied from 0.0 to 130.0 CFU ml^{-1}, with highest numbers at 30.0°C. Ojo Caliente Spring (YNP) had a uniform pH and turbidity of 8.2 and 16 JTU, respectively, at all points sampled. The highest densities of *A. hydrophila* occurred at 32.0°C. Thus, for all YNP sampling sites, *A. hydrophila* densities were highest at temperatures from 21.0 to 32.0°C (Fig. 1). There were no significant correlations between densities of *A. hydrophila* and either pH or turbidity (16).

Densities of *A. hydrophila* in all three reactor canals followed a pattern similar to that in YNP, appearing first at 45°C, reaching highest levels at 32°C, and then declining at lower temperatures (Fig. 1). Temperature was the only water-quality parameter measured that was found to vary significantly in any of the reactor canals; pH remained near 7.3 and turbidity remained near 50 JTU in all three canals during reactor operation. When reactors were not operating, *A. hydrophila* was isolated from water and from artificial and natural substrates in all parts of the canals. Within 48 h of return to normal reactor operating temperatures, *A. hydrophila* was eliminated from the water column, as well as from artificial and natural substrates, in parts of the canal where the temperature exceeded 45°C.

The densities of *A. hydrophila* in YNP and the South Carolina reactor canals were comparable to densities of other aquatic systems in the United States (8). *A. hydrophila* in reactor effluent had a thermal optimum slightly higher than that of YNP geothermal effluents (Fig. 1); however, the difference was not significant ($P > 0.5$). More than 100 attempts were made to isolate a thermophilic strain of *A. hydrophila* from reactor canal effluents, and more than 50 attempts were made at YNP; these efforts were totally unsuccessful.

Rouf and Rigney (13) reported the laboratory thermal optima for various *Aeromonas* species to be 25 to 35°C, with thermal maxima of 40 to 45°C. Thus, it is probably not coincidental that the highest densities along geothermal and man-made thermal gradients occurred at 25 to 35°C and that *A. hydrophila* could not be isolated at temperatures greater than 45°C (Fig. 1).

Epizootic outbreaks of red-sore disease may be enhanced by thermal effluent in two ways: (i) stress on individual fish, causing an increase in susceptibility to infection (3, 7), and (ii) increasing the densities of *A. hydrophila,* which then increases exposure of host populations to the pathogen (3). At temperatures of between 30.0 and 35.0°C, *A. hydrophila* is near its thermal optimum, and many warmwater fish species are commonly exposed to such temperatures during late spring, summer, and early fall; thus, epizootics of red-sore disease are more likely under these thermal conditions and during these times. As more nuclear reactors and steam-gen-

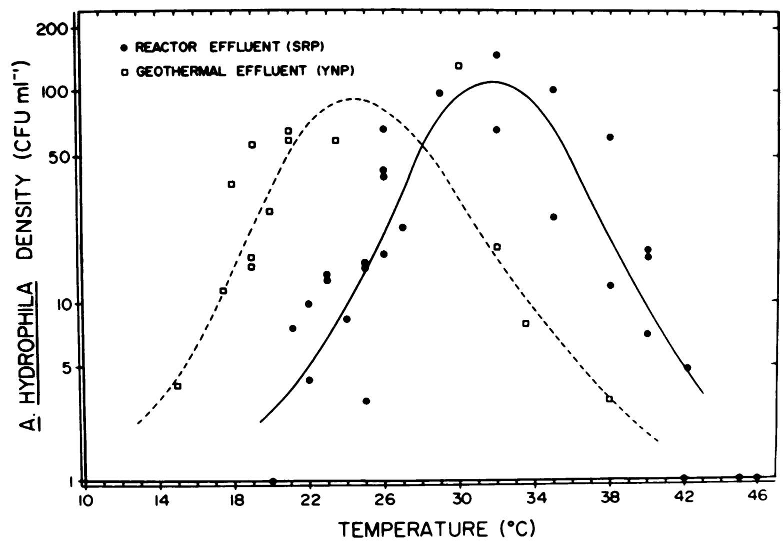

FIG. 1. *Densities of A. hydrophila by temperature for reactor effluents in South Carolina and geothermal effluents in YNP. SRP, Savannah River Plant.*

erating facilities are constructed in response to increasing energy needs, it seems probable that epizootics of red-sore disease will also occur with greater frequency.

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