Aeromonas *distribution and survival in a thermally altered lake*

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ABSTRACT

Par Pond is a thermally enriched monomictic southeastern lake which receives heated effluent from a production nuclear reactor. Fish populations in the lake have lesions of epizooty from which Aeromonas spp. *are readily isolated. Distribution and population densities of* Aeromonas *in the water column were measured along an oxygen and temperature gradient. Greater population densities of* Aeromonas *occurred below the oxygen chemocline when the lake was stratified. Survival of* A. hydrophila *under* in situ *conditions in both epilimnetic and hypolimnetic waters was determined using polycarbonate membrane diffusion chambers during two separate reactor operating conditions. Survival levels of pure cultures of* A. hydrophila *corresponded to the distribution patterns of the naturally occurring* Aeromonas*-like populations. The greater survival of* A. hydrophila *below the chemocline when the reactor was in full operation suggests that the fish populations may be exposed to* Aeromonas *for a longer period of time than when the reactor is not operating.*

INTRODUCTION

Several species of *Aeromonas* are pathogenic to fish, frogs, and a variety of reptiles, (Bullock, 1964) as well as man (Ketover, et al., 1973; Rosner, 1964). Such wide host infections are termed epizootic and often occur in combination with the peritrichous ciliate, *Epistylis*. Fish infections generally result in scale erosion and sloughing, purulent lesions, and bleeding of the fins. The disease caused by the *Aeromonas-Epistylis* complex is commonly called "red-sore disease," and under certain conditions this pathology subsequently leads to hemorrhagic septicemia and eventual death (Esch et. al., 1975). The disease is common in the southeastern U.S. and has reached epidemic proportions on occasion, causing large fish kills (McGraw, 1952; Miller and Chapman, 1953; Plumb, 1973; and Rogers, 1975).

Although *Aeromonas* appears to be an ubiquitous aquatic bacterium (Shotts et. al., 1972; Trust and Sparrow, 1974; Vezina and Desrochers, 1971), previous studies (Ross and Smith, 1974) have considered neither the survival nor the distribution of this bacterium *in situ*. This paper describes the survival of *Aeromonas hydrophila* in natural waters altered by thermal effluents discharged from a nuclear production reactor and assesses the natural distribution of *Aeromonas* in these waters.

MATERIALS AND METHODS

Study Area

Studies were conducted at the Savannah River Plant, a National Environmental Research Park operated by E. I. du Pont de Nemours & Co. The specific study site was Par Pond, a 1092-hectare monomictic lake that is used as a cooling reservoir for a nuclear production reactor. Ambient-temperature waters are used to cool the reactor, and subsequently thermal waters are discharged through a canal into a series of cooling ponds before entering Par Pond. Some areas of the pond are thermally altered while other portions reflect ambient conditions common for other Southeastern lakes. Five permanent sampling stations were established throughout the lake at various distances from the thermal discharge into Par Pond. The position of each station is shown in Figure 1.

Physical and chemical water parameters including temperature, dissolved oxygen, pH, conductivity, and redox potential were measured at each of the sampling stations on a weekly basis using a Hydrolab Surveyor multi-probe analyzer (Hydrolab Corp., Austin, Texas). These parameters were recorded during each reactor phase (Tables 1, 2, 3), i.e. when the reactor was operating and releasing thermal effluent and when it was not.

Culture Studies

Type cultures of *Aeromonas hydrophila* 7966, *A. hydrophila* 19570, *A liquefaciens* 14715, *A. proteolytica* 15338, and *A. salmonicida* 14174 were obtained from the American Type Culture Collection (ATCC) for use in comparison with cultures isolated from the natural habitat. Cultures were maintained and routinely grown on nutrient broth, while *A. hydrophila* and presumptive *A. hydrophila* cultures were routinely checked for purity using the selective R-S medium (Shotts and Rimler, 1973). Organisms utilized in pure culture studies were *A. hydrophila*, while naturally occurring aeromonads isolated from Par Pond on R-S medium are referred to as *A. hydrophila*-like.

Isolation and enumeration of *A. hydrophila*-like bacteria were determined by a membrane filter technique modified for *Aeromonas* spp. Water samples were taken at 1.0-meter depth intervals throughout the water column in both the ambient and thermally altered portions of Par Pond. Triplicate water samples from each depth were taken with an alcohol-rinsed Kemmerer bottle. The samples were immediately placed in sterile Whirl-pac bags and returned to the laboratory for processing. Known aliquots were

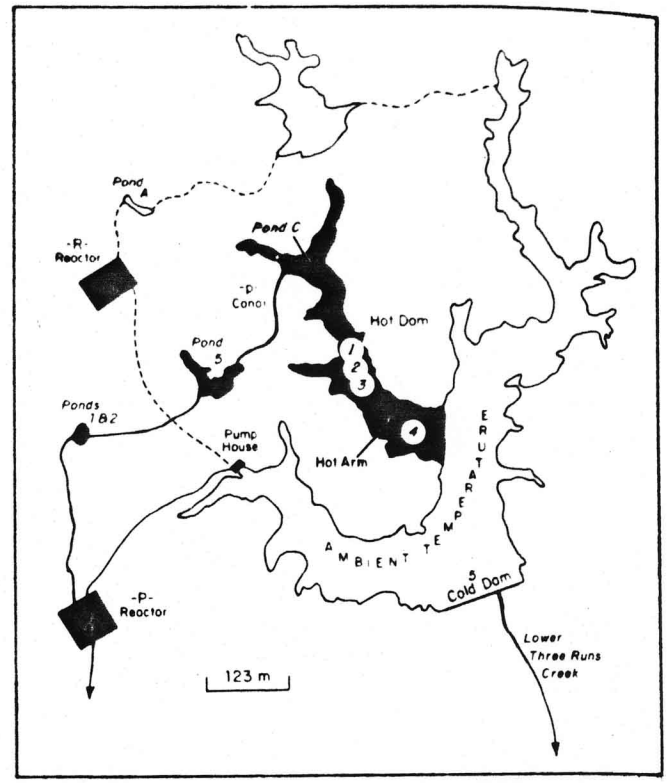

FIGURE 1 — *Par Pond system showing the ambient and thermal (shaded) temperature regions. Sampling stations are numbered.*

filtered through sterile 0.45-μ Millipore (Bedford, Mass.) filters and placed on sterile pads saturated with R-S medium and incubated for 20 hours at 30° C. The filters were then examined for yellow pigmented colonies which are presumptive *A. hydrophila*. These colonies were then tested for cytochrome oxidase (Hugh, 1970), and positive oxidase cultures were taken to be *Aeromonas hydrophila*-like organisms.

Survival Studies

Type cultures of *A. hydrophila* were checked for purity on R-S medium, transferred, and routinely grown in nutrient broth at 30° C. Cells were harvested during logarithmic growth phase, washed three times in 0.01 M phosphate-buffered saline (pH 7.2), centrifuged, and resuspended in 40.0 ml of phosphate-buffered saline to a final optical density of 0.150 at 550 nm. The cell suspension was placed into sterile membrane diffusion chambers (McFeters and Stuart, 1972) as modified for deep water studies (Fliermans and Gorden, 1977). The chambers were immediately suspended from stainless steel chains and lowered to various depths in the water column. Previous studies with modified chambers indicated that bacterial cultures inside triplicate chambers placed at the same depth in the water column had optical density readings with 5% of each other during a 2-week sampling period. Thus, single chambers were placed at four different depths with two chambers in the epilimnetic waters and two in the hypolimnetic waters at all five stations. Chambers were never placed at the surface waters, since they were readily attacked by the alligators present in the Par Pond.

Chamber Sampling

Sampling schedules were intermittent during the 2-week experiments, but generally, initial samples were taken every 3 hours for 48 hours, then once every 24 hours through the remainder of the experiment. Samples, taken aseptically using sterile plastic syringes, were measured for optical density and tested for diffusion chamber purity by immunofluorescence (Fliermans, unpublished data) and/or plating

TABLE 1 – *Chemical and physical parameters at Station 1*

Depth, m	Temperature, °C	pH	Dissolved Oxygen, ppm	Conductivity, μmho/cm²	Redox Eh, mV
			Reactor Not Operating		
0	28.6	7.9	7.0	80	ND[b]
1[a]	28.7	7.9	7.0	65	ND
2	28.7	7.8	6.8	60	ND
3	28.3	7.6	6.4	60	ND
4[a]	27.6	7.4	5.9	60	ND
5	27.2	7.4	5.4	60	ND
6[a]	25.2	7.2	2.9	70	ND
7	24.2	7.2	1.6	70	ND
8[a]	23.0	7.2	0.3	100	ND
9	22.0	7.2	0.2	110	ND
			Reactor Operating		
0	34.0	7.2	6.7	55	290
1[a]	33.8	7.4	6.3	60	290
2	30.5	7.5	6.5	60	295
3	30.0	6.9	5.9	60	310
4[a]	29.3	6.6	4.8	60	320
5	28.5	6.2	1.8	60	340
6[a]	27.8	6.0	0.3	60	355
7	25.8	6.1	0.3	70	360
8[a]	23.5	6.4	0.2	95	220
9	21.0	6.6	0.2	110	80
10	20.8	6.7	0.2	110	50

[a]Denotes chamber location.

[b]ND = Not determined.

TABLE 2 – *Chemical and physical parameters at Station 3*

Depth, m	Temperature, °C	pH	Dissolved Oxygen, ppm	Conductivity, μmho/cm²	Redox Eh, mV
			Reactor Not Operating		
0	32.0	6.7	5.4	60	300
1[a]	32.0	6.8	5.3	60	310
2	30.5	6.9	5.6	60	315
3	29.5	6.9	5.7	50	320
4	28.0	6.9	5.3	60	325
5[a]	28.0	6.7	2.8	60	340
6	27.7	6.5	0.1	60	350
7[a]	25.5	6.7	0.1	70	355
8	23.5	6.8	0.1	85	340
9[a]	22.0	6.9	0.0	100	140
			Reactor Operating		
0	38.0	7.0	6.8	60	330
1[a]	36.0	7.0	6.4	60	330
2	31.0	6.9	5.4	60	325
3	30.0	7.0	6.3	60	330
4	29.5	7.0	6.2	60	330
5[a]	29.0	6.8	3.7	60	335
6	26.5	6.4	0.3	60	350
7[a]	25.5	6.4	0.2	80	365
8	23.0	6.6	0.2	95	365
9[a]	22.5	6.7	0.2	100	190
10	20.5	6.9	0.2	110	140

[a]Denotes chamber location.

TABLE 3 – *Chemical and physical parameters at Station 5*

Depth, m	Temperature, °C	pH	Dissolved Oxygen, ppm	Conductivity, μmho/cm²	Redox Eh, mV
			Reactor Not Operating		
0	28.5	6.8	7.6	60	375
1[a]	28.5	6.9	7.0	60	375
2	28.5	6.9	6.7	60	375
3	28.5	7.0	6.5	60	375
4	28.5	7.0	6.4	60	380
5	28.5	6.9	5.9	60	380
6[a]	28.0	6.8	5.2	60	390
7	26.0	6.6	0.2	65	410
8[a]	22.5	6.8	0.2	80	420
9	21.5	6.8	0.2	80	410
10	20.5	6.8	0.2	80	395
11	20.0	6.9	0.2	85	170
12	19.0	7.0	0.2	90	110
13	18.5	6.7	0.2	90	90
14	18.5	6.7	0.2	90	60
15	18.0	6.7	0.2	90	40
16[a]	18.0	6.8	0.2	95	30
17	18.0	6.8	0.2	95	20
			Reactor Operating		
0	29.5	6.8	7.7	60	390
1[a]	29.5	6.8	7.2	60	385
2	29.5	6.9	7.0	60	385
3	29.5	6.9	6.9	60	385
4	29.5	7.0	6.8	60	385
5	29.5	7.0	6.7	65	385
6[a]	29.0	6.8	5.9	65	390
7	26.0	6.4	0.4	70	410
8[a]	23.5	6.5	0.3	80	415
9	22.0	6.5	0.2	85	415
10	21.3	6.5	0.2	95	415
11	20.0	6.8	0.2	100	200
12	19.5	6.9	0.2	100	130
13	19.0	6.9	0.2	100	100
14	18.0	7.0	0.2	100	70
15	18.0	7.0	0.2	100	55
16[a]	18.0	7.0	0.2	105	45
17	18.0	7.0	0.2	100	20

[a]Denotes chamber location.

on a selective medium. Sampling ports in the chambers were flamed with a butane cigarette lighter, a syringe was attached, aspirated five times, and then 1.0-ml samples were removed from each chamber. Samples were placed directly in sterile culture tubes, capped, and returned to the laboratory within 60 minutes for processing. Optical densities were determined at 550 nm using a Beckman Model 25 double beam spectrophotometer. Optical density measurements are expressed as a percentage of the initial cellular density, since all chambers did not contain *A. hydrophila* populations of exactly 0.150 optical density units. Each chamber was read as 100% and values greater than 100% represented an increase in optical density, while those values less than 100% represented a decrease from the initial population densities.

The utilization of membrane chambers in deep waters required separate sterile chambers containing only sterile phosphate-buffered saline as controls. These chambers were suspended alongside those containing the test bacterium and both chambers were sampled simultaneously. Such controls were necessary when measuring optical density of bacteria in deep water, since under anaerobic conditions, iron compounds are in a reduced state and remain in solution. During sampling, the chambers were pulled through oxygenated water and insoluble iron oxides were formed which interfered with optical density measurements. Thus, solutions inside the control chamber were used as reference samples in double beam spectroscopy for optical density measurements.

RESULTS

Initial population densities and survival studies for *A. hydrophila* were made after the reactor had not been operating for 30 days, subsequent measurements were conducted after the reactor had been operating for over 21 days, so that lake temperatures had stabilized (Tables 1, 2, 3). All sampling was performed during normal lake stratification, and such stratification remained stable during both phases of reactor operations.

The distribution of *A. hydrophila*-like organisms was measured indirectly using the selective medium of Shotts and Rimler (1973) who previously demonstrated the good selectivity and specificity of the medium. The population densities (expressed as the mean of triplicate samples) are shown in Table 4 for *A. hydrophila*-like bacteria in the water columns at two of the stations. Numbers of aeromonads in the hypolimnetic waters were always greater than those from epilimnetic waters and the numbers were always greater when the reactos was in full operation. A clear depth distribution gradient of the naturally occurring aeromonads was not seen in either the epilimnetic or hypolimnetic waters from any of the stations sampled.

Survival studies for *A. hydrophila* were conducted using sterile membrane diffusion chambers placed at various depths in the water column. The depth of each chamber depended on the station, the depth of thermal stratification, and the oxygen chemocline. The data for *A. hydrophila* survival, as measured by optical density, at various depths in the water column along vertical oxygen gradient and a surface temperature gradient are plotted in Figures 2 through 11.

The data in Figures 2, 4, 6, 8 and 10 demonstrate the survival of *A. hydrophila* suspended in diffusion chambers at the respective stations when the reactor was not in operation, while Figures 3, 5, 7, 9, and 11 show survival data when the reactor was operating. *A. hydrophila* populations demonstrated initial growth and/or maintenance in all the chambers regardless of the depth. Although contamination usually occurred in at least one of the chambers at each station during the experiments, bacterial cultures placed in the deeper anoxic waters always demonstrated greater survival over organisms placed in epilimnetic waters. Regardless of the depth of the chambers, or the station, *A. hydrophila* survived longer when the reactor was in full operation than when it was not. Epilimnetic cultures initially increased in optical density followed by a decline after an exposure period at Station 2 (Figures 4 and 5) and at Stations 3 and 4 (Figures 6, 7, 8, and 9, respectively). On the other hand, optical densities of hypolimnetic cultures decreased more slowly and had greater variability.

Survival measurements at Station 5 (ambient control) indicated that similar results occurred, but less dramatically than in the stations

TABLE 4 – *Distribution of Aeromonas hydrophila-like bacteria in Par Pond*

Depth, m	Aeromonas per liter						
	Reactor Operating				Reactor Not Operating		
Station 3							
0		350	400	ND[a]		20	0	0[b]
1		340	227	240		0	0	0
2		20	40	ND		0	5	20
3	($\bar{v}$ = 280)[d]	780	706	260	($\bar{v}$ = 4.3)[d]	0	8	20
4		60	220	540		0	0	0
5		60	74	160		0	0	5
6		600	724	840		0	2	20
7		800	867	780		40	8	20
8	($\bar{v}$ = 596)[d]	500	453	600	($\bar{v}$ = 226)[d]	500	323	420
9		740	667	660		380	500	500
10		200	227	280		ND		
Station 5							
0		60	40	40		120	80	100
1		6	0	0		300	280	ND
2		0	0	0		400	233	260
3	($\bar{v}$ = 472)[d]	1200	1260	1300	($\bar{v}$ = 136)[d]	120	180	120
4		640	600	492		180	100	100
5		1780	1640	1860		60	40	60
6		1640	1120	ND		0	0	0
7		0	0	0		420	520	560
8		0	0	0		120	240	140
9		0	0	0		1640	800	960
10		0	0	0		1000	1180	1040
11		3000	3600	240		740	960	1120
12		>6000	4200	5000		580	400	ND
13		>6000	>6000	>6000		260	580	ND
14	($\bar{v}$ = 3969)[d]	5200	4800	2100	($\bar{v}$ = 894)[d]	840	720	840
15		>6000	>6000	ND		1400	1360	1380
16		>6000	>6000	ND		1440	2000	1800
17		>6000	>6000	ND		ND		

[a]Not determined.

[b]Actual numbers are less than 2/liter.

[c]Dash line denotes chemocline position.

[d]$\bar{v}$ = mean of Aeromonas determinations for epilimnetic and hypolimnetic waters.

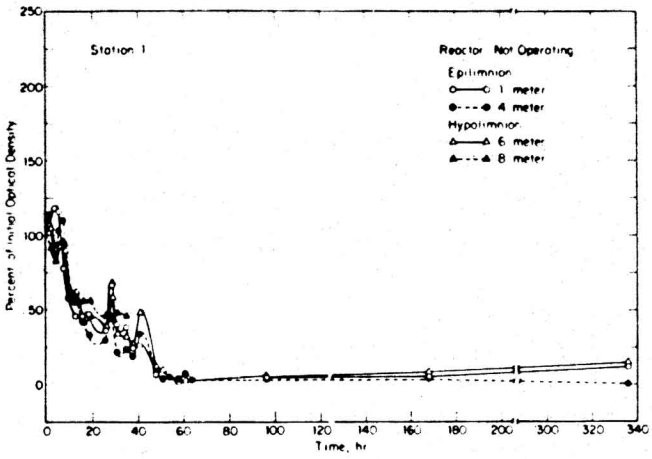

FIGURE 2 — *Survival of* A. hydrophila *at Station 1 when the reactor was not operating.*

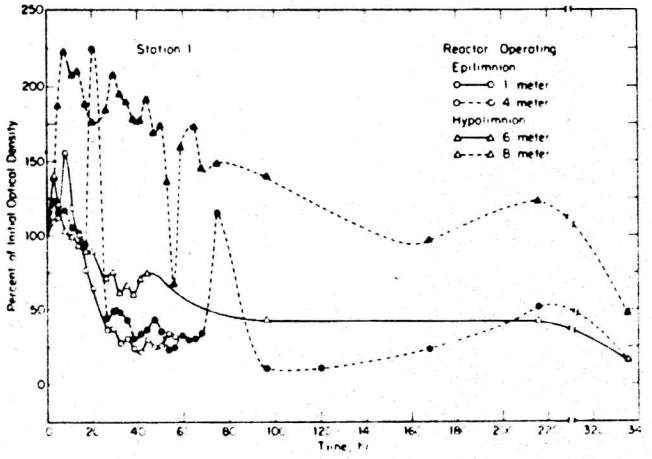

FIGURE 3 — *Survival of* A. hydrophila *at Station 1 when the reactor was operating.*

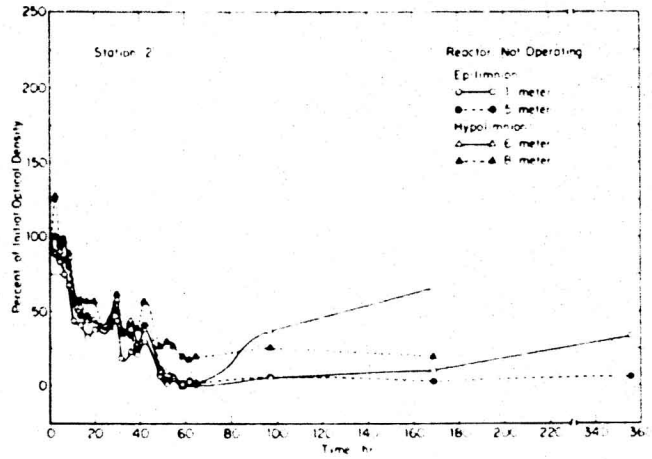

FIGURE 4 — *Survival of* A. hydrophila *at Station 2 when the reactor was not operating.*

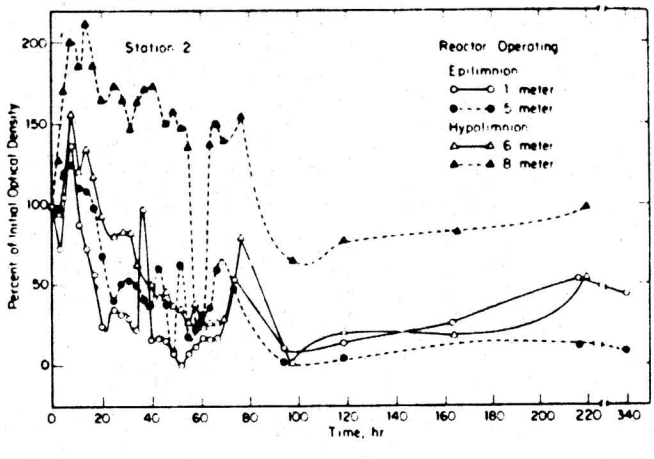

FIGURE 5 — *Survival of* A. hydrophila *at Station 2 when the reactor was operating.*

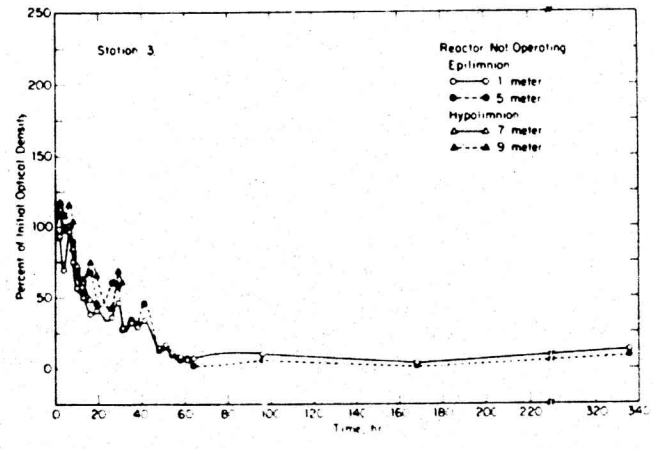

FIGURE 6 — *Survival of* A. hydrophila *at Station 3 when the reactor was not operating.*

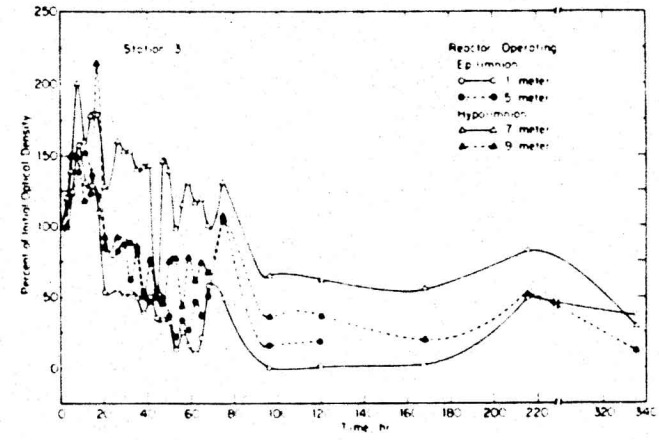

FIGURE 7 — *Survival of* A. hydrophila *at Station 3 when the reactor was operating.*

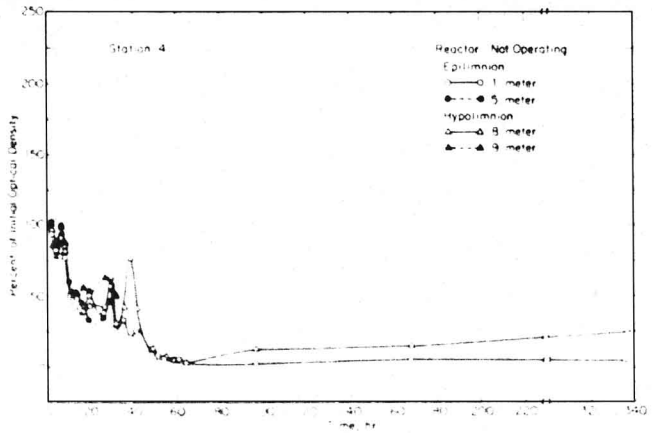

FIGURE 8 — *Survival of* A. hydrophila *at Station 4 when the reactor was not operating.*

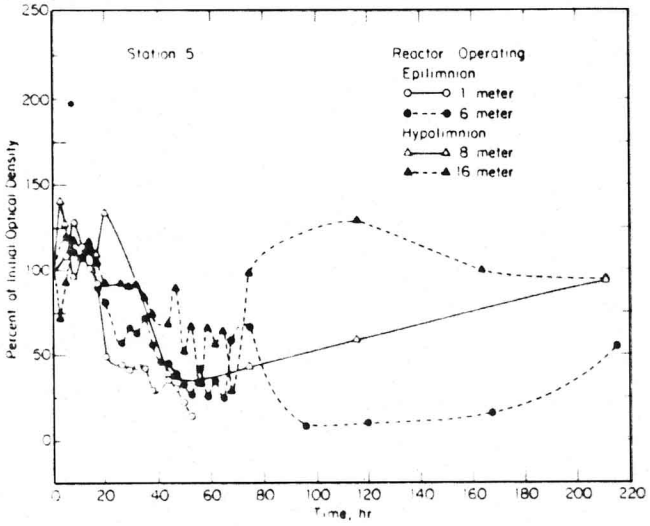

FIGURE 11 — *Survival of* A. hydrophila *at Station 5 when the reactor was operating.*

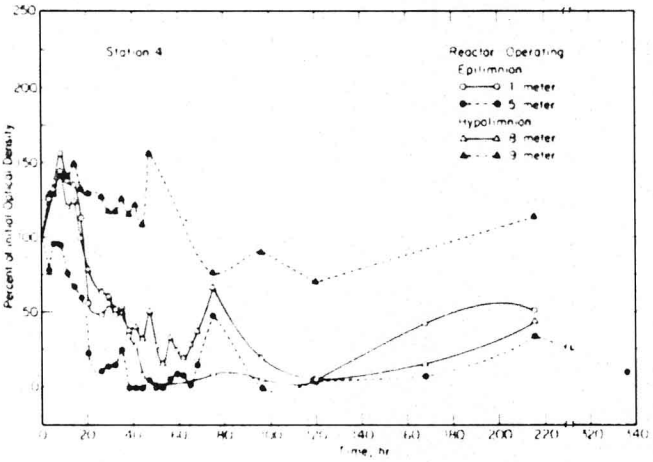

FIGURE 9 — *Survival of* A. hydrophila *at Station 4 when the reactor was operating.*

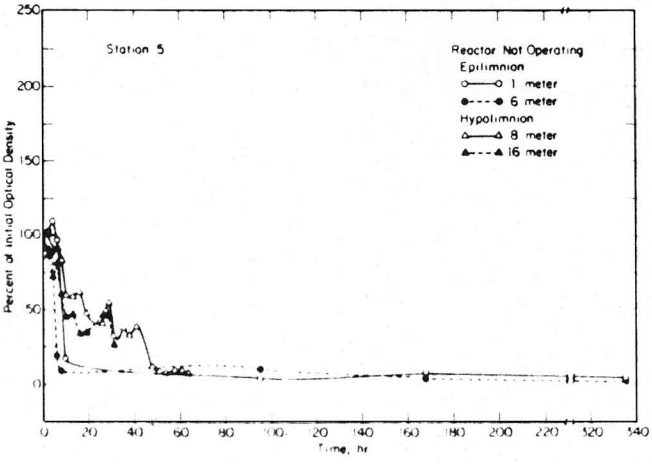

FIGURE 10 — *Survival of* A. hydrophila *at Station 5 when the reactor was not operating.*

closer to the thermal effluent. Chambers placed in the epilimnion had a rapid decrease in optical density when the reactor was not operating (Figure 10), while a slower decrease in optical density was noted at the same depths when the reactor was operating (Figure 11). Cultures in chambers placed in hypolimnetic waters were variable, but indicated that growth and survival were greater in the anoxic portions of the water column when the reactor was operating.

DISCUSSION

Previous studies on the bass populations of Par Pond (Esch *et al,* 1975,) indicated that the occurrence of "red sore disease" was significantly higher among bass captured in heated waters of Par Pond than among those captured in ambient locations. The investigations suggested that elevated temperatures may be a significant variable in the epizootiology of the disease. Esch *et al.* (1975) also demonstrated that the greatest incidence of infection occurred in larger bass. Such a distribution suggests that either the infections were lethal to smaller fish, and thus the smaller fish were not measured, or that the larger and older fish have had a longer exposure to the pathogens. Except for these studies the distribution and physiological ecology of the facultative anaerobe, *Aeromonas,* in aquatic ecosystems, and its role as a fish pathogen in thermally stressed waters is virtually unknown.

The individual techniques for measuring natural population densities, distribution, and pure culture survival produced results which permit similar conclusions to be made regarding the growth and survivorship of *A. hydrophila* in Par Pond.

Survival studies of *A. hydrophila* at various temperature and oxygen regimes indicated that the organisms maintained themselves better in the deeper hypolimnetic waters. Thus, in hypolimnetic oxygen-depleted waters, at each station in the thermal and ambient portions of the reservoir, *A. hydrophila* survived longer and increased in density to a greater extent than in epilimnetic waters at the same station.

Comparisons among stations demonstrated that when the reactor was not in operation the percentage of initial optical density approached zero after 60 hours of *in situ* incubation in every chamber at all stations. However, when the reactor was operating only chambers in the epilimnion of Station 4 were near zero percentage of their initial optical density after 60 hours of incubation. All other chambers regardless of depth or station had greater survival of A. *hydrophila.* The data are clear when a comparison is made of survival at the same station with two different reactor operations, in that

survival and growth were always better when the reactor was in operation. This was true regardless of the depth, and even at depths in the water column where the influence of reactor input could not be detected by the parameters measured. It is necessary to emphasize, however, that the sampling time between the reactor operations and thus the experiments was 21 days. Therefore, the natural stratification of Par Pond had advanced by three weeks when survival and distribution experiments were undertaken for full reactor operations. This is reflected in Table 3 by the vertical extension of the chemocline at Station 5, which is closest to the thermal input. Although the presented data are reflective of reactor operations, the described changes in survival of *A. hydrophila* in deeper waters may be confounded in part by the increase of natural stratification.

Faculative anaerobic metabolism may provide a selective advantage to *Aeromonas* for growth in the anaerobic zones of Par Pond, and that advantage is enhanced by the operation of the nuclear reactor, since the natural population densities of aeromonads are greater throughout the water column when the reactor is operating than when it is not. Other southeastern lakes do not have the high population densities of aeromonads as seen in Par Pond (Fliermans, unpublished data). Thus, the large population densities of aeromonads and the high infection levels of the largemouth bass populations in Par Pond may be due to the length of survival of *A. hydrophila* in the water column. Such survival may allow a given bass to be exposed to high levels of aeromonads for a greater portion of its life.

Although it is unclear whether the survival of aeromonads in Par Pond is due directly to thermal inputs or is indirectly related to flow rates and nutrient distributions caused by reactor operations, it is clear the aeromonads are ubiquitous throughout Par Pond and that the bass populations are heavily infected by the etiological agent(s) of "red sore disease."

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OPEN DISCUSSION OF PAPER

[*Dr. Fliermans opened the discussion with the following statement*] — We have looked at aeromonas and its distribution ... [among] bass and we have recently completed some work on the alligator population in Par Pond ... and the influence of *Aeromonas* on their distribution. These organisms have temperature optima around 33° C. Our waters are warmer than that, however, and many of the waters within the southeast that ... [receive] reactor effluents often reach temperatures that are [equivalent to human] body temperature or above and so the question comes, "what about pathogenic organisms, organisms that have the potential of causing disease in man?" Are these organisms presnt in out habitat? And if so, to what degree? We did some studies with Dr. Mike Tansey from Indiana University who has a great deal of expertise with pathogenic fungi that are thermophilic. He has spent a number of years doing research in natural ... [hot water] areas, particularly in Yellowstone National Park where he has looked at these organisms throughout the park ... [which are] associated with the hot springs. [Mike] came to our ... [Savannah River Laboratory] and we began looking at our algal mats that are very similar to the algal mats in Yellowstone. We were able to find very high numbers of these pathogens. Now the first three [Dr. Fliermans showed a slide listing pathogens. See also Table 1, this discussion] are pathogens to man through the respiratory route . . . The fourth one, *Dactylaria gelopavum* is a disease that infects turkey poults and chickens, causing encephalitis in the fowl and can indeed wipe out a turkey or a poultry flock in a very short time. ... [A conclusion from our] research was that in the algal mats which we show here in a trough . . . [containing] water coming in around 60° C [slide not available for proceedings] there is a sterilization effect in that we don't have very many organisms in this area. As we move down into cooler waters *Mastigocladus,* a blue-green alga, . . . [appears] and that is very similar to what we saw in Yellowstone. In these algal mats pathogenic fungi, particularly *Dactylaria,* are able to survive and they are able to grow. They are very numerous in these habitats, but as you go through the higher temperatures the foam that is formed contains 10^8 bacteria/ml and also high numbers of the

TABLE 1 – *Thermophilic and thermotolerant fungi isolated from samples other than water and foam collected at the Savannah River Plant*[a]

Sample Site	Temp of nearest water, °C	pH of sample[b]	Sample Description	Results of plating in YpSs (50°C) and in SDA (45°C)
P Canal	72	6.1	Bottom sediment	No fungi
P Canal	72	7.6	Green mat in splash zone at water edge	A. fumigatus, D. gallopava Thielavia terrestris Talaromyces thermophilus
P Canal	72	6.8	Greenish-black mat 6 cm above splash zone, bathed by hot vapor	A. fumigatus, D. gallopava, T. terrestris, Rhizopus rhizopodiformis
P Canal	72	5.6	Soil beneath herbs and grasses meter from water	A. fumigatus, R. rhizopodiformis, Humicola lanuginosa, Melanocarpus albomyces
P Canal	72	5.2	Soil beneath pines 10 meters from water	H. lanuginosa, Talaromyces thermophilus, T. terrestris
P Canal	68	7.0	Bottom sediment	No fungi
P Canal	68	6.6	Green mat at water edge	D. gallopava, Thermoascus crustaceus
P Canal	68	4.9	Green and black mat 3–4 cm above preceding sample	A. fumigatus, D. gallopava, R. rhizopodiformis
P Canal	68	6.2	Soil beneath grasses 1 meter from water	T. crustaceus, Rhizopus rhizopodiformis
P Canal	68	6.2	Soil beneath pines 30 meters from water	A. fumigatus, T. terrestris, R. rhizopodiformis, Thermoascus crustaceus, Scytalidium thermophilum
Pond 1	63	5.9	Bottom sediment, plant debris, algal mat	A. fumigatus, Thermoascus crustaceus
Pond 1	63	6.5	Mat and pine needles at edge of water	A. fumigatus, D. gallopava, terrestris, Thielavia heterothallica
Pond 1	63	5.7	Soil and plant roots at water edge	A. fumigatus, R. rhizopodiformis, H. lanuginosa, Humicola grisea var. thermoidea, Talaromyces thermophilus
Pond 1	63	6.0	Soil beneath grasses 10 cm directly above water (vertical bank)	A. fumigatus, D. gallopava, H. lanuginosa, T. terrestris, R. rhizopodiformis, Rhizopus nigricans
Pond 1	63	4.8	Soil beneath 10 meters from water	A. fumigatus, Talaromyces thermophilus, Thermoascus aurantiacus
P Canal	61	6.7	Drying foam on rocks at water edge	A. fumigatus, H. lanuginosa, T. terrestris, Thermoascus thermophilus
P Canal	61	6.7	Drying microbial mat at water edge	A. fumigatus, Talaromyces thermophilus
P Canal	61	5.7	Soil beneath grasses 1 meter from water	T. aurantiacus, H. lanuginosa
P Canal	61	6.2	Mat at water edge	D. gallopava
P Canal	59.5	6.1	Soil, roots, and mat at water edge	A. fumigatus, D. gallopava
P Canal	58	6.0	Dry foam and pine needles at water edge	A. fumigatus, H. lanuginosa Chaetomium thermophile var. dissitum
Pond 4	54	6.1	Mat and soil at water edge	A. fumigatus, H. lanuginosa, T. heterothallica, Scytalidium, thermophilum

[a] *Listed from highest to lowest temperature of nearby water*

[b] *The pH of a 1:1 v/v mixture of sample and distilled water was measured with an electronic pH meter*