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Aeromonas-Induced Deaths Among Fish and Reptiles in an Eutrophic Inland Lake

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SUMMARY

Aeromonas hydrophila and *Aeromonas shigelloides* were associated with mortality among fish, turtles, and alligators of Lake Apopka, Lake and Orange Counties, Florida. Factors predisposing to infection included eutrophication of the lake and low dissolved oxygen content of the water.

LAKE APOPKA, located in Lake and Orange Counties of central Florida, comprises a 31,000-acre body of water. Highly eutrophic, Apopka receives enrichment from municipal wastes, byproducts from the citrus-processing industry, runoff from citrus groves, and from water exchange with muck-farming operations.[16] During eutrophication, most of the game fish in the lake died, allowing trash fish, notably gizzard shad (*Dorosoma cepedianum*), to become the dominant species. Presently, gizzard shad constitute more than 90% of the lake's fish population.[16] Catfish (*Ictalurus* spp.) were second in abundance. A few bluegill sunfish (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) survived in and adjacent to a large freshwater spring which feeds the lake. Many inhabitants of the lake edge disappeared during the eutrophication process, e.g., frogs had virtually disappeared and the snake population was considered extremely low. Numerous soft-shelled turtles (*Trionyx ferox*) and alligators (*Alligator mississipiensis*) remained, however.

During winter and spring, 1971, the water level in Lake Apopka was lowered approximately 1.2 m. in an attempt to solidify organic matter and improve water quality. Mortality among fish, turtles, and alligators commenced during early May, 1971. Approximately 3 to 5 dead fish/acre; more than 50 soft-shelled turtles, with shell diameters in excess of 45 cm.; and 16 large (> 2.1 m.) alligators were found between May 7 and June 8. On June 10, a field laboratory was established to determine the cause of mortality among fish and reptiles of Lake Apopka.

Materials and Methods

Moribund animals and animals that had died recently were sought by search parties who traveled by airboats, aircraft, and on foot. Air surveillance was made on Lakes

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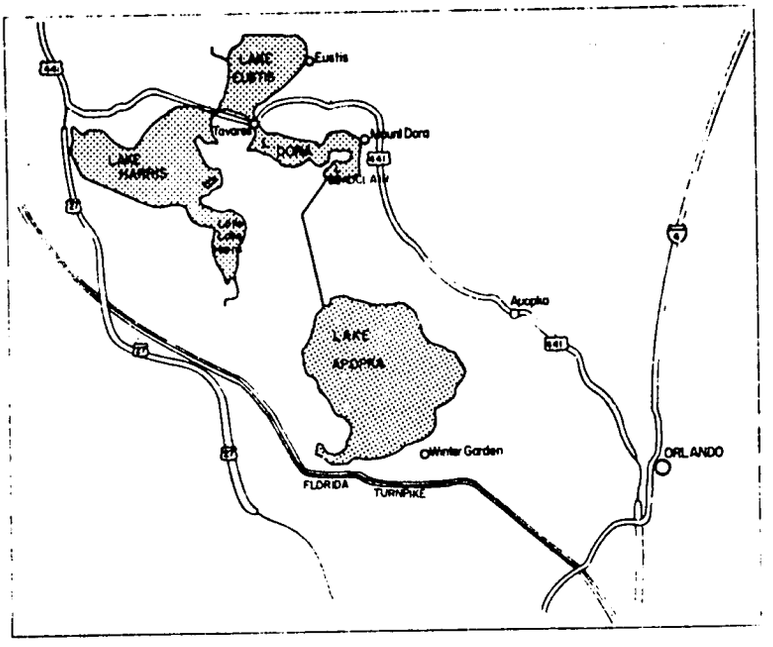

Fig. 1—Lake Apopka and surrounding lakes of the Oklawaha River drainage basin.

Apopka, Beauclair, Dora, and Eustis of the Oklawaha River basin into which Lake Apopka drains (Fig. 1). Emergent vegetation, mainly water hyacinth (*Eichornia crassipes*), was carefully searched for small reptiles. Dying fish were taken by hand from the water. Other fish were obtained from commercial fish traps near the center of the lake, and additional specimens taken at random from different parts of the lake were collected by electrical shocking techniques. For comparative purposes, large, healthy reptiles were collected at night by shooting with a dart, with a strong line attached to retrieve the specimen. To effect euthanasia, the spinal cord was severed with a knife. Small reptiles were captured by hand and visually inspected for clinical signs of disease. Most were returned to the water; a few were retained for observation. Cloacal swabs from 25 small (60 cm.) alligators were taken for bacteriologic cultures.

Cultures of water in Lake Apopka were made from 4 locations. Gauze sponges were attached to long bamboo poles and submerged in water near entry of effluents from municipal sewage and a citrus-processing plant. Sponges were submerged for 12 to 24 hours prior to culturing. Samples were taken on the weekend when there wasn't any effluent from the citrus-processing plant and again after citrus effluents were pouring into the lake. Culture mediums were exposed to aerosols produced by the prop wash from airboats at distances up to 25 m. Dissolved oxygen (DO) content of water at the periphery and center of the lake was determined by standard methods.[9]

Fish and reptiles found moribund or dead were necropsied. Representative tissues were preserved in buffered 10% formalin for histologic study. Parasitologic examinations were made on the respiratory and digestive tracts of reptiles. Major organs and blood were cultured for aerobic and anaerobic organisms. In many cases, selective mediums, including McConkey's agar, selenite and brilliant green broths, and brilliant green agar, were used in addition to 5% blood agar plates, nutrient broth, nutrient agar, and fluid thioglycollate medium in recovery of microorganisms.

In January, 1972, an obviously sick, large, bull alligator was found in Lake Beauclair, which immediately adjoins Lake Apopka. The alligator was transported to Athens, Ga., for clinical and pathologic study.

Results

Fish.—Moribund fish found in the lake as well as those stressed in fish traps had similar gross lesions, consisting of reddened abdomens and highly injected fins. Cultures from the kidneys of gizzard

shad, catfish, bluegills, and bass yielded *A. hydrophila* or *A. shigelloides*, or both. Fish collected by shocking similarly yielded *A. hydrophila* and *A. shigelloides* on culture of the kidneys. *Salmonella* spp. were recovered from a single catfish; however, this isolate was lost prior to typing.

On the last day of field studies, fish mortality was much greater than that experienced during the previous 10 days. Dissolved oxygen content of the water at this time ranged from 0.7 to 7.7 p.p.m.

Turtles.—Although many soft-shelled turtles were floating in the lake, advanced decomposition prevented detailed examinations. Only 1 intact soft-shelled turtle was retrieved. Autolysis was pronounced; however, reddening of the abdominal wall and limbs was noticed.

An alligator snapping turtle (*Macroclemys temmincki*), captured alive and presumed healthy, was necropsied. Purulent material was in the trachea and bronchi. Cultures from this material and from the kidneys yielded *A. hydrophila* and *A. shigelloides*.

Two small, hard-shelled turtles that had been maintained in captivity for 3 days were given intraperitoneal injections of 0.5 ml. of a broth culture of *Aeromonas* spp. Turtles died within 15 hours. There was reddening of the abdominal wall and limbs.

Alligators.—Searches for moribund or recently dead alligators failed to reveal suitable specimens. Badly decomposed carcasses of 3 additional alligators were found in Lakes Beauclair and Dora. Mortality of alligators continued sporadically throughout the remainder of 1971 and into 1972. To date, 25 dead alligators have been located in the lakes of the Oklawaha River drainage basin.

For comparative purposes, an apparently healthy 16.8-m. female alligator, weighing 20 kg., was captured at night. The alligator was secured with ropes and left at the field laboratory. On arrival at the laboratory the next morning, the alligator was dead. Necropsy revealed congestion and slight hemorrhage of the posterior nares, epiglottis, and lungs. The gastrointestinal tract yielded a few trematodes and nematodes. Cultures for bacteria were not made.

A 33-m. male alligator, weighing 175 kg., was found in Lake Beauclair during January, 1972. The alligator was dull and sluggish. Purulent material exuded from both nostrils and mucopurulent material was in the upper pharynx and posterior nares. Flecks of caseous exudate were found in the lungs, which had 3- to 4-mm. nodules scattered throughout. Necrotic plugs were in the bronchi. Forty-three pentastomes (*Sebekia oxycephala*) were recovered from the lungs. Many old scars, ulcers, and nodules were in the reddened stomach. The intestine and cecum were slightly inflamed. Trematodes and nematodes were recovered from the intestine. A small quantity of serous fluid was in the body cavity. Serosanguineous material was in the hip joint. There were many small wounds over the body, and a small abscess was found in the tail.

Cultures of the lungs yielded *A. hydrophila*, *Pseudomonas* sp., and *Enterobacillus* sp. *Aeromonas hydrophila* was recovered from culture of heart blood. *Aeromonas hydrophila*, *Proteus* sp., *Pseudomonas* sp., and mixed coliforms were found in cultures from the upper pharynx. The tail abscess yielded *Corynebacterium* sp., and *Salmonella typhimurium* was recovered from the gut.

Histologic examination of lung tissue revealed areas of hemorrhage, congestion, and infiltrations with heterophils beneath the alveolar epithelium. Granulomatous foci with necrotic centers were found surrounded by mononuclear and giant cells. A branching septate fungus was demonstrated in lung granulomas by using a periodic acid-Schiff stain. There were areas of heterophils in the submucosa of the stomach. Erosion of the superficial epithelium had occurred and there were infiltrations of mononuclear cells. Diffuse fatty change was observed in the liver. Many hepatic cells contained dark, intracellular pigment.

Cloacal swabs from 3 of 25 small, living alligators yielded *Salmonella braenderup*, *Salmonella anatum*, and *Arizona* spp.

Water.—Bacteriologic culture of water samples yielded *A. hydrophila*, *A. shigelloides*, *Pseudomonas* sp., and *Enterobacter* sp. At the site of entry of the citrus effluent, almost pure cultures of *A. hydrophila* were obtained. Agar plates exposed for 5 seconds to aerosols produced by airboats from distances up to 25 m. were heavily seeded with *Aeromonas* spp.

Discussion

Throughout this investigation *A. hydrophila* and *A. shigelloides* were recovered with regularity from fish, reptiles, water, and aqueous aerosols. These organisms are considered part of the normal flora of most water systems, although pathogenicity has been demonstrated in both warm- and cold-blooded vertebrates.[1,3,4,10-12,15,17,20,21] In man, *Aeromonas* spp. may cause a variety of conditions, ranging from enteritis to meningitis.[2,5,8,10-12,14,20] Abortions in cattle have been attributed to infection with *A. hydrophila*.[21] Aeromonas infections, however, are most severe in poikilothermic animals, where they are triggered by stresses, such as low DO concentrations in the water, abrupt temperature changes, and spawning.[3,11,18] Infection with *Aeromonas* spp. frequently occurs in the spring and early summer when the animal is in a weakened condition following overwintering. Extremely low DO and high temperatures were present in the spring of 1971 in Lake Apopka. Only large, fully mature alligators died and the peak of mortality appeared to be associated with the breeding season when considerable stress occurs.

Lesions in fish and turtles of Lake Apopka were similar to those described previously for aeromonas infections in these animals[1,3,10,18] and recovery of *Aeromonas hydrophila* and *A. shigelloides* leaves little doubt that these organisms were the true etiologic agents of disease among fish and turtles. Previous accounts of aeromonas infections in alligators are lacking. The finding of *Aeromonas* spp. in blood and affected organs of the 1 large alligator suggests that these organisms were the offending agents. The respiratory tract seemed to be a site of predilection for *Aeromonas* spp. in the alligator. The role of pentastomes (*Sebekia oxycephala*) in producing or accentuating this pneumonic condition is speculative; however, these arthropods frequently are associated with disease in alligators and other vertebrates.[6,7,9] Further investigations are indicated on the interactions of bacteria and pentastomes.

The widespread distribution and prevalence of *Aeromonas* spp. among the inhabitants and water of Lake Apopka were quite surprising, as was the general lack of coliforms and other sewage-related microorganisms. Recovery of almost pure cultures of *Aeromonas* spp. from the effluent of the sewage-treatment and citrus-processing plants suggested a relationship to the heavy concentrations of *Aeromonas* in the lake. This group of organisms readily utilizes citrate as a source of carbon for growth.[10,12,13] This study demonstrates that in highly eutrophic waters, microbiological assays in addition to those for fecal bacteria must be performed to more accurately determine the bacterial flora present.

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Trichinosis and Bear Meat

Of all the wildlife species known to harbor *Trichinella spiralis* in the United States, none are more important as a direct source of human infection than bears. A recent survey of 372 black bears in the northeastern United States revealed that 1.3% were infected, a prevalence 13 times greater than that for grain-fed swine and 4 times greater than that for garbage-fed swine in the United States.

Of 115 cases of trichinosis in man reported in 1971, 13 were known to be associated with consumption of bear meat.—*CDC Trichinosis Surveillance, Annual Summary, 1971, (July, 1972): 8.*