SURVIVAL AND VIABILITY OF *ESCHERICHIA COLI*
IN A THERMALLY ALTERED RESERVOIR

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Abstract—*Escherichia coli* survived and grew in a warm, monomictic reservoir receiving thermal effluent from a nuclear production reactor, which suggests that the presence of *E. coli* in these aquatic systems is not an indication of recent fecal contamination. Known populations of *Escherichia coli* were placed in sterile diffusion chambers and suspended at various depths throughout the water column. Experiments were conducted during both ambient water conditions (reactor not operating) and thermally altered water conditions (reactor operating). *E. coli* populations were observed for changes in density by using optical density and direct and indirect counting methods.

During both ambient water and thermally altered water conditions, *E. coli* survived and grew 2 to 3 weeks at each depth and at all stations. During thermal alteration, cell populations attained higher optical densities and maintained those densities for longer time periods than those during ambient water conditions. In addition, populations suspended in the hypolimnion generally maintained higher optical densities than those in the epilimnion. Data show that *E. coli* survives and grows for extended periods of time while it is in diffusion chambers in both aerobic and anaerobic portions of the water column.

INTRODUCTION

The impact of fecal pollution on the quality of water has been recognized for almost 100 years (Wolf, 1972). During much of this period, the enteric microorganism *Escherichia coli* and similar bacteria have been designated as indicators of fecal pollution. As aquatic ecosystems are continually subjected to human and animal wastes, the importance of suitable and effective indicators of recent fecal contamination is apparent (Faust, 1976).

Escherichia coli is generally accepted as an indicator organism of fecal contamination because it is assumed to be an enteric bacterium which is nonpathogenic, is easily detected, is genetically stable, is unable to grow or reproduce readily outside the

intestines of warm-blooded mammals, and dies off rapidly in fresh and marine waters (Wolf, 1972; Hendricks and Morrison, 1967). However, this acceptance has been challenged in court (Wolf, 1972) and in scientific literature (Sayler *et al.*, 1975). Several strains of *E. coli* are known enteric pathogens (Geldreich, 1972); other strains are not easily detected nor genetically stable because they are nonlactose fermenters, which fail to produce the characteristic green-sheen colonies on differential media (Koditschek and Guyre, 1974). Previous studies support the rapid dieoff of *E. coli* in a variety of aquatic ecosystems (McFeters and Stuart, 1972; Mitchell, 1968; Ogawa, 1974). The following interacting factors affect the survival and growth of *E. coli* in aquatic systems: solar radiation (Gameson and Saxon, 1967), nutrient levels (Jones, 1964; Wu and Klein, 1976), chelation (Jones, 1964), predation (Enzinger and Cooper, 1976; Jones, 1971), temperature (Hendrick and Morrison, 1967), pressure (Baross *et al.*, 1975), bacteriophage (Roper and Marshall, 1974; Verstraete and Voets, 1976), antibiosis (Verstraete and Voets, 1976), sedimentation (Ogawa, 1974), and colloids (Faust *et al.*, 1975).

In recent years, thermal discharges into streams, rivers, and lakes have provided further complicating factors. Thermal effluents, particularly in the southeastern United States, may raise the water temperatures of natural aquatic systems to a level equal to or greater than the intestinal temperature of warm-blooded animals, a level near the growth optima of many *E. coli* strains.

Such systems may provide a habitat with growth conditions suitable for these bacteria.

Much of the research relating to *E. coli* populations in aquatic ecosystems has been restricted either to the laboratory or to short-term field studies. Reported work has shown that *E. coli* populations survive from one to five days in well water (McFeters *et al.*, 1974), estuaries (Faust *et al.*, 1975), sea water, and sea water under pressure (Baross *et al.*, 1975). *E. coli* has been reported to survive up to six weeks in laboratory streams, except in the presence of *Flavobacterium lutescens*, which effectively reduce the period of survival to less than 24 days in all types of water (Cherry and Guthrie, 1975). However, Bissonnette *et al.* (1975) have shown that *E. coli* cells which appear to be dead in aquatic systems were probably injured by environmental stress, and they might revive under more favorable conditions.

The objectives of this research were to determine the ability of *E. coli* populations to survive and grow in a warm, monomictic reservoir receiving thermal effluent from a nuclear production reactor and to contribute to the evaluation of *E. coli* as an indicator of fecal contamination.

MATERIALS AND METHODS

Site

Par Pond is a warm, monomictic, nonrecreational reservoir within the boundaries of the Savannah River Plant. This

1092-hectare lake serves as a source and a discharge point for the once-through cooling water for a nuclear production reactor.

Ambient-temperature waters are used to cool the reactor; subsequently, thermal waters are discharged through a canal into an 11-km series of cooling ponds before the waters enter Par Pond. Some areas of the reservoir are thermally altered, but other areas reflect ambient water conditions common for southeastern lakes. Five permanent sampling stations were established throughout the lake at various distances from the thermal discharge into Par Pond (Fig. 1).

Physical and chemical water parameters, including temperature, dissolved oxygen, pH, conductivity, and redox potentials (E_h) were measured at each of the stations on a weekly basis with a Hydrolab Surveyor multi-probe analyzer (Hydrolab Corp., Austin, Texas). These parameters were measured and recorded (Tables 1, 2, and 3) when the reactor was not operating (RNO) and when it was operating (RO) and releasing thermal effluents into Par Pond. The survival and growth of *E. coli* were studied under ambient water conditions (July 21 through August 4, 1975) and reactor operating conditions (August 18 through August 31, 1975). The RO experiment was begun 2 weeks after the completion of the RNO. During this time interval, changes occurred in the natural stratification patterns of the lake.

Survival and Growth Studies

Bacterial isolates with an optional growth temperature of $37 \pm 1^\circ\text{C}$ were obtained from the National Communicable Disease Center (CDC, Atlanta, Georgia), where they had been repeatedly verified as *Escherichia coli*. These isolates were chosen because their genetic stability had previously been established, and such stability was necessary for concomitantly conducted studies. Cultures were grown in nutrient broth at 37°C for 24 hr, aseptically washed three times in 0.01M phosphate buffered saline of pH 7.2, and concentrated by centrifugation. Washed cells were diluted in filter-sterilized buffer and used for direct microscopic counts, optical density measurements, and all experiments measuring survival and growth.

Optical density measurements of stock cultures were made at 550 nm by using a Beckman Model 25 DB spectrophotometer (Beckman Instruments, Inc., Fullerton, California) and compared with direct microscopic counts of the suspensions by using a Zeiss Photomat III microscope, FITC stain (Fliermans and Schmidt, 1974), and a Petroff-Hausser counting chamber. Counts were made in duplicate and were correlated with optical density measurements. The resulting data for optical density and population densities thus served as standard curves which were compared with field data (Fig. 2). In subsequent experiments, direct and indirect counts were made randomly to verify optical density measurements.

Known *E. coli* population densities were placed in sterile diffusion chambers (as described by McFeters and Stuart, 1972, and modified by Fliermans and Gorden, 1977) and suspended in the lake at four different depths at each of the five permanent sampling stations. At each station, two chambers were suspended from stainless steel chains in the epilimnion and two in the hypolimnion.

Samples were taken from each chamber every 3 hr during the first 3 to 4 days, then daily for one week, and at the termination of the experiment. The chambers, after being removed from the lake, were gently shaken several times to resuspend any settled or attached cells. Sampling port plugs were removed, a sterile syringe was inserted, aspirated, and 1.0 ml of solution was removed. Samples were placed in sterile tubes, the port plugs replaced and the chambers returned to the lake. Total sampling time for all five stations was approximately 45 to 60 min. Samples taken from the chambers were periodically tested for purity by plating on nutrient agar and Eosin Methylene Blue (Difco, Detroit, Michigan) media. Any contaminated or damaged chambers were removed and replaced.

RESULTS

Water quality parameter differences were observed among stations and depths when the reactor was not operating. Differences of temperature, pH, E_h , O_2 , and conductivity were also observed among stations and depths when the reactor was operating. However,

greater parameter differences were apparent when the two reactor conditions were compared (Tables 1 and 2). Stations I, II, III, and IV showed the greatest differences while Station V had much smaller differences in water quality (Table 3). Station V, which appeared to be essentially unaffected by the thermal effluent into Par Pond, probably reflected the natural progression of lake stratification and was designated a control station.

Measurements also show the presence of a thermocline and a chemocline at each station. *E. coli* populations placed in the epilimnion were generally exposed to warm, aerobic waters which have a neutral pH and a conductivity of 50 to 80 $\mu\text{mho}/\text{cm}^2$. Populations in the hypolimnion were exposed to anaerobic, cool waters which have a low redox E_h and a conductivity of 60 to 110 $\mu\text{mho}/\text{cm}^2$ (Tables 1, 2, and 3).

The density of *E. coli* populations in the chambers was measured with optical density procedures and verified by plate counts and microscopic counts of the populations. Therefore, the percent of the initial optical density of the *E. coli* population placed in the chamber is regarded as the survival of that population.

Data indicate that essential growth factors were not significantly different for *E. coli* among stations, and *E. coli* survived equally well at all experimental sites when the reactor was not operating. (Table 4). During RNO conditions, the curves of *E. coli* population densities at a given depth were essentially the same among all

stations. Depth differences were observed, however, with population densities of the hypolimnion surpassing those of the epilimnion. Also, no obvious gradients or differences were observed in physical parameters among stations.

Reactor Not Operating (RNO)

E. coli population densities in 18 of the 20 sampling sites decreased below initial levels to about 80% survival within 24 hr. At Station III, depths 7 and 9 m, the densities increased slightly to 120%. At all stations and all depths, the population densities fluctuated about a mean of 55% for 48 hr after which they remained at a level near 25% of the original until the termination of the experiment. During RNO conditions, the population densities in the chambers at all depths and all stations were very similar, especially for the first 48 hr of incubation (Table 4). Population density curves for all depths at a typical station (Station II) are given in Fig. 3.

Reactor Operating (RO)

E. coli population densities showed measurable differences among stations and depths, especially for the first 48 hr of the experiment. The average population densities of epilimnion chambers were significantly lower than those of the hypolimnion at every station. Population densities of epilimnion chambers were higher at Stations II through IV than those at Stations I and V. Population densities of the hypolimnion were significantly

higher at Stations I through IV than those at Station V ($\chi^2 = 16.27$ $P < 0.001$). Furthermore, the population densities at 19 of the 20 sampling sites were significantly higher during RO conditions than those during RNO conditions ($P < 0.01$, Sign Test, Siegel, 1956) and ranged from 120 to >270% survival. Subsequently, the densities decreased, fluctuated, and ranged from <10 to 100% after 340 hr. Station differences between survival and growth of *E. coli* were observed. During the first 48 hr, the average percent of survival in epilimnion chambers was 54% at Station I, 124% at Station II, 106% at Station III, 144% at Station IV, and 65% at Station V. During the first 48 hr, hypolimnion chamber densities of Stations I through IV were within a range of 143 to 166% survival. Data for typical stations are presented in Figs. 4 and 5.

The mean population survival of all chambers at Stations I through IV for the first 48 hr was 55% during RNO and 129% during RO conditions, or an average difference (RO minus RNO) of 74%. At Station V, the average difference was 23% (Table 4). Therefore, a difference of 51% may be attributed to reactor operation influence on the total water column along the vertical and horizontal gradients. *E. coli* growth differences among stations and depths (Table 4) were quite striking because Stations I and III had small differences among chambers in the epilimnion, but greater differences were recorded throughout the remainder of the water column. If data for these epilimnion chambers are

eliminated, the mean difference is an even more substantial 78% survival.

Further information may be gained by considering epilimnion and hypolimnion chamber populations separately. *E. coli* population densities suspended in the hypolimnion at Stations I through IV were able to survive at 93% which was higher during RO than that during RNO conditions for the first 48-hr period. The difference at Station V was 32%; therefore, at Stations I through IV, the mean difference was 61% greater than that at control station V (Figs. 4c, 4d, 5c, and 5d). In contrast, epilimnion populations at Station I survived at a lower percent than those at Station V (Table 4). However, populations of *E. coli* in epilimnion chambers at Stations II, III, and IV survived at a mean difference of 74% or 60% greater than epilimnion populations at Station V (Figs. 4a, 4b, 5a, and 5b).

DISCUSSION

Many of the previous studies involving *E. coli* survival have been limited by one or more of the following problems: (1) The studies were generally of short duration, less than one week (McFeters and Stuart, 1972). (2) Artificially induced temperature and laboratory conditions prevailed (Cherry and Guthrie, 1975). (3) The same bacterial populations were not followed throughout the study because of movement within the body of water (Geldreich, 1972). (4) Survival in deep water lakes had not been

considered. In this study, each of these problems was eliminated by conducting long-term observations of known *E. coli* population densities in a thermally altered reservoir under a variety of physical and chemical conditions.

To assess the survival, growth, and attrition of *E. coli* in the chambers, 27 samples of *E. coli* were taken. These samples showed a population depletion of 50%, which generally occurred after the fifth day. A theoretical dilution curve was established so that a known percent of initial optical density corresponded to the number of samples taken (Fig. 2). Thus, those populations which maintained densities above the theoretical levels showed survival and growth, and those population densities below the theoretical levels showed natural attrition.

Control chambers containing only buffered saline or filtered and sterilized lake water were suspended in the epilimnion and hypolimnion at several stations as controls to measure the turbidity of the lake water. The optical densities of hypolimnion waters surpassed that of epilimnion waters regardless of the reactor operating conditions but represented no more than 25% of the initial optical density of the chamber populations. Therefore, major differences between optical density in the diffusion chambers during RNO and that during RO conditions were caused by the survival and growth of bacterial populations.

The increases in optical density for the first 24 to 48 hr were probably due to favorable growth conditions for *E. coli* and

not the presence of associated nutrients because cells were washed three times in sterile buffer to remove extraneous substrate. The rapid diffusion of materials through the membranes, less than 2 hr, indicates that cryptic growth conditions were probably not a factor in the chambers.

Reactor operating conditions resulted in physical and chemical gradients becoming established in the warm arm of Par Pond. These gradients were reflected by changes in surface temperature, nutrients, and water chemistry. The gradients were not, however, linear from Stations I through V. Rather, Stations II and III received the highest temperatures due to a "teardrop effect" caused by the movement of the water from the inflow around the sides of the lake with the confluence of the two flows appearing near Stations II and III. Thus, Stations I through IV were most directly affected by the reactor operations. Station V was about 5.6 km from the inflow and served as a control station; Station I was less affected by the effluent flow than Stations II, III, and IV (Fig. 1).

When the reactor is operating and the thermal effluent is flowing into the lake, both dissolved and particulate organic matter passes into Par Pond. The sources of this organic matter are (1) the Savannah River, (2) Par Pond recycled water, and (3) algae-bacterial production from the P-canal system. Although Par Pond waters do not contain a high standing crop of

dissolved nutrients, the lake is productive and richer in nutrients when compared with many other southeastern lakes. The temperature, nutrients, and other factors associated with the thermal effluent contributed to the increased survival and growth of *E. coli* and to the apparent gradient of survival from station to station.

Data in Table 4 show that *E. coli* populations at Station V had only a 23% increase in survival during RO conditions as compared to RNO conditions. In contrast, Stations I through IV showed a mean of 74% survival or a 51% greater survival than that at Station V when the reactor was operating. These data indicate that survival and growth conditions for *E. coli* were more favorable in the water column at the other stations than those conditions at Station V.

Conditions for *E. coli* survival and growth in the epilimnion at Stations I and III were inhibitory, possibly because of high RO temperatures; the hypolimnion conditions were favorable at Stations I, II, III, and IV. Epilimnion optical densities were generally lower than those of the hypolimnion. These results indicate that the influences of the thermal effluent on survival and growth conditions were well distributed throughout the water column and were not localized either above or below the thermocline.

The following generalizations may be made regarding *E. coli* survival and growth in Par Pond: (1) *E. coli* is able to survive and grow for two to three weeks during both ambient and thermally

altered water conditions. (2) At all stations and at all depths, *E. coli* populations maintained themselves better during thermally altered conditions than ambient water conditions. (3) Populations in the hypolimnion survived better (at higher densities) and for longer periods than those in the epilimnion. (4) During thermally altered lake conditions, the populations at Stations II, III, and IV maintained higher densities than those at Stations I and V.

Our data contradict those studies which indicate that *E. coli* populations die off rapidly in warm water (McFeters and Stuart, 1972; Cherry and Guthrie, 1975). Thermally altered aquatic ecosystems are often highly productive and may therefore supply nutrients capable of supporting bacterial growth. Such conditions may prevail in waters which approach human body temperature, the optimum growth temperatures for *E. coli* and several other human pathogens.

Geldrich (1972) and Brock (1975) have discussed in some detail the possible ecological consequences of thermal stress on natural aquatic ecosystems. Our data support their suggestions that nutrient-rich waters, even those without a high standing crop of nutrients, when exposed to thermal stress, may serve as a favorable survival and growth medium for *E. coli* and possibly other enteric bacteria. Eutrophic patterns of Par Pond have changed dramatically from 1965 to 1969 and have shown a sixfold increase in phytoplankton (Tilly, 1975). This primary productivity was influenced by nutrient increases and increased evaporation because

of reactor operations. In addition, Grace and Tilly (1976) have shown that the total standing crop of submerged macrophytes is three times as great in the warm portion of Par Pond (near Stations I, II, and III) as that at the cold end (near Station V). They concluded, "temperature may be the most important single factor involved, but such factors as light, turbulence, nutrients, substrate, competition, and sociological features must be considered." Many of these factors are affected by reactor operations in Par Pond, a system rich in available nutrients.

Possible reasons for fluctuations in the optical density measurements at several stations are (1) *E. coli* populations might have been exposed to several different nutrients requiring enzyme induction for assimilation. If so, then the populations may fluctuate rapidly as each nutrient is consumed and then depleted. (2) These populations might have experienced a situation similar to that observed by Orlab (1956) in which *E. coli* in seawater rapidly died, but resistant cells developed, and the populations then increased only to develop cyclic oscillations about a mean which is characteristic of natural populations (Odum, 1971).

The survival and growth of *E. coli* in Par Pond during both ambient and thermally altered water conditions show that this organism is capable of survival and growth for longer time periods than is generally suggested in scientific literature. The data further indicate that *E. coli* may not be a suitable indicator of recent fecal contamination in thermally altered aquatic ecosystems

because: (1) *E. coli* survives for long periods in water. (2) Genetic changes such as lac + to lac -, which mask the true numbers of *E. coli* present, may occur (K. Kasweck, Florida Institute of Technology, Melbourne, Florida, and the authors, personal communications). (3) Low numbers of *E. coli* may simply show that the populations are at a low point in the oscillation cycle and may respond rapidly to favorable growth conditions.

Subtle differences in temperature, pH, redox potentials (E_h), conductivity, O₂ levels, and other unmeasured parameters may be responsible for differences between survival and growth at each station and depth. Although the two phases of the experiment were conducted successively rather than simultaneously, the period of time between reactor conditions was of such duration that similar lake stratification patterns existed. In addition, the result of reactor operations affects the thermal stability of the system more dramatically than the natural stratification process. Thus, the data reported here are a result of reactor operations rather than the natural stratification processes.

The differences between *E. coli* population survival and growth during thermal alterations suggest that care must be taken when *E. coli* is regarded as an indicator of recent fecal contamination in warm aquatic ecosystems. In addition, the data indicate that the combination of thermal alterations and nutrient enrichment may provide suitable habitat conditions for the survival and growth of *E. coli* and possibly other pathogens. Such studies

further indicate that wise management of thermally altered aquatic ecosystems is essential.

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LEGENDS FOR FIGURES

- Fig. 1 Map of Par Pond and associated canal system showing permanent sampling stations in the thermally altered region of the reservoir (shaded zone) and Station V near the cold dam.
- Fig. 2 Theoretical curve for diluting bacteria from the diffusion chambers because of the sampling process. Measured optical densities were compared to the theoretical curve to determine the extent of survival.
- Fig. 3 *E. coli* survival and growth in diffusion chambers placed at four different depths at a typical station in Par Pond. Measurements were made when the reactor was not operating.
- Fig. 4 *E. coli* survival and growth in diffusion chambers placed at four different depths at a single station in Par Pond. Measurements were made when the reactor was not operating and when it was operating.
- Fig. 5 *E. coli* survival and growth in diffusion chambers placed at four different depths at a single station in Par Pond. Measurements were made when the reactor was not operating and when it was operating.

Fig. 1

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Table 1. Chemical and physical parameters at Station I

Depth, m	Temperature, °C	pH	Dissolved Oxygen, ppm	Conductivity, $\mu\text{mho}/\text{cm}^2$	E _h , 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 Chamber location.^b ND = not determined.

Table 2. Chemical and physical parameters at Station III

Depth, m	Temperature, °C	pH	Dissolved Oxygen, ppm	Conductivity, $\mu\text{mho}/\text{cm}^2$	E _h , 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 Chamber location.

Table 3. Chemical and physical parameters at Station V

Depth, m	Temperature, °C	pH	Dissolved Oxygen, ppm	Conductivity, µmho/cm ²	E _h , 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 Chamber location.

Table 4. Comparison of optical density readings during reactor not operating and reactor operating conditions

Percent of initial optical density. Numbers represent the means for each depth during the first 48 hr of the study						
Station	Depth, m	Reactor Operating, %	Reactor Not Operating %	Difference RO > RNO, %	χ^2 Probability Values	
					Station Depth ^a	Control Depth ^b
I	1	51	54	-3	0.99	0.50
	4	59	52	5	0.95	0.80
	6	153	48	105	0.001	0.001
	8	134	57	77	0.001	0.001
	Mean →	99	52	47		
II	1	166	56	110	0.001	0.001
	4	82	54	38	0.01	0.95
	7	134	47	87	0.001	0.001
	9	152	60	92	0.001	0.001
	Mean →	133	54	79		
III	1	65	50	15	0.30	0.001
	5	149	41	108	0.001	0.001
	7	88	68	14	0.50	0.50
	9	221	109	113	0.001	0.001
	Mean →	130	67	62		
IV	1	146	60	86	0.001	0.001
	5	142	46	96	0.001	0.001
	8	148	45	103	0.001	0.001
	10	184	32	153	0.001	0.001
	Mean →	155	46	109		
V	1	55	52	3	0.99	CONTROL
	6	75	51	24	0.01	"
	8	95	52	44	0.001	"
	16	85	64	21	0.10	"
	Mean →	77	54	23		

- a. χ^2 probability values show the probability of a significant difference between the optical densities for the same station and the same depth when the reactor is operating as compared to the reactor not operating.
- b. χ^2 probability values show the probability of a significant difference between the control station and each depth.