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Ecology of *Aeromonas hydrophila* in a South Carolina Cooling Reservoir

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Abstract. Densities of *Aeromonas hydrophila* were determined monthly from December 1975 to December 1977 in a South Carolina cooling reservoir which receives heated effluent from a single nuclear production reactor. Selected water quality parameters and prevalence of red-sore disease among largemouth bass were monitored simultaneously.

Higher densities of *A. hydrophila* were observed in areas of the reservoir receiving effluent from the reactor. Densities of *A. hydrophila* generally were heterogeneous in the water column. The sediments had lower densities of *A. hydrophila* than water immediately above. *A. hydrophila* could not be isolated from sediments greater than 1 cm from the water interface. Temperature, redox potential, pH, and conductivity were all significantly correlated with densities of *A. hydrophila* in the water column. The temporal and spatial distribution and abundance of *A. hydrophila* in water were not related to total organic carbon, dissolved organic carbon, particulate organic carbon, inorganic carbon, or dissolved oxygen. High densities of *A. hydrophila* were observed in mats of decomposing *Myriophyllum spicatum* and, enterically, in largemouth bass, several other species of fish, turtles, alligators, and snails. The greatest densities of *A. hydrophila* in water occurred during March and June with a second peak in October. The mean monthly densities of *A. hydrophila* were positively correlated with the incidence of infection in largemouth bass. Largemouth bass from thermally altered parts of the reservoir had a significantly higher incidence of infection. It is concluded that thermal effluent significantly affects the ecology of *A. hydrophila* and the epizootiology of red-sore disease within Par Pond.

Introduction

Aeromonas hydrophila has long been known as a pathogen of amphibians (4, 33), reptiles (23, 33), fish (5, 13, 33, 36), snails (24), and cows (41). There are also

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a number of reports of fatal human septicemias caused by *A. hydrophila* (3), although in nearly all cases the patient was debilitated by some other condition. Recently, however, there have been reports of apparently healthy individuals contracting infections during water activities (8, 14).

In the southeastern United States, *A. hydrophila* is the etiological agent for "red-sore" disease in fish (19). This disease, the external pathology of which includes scale erosion and hemorrhaging of the purulent lesions, is common among centrarchids and occasionally reaches epidemic proportions, resulting in massive fish kills (16, 29). Miller and Chapman (25) reported that 37,500 fish died of the disease in Badin Lake, North Carolina, over a single 13-day period. Epizootics caused by *A. hydrophila* occur not only in natural fish populations but also in fish hatcheries where losses often are exceedingly high (13, 26).

It is surprising that little is known about the prevalence and distribution of *A. hydrophila*, considering its obvious importance. Although *A. hydrophila* was assumed to be cosmopolitan, only recently was this confirmed (18). *A. hydrophila* is found in all aquatic habitats except those that are considered extreme, e.g., hypersaline lakes, hot springs; highest densities are reported from lotic habitats. Generally, *A. hydrophila* has been considered as a freshwater bacterium (10). However, Hazen et al. (18) recently isolated *A. hydrophila* from marine systems; the same study also reported that densities of *A. hydrophila* were positively correlated with conductivity, but not pH or temperature.

Since preliminary studies have revealed that increased temperature was related to the incidence of red-sore disease in largemouth bass *(Micropterus salmoides)* and that the density of *A. hydrophila* may affect the incidence of red-sore disease (6), the objectives of the present study were (a) to measure the distribution and abundance of *A. hydrophila* at selected sites in a thermally altered reservoir while simultaneously monitoring a series of selected water quality parameters and (b) to determine if the ecology of *A. hydrophila* is integral to the epizootiology of red-sore disease in largemouth bass.

Materials and Methods

Study Site

The primary area of study was Par Pond (81° 31'N, 33°, 14'W), located on the Savannah River Plant near Aiken, South Carolina (Fig. 1). Par Pond is an 1012 ha artificial impoundment with a mean depth of 6.2 m, a maximum depth of 17 m, a shoreline length of 53 km, and a total volume of 6.2×10^7 m^3. The volume replacement time is 6 months and the watershed covers 8324 ha. The impoundment was created by damming Lower Three Runs Creek in 1958. Another dam separates Par Pond from Pond C (PC), a 57 ha precooling pond. The latter is connected to Par Pond via a 3 × 3 m culvert; water is drawn from Pond C at a depth of 10 m and forced out over the surface of Par Pond. Pond C has a mean depth of 4 m, a maximum depth of 10 m, and a shoreline length of 7.5 km; it receives water (6.81×10^5 liters min^{-1}) from a single canal system connected to P reactor (Fig. 1). P reactor is a second generation heavy water nuclear production facility. A series of canals and small ponds, 6.8 km long and covering 35 ha, connect the reactor to Pond C. Most of the water used to cool P reactor is pumped from the west arm of Par Pond at a depth of 6 m; however, Savannah River water (2.84×10^1 liters min^{-1}) is added to maintain a constant volume in the reservoir. Water exits Par Pond via a surface skimmer in the main dam; it then enters Lower Three Runs Creek (L3R) and courses 30 km before merging with the Savannah River (22, 26, 27).

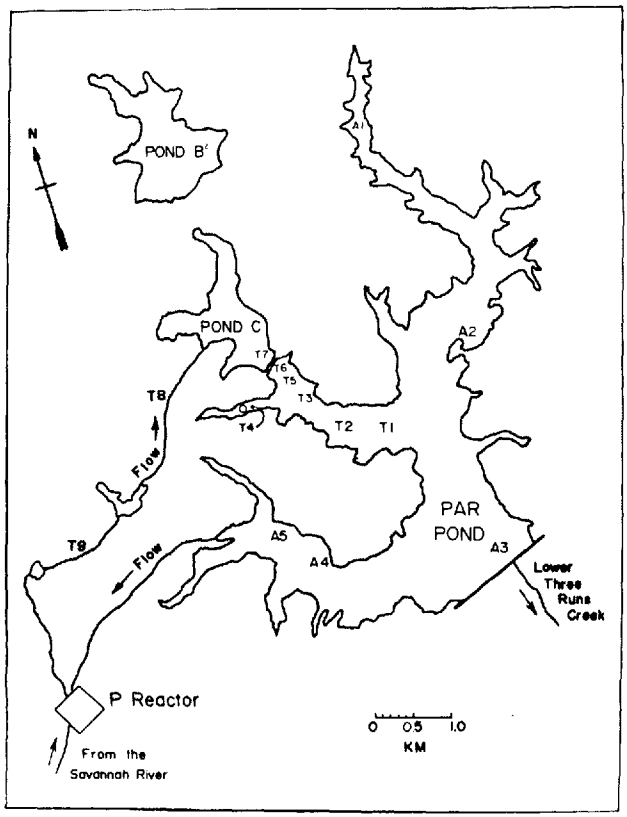

Fig. 1. Sampling sites on Par Pond, near Aiken, South Carolina (A1–A5 = ambient sites; T1–T9 = thermally altered sites).

Sampling

Water samples were collected using a 1 liter vertical lucite Kemmerer sampling bottle (Wildlife Supply Co., Saginaw, Mich.). The bottle was washed with 70% ethanol after each sample was taken. Each water sample was placed in a sterile 180 ml whirl-pak bag (NASCO) and kept on ice for transport to the lab; the time from collection site to the lab never exceeded 4 h.

Sediments were collected quarterly with heavy duty and lightweight Wildco core samplers equipped with inserts (Wildlife Supply Co., Saginaw, Mich.); core samplers were sterilized with 70% ethanol between samples. Centers of cores were removed at 5 cm intervals from the sediment-water interface to a minimum depth of 25 cm, placed in whirl-pak bags and kept on ice during transit to the lab.

The largemouth bass population was monitored monthly as described by Esch et al. (6). A minimum of 200 bass were examined monthly for evidence of red-sore disease. The disease was confirmed by culture from randomly selected lesions and anti-*A. hydrophila* fluorescent antibody staining of characteristic lesions [see Hazen et al. (19) for details].

Abundance and distribution of *A. hydrophila* were measured by monthly and seasonal sampling. Three samples were taken at the surface and at 1 m intervals in vertical profile at each station. Water samples were taken monthly at sites A3, A5, T3, and T5 (Fig. 1). Additional samples were taken seasonally at T1, T2, T4, T6, T7, T8, T9, A1, A2, and A4 (Fig. 1). Quarterly samples were also taken from the Savannah River (SR), the source for Par Pond make-up water and Lower Three Runs Creek, the outflow of Par Pond.

Bacteriological Methods

Aeromonas hydrophila density was estimated by viable cell count using Rimler-Shotts (R-S) medium (34). All density estimates were made in triplicate. A specific volume of sample water was filtered through a sterile, gridded 47 mm membrane filter with pore diameter of 0.45 μm. The filter was then placed on R-S medium and incubated at 35°C for 20–24 h. Following incubation, yellow colonies were counted with the aid of a magnifying lens, as previously described by Hazen et al. (18).

Sediments were analyzed as follows: 1 g samples of sediments were carefully removed from each whirl-pak bag, placed into capped, sterile 20 ml culture tubes with 9 ml of sterile 0.1 M phosphate-buffered saline (PBS) (pH 7.0), and mixed for 1 min with a vortex mixer. Subsequently, nine 10-fold serial dilutions were performed. CFU counts were then determined for each dilution as described above for water. The sediment analysis methods were also used to determine the presence of enteric *A. hydrophila* in the biota.

Water Quality

Five water quality parameters were measured simultaneously with *A. hydrophila* density. Dissolved oxygen, pH, conductivity, temperature, and redox potential were monitored using a Hydrolab surveyor Model 5901 (Hydrolab Corp., Austin, Texas). Standard APHA methods (29) were followed for all in situ measurements. These measurements were also taken at weekly intervals at 10 sites (A1, A2, A3, A4, A5, T1, T2, T3, T4, and T5; see Fig. 1).

Total organic carbon (TOC), inorganic carbon (IOC), particulate organic carbon (POC), and dissolved organic carbon (DOC) were measured seasonally at Stations A3, A5, T3, T5, T6, and T7 (Fig. 1), as well as the Savannah River and Lower Three Runs Creek. TOC and DOC were measured with an Ionics combustion infrared carbon analyzer, Model 445 (Ionics Corp., Watertown, Mass.), according to APHA standard methods (28). Water samples were filtered through carbon-free AP 40 microfiber glass filters (Millipore, Bedford, Mass.) and the filtrate analyzed to obtain DOC estimates. Particulate organic carbon was estimated by subtracting DOC from TOC. Samples were stored for analysis by freezing (−20°C) until processed. This storage technique does, however, underestimate inorganic carbon of samples in which inorganic carbon is primarily carbon dioxide.

Data Analysis

A Hewlett-Packard 3000 series computer was used for all statistical tests. Some data were analyzed using IDA (Interactive Data Analysis, University of Chicago) and modifications of programs by Davies (2). Factorial analysis of variance, paired and unpaired Student's *t* tests were used to test for differences between sites, depths, seasons, months, and years. Multiple regressions were used to determine correlations of *A. hydrophila* densities with water quality parameters. Differences between regressions were determined by F test. *A. hydrophila* densities, total organic carbon, dissolved organic carbon, and particulate organic carbon were found to be heteroscedastic by determining skewness and kutosis against a normal probability plot. The heteroscedasticity was reduced by transforming each of these measurements with Log (x + 1) prior to analysis (43). Percent data used for red-sore infections were subjected to arcsine transformation. Any statistical probability equal to or less than 0.05 was considered significant.

Results

Water Quality

Physical and chemical parameters for site A3 are given in Table 1. Only data for each season at 1 and 15 m are presented; for complete depth-time isopleths for each parameter at each site, see Hazen (15). Temperature and organic carbon

Table 1. Water quality in Par Pond at site A3[a]

Date	Depth (m)	Temp. (°C)	DO (mg liter^{-1})	pH	Redox (mv)	Cond. (μmho)	TOC (mg liter^{-1})	DOC (mg liter^{-1})	POC (mg liter^{-1})	IC(mg liter^{-1})
12-75	1	18.0	8.5	6.9	370	50	N.D.	N.D.	N.D.	N.D.
	15	15.8	7.9	7.1	400	55	N.D.	N.D.	N.D.	N.D.
3-76	1	18.5	9.3	7.4	355	65	N.D.	N.D.	N.D.	N.D.
	15	13.0	4.4	6.2	410	70	N.D.	N.D.	N.D.	N.D.
7-76	1	30.0	7.1	7.5	310	55	265.5	N.D.	N.D.	2.3
	15	18.0	0.0	6.1	−10	85	5.5	N.D.	N.D.	4.4
10-76	1	24.0	6.9	6.3	390	70	74.1	N.D.	N.D.	2.0
	15	19.0	0.3	5.7	230	130	9.0	N.D.	N.D.	3.4
12-76	1	10.5	10.8	6.4	435	60	343.7	N.D.	N.D.	2.9
	15	10.5	10.3	6.4	435	70	5.0	N.D.	N.D.	2.5
3-77	1	13.5	10.5	7.3	410	65	193.1	102.5	90.6	0.5
	15	12.0	9.2	7.1	480	80	2.9	0.5	2.4	0.5
7-77	1	31.0	7.9	8.2	320	85	4.7	1.6	3.1	0.5
	15	17.0	0.3	6.9	360	110	4.5	2.0	2.5	0.5
10-77	1	19.5	7.4	6.8	390	60	3.4	2.4	1.0	3.5
	15	18.5	6.1	6.7	390	60	6.4	3.3	3.1	3.3
12-77	1	15.5	10.0	7.5	350	60	3.5	3.5	0	3.1
	15	12.0	8.6	7.0	390	60	4.5	4.5	0	3.0

[a] DO, dissolved oxygen; Cond., conductivity; TOC, total organic carbon; DOC, dissolved organic carbon; POC, particulate organic carbon; IC, inorganic carbon; N.D., not determined.

were significantly higher and dissolved oxygen was lower at thermal stations as compared with stations A1–A5; none of the other physicochemical parameters were different.

Temporal and Spatial Distribution of A. hydrophila

Sites T1–T5 and A1–A5 in Par Pond were all sampled quarterly for *A. hydrophila*. Factorial analysis of variance (FANOVA) indicated significant differences in *A. hydrophila* densities between seasons and sites but not between depths (Table 2). The analysis also revealed significant interactions between seasons and sites; on the other hand, interactions were not significant for sites and depths, seasons and depths, and seasons, sites, and depths. Further analysis indicated that density differences were not significant within ambient or thermal sites. Thermal sites always had higher densities when the reactor was operating; however, variance of means prevented differences from being significant at all times (Fig. 2).

Densities of *A. hydrophila* were also measured monthly at sites A3, A5, T3, and T5. Differences between 1976 and 1977 were not significant for any depth at any site. FANOVA indicated significant differences between months (Fig. 3) and sites (Fig. 4), but not between depths (Fig. 5). There was also a significant interaction between months and sites, but no significant interactions between months and depths, sites and depths and months, sites and depths. Differences in density of *A. hydrophila* between A3 and A5 and between T3 and T5 were not signifi-

Table 2. FANOVA of *A. hydrophila* densities[a]

		cfu[b]			Cfu[c]
Quarters	F	26.73[d]	Months	F	12.75
(seasons)	df	7;447		df	23;671
	P	<0.0001		*P*	<0.0001
Sites	F	4.17	Sites	F	8.74
	df	7;447		df	3;671
	P	<0.0005		*P*	<0.0005
Depths	F	1.13	Depths	F	1.15
	df	6;447		df	6;671
	P	>0.5		*P*	>0.5
Q × S	F	2.31	M × S	F	2.89
	df	49;447		df	69;671
	P	<0.0001		*P*	<0.0001
Q × D	F	0.35	M × D	F	0.40
	df	42;447		df	138;671
	P	>0.5		*P*	>0.5
S × D	F	0.34	S × D	F	0.37
	df	42;447		df	18;671
	P	>0.5		*P*	>0.5
Q × S × D	F	0.28	M × S × D	F	0.20
	df	294;447		df	414;671
	P	>0.5		*P*	>0.5

[a] F, F statistic; df, degrees of freedom; *P*, probability; Q, quarters; S, sites; D, depths; cfu, colony forming units ml^{-1}.
[b] Sites A2, A3, A4, A5, T1, T2, T3, and T5.
[c] Sites A3, A5, T3, and T5.
[d] Significant F statistics are underlined.

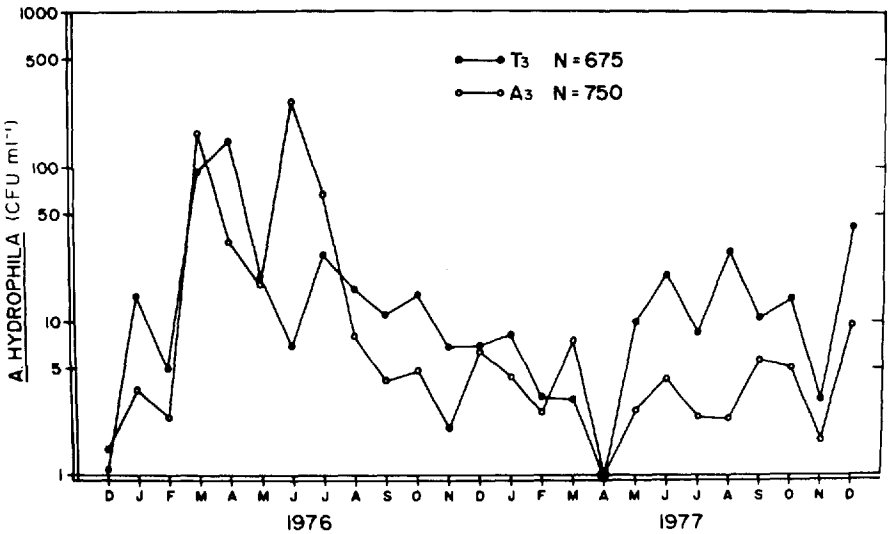

Fig. 2. Density of *A. hydrophila* at T3 and A3 by month.

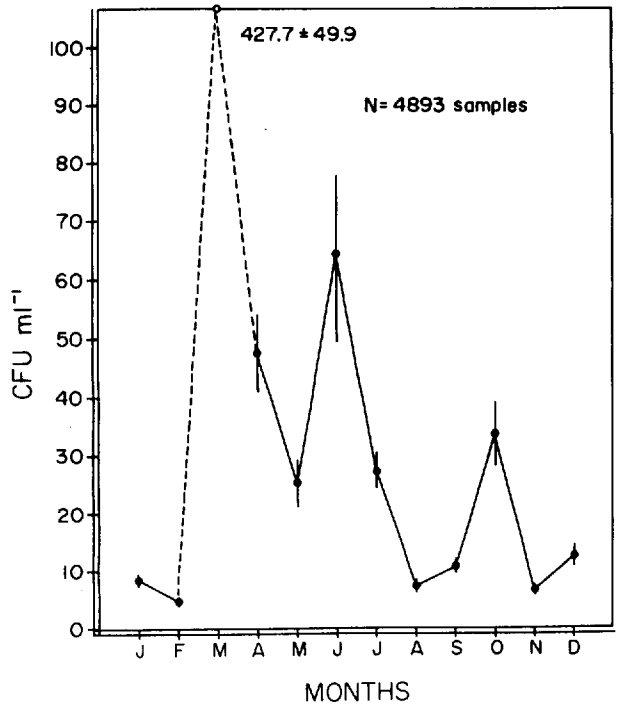

Fig. 3. Mean density of *A. hydrophila* by month (mean ± 1 SE).

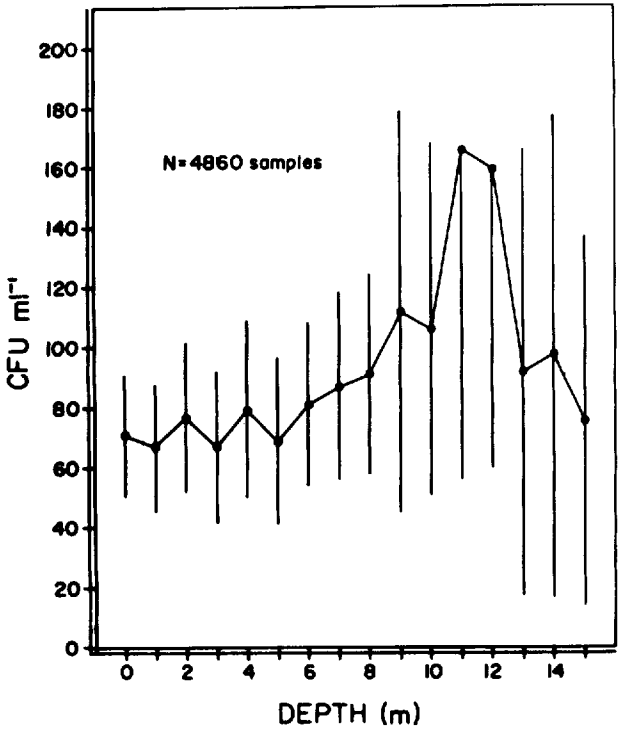

Fig. 4. Mean density of *A. hydrophila* by depth (mean ± 1 SE).

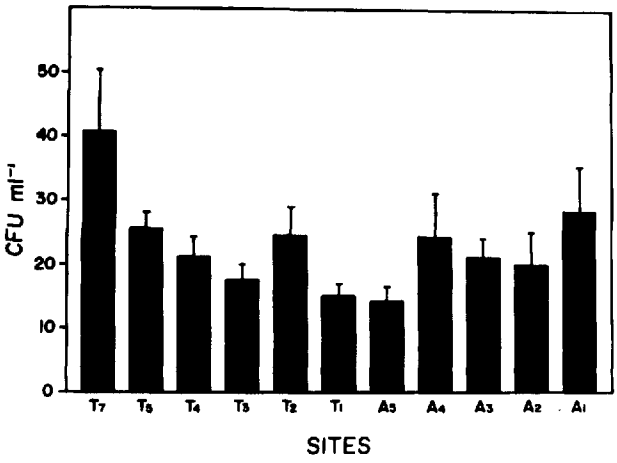

Fig. 5. Mean density of *A. hydrophila* by site (mean ± 1 SE).

cant. Again, thermal sites were significantly higher in density of *A. hydrophila*, but only when the reactor was operating.

Correlations Between A. hydrophila *and Water Quality*

The total multiple regression for all data sets in which carbon data were available was found to be highly significant (Table 3); 47% of the error in the regression was attributed to the measured variables. The correlation matrix shows a significant negative correlation between *A. hydrophila* density and temperature, redox potential, and pH. Dissolved oxygen, conductivity, TOC, IC, POC, and DOC were not significantly correlated with *A. hydrophila* density (Table 3).

All parameters except carbon (IC, TOC, POC, and DOC) were examined monthly at sites A3, A5, T3, and T5. The overall multiple regression was significant, with the variables explaining 10% of the variation in the regression (Table 4). Because this second data set was so large, it was partitioned into thermal and ambient sites, seasons, epilimnion and hypolimnion, stratification and mixis periods and all other possible combinations. Regression and correlation analysis were performed on individual and combinations of data sets.

There were significant differences between regressions at ambient and thermal sites, seasons, stratification and mixis periods, thermal sites during mixis, ambient sites during mixis periods, thermal sites during stratification periods, ambient sites during stratification periods, and all sites combined for 1976 and 1977. There were no significant differences between regressions for data from hypolimnion and epilimnion. All regressions were highly significant, with variation in the parameters explaining a minimum of 18% and maximum of 61% of the error in the regression. Correlation matrices indicated a strong negative correlation for redox potential and pH with densities of *A. hydrophila*, in all data sets. Temperature was negatively correlated with *A. hydrophila* densities except during the time of stratification at the thermal site in 1977.

Table 3. Correlation matrix and multiple regression of seasonal samples

	Temp	DO	Redox	Cond	pH	IC	TOC	DOC	POC
Temp	1.0000								
DO	0.2562[a]	1.0000							
Redox	0.4998	−0.0689	1.0000						
Cond	−0.2853	0.7070	−0.1973	1.0000					
pH	−0.0932	−0.6725	0.6042	−0.4271	1.0000				
IC	−0.0313	0.0353	−0.1949	0.1128	0.0143	1.0000			
TOC	−0.0251	−0.1307	0.1310	−0.0066	0.0961	−0.4908	1.0000		
DOC	−0.0293	0.1171	−0.0629	0.0942	−0.1200	−0.0154	−0.0489	1.0000	
POC	0.0649	0.0450	0.0231	0.0028	0.0044	0.0132	0.0697	−0.0360	1.0000
A. hydrophila	−0.3072	0.1528	−0.9226	0.1014	−0.7763	0.0280	−0.1365	0.0150	−0.0943

$N = 120$.
$Y = 12.33 - 0.022xt + 0.040xd - 0.015xr + 0.004xc - 0.940xp + 0.129xic - 0.001xtc + 0.026xdc + 0.010xpc$.
$r = 0.6825$; $r^2 = 0.4658$; $F = 10.66$; df = 9110; $P < 0.0001$.
[a] Significant correlations are underlined.

A. hydrophila *and Other Biota*

Large mats of *Myriophyllum spicatum* (Asian milfoil) were present from June through November in Par Pond. Densities of *A. hydrophila* within these mats during summer were not different from those in open water from nearby sites. However, when the mats floated free and began to decompose in the fall, the density of *A. hydrophila* in the surface microlayer of these mats was exceedingly high. In one sample, the *A. hydrophila* density was 7950 ± 12 cfu ml^{-1} (mean ± 1 SE, $N = 20$), whereas 10 m away from the mat, density in the surface microlayer was only 46 ± 12 cfu ml^{-1} (mean ± 1 SE $N = 20$). To determine if *A. hydrophila* was in direct contact with the milfoil, 10 g quantities of *M. spicatum* were homogenized in a Waring blender for 10 min and the homogenates filtered;

Table 4. Correlation matrix and multiple regression of monthly samples (A3, A5, T3, T5)

	Temp	DO	Redox	Cond	pH
Temp	1.0000				
DO	0.5066[a]	1.0000			
Redox	0.1617	−0.2481	1.0000		
Cond	−0.0488	−0.3708	−0.1471	1.0000	
pH	−0.4800	−0.6187	0.2876	−0.1249	1.0000
A. hydrophila	0.0144	0.1270	−0.5606	−0.3245	−0.0721

$N = 711$.
$Y = 4.198 - 0.023xt - 0.127xd - 0.004xr + 0.007xc + 0.157xp$.
$r = 0.3166$; $r^2 = 0.1003$; $F = 15.71$; df = 5,705; $P < 0.0001$.
[a] Significant F statistics are underlined.

A. hydrophila could not be isolated after 24 h incubation of filtered aliquots cultured on R-S medium.

The gastrointestinal contents were analyzed from nine species of fish, two species of snails, one species of turtle, and the American Alligator; *A. hydrophila* was identified as a part of the enteric floral community in each species (Table 5). In addition, at least 25 largemouth bass *(Micropterus salmoides)* were collected quarterly over a period of 1 year from sites T6 and A3, and the gut contents examined for the presence of *A. hydrophila*. At T6, 66 of 127 bass were positive for enteric *A. hydrophila,* whereas at A3, 51 of 103 bass examined were positive. The differences between bass at the two sites are not significant ($X^2 = 0.1$; df = 1; $P = 0.75$). To obtain an estimate of the relative density of *A. hydrophila* within the intestine of largemouth bass, 1 g of fecal material was taken from 8 different adults; the mean *A. hydrophila* density was 7.7×10^5 cfu g^{-1} (coefficient of variation = 208%) of feces.

The extent of red-sore disease within the largemouth bass population was measured monthly, from September 1974 thru December 1977. During most seasons, bass collected in thermal areas had higher infection percentages than those collected in ambient areas (Fig. 6). The highest infection levels occurred in the spring and the lowest in the winter, during each of 3 years. There was a significant correlation between *A. hydrophila* densities in the water column at all sites and the levels of infection among bass (Fig. 7) ($r = 0.5008$; df = 27; $P < 0.01$).

The data were also analyzed using a 1 month lag period since it appeared that each increase in *A. hydrophila* density in the water column was followed within a month by increases in infection percentages of red-sore disease. Indeed, the lagged regression appeared to provide a better fit ($r = 0.6459$; df = 26; $P < 0.001$). When analyzed, however, by Fisher's Z statistic, it was determined that

Table 5. Incidence of *Aeromonas hydrophila* in GI tract of Par Pond animals

Species	Number positive for	% positive for	Number examined
Snails			
Physa sp.	6	20	30
Helisoma sp.	6	20	30
Reptiles			
Pseudomys scripta	5	100	5
Alligator mississippiensis	15	100	15
Fishes			
Micropterus salmoides	176	56	314
Lepomis macrochirus	102	74	138
L. auritus	71	75	95
L. gulosus	17	89	19
L. punctatus	4	80	5
Amia calva	1	25	4
Pomoxis nigromaculatus	3	100	3
Alosa aestivalis	44	44	100
Erimyson oblongus	4	100	4

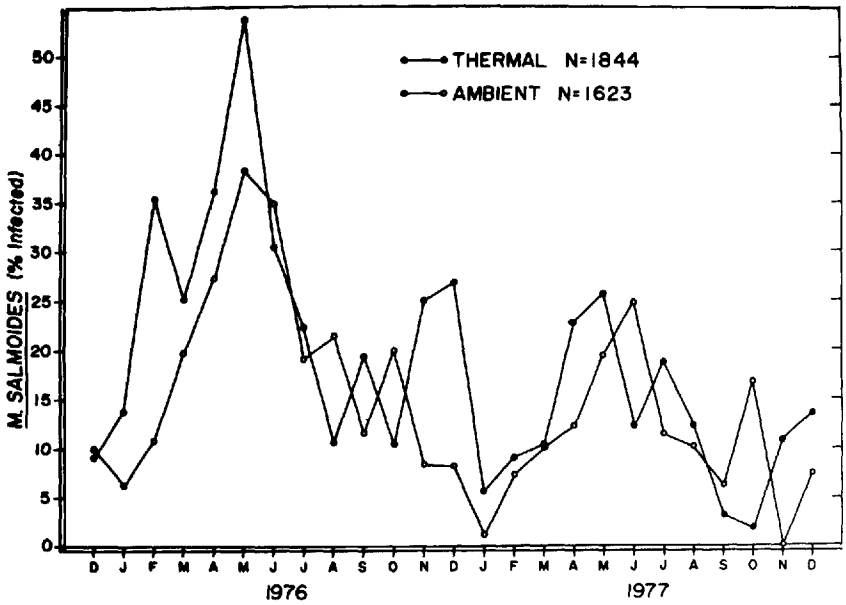

Fig. 6. Red-sore disease infection rate in *M. salmoides* from thermal and ambient areas by month.

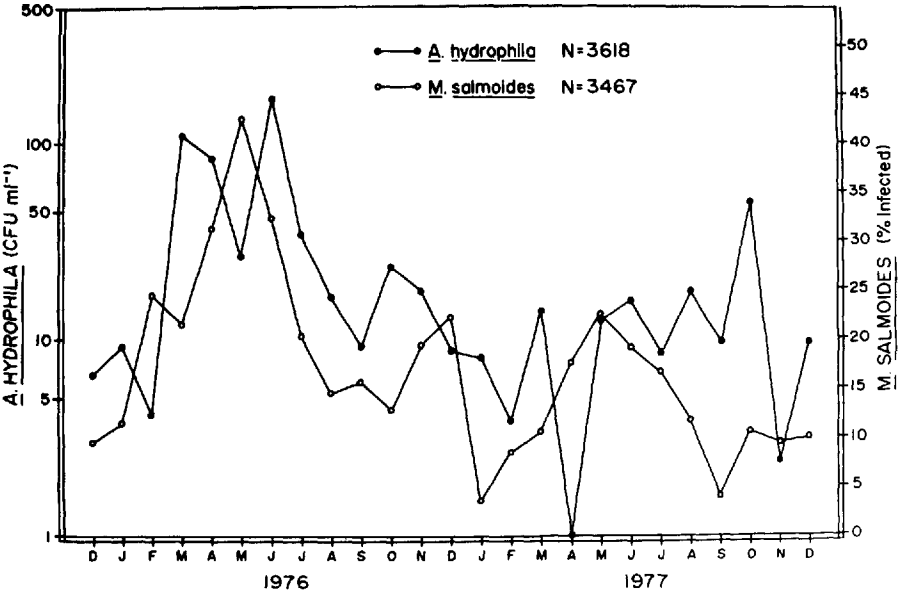

Fig. 7. Mean density of *A. hydrophila* and red-sore disease incidence in *M. salmoides* by month.

the regression of the lagged data was not significantly different from that which has not been lagged ($A = 0.778$; $P = 0.2$).

Discussion

Reactor effluent in Par Pond is associated with large standing crops of periphyton (38), submerged macrophytes (12), zooplankton (40), emergent macrophytes (32), and aquatic vertebrates (1, 9). The various water quality characteristics reported in the present study support results and conclusions of these investigations as regards relative levels of production within ambient and thermal areas of Par Pond. Based on the present observations, and those of Tilly (37), Par Pond can be characterized as a highly eutrophic, warm monomictic lake in which productivity, respiration, and water quality have been substantially altered by the input of thermal effluent from a nuclear production reactor.

Spatial and Temporal Distribution of A. hydrophila

The densities of *A. hydrophila* in Par Pond are only slightly elevated when Par Pond is compared to other southeastern lakes and are well within the normal range for most lakes and rivers in the United States (18).

Unlike those of many other bacteria, *A. hydrophila* densities did not vary with depth in a consistent manner. Thus, although mean density increased with depth, variance also increased. It would seem that the hypolimnion represents a more heterogeneous habitat than the epilimnion for *A. hydrophila*.

The density of *A. hydrophila* did not conform to the general pattern believed to occur for bacteria in sediments. Heterotrophic bacteria normally increase by several orders of magnitude at the sediment-water interface and then decline slowly with depth in the sediments (20). However, *A. hydrophila* densities were less than 1 cfu/g at a depth of 1 cm or greater from the silt surface. The importance of *A. hydrophila* as a mineralizer is, therefore, questionable.

The seasonal changes in densities of *A. hydrophila* in Par Pond were relatively similar from year to year, with the highest numbers occurring during the spring and the lowest in the winter (Fig. 3). There was also a slight increase in overall density when the reservoir turned over in October of each year. This observation is not surprising since it is known that during fall mixis, nutrients in hypolimnetic water are freed for mixing throughout the water column. Although a similar seasonal pattern was observed during each year, the amplitude was quite different from year to year. The highest densities during the 3 years of study (an order of magnitude above what was normally found) were observed during spring 1976. The only water quality parameter significantly different during this period was redox potential.

Aeromonas hydrophila does not maintain consistent relationships with water quality parameters across time. This conclusion was arrived at after statistical comparison of multiple regressions of data from different years, different stratification periods, different mixis periods, and the hypolimnion and epilimnion. Only the multiple regressions for the hypolimnion and epilimnion were not sig-

nificantly different. Thus *A. hydrophila* maintains a consistent spatial, but not a temporal, relationship with water quality.

Multiple correlation analysis indicates that densities of *A. hydrophila* were not correlated with TOC, DOC, POC, or IC. Carbon is, therefore, probably not limiting to *A. hydrophila* in natural habitats. Giesy and Paine (11) observed that growth of *A. hydrophila* in vitro was not stimulated by various carbon fractions from water collected in a pond near the study reservoir. In addition, Wright and Hobbie (42) reported that carbon (at concentrations as low as 1 mg liter^{-1}) does not appear to be limiting for heterotrophic bacteria in water. Observed positive spikes in the vertical profiles of TOC, DOC, POC, and carbon fixation (37) further indicate that *A. hydrophila* densities are unrelated not only to total carbon but also to vertical primary production. The lack of significant correlations between densities of *A. hydrophila* and dissolved oxygen does not suggest a relationship between *A. hydrophila* and total community respiration and/or total community productivity. Specific niche requirements, however, seem to be evident from the strong association between densities of *A. hydrophila* and decomposing *M. spicatum* and the gastrointestinal canal of several species of aquatic animals. These habitats may be ideal since they provide specific nutrients and/or refuge from predators and competitors.

The association of *A. hydrophila* with decaying mats of *M. spicatum* suggests that *A. hydrophila* may play an important role in degradative processes. However, because *A. hydrophila* was not found in significantly higher densities even 10 m from mats of decaying *M. spicatum*, it can be assumed that degradation of aquatic macrophytes does not represent an important source of *A. hydrophila* in open water.

Three water quality parameters in Par Pond (redox potential, temperature, and pH) were consistently and significantly correlated with densities of *A. hydrophila*. Redox potential was always negatively correlated with densities of *A. hydrophila*. Since formation of active reducing agents by heterotrophic bacteria decreases redox potential, it seems reasonable that higher densities of *A. hydrophila* and/or other similar heterotrophic bacteria could account for the negative correlation in Par Pond. Although optimal pH values for *A. hydrophila* growth have not been reported, *A. hydrophila* grows well at pH 5–9, with little or no growth at pH 4 and pH 10 (18). Since Par Pond has a relatively poor buffering capacity due to its relative softness (37), increased densities of *A. hydrophila* and/or similar heterotrophic bacteria could lower the pH and produce the observed negative correlation. It would thus seem likely that changes in both pH and redox potential are a consequence of increased *A. hydrophila* density rather than the reverse.

Temperature was negatively correlated with densities of *A. hydrophila* in Par Pond. This observation is perplexing since densities of *A. hydrophila* are significantly higher in thermally altered parts of the reservoir. In addition, densities of *A. hydrophila* along thermal gradients (17) are highest at the thermal optimum (35°C) of *A. hydrophila* (31). The overall rapid increase in *A. hydrophila* numbers during the early spring, and subsequent decline throughout the summer, is undoubtedly the basis for the negative correlation with temperature. The decline in densities of *A. hydrophila* as water temperatures increase may be brought on by (a) mortality which increased faster than the doubling time decreased, and/or

(b) increased predation by zooplankton, and/or (c) competition with algae and other bacteria for available nutrients.

Kuznetsov and Romanenko (21) showed that the generation time of bacteria in an artificial reservoir changed inversely with water temperature. Ross and Smith (30) reported that *A. hydrophila* mortality was twice as high in river water at 32.2°C as compared to 21.1°C. These observations would seem to indicate that mortality and doubling time are of great importance in the decline of *A. hydrophila* densities as temperature increases. However, Fliermans et al. (7) found that densities of *A. hydrophila* maintained in chambers suspended at various depths and sites within Par Pond declined rapidly at first and then stabilized. Densities in chambers at thermal sites stabilized at higher levels than at ambient stations, with cooler hypolimnetic waters supporting higher densities at all sites. Thus predation and/or competition, as inferred by these exclusion experiments, may be of greater significance in regulating densities of *A. hydrophila* in Par Pond than direct effects of temperature on generation time or senescence-related mortality. Temperature must, however, be considered as an important limiting factor since densities of *A. hydrophila* are always higher in thermally altered areas, and because densities of *A. hydrophila* are always highest at the thermal optimum of *A. hydrophila* along a variety of natural and man-made thermal gradients (17).

Epizootiology of Red-Sore Disease versus the Ecology of A. hydrophila

The primary etiological agent in red-sore disease is *A. hydrophila,* and the most probable route of infection is the surface epithelium of the fish (19). Esch et al. (6) found that all centrarchid fish species in Par Pond, with the exception of the black crappie *(Pomoxis nigromaculatus),* can be infected, and that largemouth bass *(Micropterus salmoides)* have the highest levels of infection.

Vezina and Desrochers (39); Shotts et al. (33); Haley et al. (13), and Snieszko (35) all suggested that epizootic outbreaks of red-sore disease coincide with lowered dissolved oxygen and increased organic loading. The same investigators suspected densities of *A. hydrophila* to be higher in their study sites because of lowered dissolved oxygen and increased organic loading. However, the present study has conclusively shown that *A. hydrophila* densities in Par Pond are not related to either dissolved oxygen or organic carbon.

The present study and others (5, 6) have reported that bass have a higher incidence of infection in the effluent arm during most seasons. Higher levels of red-sore infection are significantly correlated with higher densities of *A. hydrophila* in the water column. However, the relationship between the incidence of red-sore disease and densities of *A. hydrophila* may not be direct, since Esch et al. (6) and Esch and Hazen (5) have also shown that there is a significant correlation between the probability of bass acquiring red-sore disease and the body condition, or K-factor, of a given bass. Moreover, body condition is strongly correlated with health of the fish, as indicated by hematological parameters (5, 16). Bass from thermally altered parts of Par Pond have a significantly lower mean body condition (5). Thus, while an increase in temperature elevates density of *A. hydrophila* in the water column, it simultaneously increases susceptibility

of bass to infection by *A. hydrophila*. Although changes in vulnerability alone could account for increased incidence of disease, it is more probable that a combination of increased susceptibility of bass and increased density of *A. hydrophila*, each promoted by increased water temperature, is responsible for affecting the epizootiology of red-sore disease in the thermally altered arm of Par Pond.

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