A Two-Year Study of Bacterial Populations in Indiana Farm Pond Waters[1]

L. B. HUGHES and H. W. REUSZER, Purdue University

Abstract

A two-year study of bacterial populations in farm pond waters was conducted on three farm ponds on the Southern Indiana Purdue Agricultural Center. Samples of water from the surface and near the bottom were taken approximately once per month from each pond. Bacterial numbers were determined by colony counts. Great differences in bacterial numbers were found between ponds and large seasonal variations in bacterial numbers were found within ponds. Maximum bacterial numbers occurred in late spring or early summer in all three ponds. Consistently higher numbers of bacteria were present in Pond C and lowest numbers were present in Pond B. Water temperatures were quite similar in all three ponds, although a significant correlation between water temperature and bacterial numbers was found only in Pond C. Organic carbon content was consistently highest in Pond C and lowest in Pond B. Significant positive correlation coefficients between organic carbon and baterial numbers were found in Ponds A and B, but a significant negative correlation was found in Pond C.

Introduction

Farm ponds have many uses in our day including serving as sources of recreation, sport and commercial fishing, livestock water, and even water for human consumption and general household use. Consequently, the chemical and biological nature of farm pond waters is of extreme importance. A thorough search of the literature revealed few microbiological studies in farm pond waters. The purpose of this study was to determine the changes in numbers of bacteria in farm pond waters over a 2-year period and to investigate some of the factors possibly influencing the bacterial numbers.

Literature

Many workers have reported large fluctuations in bacterial numbers in bodies of water. Fred *et al.* (1) and Snow and Fred (4) found large fluctuations of bacterial numbers in Lake Mendota, Wisconsin, in studies covering 4 years and extreme fluctuations could not always be explained (1). In a brief study of Flathead Lake, Montana, Graham and Young (2) found maximum bacterial numbers below 8,000 per ml. Lower numbers of bacteria were found in the surface water than in water below the surface. Stark and McCoy (5) reported striking differences in bacterial numbers in the surface water at different locations on the same lake.

Irwin and Claffey (3) found wide variations in numbers of bacteria in the waters of 20 ponds in Oklahoma with fewer bacteria found in ponds with higher turbidity. Wilson *et al.* (7) reported 5,000 to

[1] Journal Paper No. 3889, Purdue University Agricultural Experiment Station, Department of Agronomy, Lafayette, Indiana. This study was supported in part by the Office of Water Research, U. S. Department of the Interior.

30,000 bacteria per ml in 6 West Virginia ponds. Little differences in numbers of bacteria were detected at different depths. Water temperature and pH showed no correlation to the bacterial numbers.

Procedures

The three farm ponds studied were constructed in 1953 or 1954 on the Southern Indiana Purdue Agricultural Center. The ponds were arbitrarily labeled A, B, and C. Pond A was the largest with a surface area of 0.96 acre, a maximum depth of 12 feet, and a watershed area of 7 acres. Substantial aquatic plants in the edge of Pond A contributed organic matter to the water. Pond B had a surface area of 0.66 acre, a maximum depth of 12 feet and a watershed area of 2 acres. Very little aquatic plant growth occurred in this pond. Pond C had a surface area of 0.30 acre, a maximum depth of 15 feet and a watershed area of 3 acres. Dense growth of aquatic plants extended several feet into the edge of the water. Substantial algal growth was noted in Ponds A and C in late spring and early summer, but little algal growth was noticed in Pond B. A mixture of alfalfa and orchard grass was the common forage crop growing on the three watersheds and provided good protection against erosion of the soil.

Samples of water were taken from a raft at the surface and 6 inches from the bottom at 4 different locations on each pond approximately once per month over a 2-year period (April, 1967 to March, 1969). Samples of water were immediately placed in ice and transported back to the laboratory (4 hours travel time). The water samples were then stored overnight at 5° C and plated the following day on an agar medium containing 1.0 gm glucose, 1.0 gm peptone, 0.5 gm yeast extract, 0.25 gm K_2HPO_4, 12.0 gm agar, and 1,000 ml deionized water. Bacterial numbers were determined by colony counts made after 14 days incubation in the dark.

Water temperatures were obtained four times daily by E. J. Monke and P. R. Goodrich of the Purdue University Agricultural Engineering Department using thermocouples at various depths. Data used in this paper are the means of the four temperatures recorded on the day of sampling. Water temperatures were available only from April to December, 1967. Aliquots of water were evaporated and organic carbon was determined using the manometric procedure described by Van Slyke and Folch (6). Data on pH, nitrate concentration, and water turbidity were obtained from the Indiana State Board of Health.

Results and Discussion

The factors possibly influencing the bacterial numbers that were studied include organic matter content, water temperature and to a lesser extent, pH, nitrate concentration, and turbidity of the water.

Nitrate concentration, pH, and water turbidity of the three ponds are shown in Table 1. The highest average pH found in Pond B would seem to be farther from the optimum pH for maximum bacterial growth than the pH in either Ponds A or C. Water turbidity was also highest in Pond B. The small variations in nitrate concentrations would not be expected to have significant effect on bacterial numbers.

TABLE 1. *Turbidity, pH and nitrate concentration of the pond waters at different times.*[2]

	April 1967	August 1967	December 1967	April 1968	July 1968	Avg.
			pH			
Pond A	7.4	6.8	8.0	7.1	7.0	7.3
Pond B	7.4	8.0	7.4	7.7	7.8	7.7
Pond C	7.3	7.1	7.4	7.6	7.3	7.3
			Nitrate Concentration (ppm)			
Pond A	0.1	0.1	0.3	0.1	0.1	0.14
Pond B	0.1	0.2	0.2	0.1	0.1	0.14
Pond C	0.1	0.2	0.3	0.1	0.2	0.18
			Turbidity			
Pond A	0.3	0.1	10	2	0.3	2.5
Pond B	15	0.1	3	15	3	7.2
Pond C	0.7	0.2	3	1	0.6	1.1

[2] The authors express gratitude to the Indiana State Board of Health for use of these data.

Water temperatures of the 3 ponds for a 9-month period are shown in Figure 1. Surface water temperatures were quite similar in all 3 ponds, reaching a maximum near 80°F in June, and a minimum near 40°F in December. The maximum bottom temperatures in Ponds A and B were above 70°F in August. Cooling began one month earlier in Pond C. Minimum bottom water temperatures occurred in December and were about equal to surface temperatures at that time.

Seasonal variations in organic carbon content are shown in Figure 2. The highest organic carbon content generally occurred in late spring or early summer in both years. Pond B had the least seasonal variation of organic matter content, with a range of 7.1 to 14.8 mg of organic carbon per liter of water with similar quantities of organic carbon in the surface water and in the water near the bottom. Pond A showed larger seasonal variation of organic matter at both depths with organic carbon quantities ranging from 8.0 to 24.0 mg per liter. Higher organic matter content generally was prevalent near the bottom than at the surface in Pond A. Both depths of Pond C contained the highest organic matter content and the largest seasonal variation, ranging from 11.1 to 29.2 mg of organic carbon per liter of water.

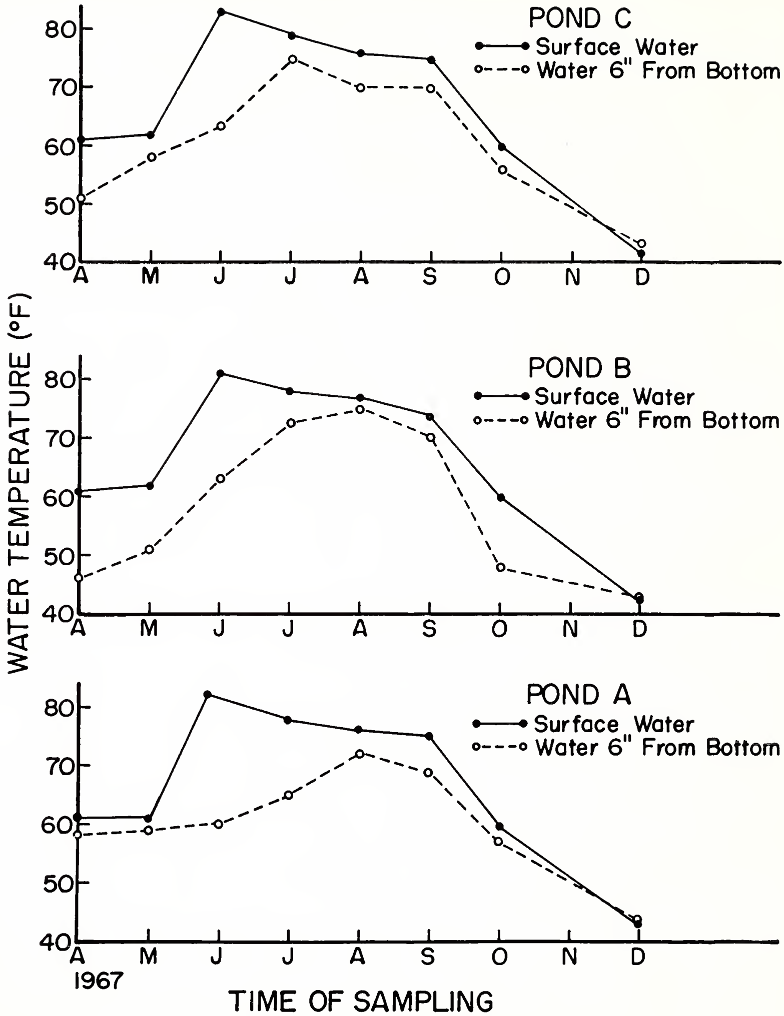

FIGURE 1. *Seasonal variations in water temperature for a nine-month period. (The authors express gratitude to P. R. Goodrich and E. J. Monke for use of these data.)*

Bacterial numbers are given in Table 2. Seasonal variations in bacterial numbers are shown in Figure 3. The highest numbers of bacteria generally were present in late spring or early summer in both years of the study. High numbers of bacteria seemed associated with the active growth period of aquatic vascular plants rather than the autumn period of plant death and decay. The role of algae with respect to numbers of bacteria was not clear. Pond A had bacterial maxima at the same time each year. The surface water usually had fewer bacteria than the bottom water. Bacteria in Pond A ranged from 4,400 to 102,700

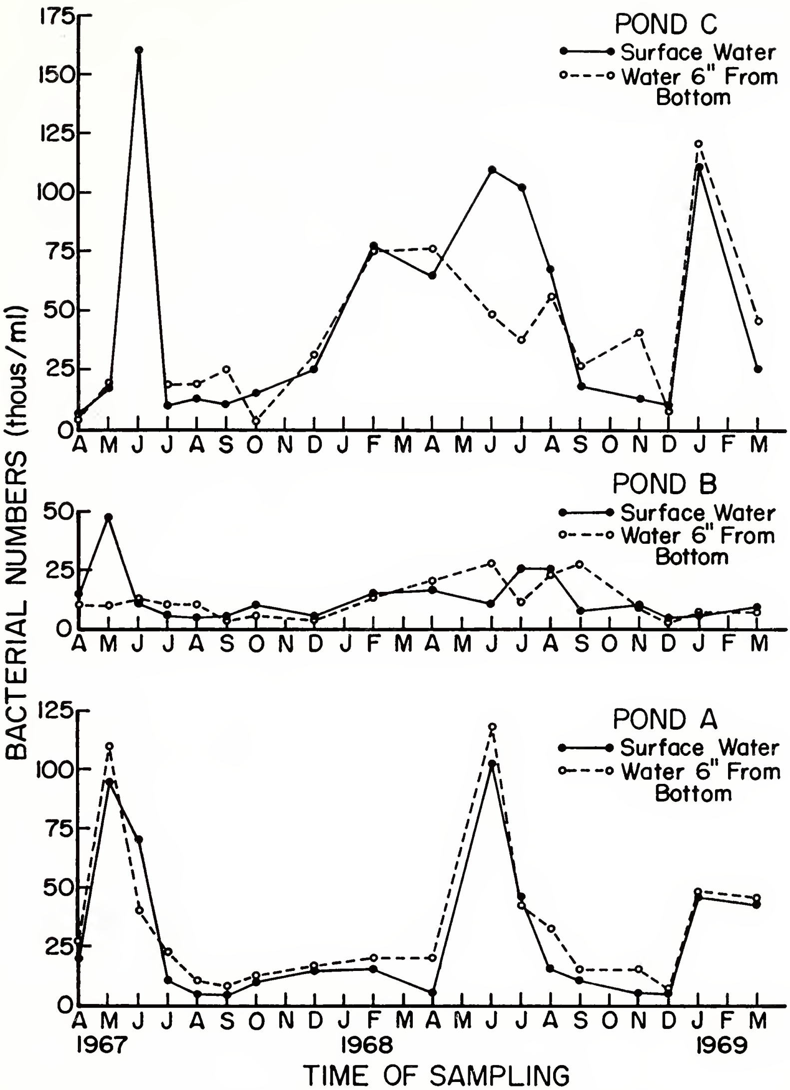

FIGURE 2. *Seasonal variations in organic carbon content of the pond waters for a two-year period.*

per ml in the surface water and from 5,100 to 118,100 in the bottom water. Pond B had only small variations in bacterial numbers with similar numbers of bacteria at both depths throughout the 2-year period. Bacteria in Pond B ranged from 4,200 to 48,000 per ml in the surface water and from 3,400 to 28,300 per ml in the water near the bottom. Pond C had the highest numbers of bacteria and the largest seasonal variations of bacterial numbers. In Pond C, the bacteria ranged

TABLE 2. *Seasonal variations of bacterial numbers in thousands per ml for a two-year period.*

Month	Pond A		Pond B		Pond C	
	Upper	Lower	Upper	Lower	Upper	Lower
April 1967	20.7	28.3	14.2	10.4	7.7	3.2
May	94.1	110.6	48.0	10.8	17.0	18.2
June	70.6	40.1	11.8	12.5	160.0	---
July	11.1	22.4	6.2	11.6	9.9	17.6
August	4.8	9.7	5.3	10.2	13.3	19.2
September	4.4	7.8	5.0	4.2	10.9	24.5
October	10.4	12.2	9.6	6.9	15.0	3.6
December	15.1	16.3	5.7	6.4	25.2	31.3
February 1968	15.9	20.1	15.9	15.4	77.8	75.1
April	6.8	20.1	16.9	20.6	64.6	75.6
June	102.7	118.1	10.6	28.3	109.3	48.4
July	46.7	42.8	25.2	12.0	102.5	37.3
August	16.6	32.7	25.1	24.1	68.4	56.4
September	11.6	14.3	7.1	27.7	18.8	26.7
November	5.3	15.5	10.2	9.6	13.1	41.3
December	4.5	5.1	4.2	3.4	10.4	8.8
January	45.2	47.2	5.8	6.5	111.2	121.0
March	42.7	44.1	9.2	8.5	25.7	45.8

from 7,700 to 160,000 per ml in the surface water and from 3,200 to 121,000 per ml in the bottom water.

Many chromogenic bacterial colonies appeared on the plates including red, pink, shades of yellow and orange, white and cream colored.

A statistical analysis of variance of the bacterial numbers is given in Table 3. In each of the ponds, the numbers of bacteria varied significantly with time of sampling, location of sampling, and depth of

TABLE 3. *Analysis of variance of bacterial numbers.*

Source of Variation	F Values		
	Pond A	Pond B	Pond C
Time of Sampling	859.41**	276.68**	681.51**
Location of Sampling	55.58**	49.72**	23.15**
Depth of Sampling	74.58**	3.49**	20.73**
Time x Location	40.72**	55.07**	19.76**
Time x Depth	24.61**	186.74**	100.26**
Location x Depth	24.87**	47.92**	6.76**
Time x Depth x Location	18.67**	61.62**	14.79**

** Significant at the 1% level.

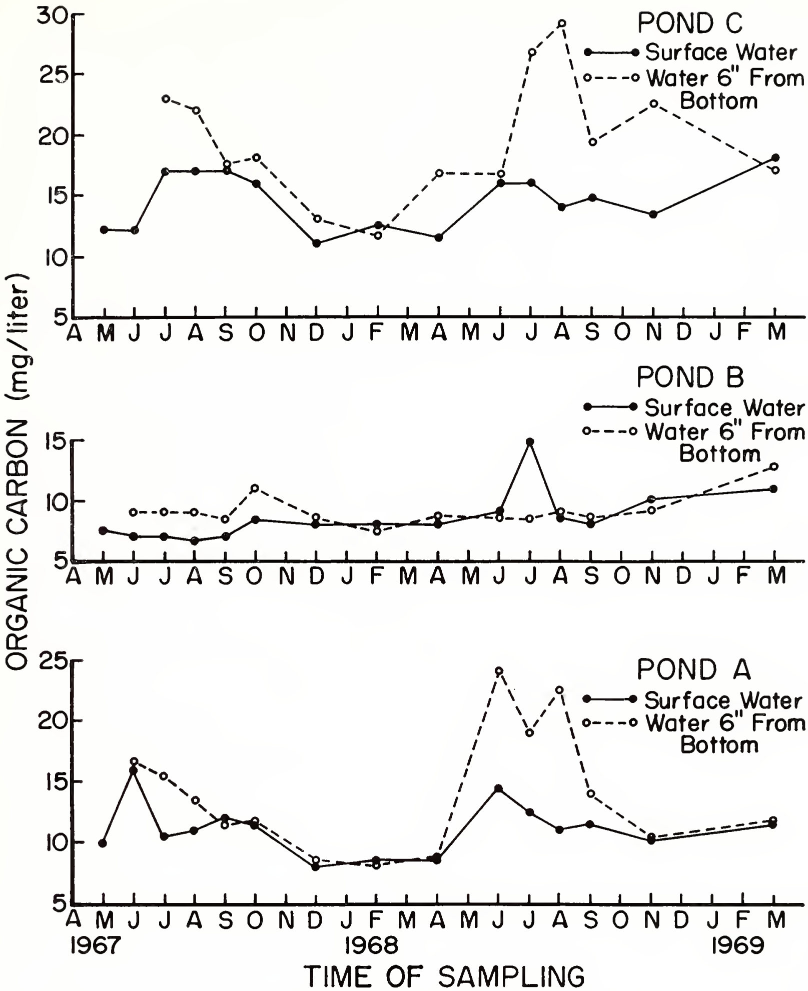

FIGURE 3. *Seasonal variations in bacterial numbers for a two-year period.*

sampling. Table 4 shows correlation coefficients for organic carbon and water temperature with bacterial numbers in each of the ponds. Correlation coefficients between water temperature and bacterial numbers were not significant in Ponds A and B but were highly significant in Pond C. The correlation coefficients between organic carbon and bacterial numbers were highly significant in both Ponds A and B while a negative correlation was significant at the 5% level in Pond C.

TABLE 4. *Correlation coefficients*

	Organic Carbon	Water Temperature	Bacterial Numbers
		Pond A	
Organic Carbon	1.000 (580)	.445 (260)	.440** (580)
Water Temperature	.445 (260)	1.000 (320)	.042 (320)
Bacterial Numbers	.440** (580)	.042 (320)	1.000 (720)
		Pond B	
Organic Carbon	1.000 (580)	.122 (260)	.299** (580)
Water Temperature	.122 (260)	1.000 (320)	—.005 (320)
Bacterial Numbers	.299** (580)	—.005 (320)	1.000 (720)
		Pond C	
Organic Carbon	1.000 (560)	.355 (260)	—.115* (560)
Water Temperature	.355** (240)	1.000 (300)	.325** (300)
Bacterial Numbers	—.115* (560)	.325** (300)	1.000 (700)

* Significant at 5% level.
** Significant at 1% level.
Values in parentheses indicate number of samples.

Summary

Seasonal variations of bacterial numbers were found in a 2-year study of three Indiana farm pond waters. Statistical analysis showed significant variation of numbers of bacteria in each pond with time of sampling, location of sampling, and depth of sampling. In addition, correlation coefficients for water temperature with bacterial numbers were significant in Pond C and not significant in Ponds A and B. Correlation coefficients for organic matter content with bacterial numbers were significant in Ponds A and B, but a significant negative correlation co-efficient was found in Pond C.

Literature Cited

1. FRED, E. B., F. C. WILSON, and A. DAVENPORT. 1924. The distribution and significance of bacteria in Lake Mendota. Ecology 5:322-339.

2. GRAHAM, V. E., and R. T. YOUNG. 1934. A bacteriological study of Flathead Lake, Montana. Ecology 15:101-109.

3. IRWIN, W. H., and J. CLAFFEY. 1968. Soil turbidity, light penetration and plankton populations in Oklahoma ponds and lakes. Proc. Okla. Acad. Sci. 47:72-81.

4. SNOW, LAETITIA M., and E. B. FRED. 1926. Some characteristics of the bacteria of Lake Mendota. Wis. Acad. Sci., Arts and Letters. 22:143-154.

5. STARK, W. H., and ELIZABETH MCCOY. 1938. Distribution of bacteria in certain lakes of northern Wisconsin. Zentrbl. Bakt. Abt. II. 98:201-209.

6. VAN SLYKE, D. D., and J. FOLCH. 1940. Manometric carbon determination. J. Biol. Chem. **136**:509-551.

7. WILSON, H. A., T. MILLER, and ROSA THOMAS. 1966. Some microbiological, chemical and physical investigations of farm ponds. West Virginia Agriculture Experiment Station Bull. 522T. p. 1-17.