Microb Ecol (1983) 9:137–153

MICROBIAL ECOLOGY

A Model for the Density of *Aeromonas hydrophila* in Albemarle Sound, North Carolina

Terry C. Hazen

Department of Biology, Faculty of Natural Sciences, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

Abstract. The abundance of *Aeromonas hydrophila* was measured monthly at 29 sites in Albemarle Sound, North Carolina and its tributaries from April 1977 through July 1979. Simultaneous measurements included heterotrophic plate count bacteria, fecal coliform bacteria, and 18 physical and chemical parameters. Using only 6 water quality parameters, multiple correlation and regression analysis of the data produced a best-fit regression which explained 38% of the variation observed in *A. hydrophila* density. The 6 water quality parameters included dissolved oxygen, temperature, orthophosphate, chlorophyll A trichromatic, total Kjeldahl nitrogen, and ammonia. Heterotrophic plate count bacteria and fecal coliform densities were highly correlated with *A. hydrophila* density, but made the model very unstable. The model was successfully tested against similar data collected for 2 other North Carolina reservoirs, Lake Norman and Badin Lake. Data from 10 sites in Badin Lake over 18 months and from 7 sites on Lake Norman over 5 months were not significantly different from the Albemarle Sound model. Conditions of water quality that may give rise to "blooms" of *A. hydrophila* will simultaneously contribute to the probability of increased epizootics in fish in the southeastern United States.

Introduction

Aeromonas hydrophila is a ubiquitous facultative pathogen. It has been reported throughout the United States in all but the most extreme habitats [20, 21]. Indeed, it has been isolated in high numbers from pristine alpine lakes [21], Louisiana bayous [21], and the aphotic zones of the Atlantic Ocean (1,000 m isolation 5 miles southeast of Puerto Rico; T. C. Hazen, unpublished observations).

A wide range of poikilothermic and homeothermic animals, including man, can be infected by *A. hydrophila* [6, 7, 10, 15, 23, 24, 30, 33, 34]. In the southeastern United States, *A. hydrophila* causes extensive losses to commercial and sport fisheries as the etiological agent for red-sore disease [22]. In one documented case, 37, 500 fish were killed over a single 13-day period in one North Carolina reservoir, Badin Lake [25]. During the fall of 1976, approximately 95% of the white perch (*Roccus americanus*) population was killed by

red-sore disease in Albemarle Sound, North Carolina; during this epizootic, approximately 50% of the commercial fish catch for Albermarle Sound was discarded because of unsightly surface lesions.

In view of the serious implications for the commercial and sport fishing industries in the southeastern U.S., and with inadequate information available regarding the ecology of *A. hydrophila* in North Carolina, a study was undertaken to comprehensively examine the correlation of selected water quality parameters and the density of *A. hydrophila* in Albemarle Sound and in Lake Norman and Badin Lake, North Carolina. Since the relationship between density of *A. hydrophila* and prevalence of red-sore disease within largemouth bass had been previously shown to be so strong [8], it was believed that the present approach would be useful in identifying those water quality parameters that may increase the probability of red-sore epizootics.

Materials and Methods

Study Site

The primary area of study was Albemarle Sound (76°N, 36°10′W), located in the northeast corner of North Carolina (Fig. 1). Albemarle Sound is a natural estuary with a mean depth of 3 m, a maximum depth of 20 m, and a shoreline of 600 km. The total watershed covers 45, 695 km^2. Albemarle Sound has 2 major tributaries, accounting for 83% of the total watershed: the Roanoke River (25, 123 km^2) and the Chowan River (12, 872 km^2). The nearest connection to the Atlantic Ocean is Oregon Inlet near Roanoke Island, site 23. The annual mean tidal range at Oregon Inlet is 0.6 m whereas tides in Albemarle Sound are less than 0.3 m. The characteristic diurnal cycle of tides is approximately 24.8 hours. Average annual rainfall in the area is 114 cm. River flow into Albemarle Sound is greatest during the winter (400 $m^3\ s^{-1}$) and lowest during the summer (>30 $m^3\ s^{-1}$). At times, flow can even reverse briefly during the summer [32]. In the lower Chowan River, flushing times range from more than 50 days during the summer to less than 10 days during the winter [32]. The entire basin supports a rural economy of 500,000 (estimated from the 1970 census). In 1972, commercial fishing was estimated to be producing $5 million annually [2, 4].

Sampling

Water samples were collected using a 2 liter vertical lucite Kemmerer sampling bottle (Wildlife Supply Co., Saginaw, MI). 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, Ft. Wilkinson, WI) and kept on ice for transport to the lab; the time from collection site to the lab never exceeded 1 hour.

Abundance and distribution of *A. hydrophila* were measured monthly. Three samples were taken at the surface and at 1 m intervals in vertical profile at each station (Fig. 1).

Bacteriological Methods

Aeromonas hydrophila density was estimated by viable cell count using Rimler-Shotts (R-S) medium [31]. All density estimates were made 4 times on the same sample. A specific volume of sample was filtered through a sterile, gridded, 47 mm membrane filter with a pore diameter of 0.45 μm (Millipore Corp., Bedford, MA). The filter was then placed on R-S medium and incubated at 35°C for 20–24 hours. Following incubation, yellow colonies were counted with the aid of a

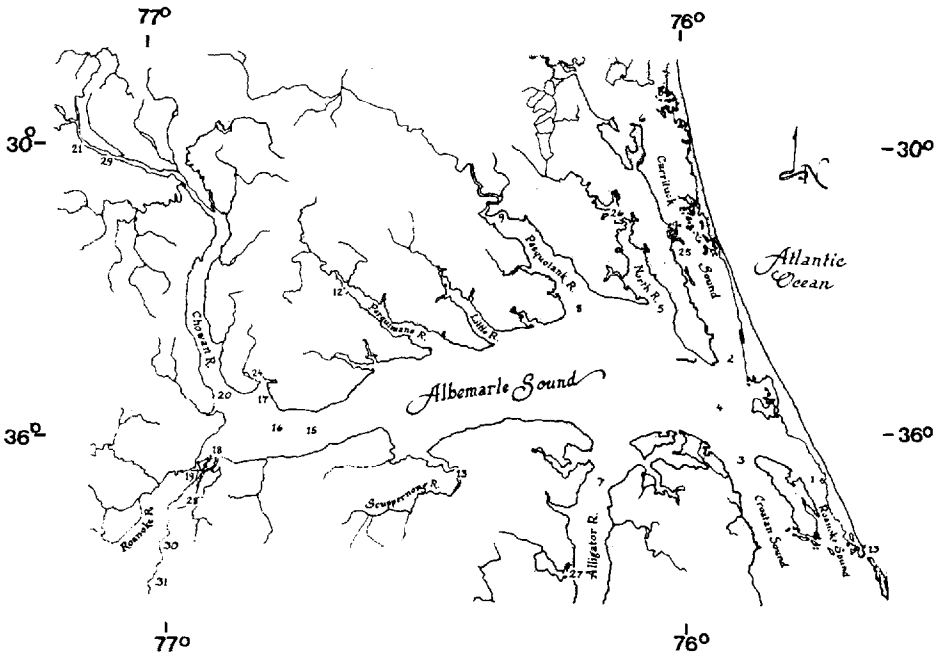


Fig. 1. Albemarle Sound study sites.

magnifying lens; each colony was assumed to represent one colony forming unit (CFU). Periodically, colonies were isolated from membrane filters and confirmed as *A. hydrophila* using API-20E (Analytab Products, Plainview, NY), oxidase tests, the vibriostatic agent 0/129, and *A. hydrophila* specific, fluorescent antibody. All techniques are as previously described by Fliermans and Hazen [12], Hazen [17] and Hazen et al. [21].

Fecal coliform estimates were obtained from 4 aliquots from each sample. A specific volume of sample water was filtered through a sterile, gridded, 47 mm membrane filter with a pore diameter of 0.7 μm (Millipore Corp., Bedford, MA). The filter was then placed on m-FC medium (Difco, Detroit, MI) and incubated at 44.5°C for 24 hours. Following incubation, blue colonies were counted with the aid of a magnifying lens according to APHA standard methods [1].

Heterotrophic plate count bacteria were also estimated from 4 aliquots of each sample. A specific volume of sample water was filtered through a sterile, gridded, 47 mm membrane filter with a pore diameter of 0.45 μm (Millipore Corp., Bedford, MA). The filter was then placed on TGE medium (Difco, Detroit, MI) and incubated at 35°C for 24 hours. Following incubation, all colonies were counted with the aid of a magnifying lens, according to APHA standard methods [1].

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, TX). APHA standard methods were followed for all in situ measurements. Four liters of water were collected, divided into various bottles, and small amounts of the following preservatives added: nitric acid, sulfuric acid, zinc acetate, and mercuric chloride. All samples were then placed on ice for transport to the laboratory. The ap-

propriately preserved samples were analyzed for the following parameters: ammonia, total Kjeldahl nitrogen, nitrates, nitrites (after APHA [1] except that samples were dialyzed instead of filtered); sulfates (turbidimetric method); orthophosphates, total phosphorus (ascorbic acid method and samples were dialyzed); mercury, total organic carbon (using an Oceanographic International Corporation Model 524B equipped with a Lira 303 IR detector); sulfides (methylene blue method), chlorophyll A trichromatic, chlorophyll A corrected, and pheophytin A. APHA [1] standard methods were used for all determinations; for more details see Esch and Hazen [9].

Data Analysis

A Hewlett-Packard, 3000 series, or an IBM 370-148 computer, was used for all statistical analyses. Some data were analyzed using IDA (Interactive Data Analysis, University of Chicago), and modifications of programs by Davies [5]. Factorial analysis of variance was used to test for differences between sites and seasons. Multiple correlations were used to determine relationships of *A. hydrophila* densities with water quality parameters against densities of *A. hydrophila.* Parameters were then removed in a stepwise manner until all the remaining parameters showed t statistics that indicated they significantly affected the density of *A. hydrophila.* Some data (bacteria counts, orthophosphates, total phosphorus, nitrate, nitrite, total organic carbon, ammonia, and chlorophyll A) were found to be heteroscedastic by determining skewness and kurtosis against a normal probability plot. Heteroscedasticity was reduced by transforming each of these measurements with $\text{Log}(x + 1)$ or $(x + 0.1)$ or $(x + 0.01)$, prior to analysis [35]. Any statistical probability ≤ 0.05 was considered significant.

Results

Data for all parameters measured were computed separately by month and site. Physical and chemical parameters for 9 sites during January 1978, August 1978, and July 1979 are presented in Tables 1, 2, and 3, respectively. All sites consistently showed complete mixis at all depths for all parameters measured. Only the 9 most representative sites are shown.

Sites 1, 2, and 3 were always brackish with salinities greater than 10 ppt. Site 27 received runoff from intensive agriculture and had significantly higher total nitrogen and significantly lower chlorophyll A trichromatic. Site 29 is at the point source of effluent from a nitrogen fertilizer factory and had significantly higher ammonia and total nitrogen than adjacent sites. Site 30 is at the point source of effluent from a pulp mill and had significantly higher conductivity, pH, turbidity, ammonia, total nitrogen, and total organic carbon, but a significantly lower redox potential and dissolved oxygen. For the complete data set on each parameter, site, and depth, see Esch and Hazen [9].

Bacteria Distribution and Density

Factorial analyses of variance indicate significant differences in heterotrophic plate count bacteria densities by site ($F = 5.59$; $\text{df} = 20$ & 104; $P < 0.0001$), but not by season. The highest densities of heterotrophic plate count bacteria occurred in November ($> 10^5$ CFU ml^{-1}); the spring and summer months were quite variable (10^2–10^5 CFU ml^{-1}). The brackish water sites had moderate

Table 1. Water quality in Albemarle Sound during January 1978 at 1 m depth

Site	1	2	3	7	9	12	13	15	20
Temperature[a]	8.0	6.5	6.2	6.4	5.2	4.3	8.0	4.5	4.0
Conductivity[b]	18,000	5,000	530	3, 520	118	1, 162	1, 200	110	120
Dissolved oxygen[c]	11.7	12.6	12.7	12.9	10.0	10.3	12.9	12.9	11.2
pH	7.9	7.8	7.7	7.5	6.2	6.5	6.0	7.6	7.1
Redox potential[d]	440	450	415	430	450	455	420	387	300
Turbidity[e]	68	68	68	61	92	68	134	68	92
CAT[f]	20	28	29	23	14	13	15	19	13
CAC[f]	16	21	16	16	5	5	5	5	5
PA[f]	10	14	25	16	14	17	21	22	22
Sulfate[c]	890	220	44	160	34	33	38	25	39
Sulfide[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ammonia[c]	0.02	0.05	0.02	0.02	0.12	0.22	0.12	0.02	0.13
TKN[c]	0.4	0.7	0.4	0.5	0.8	0.8	0.7	0.4	0.5
$NO_3 + NO_2$[c]	0.05	0.02	0.09	0.12	0.75	0.95	1.50	0.27	0.33
Phosphates[c]	0.02	0.02	0.02	0.05	0.02	0.02	0.05	0.02	0.06
Total phosphorus[c]	0.05	0.06	0.06	0.06	0.13	0.10	0.15	0.10	0.13
TOC[c]	5	17	14	23	38	34	28	13	14
Mercury[f]	ND	ND	ND	ND	ND	ND	ND	ND	ND
AH[g]	0.9	2.8	0	0.43	40	60	250	8	4
FC[g]	0.19	0	0.005	0.026	0.75	1.3	3.9	0.2	0.45
HPC[g]	320	1,000	160	58	3, 300	2, 400	6, 200	630	1, 400

[a] = °C; [b] = μmho cm^{-1}; [c] = mg $liter^{-1}$; [d] = mv; [e] = Jackson turbidity units; [f] = μg $liter^{-1}$; [g] = CFU ml^{-1}; CAT= chlorophyll A trichromatic; CAC = chlorophyll A corrected; PA = pheophytin A; TKN = total Kjeldahl nitrogen; TOC = total organic carbon; AH = *A. hydrophila*; FC = fecal coliforms; HPC = heterotrophic plate count; ND = not determined

Table 2. Water quality in Albemarle Sound during August 1978 at 1 m depth

Site	1	2	3	7	9	12	13	15	20
Temperature[a]	28.0	30.5	30.0	29.5	30.0	29.0	32.0	29.0	30.0
Conductivity[b]	94.0	660	1, 150	1,000	190	2,000	145	80	85
Dissolved oxygen[c]	8.1	10.1	9.2	8.4	4.5	7.5	7.8	8.6	9.5
pH	7.6	8.9	7.5	7.0	6.0	6.6	6.6	7.0	7.9
Redox potential[d]	340	340	415	450	515	475	490	500	370
Turbidity[e]	36	47	20	4	26	26	12	36	88
CAT[f]	17	45	15	5	20	5	43	18	83
CAC[f]	28	41	5	5	13	5	27	14	80
PA[f]	5	5	13	5	12	5	29	5	5
Sulfate[c]	480	2	56	53	11	13	13	ND	12
Sulfide[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ammonia[c]	0.02	0.02	0.02	0.2	0.2	0.29	0.02	0.02	0.02
TKN[c]	0.6	1.0	0.2	0.2	0.4	1.3	1.1	0.4	0.8
$NO_3 + NO_2$[c]	0.02	0.02	0.02	0.16	0.08	0.31	0.15	0.02	0.02
Phosphates[c]	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.02	0.02
Total phosphorus[c]	0.02	0.02	0.02	0.02	0.06	0.24	0.09	0.02	0.06
TOC[c]	5	10	5	13	5	37	24	5	5
Mercury[f]	0.2	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2
AH[g]	1.5	11	3.7	1.4	6	2	4.1	78	6
FC[g]	0.06	0.05	0.03	0.91	1.0	1.0	0.65	0.05	0.4
HPC[g]	52	240	76	180	430	370	390	1, 700	430

See Table 1 for notations and abbreviations

Table 3. Water quality in Albemarle Sound during July 1979

Site	1	2	3	7	9	12	13	15	20
Temperature[a]	28.0	27.5	28.0	28.0	30.0	30.0	26.5	28.0	27.5
Conductivity[b]	15,000	3, 660	10,000	170	200	200	210	100	80
Dissolved oxygen[c]	7.3	7.1	7.1	6.9	4.5	8.9	3.7	7.7	8.0
pH	7.8	7.8	7.2	6.7	5.8	6.8	6.0	6.8	7.8
Redox potential[d]	300	300	335	340	340	300	210	350	290
Turbidity[c]	96	94	94	97	81	92	99	97	92
CAT[f]	14	18	13	5	21	44	5	16	59
CAC[f]	11	16	5	5	14	34	5	10	56
PA[f]	5	5	5	5	12	16	5	12	5
Sulfate[c]	680	150	430	73	11	14	13	9	8
Sulfide[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ammonia[c]	0.13	0.02	0.02	0.02	0.5	0.02	0.12	0.02	0.02
TKN[c]	0.5	0.5	0.4	0.4	1.8	0.7	0.7	0.4	0.8
$NO_3 + NO_2$[c]	0.02	0.02	0.02	0.11	0.20	0.15	0.19	0.11	0.02
Phosphates[c]	0.02	0.02	0.02	0.02	0.12	0.02	0.02	0.02	0.02
Total phosphorus[c]	0.02	0.02	0.02	0.02	0.33	0.13	0.09	0.06	0.08
TOC[c]	5	12	5	16	82	26	25	12	14
Mercury[f]	1.0	0.2	1.5	0.2	0.6	0.2	0.2	0.2	0.9
AH[g]	1,000	1,000	700	ND	30	24.3	ND	100	10,000
FC[g]	0.10	ND	1.40	0.10	1.0	0.20	200	0.03	4.83
HPC[g]	50,000	10,000	20,000	30,000	5,000	1, 900	100,000	15,000	5,000,000

See Table 1 for notations and abbreviations

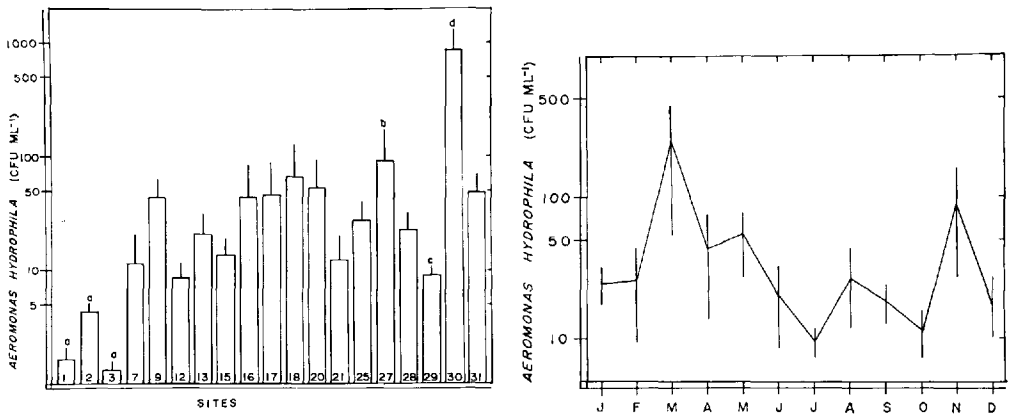


Fig. 2. Density of *A. hydrophila* by site; mean ± 1 standard error. *a* = brackish sites, *b* = intensive agriculture runoff, *c* = nitrogen fertilizer factory, *d* = pulp mill.
Fig. 3. Density of *A. hydrophila* by month; mean ± 1 standard error.

densities of heterotrophic plate count bacteria (10^4 CFU ml^{-1}); site 29 had significantly lower densities ($<5 \times 10^3$ CFU ml^{-1}) than all other sites.

Fecal coliform densities were not significantly different between sites; however, differences by season were significant ($F = 21.67$; df = 8 & 104; $P < 0.0001$). Densities of fecal coliforms at the brackish sites were low (<1 CFU ml^{-1}) whereas those sites receiving effluent were noticeably higher. Indeed, densities at site 30 (receiving pulp mill effluent) were above recommended limits [3] at all times ($>10^2$ CFU ml^{-1}).

Aeromonas hydrophila densities (Fig. 2 and 3) were significantly different by season ($F = 5.87$; df = 8 & 104; $P < 0.01$) and by site ($F = 6.60$; df = 20 & 104; $P < 0.0001$). Densities of *A. hydrophila* were highest during the spring and fall. The brackish water sites had significantly lower densities than freshwater sites, whereas site 27 and site 30 had significantly higher densities of *A. hydrophila* than at other stations. The *A. hydrophila* densities at site 29 were significantly lower (Fig. 2) than adjacent sites.

Correlation and Regression of A. hydrophila *Densities with Water Quality*

The multiple correlation half-matrix (Table 4) shows significant positive correlations between densities of *A. hydrophila* and site, month, temperature, chlorophyll A trichromatic, chlorophyll A corrected, total Kjeldahl nitrogen, orthophosphate, total phosphorus, total organic carbon, heterotrophic plate count bacteria, and fecal coliform bacteria densities. Significant negative correlations were observed between densities of *A. hydrophila* and dissolved oxygen, pH and ammonia concentrations. The other parameters were not significantly correlated with *A. hydrophila* density.

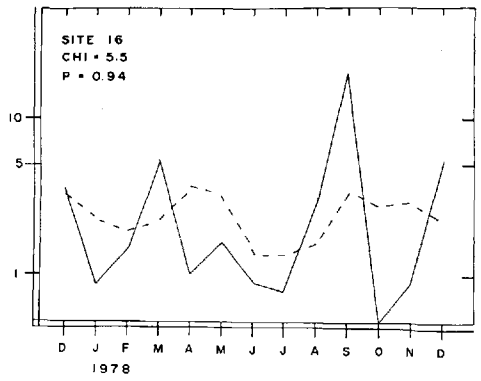
The best-fit regression of the first year of data using dissolved oxygen, temperature, orthophosphates, chlorophyll A trichromatic, total Kjeldahl nitrogen,

Table 4. Correlation half-matrix

	Site	Month	Temp	Cond	DO	pH	Redox	CAT	CAC	PA	SO_4	SO_2	NH_3	TKN	NO_{3+2}	PO_4	TP	TOC	HPC	FC	AH
Site	1.000																				
Month	.068	1.000																			
Temp	.032	*.571*	1.000																		
Cond	*−.406*	−.041	−.044	1.000																	
DO	*−.276*	*−.455*	*−.618*	*.120*	1.000																
pH	*−.264*	−.057	−.055	*.338*	*.359*	1.000															
Redox	*−.113*	*.118*	−.042	*−.118*	*.135*	*−.388*	1.000														
CAT	.040	−.056	*.152*	.016	−.036	*.206*	*−.201*	1.000													
CAC	.053	.098	*.235*	−.037	−.070	*.180*	−.107	*.802*	1.000												
PA	.051	*−.474*	*−.274*	.097	.048	*.117*	*−.341*	*.413*	.063	1.000											
SO_4	−.027	.040	.012	*.197*	.008	*.261*	−.048	.044	.032	.007	1.000										
SO_2	−.017	−.097	−.099	.000	.081	−.006	−.059	.013	.015	.042	.002	1.000									
NH_3	*.182*	.061	*.115*	−.025	*−.494*	−.040	*−.276*	.088	.061	*.183*	−.023	.002	1.000								
TKN	*.194*	.082	.106	−.011	*−.415*	−.057	*−.227*	*.125*	.088	*.215*	−.004	.008	*.657*	1.000							
NO_{3+2}	−.058	−.037	*−.231*	*−.211*	.045	*−.370*	*.271*	*−.194*	*−.125*	*−.137*	−.087	.049	.032	.024	1.000						
PO_4	.106	*.141*	−.089	−.009	*−.217*	−.065	−.103	.031	*−.018*	.099	−.006	.000	*.295*	*.248*	−.046	1.000					
TP	*.250*	.027	.027	−.094	*.481*	.025	*−.220*	*.154*	.102	*.271*	−.053	.020	*.789*	*.690*	.029	*.231*	1.000				
TOC	.093	−.045	.068	−.022	*−.267*	−.031	*−.197*	.083	.019	*.258*	−.021	.003	*.460*	*.390*	−.026	*.147*	*.447*	1.000			
HPC	.099	*−.131*	*−.301*	.050	−.017	−.009	−.101	.002	−.024	*.183*	.041	.060	*.238*	*.172*	.066	.046	*.281*	.100	1.000		
FC	*.353*	*.194*	*.135*	*−.113*	*−.310*	−.099	−.003	−.021	−.004	.005	−.019	−.000	*.210*	*.186*	.037	.069	*.313*	.069	*.229*	1.000	
AH	*.307*	*.213*	*.142*	−.074	*−.402*	*−.191*	−.077	*.126*	*.128*	.051	.050	−.078	*−.199*	*.235*	−.027	*.135*	*.357*	*.160*	*.230*	*.515*	1.000

N = 322, $P < .05$ when $r > .109$.

See Table 1 for notations and abbreviations



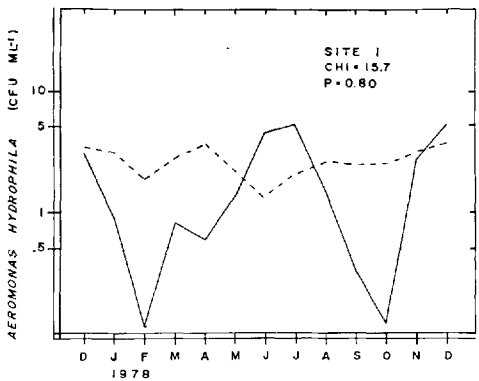


Fig. 4. Predicted (broken line) and observed (solid line) densities of *A. hydrophila* at an undisturbed site.

Fig. 5. Predicted (broken line) and observed (solid line) densities of *A. hydrophila* at brackish site.

Table 5. Best-fit regression statistics

Summary	Multiple r	r^2
Unadjusted	.6244	.3899
Adjusted[a]	.6151	.3783[b]

Analysis of variance:

Source	Sum of squares	Degrees of freedom	Mean square	F statistic
Regression	3.276	6	0.546	33.55[b]
Residuals	5.126	315	0.016	
Total	8.402	321	0.026	

Analysis of coefficients:

Variable	B (Std. V.)	B[d]	Standard error[b]	T statistic[c]
DO	−.4144	−.1201	.0222	−5.413
Temp	−.2804	−.0239	.0058	−4.119
PO_4	.4772	10.8710	1.5135	7.183
CAT	.1421	.0071	.0026	2.714
TKN	.1523	.1149	.0456	2.522
NH_3	−.3101	−.6685	.1591	−4.202
AH	0	1.8135	.2976	6.094
N = 322				

[a] = where the correlation coefficient is adjusted to account for the biased estimator of the population parameters; [b] = $P < 0.0001$; [c] = $P < 0.05$, when $T > 1.968$; [d] = slope

and ammonia explains 37.83% of the variation in densities of *A. hydrophila* (Table 5). The analysis of variance is highly significant ($P < 0.0001$) and each of the independent variables has a slope significantly different from 0 as seen by the analysis of coefficients (Table 5).

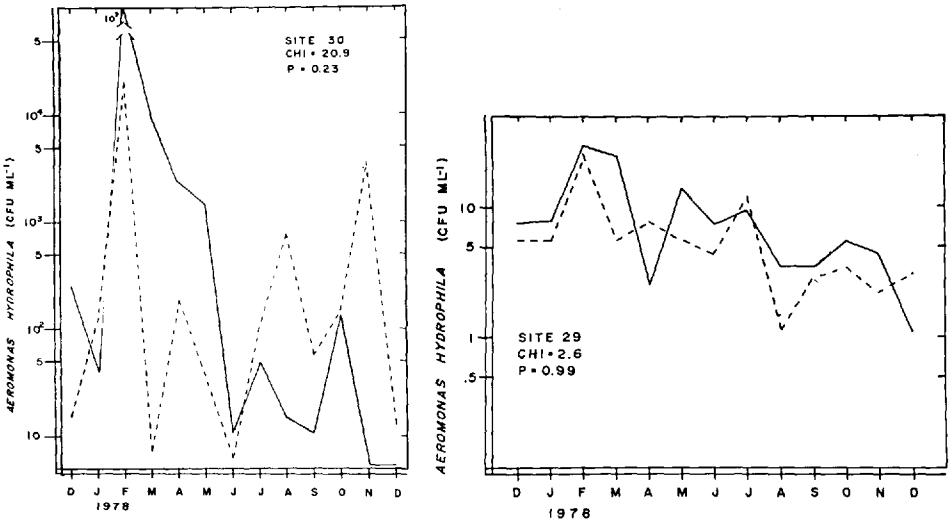


Fig. 6. Predicted (broken line) and observed (solid line) densities of *A. hydrophila* at a site receiving pulp mill effluent.

Fig. 7. Predicted (broken line) and observed (solid line) densities of *A. hydrophila* at a site receiving nitrogen fertilizer factory effluent.

Predictions of A. hydrophila *Densities*

Water quality parameters not used to develop the model were used to predict densities of *A. hydrophila* at all sites, using the regression model (Table 5). Predicted values were compared to actual *A. hydrophila* estimates using chi-square goodness-of-fit. None of the sites demonstrated significant differences between predicted and observed densities of *A. hydrophila.* The observed densities of *A. hydrophila* were very similar to predicted values at undisturbed sites (Fig. 4), brackish sites (Fig. 5), the pulp mill site (Fig. 6) and the fertilizer factory site (Fig. 7). For not more than 3 of the 13 months tested for each site, were predicted densities significantly different from the observed densities. For a total of 345 predictions, the chi-square value was 216.9 ($P > 0.999$).

The model was also used to predict densities of *A. hydrophila* at the 10 sites for 13 months in Badin Lake, NC. Again, observed densities were not significantly different for any of the 10 sites. For 175 predictions in Badin Lake, the chi-square was 121.6 ($P > 0.999$). Typically, fewer than 3 months were significantly different at any site (Fig. 8). In addition, the model was tested at 7 sites in Lake Norman, NC over 5 months and again differences between observed and predicted densities of *A. hydrophila* were not significant. For 27 predictions in Lake Norman, the total chi-square was 16.6 ($P > 0.90$). For 547 predictions in all 3 bodies of water, the total chi-square was 355.2 ($P > 0.999$).

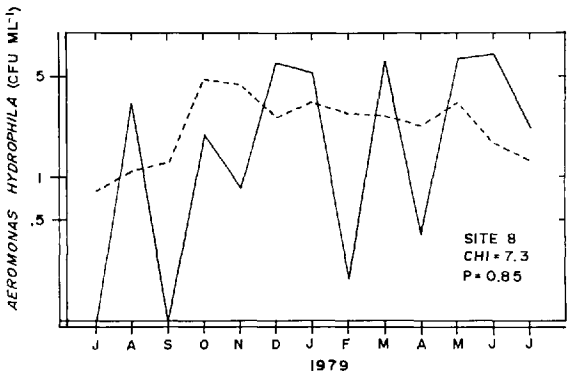


Fig. 8. Predicted (broken line) and observed (solid line) densities of *A. hydrophila* at an undisturbed site in Badin Lake.

Table 6. Fish infections and density of *Aeromonas hydrophila* in the water column

Date	Location	Percent prevalence of red-sore disease	Water column *A. hydrophila*[b]
10/77	Albemarle Sound	20.0 (85)[a]	2.5 ± 0.5
4/77	Lake Hickory	23.0 (109)	42.5 ± 13.2
4/78	Lake Hickory	4.5 (66)	5.0 ± 3.4
5/77	Lake Gaston	14.3 (315)	17.4 ± 4.6
6/77	Badin Lake	29.0 (62)	57.2 ± 21.2
4/78	Badin Lake	2.4 (207)	1.7 ± 0.7
5/78	Badin Lake	2.8 (145)	20.7 ± 6.9
7/78	Badin Lake	8.7 (115)	9.4 ± 7.8
10/78	Chowan River	3.8 (53)	8.2 ± 4.1
12/78	Lake Norman	15.4 (39)	12.8 ± 11.0

[a] Numbers in parentheses refer to numbers of fish sampled
[b] Mean CFU ml^{-1} ± 1 standard error

Fish Disease and A. hydrophila *Density*

Densities of *A. hydrophila* were estimated at several sites in open water simultaneous with collection and examination of largemouth bass (*Micropterus salmoides*) for the presence of red-sore disease (Table 6). Densities of *A. hydrophila* in 5 different bodies of water in North Carolina sampled at different times were significantly correlated with prevalence of infected fish ($r = 0.73$; $P < 0.02$).

Discussion

Spatial and Temporal Distribution of A. hydrophila

The densities of *A. hydrophila* in Albemarle Sound are elevated when compared with other southeastern reservoirs [17], but are well within the normal range

for most lakes, rivers, and estuaries in the United States [21]. As has been demonstrated previously, densities of *A. hydrophila* were not significantly different between depths [17].

Seasonal densities of *A. hydrophila* in Albemarle Sound exhibit a striking periodicity (Fig. 3). The highest numbers occur early in spring, decline to low levels in summer and then increase briefly in the fall. The spring maximum, followed by a weaker fall peak, corresponds to general patterns of phytoplankton density and productivity observed in many temperate lakes [13] and this system [32]. The strong positive correlation between *A. hydrophila* density and chlorophyll A (Table 4) provides additional support for the relationships between seasonal changes in *A. hydrophila* and productivity in the Sound. The seasonal pattern for *A. hydrophila* density in Albemarle Sound parallels that reported for a South Carolina cooling reservoir [16]. Moreover, the same study [16] also revealed an association between carbon fixation in the water column and densities of *A. hydrophila.*

Densities of *A. hydrophila* were significantly lower in brackish water when compared with freshwater sites (Fig. 2); brackish stations were also low in phosphate, nitrate and chlorophyll A. On the other hand, site 30 (pulp mill effluent), site 27 (intensive agriculture runoff), and site 29 (nitrogen fertilizer factory effluent) all had significantly higher densities of *A. hydrophila* as well as elevated concentrations of phosphate, nitrate, and chlorophyll A.

Other Bacteria

The highest densities of heterotrophic plate count bacteria were observed at site 27 (intensive agriculture runoff) and site 30 (pulp mill effluent). The lowest densities of heterotrophic plate count bacteria occurred at site 29 (nitrogen fertilizer factory effluent); however, low densities were also observed at sites 16 and 17 (10^3 CFU ml^{-1}). The distribution of heterotrophic plate count bacteria is thus quite unlike the patterns observed for *A. hydrophila.* The large variability observed between sites during each month produced significant differences between months. However, the large variability also made it impossible to perceive the nature of a seasonal pattern for heterotrophic plate count bacteria.

Densities of fecal coliform bacteria showed a pattern more similar to that of *A. hydrophila* than to heterotrophic plate count bacteria. Brackish sites had very low densities of fecal coliforms whereas the pulp mill effluent site had the highest numbers. Unlike *A. hydrophila,* however, densities of fecal coliform bacteria were high at site 29 (nitrogen fertilizer factory effluent) and low at site 27 (intensive agriculture runoff). Site 21 also had high densities of fecal coliform bacteria, but low densities of *A. hydrophila*; the reason for the large numbers of fecal coliforms at site 21 is unknown. A regular seasonal periodicity in densities of fecal coliforms was unapparent.

Correlations Between A. hydrophila *and Water Quality*

The abundance of *Aeromonas hydrophila* was positively correlated with temperature, a factor that has been previously shown as important in limiting the

densities of *A. hydrophila* in both natural and thermally altered environments [20]. Thus, the number of *A. hydrophila* are always highest between 30° and 35°C, and then decline with increasing temperature until the thermal maximum of 45°C is reached [11, 28].

This study, similar to that of Hazen [17], indicates a significant negative relationship between *A. hydrophila* and dissolved oxygen and pH. Apparently, *A. hydrophila* has a slight competitive advantage over other bacteria when levels of dissolved oxygen decline. This is indicated by the lack of correlation between dissolved oxygen and heterotrophic plate count bacteria. Water pH is apparently not directly correlated with densities of *A. hydrophila* because of a strong positive correlation between pH and dissolved oxygen and since the pH optimum for *A. hydrophila* is slightly basic (T. C. Hazen, unpublished observations).

The significant positive correlations between densities of *A. hydrophila* and total Kjeldahl nitrogen, orthophosphate, and total phosphorus may be influenced by the strong correlation between these parameters and chlorophyll A. Since each of these factors is known to affect the density of phytoplankton [13], it is reasonable to expect that they would indirectly indicate *A. hydrophila.* An explanation for the negative correlation between ammonia and densities of *A. hydrophila* is not apparent; however, it may be a direct influence since survival of *A. hydrophila* in diffusion chambers at site 29 (nitrogen fertilizer factory) is significantly lower that at other sites where ammonia is lower [19]. This may also explain the low densities of *A. hydrophila* observed at site 29.

Strong positive correlations were also observed between densities of *A. hydrophila,* fecal coliforms, and heterotrophic plate count bacteria. Caution should be exercised in assessing the importance of these correlations, since sewage effluents would be expected to have high fecal coliform densities, heterotrophic plate count densities, phosphates, nitrates and total organic carbon [14]. This is especially apparent at sites 21 and 29 which are very high in fecal coliform densities but low in *A. hydrophila,* and site 27 which is low in fecal coliforms but high in *A. hydrophila.*

The Model and Predictions of A. hydrophila *Densities*

The best-fit regression produced a model that predicted densities of *A. hydrophila* using only 6 water quality parameters: temperature, dissolved oxygen, orthophosphate, chlorophyll A trichromatic, total Kjeldahl nitrogen, and ammonia. All of these parameters except ammonia, dissolved oxygen, and temperature had a positive effect on the density of *A. hydrophila* (Table 5). Sites receiving significant nitrogen input (Fig. 7) and organic loading (Fig. 6) were predicted as well as those that were not disturbed (Fig. 4). Indeed, large variation in magnitude of *A. hydrophila* density can be predicted by the model (Fig. 6). The predictability of the very low densities of *A. hydrophila* observed at brackish sites suggests that low nutrient levels, not salinity, are contributing to low densities of *A. hydrophila* observed in marine habitats. Recent reports [29] have suggested that what is being identified as *A. hydrophila* in marine habitats is probably Group F bacteria. The ability of the model to explain densities of

A. hydrophila at brackish sites demonstrates that *A. hydrophila* is indeed a normal floral constituent of brackish and marine habitats [26].

The densities of heterotrophic plate count bacteria and fecal coliforms were also incorporated into another version of the model. In this version, nearly all sites in Albemarle Sound had significant differences between observed and predicted densities of *A. hydrophila.* Thus, although sewage effluent may contribute to high densities of *A. hydrophila,* it is probably not as much due to direct fecal contamination as it is to the increase in nutrients necessary for growth and survival.

Other studies have demonstrated correlations between a relative eutrophic index and densities of *A. hydrophila* [27]. The model developed for Albemarle Sound and the relative eutrophic index are both useful since the index also incorporates the following parameters: total phosphorus, dissolved phosphorus, inorganic nitrogen, secchi depth, chlorophyll A and hypolimnetic dissolved oxygen. The index cannot, however, be used as a predictive model.

From the model it has been shown (Table 5) that small increases in total nitrogen and orthophosphate stimulate density increases in *A. hydrophila,* whereas large increases are needed in temperature, dissolved oxygen, chlorophyll A and ammonia. Nitrogen and phosphorus are nearly always limiting in aquatic systems. As has been shown in numerous studies, small changes in these 2 nutrients will cause large increases in phytoplankton densities. It is suggested that *A. hydrophila* is probably receiving the major nutrients it requires from these "leaky" phytoplankton.

The utility of this model is seen by its application to 2 North Carolina reservoirs. Thus, at every site tested in Lake Norman and Badin Lake, the differences between observed and predicted densities of *A. hydrophila* were not significant. Indeed, the overall difference probability for the goodness-of-fit of the Albemarle Sound model, tested against 547 different predictions, was a remarkable 0.999. Studies in progress in a tropical rain forest watershed in Puerto Rico also indicate predictability of densities of *A. hydrophila* using the Albemarle Sound model [18].

Densities of A. hydrophila *and Fish Disease*

Studies have shown strong positive correlations between density of *A. hydrophila* in the water column and incidence of red-sore disease in fish over a 4-year period in a South Carolina reservoir [8, 17]. During the course of the present investigation, 6 North Carolina reservoirs were examined at different times for density of *A. hydrophila* and prevalence of red-sore disease in largemouth bass; the correlation was again highly significant.

Acknowledgments. The author is grateful to Dr. Gerald W. Esch for reviewing the manuscript, for guidance throughout the study, and administration of monies; and to Dr. Robert P. Hirsch for technical assistance in various aspects of the initial part of this study. I am also grateful to William Crawford, Andy Roberts, Edgar Roberts, James Matthews, Anne Mansfield, Mark Mondl, Joe Camp, Cecile Smith, Greg Olson, Gayle Hazen and Ruth Melendez for their technical assistance.

Throughout this study, I was given extremely generous technical support by Steve Tedder and his associates within the Division of Environmental Management of the North Carolina Depart-

ment of Natural Resources and Community Development. The personnel at Duke Power Company also provided help for work on Lake Norman.

A special thanks is due to the Twin Cities Bass Masters Club of Winston-Salem, North Carolina and the B.A.S.S. club of Franklin, Virginia for helping us obtain the largemouth bass needed for this study; their interest and enthusiasm was inspirational.

This work was supported by a grant, B-112-NC, from the North Carolina Water Resources Research Institute. It was also supported in part by a grant from the North Carolina Board of Science and Technology and by Contract DE-AS09-76EY-0900 between Wake Forest University and the Department of Energy. I also acknowledge support through a grant from the Oficina de Coordinacion de Estudios Graduados y Investigaciones of the University of Puerto Rico, grant RR-8102 from the National Institutes of Health to the University of Puerto Rico, and Sea Grant 04F15844030 project EN/P-45 from the National Oceanographic and Atmospheric Administration to the University of Puerto Rico.

References

1. American Public Health Association (1975) Standard methods for the examination of water and wastewater, 14th edn. American Public Health Association, Washington, DC
2. Bond S, Cook B, Howells DH (1978) The Chowan River project. Summary report for the water resources research institute of the University of North Carolina, Raleigh, North Carolina
3. Bonde GJ (1977) Bacterial indicators of water pollution. Adv Aquatic Microbiol 1:273–367
4. Daniel CC (1977) Digital flow model of the Chowan River estuary, North Carolina. U.S. geological survey water resources investigations 77-63 National Technical Information Service, Springfield, Virginia
5. Davies RG (1971) Computer programming in quantitative biology. Academic Press, New York
6. Davis WA, Kane JG, Garagusi VG (1978) Human *Aeromonas* infections: a review of the literature and a case report of endocarditis. Medicine 57:267–277
7. Emerson H, Norris C (1905) "Red Leg"—an infectious disease of frogs. J Exp Med 7:32–60
8. Esch GW, Hazen TC (1978) Thermal ecology and stress: a case history for red-sore disease in largemouth bass (*Micropterus salmoides*). In: Thorpe JH, Gibbons JW (ed) Energy and environmental stress in aquatic systems. Department of Energy Symposium Series CONF-7711114. National Technical Information Service, Springfield, Virginia
9. Esch GW, Hazen TC (1980) The ecology of *Aeromonas hydrophila* in Albemarle Sound, North Carolina. Water Resources Research Institute of The University of North Carolina. Report 153, Raleigh, North Carolina
10. Esch GW, Hazen TC, Dimock Jr RV, Gibbons JW (1976) Thermal effluent and the epizootiology of the ciliate *Epistylis* and the bacterium *Aeromonas* in association with centrarchid fish. Trans Am Microsc Soc 95:687–693
11. Fliermans CB, Gorden RW, Hazen TC, Esch GW (1977) *Aeromonas* distribution and survival in a thermally altered lake. Appl Environ Microbiol 33:114–122
12. Fliermans CB, Hazen TC (1980) Immunofluorescence of *Aeromonas hydrophila* as measured by fluorescence photometric microscopy. Can J Microbiol 26:161–168
13. Fogg GE (1965) Algal cultures and phytoplankton ecology. University of Wisconsin Press, Madison, Wisconsin
14. Grabow WOK, DuPreez M (1979) Comparison of m-Endo, LES, MacConkey and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl Environ Microbiol 38:351–358
15. Haley R, Davis SP, Hyde JM (1967) Environmental stress and *Aeromonas liquefaciens* in American and threadfin shad mortalities. Prog Fish-Cult 29:193
16. Hazen TC (1978) The ecology of *Aeromonas hydrophila* in a South Carolina cooling reservoir. PhD thesis, Wake Forest University, Winston-Salem, North Carolina
17. Hazen TC (1979) The ecology of *Aeromonas hydrophila* in a South Carolina cooling reservoir. Microb Ecol 5:179–195

18. Hazen TC, Aranda CF (1981) The relationship between the distribution and abundance of bacteria and water quality in the Rio Mameyes watershed. Septimo Simposio de Recursos Naturales. Puerto Rico, San Juan, pp 87–111
19. Hazen TC, Esch GW (1983) Effect of effluent from a nitrogen fertilizer factory and a pulp mill on the distribution and abundance of *Aeromonas hydrophila* in Albemarle Sound, North Carolina. Appl Environ Microbiol 45:31–42
20. Hazen TC, Fliermans CB (1979) Distribution of *Aeromonas hydrophila* in natural and man-made thermal effluents. Appl Environ Microbiol 38:166–168
21. Hazen TC, Fliermans CB, Hirsch RP, Esch GW (1978) The prevalence and distribution of *Aeromonas hydrophila* in the United States. Appl Environ Microbiol 36:731–738
22. Hazen TC, Raker ML, Esch GW, Fliermans CB (1978) Ultrastructure of red-sore lesions on largemouth bass *Micropterus salmoides:* the association of the peritrich *Epistylis* sp. and the bacterium *Aeromonas hydrophila.* J Protozool 25:351–355
23. Marcus LC (1971) Infectious diseases of reptiles. J Am Vet Med Assoc 159:1626–1631
24. Mead AR (1969) *Aeromonas liquefaciens* in the leukodermia syndrome of *Achatina fulica.* Malacologia 9:43
25. Miller RM, Chapman WR (1976) *Epistylis* sp. and *Aeromonas hydrophila* infections in fishes from North Carolina reservoirs. Prog Fish-Cult 38:165–168
26. Peele ER, Singleton FL, Penning JW, Cavari B, Colwell RR (1981) Effects of pharmaceutical wastes on microbial populations in surface waters of the Puerto Rico dump site in the Atlantic Ocean. Appl Environ Microbiol 41:873–879
27. Rippey SR, Cabelli VJ (1980) Occurrence of *Aeromonas hydrophila* in limnetic environments: relationship of the organism to trophic state. Microb Ecol 6:45–54
28. Rouf MA, Rigney MM (1971) Growth temperatures and temperature characteristics of *Aeromonas.* Appl Microbiol 22:503–506
29. Seidler RJ, Allen DA, Colwell RR, Joseph SW, Daily OP (1980) Biochemical characteristics and virulence of environmental group F bacteria isolated in the United States. Appl Environ Microbiol 40:715–720
30. Shotts Jr EB, Gaines JL, Martin C, Prestwood AK (1972) *Aeromonas*—induced deaths among fish and reptiles in an eutrophic inland lake. J Am Vet Med Assoc 161:603–607
31. Shotts Jr EB, Rimler R (1973) Medium for the isolation of *Aeromonas hydrophila.* Appl Microbiol 26:550–553
32. Stanley DW, Hobbie JE (1981) Nitrogen recycling in a North Carolina coastal river. Limnol Oceanogr 26:30–42
33. Trust TJ, Chipman DC (1979) Clinical involvement of *Aeromonas hydrophila.* Can Med Assoc J 120:942–947
34. Wohlegemuth D, Pierce RL, Kirkbride CA (1972) Bovine abortion associated with *Aeromonas hydrophila.* J Am Med Assoc 160:1001
35. Zar JH (1974) Biostatistical analysis. Prentice Hall, Englewood Cliffs, New Jersey