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SURVIVAL AND DISTRIBUTION OF *AEROMONAS HYDROPHILA* IN NEAR-SHORE COASTAL WATERS OF PUERTO RICO RECEIVING RUM DISTILLERY EFFLUENT

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Abstract—For a period of 1 year, monthly water samples were taken for estimates of *Aeromonas hydrophila* density at 6 sites in Ensenada de Boca Vieja near San Juan, Puerto Rico. Five sites were associated with the effluent plume of the world's largest rum distillery, the sixth site was 177 m upcurrent. Fifteen water quality parameters were monitored concurrently with *A. hydrophila* estimates. The toxic and stimulatory nature of the effluent made correlations with any physicochemical parameter difficult. However, a significant multiple regression was obtained against density of *A. hydrophila* using temperature, total phosphorus, total organic carbon and orthophosphates. Densities of *A. hydrophila* were always higher in the effluent plume and usually highest at the site closest to effluent outfall. Suspensions of *A. hydrophila* placed in diffusion chambers at the effluent point source not only survived, but gradually increased in density, while 500 m upcurrent densities of *A. hydrophila* in diffusion chambers rapidly declined. Significant differences in *A. hydrophila* density between the two sites could be detected after only 18 h. The diffusion chamber studies confirm natural correlations with water quality that indicate *A. hydrophila* densities in marine habitats can become elevated under the appropriate conditions. Higher densities of other potential pathogens e.g. *Klebsiella pneumoniae*, *Vibrio cholerae*, were also observed in the rum distillery effluent plume.

INTRODUCTION

Aeromonas hydrophila is a gram negative, facultatively anaerobic rod, 2–3 μm long with a single polar flagellum. This bacterium is commonly observed in sewage effluents (Grabow & DuPreez, 1979) and thermal effluents (Hazen & Fliermans, 1979; Hazen, 1979). However, Hazen *et al.* (1978a) demonstrated that *A. hydrophila* is found in all but the most extreme aquatic habitats, i.e. hypersaline and hyperthermal waters. Indeed, similar densities were reported for cold, pristine alpine lakes in Wyoming and eutrophic Louisiana bayous. *Aeromonas hydrophila* has been found to cause disease in fish (Miller & Chapman, 1976), frogs (Emerson & Norris, 1905), lizards (Marcus, 1971), alligators (Gorden *et al.*, 1979), turtles (Shotts *et al.*, 1972), snails (Mead, 1969) and cattle (Wohlegemuth *et al.*, 1972). In addition, many cases of gasteroenteritis and wound infections in man have been recently reported (Davis *et al.*, 1978). Trust & Chipman (1979) felt that as much as 13% of all acute gasteroenteritis cases report by hospitals were caused by *A. hydrophila*. Since *A. hydrophila* normally causes only a mild, self-limiting gasteroenteritis, the number of unreported human cases must be enormous.

In the southeastern United States *A. hydrophila* is the primary etiological agent for red-sore disease in fish (Hazen *et al.*, 1978b). Red-sore disease losses to commercial and sports fisheries in the southeast are tremendous, one recent report documented the death of over 35,000 fish over one 13 day period in one North Carolina reservoir (Miller & Chapman, 1976). Recently, red-sore disease has been observed in Puerto Rico in wild populations of largemouth bass, introduced from Georgia (Hazen, Unpublished data).

Large rum distilleries occur on several Carribean Islands, and nearly all discharge effluents directly into near-shore coastal waters, without prior treatment. The largest rum distillery in the world is located at Ensenada de Boca Vieja, a bay adjacent to San Juan harbor in Puerto Rico. This one industry pumps more than 1.4×10^6 l day^{-1} of untreated effluent, into this bay more than 300 days each year (Costle, 1979).

Rum distillery effluent (slops) are composed of cooling waters from stills, fermentation vats, hot spent wash, and water used for cleaning the fermentation vats and distillery (Sheehan & Greenfield, 1980). The slops are an odorous, reddish brown, viscous, complex mixture of organic acids, aldehydes, proteins, amino acids, polysaccharides and heavy metals. The pH is usually near 4.7 and the BOD_5 is usually close to 32,000 mg O_2 l^{-1} (Costle, 1979). At Ensenada de Boca Vieja, the effluent plume can extend for several kilometers parallel to the coast. The plume is normally anoxic and has been shown to kill fish, crabs, snails and many other forms of marine life in the surrounding water (González *et al.*, 1979). High concentrations of the rum slop are bacteriostatic; how-

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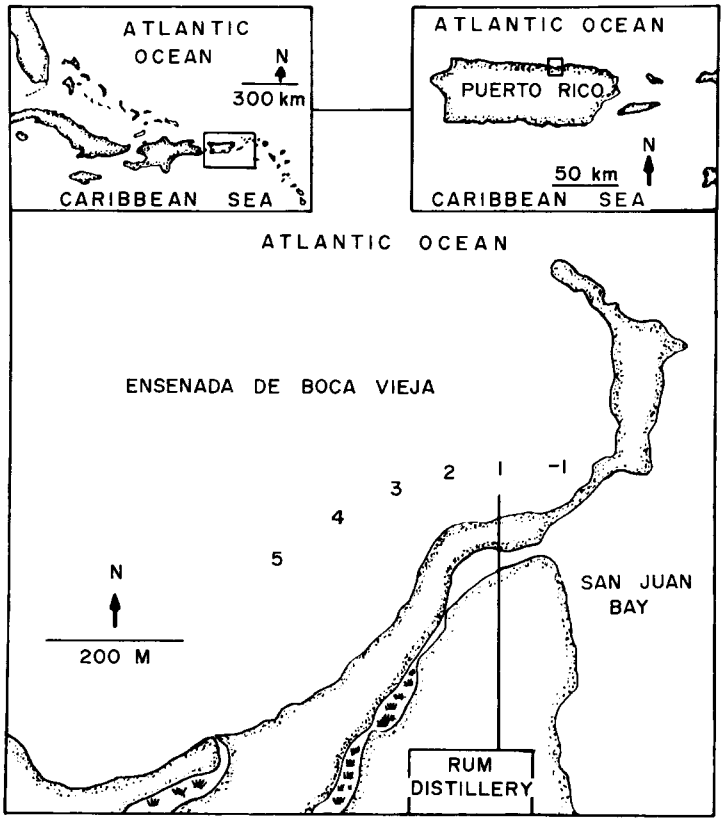

Fig. 1. Ensenada de Boca Vieja study sites.

ever, dilutions of the slop can stimulate growth of some bacteria which, in turn, detoxify the effluent for certain algae and other bacteria (Tosteson *et al.*, 1973; Tosteson & Hale, 1978). Preliminary studies done in our laboratory indicated that rum distillery effluent, supported high densities of potentially pathogenic bacteria like *A. hydrophila.*

The objectives of the present study were (1) to determine the distribution and abundance of *A. hydrophila* in a bay receiving rum distillery effluent; (2) to ascertain the *in situ* survival characteristics of *A. hydrophila* in marine waters contaminated with rum slops; and (3) to find if other potentially pathogenic bacteria and/or indicator bacteria were affected by rum distillery effluent in tropical marine environments.

MATERIALS AND METHODS

Study site

Ensenada de Boca Vieja (18 27′ 48″ N, 66 08′ 42″ W) is a protected cove immediately adjacent to San Juan Bay, Puerto Rico. This cove has a tidal range of 0.5 m, shoreline length of 855 m, mean depth of 4 m, surface area of 94,643 m^2, and receives 1.4×10^6 l day^{-1} of untreated effluent from a single rum distillery (Costle, 1979). An underground pipe connects the discharge point with the rum distillery which is 1 km to the southwest. Shore currents move the effluent plume in a westerly direction, parallel with the shoreline. On rare occasions the wind is from the northwest and creates a current that moves the waste plume in an easterly direction so that it tends to accumulate in the apex of the cove.

Six sites, at different distances from the outfall, were chosen for water sampling. Site −1 was upcurrent, 177 m from the discharge point while site 1 was at the point source. Sites 2, 3, 4 and 5 were downcurrent directly in the effluent plume at distances of 5, 128, 421 and 713 m respectively from the point source (Fig. 1). Sampling sites −1, 1, 2 and 3 were 50 m from the edge of the mean high tide shoreline, while sites 4 and 5 were 335 m from the mean high tide shore.

Sampling procedures

Water samples were collected from March 1980 to February 1981 at monthly intervals. Samples for bacteriological analysis were taken by grab sampling, placed into sterile, 180 ml Whirl Pak bags (Nasco International, Fort Wilkinson, WI) and kept on ice until processed. Time from collection to analysis never exceeded 4 h. Water for physicochemical analysis was taken by grab sampling using 500 ml bottles containing appropriate preservatives; all were chilled until analyzed.

Bacteriological procedures

Densities of *A. hydrophila* were estimated using Rimler–Shotts (R–S) media (Shots & Rimler, 1973). A minimum of 1 ml was filtered through a 0.45 μm, gridded, 47 mm dia., HA type membrane filter (Millipore Corp., Bedford, MA). Dilutions were made using filter-sterilized, phosphate buffered saline, pH 7. After filtration, filters were placed on sterile pads soaked with R–S medium in sterile tight-fitting petri dishes and incubated at 35°C for 20 h. When incubation was complete the number of yellow colonies were counted with the aid of a magnifying lens and the number

of colony forming units per ml (CFU ml^{-1}) recorded, as described by Hazen (1979) and Hazen *et al.* (1978a).

Samples were also analyzed for fecal coliform bacteria using m-FC media (Difco, Detroit, MI). Dilutions were made when necessary (as above) and aliquots filtered through 0.7 μm, gridded, 47 mm dia., HC type membrane filters (Millipore Corp., Bedford, MA). Colony forming units were estimated by counting blue colonies after 24 h incubation at 44.5°C (APHA, 1975).

Standard bacteria counts were determined using m-Plate Count media (Difco, Detroit, MI). Again, dilutions were made with filter-sterilized phosphate buffered saline, pH 7.0 and filtered through 0.45 μm, gridded, 47 mm dia., HA type membrane filters (Millipore Corp., Bedford, MA). All colonies were counted after incubation at 35°C for 24 h (APHA, 1975). Bacteria were identified using the API-20E (Analytab Products, Plainview, NY), oxidase, 0/129 sensitivity, and fluorescent antibody or serology (see Fliermans & Hazen, 1980; Hazen, 1979 for details).

Survival of Aeromonas hydrophila *in situ*

Pure cultures of *A. hydrophila* (ATCC 7966) were grown in nutrient broth at 35°C for 24 h. Cells were harvested by centrifugation and washed in filter-sterilized, phosphate buffered saline (pH 7). The number of cells ml^{-1} was determined with a model ZF Coulter Counter (Coulter Electronics, Hialeah, FL) and then adjusted to 10^8 cells ml^{-1}.

The final bacteria suspension was then placed into a sterile diffusion chamber just prior to immersion at the study site. The chambers used had a capacity of 100 ml, a total diffusion surface area of 16,515 mm^2 and were a modification of the MSU-DME chamber (McFeters & Stuart, 1972). O-rings and a different sampling port were added to each chamber to reduce contamination and leakage. A 0.45 μm, 142 mm dia., nylon reinforced, Acropor membrane filter (Gelman Instrument Co., Ann Arbor, MI) was used to create the diffusion surface.

In January, 1981, five chambers were suspended 1 m below the surface, 500 m upcurrent from the effluent plume. Another five chambers were suspended 1 m below the surface. 5 m from the point source and in the effluent plume. One-half ml samples were taken from each chamber with a sterile syringe at regular intervals for 72 h. Each sample was immediately fixed in 1.5 ml of 10% phosphate buffered formalin (pH 7) and refrigerated for later enumeration with the Coulter Counter. This technique satisfactorily preserves *A. hydrophila* cells used in counting for more than two weeks (Hazen, unpublished data).

Water quality analysis

Each time bacterial sampling was conducted, several physicochemical parameters were measured *in situ* at each of the sampling stations. Salinity and conductivity were measured with a model 33 salinity–conductivity–temperature meter (Yellow Springs Instrument Co., Yellow Springs, OH). Temperature and dissolved oxygen were determined with a model 57 dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, OH). A digital pH meter, model 201 (Orion Research, Cambridge, MA) was used for pH determinations in the field. Orthophosphates, total phosphorus, nitrates, sulfates, chlorophyll A trichromatic and turbidity were determined using APHA Standard Methods (APHA, 1975). Total organic carbon (TOC) was measured with a model 524-B infrared carbon analyzer (Oceanography International Corp., College Station, TX), using the ampulla method (Menzel & Vaccaro, 1964).

Data analysis

Programs developed on a IBM 370-148 computer were used for all statistical tests. Two factor analysis of variance (FANOVA) was used to test differences between sites and time. Multiple correlation and regression were used to determine relationship between densities of *A. hydrophila* and water quality parameters. Heteroscedastic data, as determined by skew and kurtosis, were made more homoscedastic by transformation with $\text{Log}(X + 1)$. Any statistical probability greater than or equal to 0.05 was considered significant (Zar, 1974).

RESULTS

Water quality

The rum distillery in this study normally operates 24 h a day for 300 days each year. The factory closes only during December and part of January for repairs and holidays. During the study period; however, the facility also ceased operations in March, April and May because of an employee strike. Therefore, during the strike, sampling was discontinued.

Representative physical and chemical data for each site are presented in Tables 1 and 2. Table 1 shows measurements made during November 1980 when rum slops were being discharged. Table 2 shows the same sites and measurements but one month later when slops were not being discharged. Nitrates, phosphates, total phosphorus, sulfates, total organic carbon and chlorophyll *a* concentrations were all significantly higher at the first four sites in the effluent plume. Turbidity (percent transmittance), dissolved oxygen and pH were significantly lower at these sites (1–4) during effluent discharge. In addition, a thermal gradient was observed beginning at the effluent point source (60°C) and extending for approx. 10 m down current (40°C).

Table 1. Water quality during rum distillery discharge; November 1980

Site	−1	1	2	3	4	5
Distance (m)	−177	0	5	128	421	713
Temp (°C)	27	33	30	27	27	27
DO	6.9	3.4	5.8	7.8	8.2	8.9
Cond (μmhos)	ND	ND	ND	ND	ND	ND
pH	8.0	4.8	5.3	7.8	8.0	8.0
Turb (%T)	100	4	30	100	100	100
Salinity (‰)	39	43	27	19	39	33
NO_3	ND	0.609	0.250	0.007	0	0.008
SO_4	2867	3752	3018	2608	2987	2908
PO_4	0.020	2.780	0.774	0.061	0.011	0.011
TP	0.039	8.940	2.760	0.034	0.030	0.029
Chl. *a*	198	22780	22780	150	64	113
TOC ($\mu g\, l^{-1}$)	1.57	1244	391	5.37	3.52	1.10

ND = not determined, all values not indicated are in $mg\, l^{-1}$.

Table 2. Water quality with rum distillery closed; December, 1980

Site	−1	1	2	3	4	5
Distance (m)	−177	0	5	128	431	213
Temp (°C)	26	26	26	26	26	26
DO	5.0	5.2	4.9	5.4	6.0	5.8
Cond (μmhos)	ND	ND	ND	ND	ND	ND
pH	8.05	8.05	8.05	8.05	8.05	8.05
Turb (%T)	96	96	100	90	96	100
Salinity (‰)	39	32	32	38	41	41
NO_3	0.154	0.215	0.221	0.130	0.179	0.161
SO_4	1335	1233	1485	1271	886	1014
PO_4	0.015	0.015	0.023	0.012	0.007	0.049
TP	0.047	0.032	0.05	0.033	0.113	0.047
Chl. *a*	75.04	37.52	69.68	0	0	0
TOC ($\mu g\,l^{-1}$)	1.76	1.35	1.32	1.76	2.45	2.16

ND = not determined, all values not indicated are in $mg\,l^{-1}$.

Bacteria distribution and abundance

Densities of *A. hydrophila* were significantly different by site (Fig. 2; $F = 5.9$; d.f. = 5, 36; $P < 0.001$) and month (Fig. 3; $F = 4.5$; d.f. = 11, 36; $P < 0.001$) as determined by FANOVA. Densities as high as 10^6 CFU ml^{-1} were observed at site 1, the point source and as low as 10 CFU ml^{-1} at site −1, the non-polluted sampling station. During the distillery down times (December, March–May) the densities of *A. hydrophila* were generally low (Fig. 2). As distance from the discharge point increased density of *A. hydrophila* decreased. All plume sites (1–5) had significantly higher densities of *A. hydrophila* than the control site $P < 0.01$).

Consistently higher densities of fecal coliform bacteria were also associated with the effluent plume (Fig. 4). Densities of fecal coliform bacteria were highest during discharge periods at all sites in the effluent plume; however, rapid and significant changes were observed from one sampling period to another (Fig. 5).

Standard count bacteria were highest at sites 2–5; however, all effluent sites (1–5) had significantly higher densities than the control site −1 (Fig. 6). No seasonal pattern of density differences were apparent in the standard count bacteria, other than differences correlated with the operation of the distillery (Fig. 5).

Identification of bacteria from differential media at various times and sites in the effluent plume indicated the presence of: *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Staphylococcus aureus*, *Klebsiella ozaenae*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Vibrio* spp, *Pseudomonas* spp and *Citrobacter freundii*.

Multiple correlation and regression of A. hydrophila *with water quality*

The multiple correlation (Table 3) demonstrated significant correlations between *A. hydrophila* and total organic carbon (positive) and turbidity (negative). In addition, positive correlations were also observed between *A. hydrophila* and temperature, total phosphorus, phosphates and chlorophyll *a*. No correlations were indicated between standard count bacteria, fecal coliform bacteria and densities of *A. hydrophila*.

A best-fit multiple regression analysis revealed that temperature, phosphates, total phosphorous and total

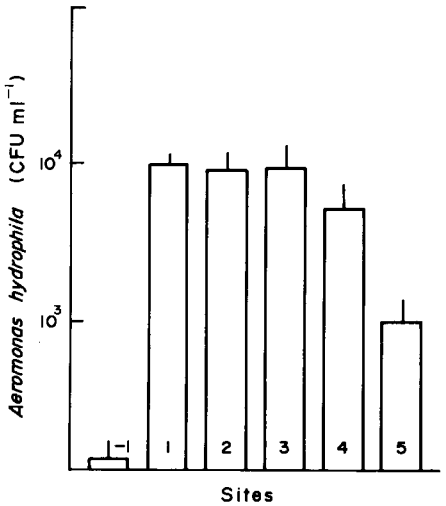

Fig. 2. Density of *A. hydrophila* by site; mean ± 1 SE.

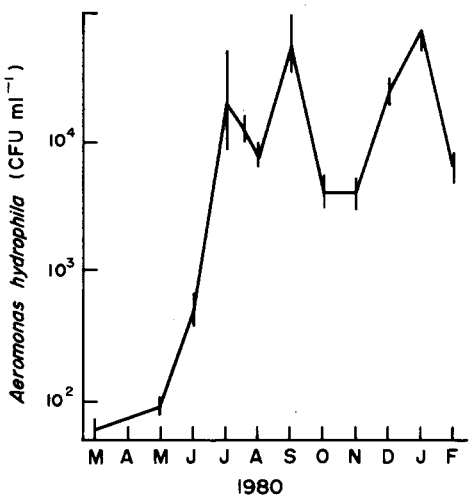

Fig. 3. Density of *A. hydrophila* by month; mean ± 1 SE.

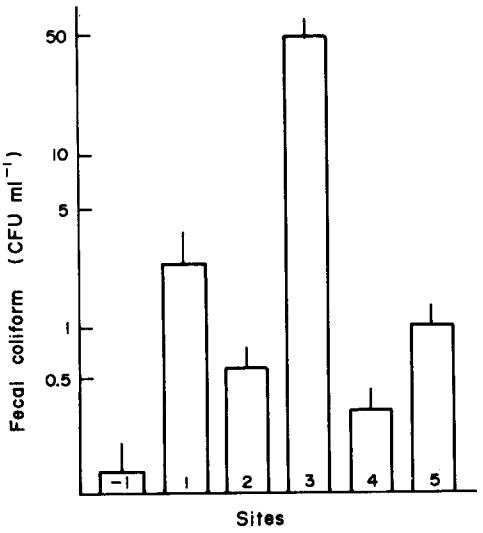

Fig. 4. Density of fecal coliform bacteria by site; mean ± 1 SE.

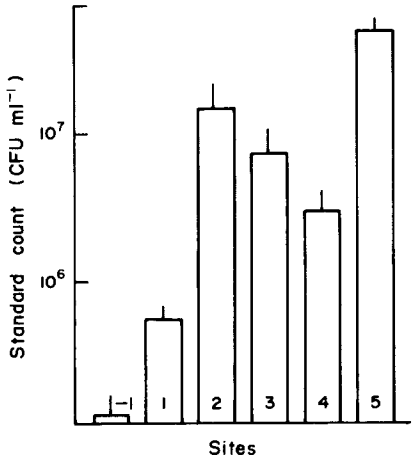

Fig. 6. Density of standard count bacteria by site; mean ± 1 SE.

organic carbon explained 41.2% of the variation in densities of *A. hydrophila* at the sites sampled (Table 4). The regression analysis of variance was also highly significant (Table 4).

A. hydrophila *survival* in situ

The density of *A. hydrophila* placed in diffusion chambers at the effluent point source not only survived but increased (Fig. 8). The upcurrent chambers; however, had significantly lower densities of *A. hydrophila* than the point source chambers throughout the entire study (Table 5). Densities of *A. hydrophila* in diffusion chambers at the control site declined rapidly, becoming significantly different from the point source chambers after 18 h.

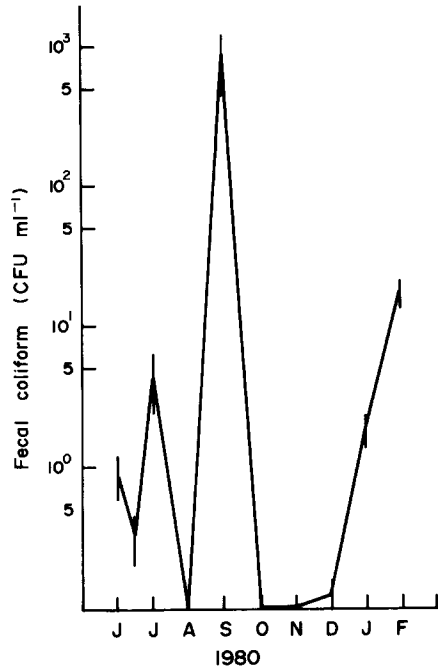

Fig. 5. Density of fecal coliform bacteria by month; mean ± 1 SE.

DISCUSSION

Rum distillery effluent in Ensenada de Boca Vieja creates an effluent plume 1000 m long which significantly increases the concentration of nitrates, phosphates, total phosphorus, sulfates, total organic carbon and temperature of adjacent marine waters. In addition, the effluent plume significantly lowers turbidity (percent transmittance), dissolved oxygen and pH. At the outfall (site 1) the concentration of organic acids, aldehydes, alcohol, high temperature, low pH and low dissolved oxygen appear to have a striking toxic effect on all marine life including bacteria and cyanobacteria. However, after slight dilution and cooling (2–3 m), bacteria flourish in the nutrient soup created by the effluent (sites 2–5). The bacteria also detoxify many compounds, thus enabling cyanobacteria and green algae to flourish. The stimulatory effect of the normally limiting nutrients (organics, phosphates, nitrates) cause bacterial and algae blooms which increase chlorophyll *a* concentration and decrease dissolved oxygen. The toxic nature of the effluent for nearly all higher organisms remains apparent along the entire plume (González *et al.*, 1979). It should be noted however, that since this cove faces the Altantic Ocean, heavy seas (3 m swells) strong counter-currents created by high winds, and tidal action can alter the position and the size of the plume to such an extent that the area affected is probably much greater than that observed during calm days.

The present study reports densities of *A. hydrophila* which are the highest reported for marine environments (Hazen *et al.*, 1978a). Though *Aeromonas* spp have not been recorded previously from coastal

Table 3. Correlation matrix for water quality and densities of bacteria

	AH	DIST	TEMP	DO	PH	COND	TURB	SALIN	NO_3	SO_4	PO_4	TP	CHL A	TOC	SCB	FC
AH	1.000															
DIST	−0.043	1.000														
TEMP	0.218	−0.326	1.000													
DO	−0.127	0.059	−0.476	1.000												
PH	−0.149	0.001	−0.180	0.259	1.000											
COND	−0.021	−0.017	0.196	−0.209	−0.120	1.000										
TURB	−0.319	0.462	−0.667	0.403	0.824	0.110	1.000									
SALIN	0.044	0.139	0.237	−0.306	−0.196	−0.533	−0.153	1.000								
NO_3	0.088	−0.238	0.509	−0.263	−0.412	0.149	−0.436	0.251	1.000							
SO_4	−0.065	−0.274	0.605	−0.243	−0.605	0.095	−0.515	0.262	0.362	1.000						
PO_4	0.226	−0.353	0.757	−0.261	−0.639	0.142	−0.664	0.170	0.574	0.542	1.000					
TP	0.238	−0.335	0.706	−0.244	−0.617	0.102	−0.647	0.090	0.469	0.456	0.965	1.000				
CHL A	0.202	−0.245	0.342	0.093	−0.446	−0.491	−0.442	0.101	0.093	0.205	0.625	0.704	1.000			
TOC	0.394	−0.272	0.414	−0.073	−0.481	0.533	−0.561	−0.009	0.131	0.139	0.605	0.729	0.727	1.000		
SCB	0.143	−0.013	0.206	0.028	−0.134	0.377	−0.260	0.244	0.157	0.106	0.183	0.185	0.181	0.223	1.000	
FC	0.120	−0.011	−0.046	−0.062	−0.008	0.303	0.002	−0.174	−0.111	−0.045	−0.134	−0.129	−0.181	0.205	0.091	1.000

AH = *Aeromonas hydrophila*, DIST = Distance, TEMP = Temperature, DO = Dissolved Oxygen, COND = Conductivity, TURB = Turbidity, SALIN = Salinity, NO3 = Nitrates, SO4 = Sulfates, PO4 = Phosphates, TP = Total Phosphorus, CHL A = Chlorophyll *a* trichromatic, TOC = Total Organic Carbon, SCB = Standard Count Bacteria, FC = Fecal Coliform Bacteria.
* $P \leqslant 0.05$ when $T \geqslant 0.259$.
All underlined values are significant.

waters of Puerto Rico, it has been recently reported as a dominant floral constituent of open ocean water just north of Puerto Rico (Peele *et al.*, 1981). Other authors (Siedler *et al.*, 1980) have suggested that *A. hydrophila* is not found in marine habitats. but rather that group F bacteria are mistakenly identified as *A. hydrophila.* The present study and others (Peele *et al.*, 1981; Hazen, In press) have shown that not only does *A. hydrophila* survive and grow in marine waters, it can become the dominant bacterium and can reach very high densities in polluted marine waters.

The seasonal periodicity in densities of *A. hydrophila* observed in other habitats (Hazen, 1979, In press) was not observed in the present study. This is not unreasonable considering the strong positive relationship previously reported between densities of *A. hydrophila* and temperature (Hazen & Fliermans, 1979). Thus, temperatures at the Puerto Rico study site ranged from 26 to 45°C, which are within the optimum range for growth of *A. hydrophila* (Hazen & Fliermans, 1979).

Aeromonas hydrophila, as has been shown for the southeastern (Hazen, 1979, In press) and the northeastern United States (Rippey & Cabelli, 1980), is strongly correlated to concentrations of phosphates, nitrogen, primary productivity (Chlorophyll *a*), organics and temperature. The present study shows that when a best fit regression includes temperature, total phosphorus, total organic carbon phosphates, as much as 41.2% of the variation in densities of *A. hydrophila* in a polluted tropical marine habitat can be explained. Though not included in the model, because of instability, correlations were also observed between densities of *A. hydrophila* and turbidity, and chloro-

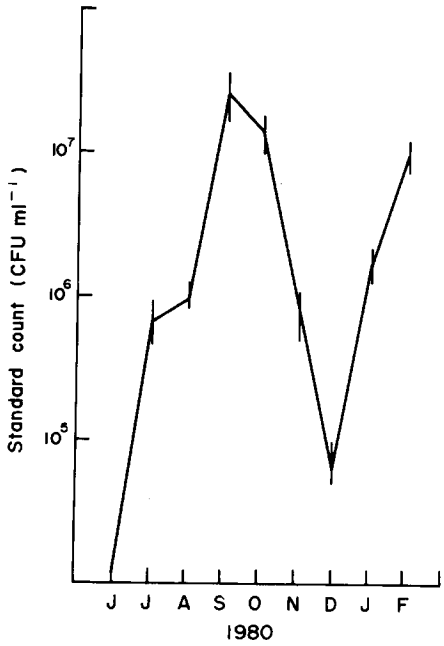

Fig. 7. Density of standard count bacteria by month; mean ± 1 SE.

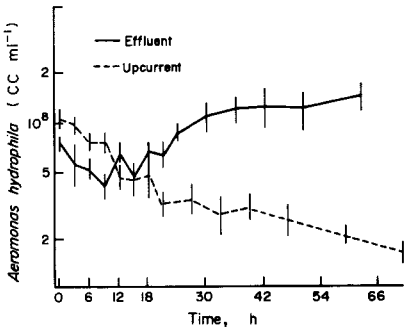

Fig. 8. Survival of *A. hydrophila* in diffusion chamber overtime; mean ± 1 SE (densities in chambers at the effluent).

phyll *a*. The very highly significant correlations between chlorophyll *a* and phosphates, and total phosphorus, and total organic carbon, suggest that correlations between densities of *A. hydrophila* and phosphates, and total phosphorus, and total organic carbon are probably indirect. It is much more likely that algae are providing specific nutrient requirements for *A. hydrophila* and that the algae are in turn using the phosphorus compounds directly. This observation was also made for a study in a North Carolina estuary (Hazen, In press).

The strong positive correlations between *A. hydrophila* and phosphates, organics, total phosphorus and primary productivity suggest that densities of *A. hydrophila* are sometimes extremely low in marine environments because of the concurrent scarcity of these nutrients. Similar observations have been made for a freshwater lotic system in a tropical rain forest (Hazen, Unpublished data). Lack of correlation between densities of *A. hydrophila* and fecal coliform bacteria, and standard count bacteria, suggest that the relationship between the above nutrients and *A. hydrophila* is not due simply to pollution or sewage contamination, i.e. *A. hydrophila* is not solely a constituent of the sewage effluent. The correlations between water quality and densities of fecal coliform bacteria and densities of standard count bacteria demonstrates that none of the three bacterial counts were related to water quality in exactly the same way and moreover, that densities of the three bacteria parameters were not significantly correlated with each other.

The survival and growth of *A. hydrophila* sequestered *in situ* at the effluent point source, and concurrent decline 500 m upcurrent, confirms the correlations between natural densities of *A. hydrophila* and water quality discussed above. Indeed, it also unequivocally demonstrates the ability of *A. hydrophila* to achieve high densities, given the appropriate water quality conditions.

This study, combined with others (Hazen, 1979;

Table 4. Best fit regression statistics

Summary				
		Multiple r	r^2	
Unadjusted		0.0720	0.4600	
Adjusted*		0.6655	0.4166‡	
Analysis of variance				
Source	Sum of squares	Degrees of freedom	Mean square	F statistic
Regression	5.71×10^{11}	4	1.43×10^{11}	16.92§
Residuals	4.38×10^{11}	52	8.43×10^{9}	
Total	1.01×10^{12}			
Analysis of coefficients				
Variable	B¶	Standard error (B)	T statistic†	
Temp	1.18×10^{4}	4.84×10^{3}	2.43	
TP	-5.04×10^{4}	1.07×10^{4}	−4.68	
TOC	3.49×10^{2}	4.49×10^{1}	7.77	
PO4	6.34×10^{4}	2.31×10^{4}	2.74	
Y intercept = -3.29×10^{5}				

$N = 57$.
*Where the correlation coefficient is adjusted to account for the biased estimator of the population parameter.
†$P < 0.01$ when $T \geqslant 2.00$.
‡$P < 0.001$.
§$P < 0.0001$.
¶Slope.

Table 5. Two factors ANOVA for survival of *A. hydrophila* in rum distillery effluent

Source of variance	Sum of square	Degrees of freedom	Mean square	F statistic	Probability
Site	363.79	1	363.79	58.73	0.0001
Time	176.82	12	14.73	2.37	0.05
Site + time	1025.67	12	85.47	13.80	0.0001
Error	483.08	78	6.19		
Total	2049.37	103			

Hazen & Fliermans, 1980; Rippey & Cabelli, 1980) suggest that the density of *A. hydrophila* in all unperturbed aquatic and marine environments is related to certain water quality parameters, namely: phosphorus, chlorophyll *a*, organic carbon and temperature. However, temperature and chlorophyll *a* are probably the only direct effectors since the others are intimately related to the concentration of chlorophyll *a*. In addition this study has shown that densities of potentially pathogenic bacteria (*A. hydrophila*, *Vibrio chlolerae* and *Klebsiella pneumoniae*) are increased by rum distillery effluent. Indeed, rum distillery effluent has been shown to have a stimulatory effect on the entire bacterial community.

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