INTERNAL REPORT 88

DEGRADATION OF ORGANIC COMPOUNDS IN FRESHWATER SEDIMENTS BY BACTERIA

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INTRODUCTION

In fresh water aquatic ecosystems the sediment water interface has been found as a site of intense microbial acitvity and, not surprisingly, is rich in bacterial biomass. Hobie and Crawford (1969) found that the heterotrophic bacteria in these systems play an important and basic role in the mineralization and transformation of carbon compounds into forms that are available to higher organisms.

Cellulose and to a lesser extent, chitin, and their subsequent degradation products, including glucose, are perhaps the greatest carbon sources in the aquatic environment. These compounds are rapidly utilized in the aquatic environment and, as a result, the levels of glucose at any given time remain very low. These dissolved organic compounds existing in natural waters in low concentration can only be accurately detected by radioactive tracer techniques. Accordingly, the rates of glucose mineralization by indigenous bacteria have been studied in our laboratory since June 1972. Sediment samples have been collected and analyzed from the four lakes under study: Lakes Washington, Sammamish, Chester Morse, and Findley.

MATERIALS AND METHODS

Sampling

Samples of the fine bacteria-rich sediment at the sediment-water interface were collected aseptically with a suction type sampling device. The samples were transferred to plastic or glass containers with screw cap lids. At the time of sampling, a temperature profile was obtained from the sediment to the surface of the lake, using a Tri-R electronic thermometer. Collected samples were either refrigerated or kept on ice, returned to the laboratory, and analyzed within 12 hours after collection.

Bacterial Counts

Estimation of the bacterial biomass in the samples of sediment-water interface were made by the plate count method. All inoculated plates were incubated at the *in situ* temperature. The composition of the medium used for the plate counts was obtained from Dr. Bruce Lighthart, Institute for Freshwater Studies, Western Washington State College, Bellingham, Washington and contained the following: yeast extract, 0.2 gm; sodium caseinate, 0.5 gm; peptone, 0.5 gm; soluble starch, 0.5 gm; glycerol, 1.0 gm; K_2HPO_4, 0.2 gm; $MgSO_4$, 0.05 gm; $FeCl_3$, trace; agar, 15 gm; distilled water, 900 ml; and lake water (collected from sampling area), 100 ml. The medium was sterilized at 121°C for 15 minutes.

For the estimation and isolation of chitinoclastic bacteria, approximately 60 grams of reprecipitated chitin (Chan 1969) were added to the above medium. Bacteria capable of digesting chitin were easily detected by the presence of a clear zone of digested chitin surrounding the colonies.

Glucose Utilization

The rate of glucose utilization by bacteria was measured by incubating sediment or water with uniformly labeled 14C glucose at the *in situ* temperature in 50 ml serum bottles in a refrigerated water bath. Turnover time and maximum velocity were determined by the method of Harrison et al. (1971). A typical plot of t/f versus A is shown in Figure 1. (t is the incubation time in hours, f is the fraction of available substrate mineralized, and A is the concentration of added substrate. Extrapolation back to the y intercept, where $A = 0$, gives the turnover time for mineralization at the natural substrate concentration. The inverse of the slope of the line is a measure of V_{max}, the maximal velocity of mineralization).

All values for turnover times and maximum velocities given in this report are preliminary estimates. A computer program was completed in January 1972. The data are now being processed for linear regression analysis and submission to the information bank. The program is reported in an IBP progress report, Automated Data Acquisition: An Example, by E. R. Hamerly and Pearl Knopf.

RESULTS AND DISCUSSION

As expected, bacteria associated with the sediment near the shore are present in higher numbers than those found at the greater depths. Consequently glucose turnover times are much lower in the shore samples (Tables 1 and 2). Higher mineralization rates for inshore waters have also been detected in the marine environment (Okutani et al. 1972).

The sediment organisms are also capable of mineralizing more glucose than bacteria in the overlying water column. For example, turnover time and V_{max} obtained from a Findley Lake sediment sample (shore, 8/30/72, Table 1) were 2 hours and 5 µg of glucose per gram (dry weight) per hour. The same parameters measured from lake water on the same day were 250 hours for turnover time and a maximal velocity of 1.1×10^{-3} µg of glucose per ml per hour. Greater numbers of bacteria are found in the sediment-water interface than in the water column, which may account for these differences.

Glucose turnover time has been found to be inversely proportional to sediment temperature. Results from shore sediment samples are shown in Figure 2. In both Lakes Findley and Washington the rates of glucose turnover (time in hours required for the bacteria to completely remove the substrate from the samples) is rapid at the higher temperatures of approximately 20°C but drops off rapidly at the lower temperatures. The data for Lake Sammamish in Figure 2 shows the opposite; however, insufficient data had been collected to indicate if this is to be expected. Similar glucose turnover data have been obtained from sediment samples collected at greater depths. These data are also shown in Tables 1 and 2.

The rate of glucose utilization also varies with the season, as expected from the temperature data given above. The rate of glucose utilization increases during the spring, reaching a maximum during the summer, and then decreases during the fall and winter. This seasonal variation is shown for Findley Lake (Figure 3). The initial sample (27 meters) collected 27 July 1972 was obtained approximately one week after the snow and ice cover had melted from the lake. This shorter turnover time presumably was due to the flux of nutrients as a result of the melt. The shore sediment values for glucose turnover time also do not fluctuate with the seasons as much as do the samples collected at 27 meters.

Differences in glucose turnover time during the seasons of the year for samples collected from Lake Washington were studied. A comparison was made between turnover time of samples collected at different seasons (samples run at the different *in situ* temperatures) with samples collected during the winter and tested at different temperatures. The data are shown in Figure 4. The very close correlation between these two plots is evident. These data show that the rate of glucose turnover in Lake Washington sediments is dependent upon temperature. Samples collected during the summer months when the water is warm and run at the *in situ* temperatures compare very well with samples collected during the winter and run at comparable temperatures. These data indicate that the populations of bacteria responsible for glucose turnover do not fluctuate during the year but remain fairly constant and are influenced greatly by the prevailing temperatures. A plot of the turnover time at three temperatures for the winter sample run at 3.0, 9.4, and 19.0°C is shown in Figure 5. These data also clearly indicate the influence of temperature on glucose turnover time.

During these studies, no attempt has been made to identify bacteria in sediments collected from the four lakes. Aerobic plate counts have been run on most of the samples, and anaerobic plate counts have been run on a Lake Findley sample. These data are shown in Tables 1 and 2. Results of counts of bacteria digesting chitin show that Lakes Washington and Findley have bacterial populations, one to two per cent of which are capable of digesting chitin. These organisms are important from the standpoint of digestion of chitin from insect exoskeletons. These chitinoclastic bacteria have been isolated and are being maintained for future work.

Enrichment cultures are now under way for the isolation of bacteria capable of digesting cellulose in Lakes Washington and Findley. These studies will be expanded to include Lakes Chester Morse and Sammamish. Preliminary studies with Lake Findley shore sediments indicate that cellobiose (a product of cellulose hydrolysis or breakdown) is metabolized by bacteria to CO_2, but at a much slower rate than glucose. These studies are being expanded to include sediments from the other three lakes. When the rate of breakdown or turnover of cellobiose has been determined, the rate of cellulose decomposition will be studied, using ^{14}C labeled cellulose.

During the sampling trips the temperature profiles have been determined in the four lakes. The temperature measuring equipment was not available during the early part of this year's work. However, the data that have been collected are shown in Table 3. The most interesting and significant data are those obtained at Findley Lake and are shown in Figure 6. On this

figure are plotted the temperature profiles obtained on 30 August, 12 October, and 1 November 1972. It can be seen that on 30 August 1972 the temperature varied from a high of 16.9°C down to 4.3°C at 27 meters. This temperature had changed by 12 October to a high of 7.8°C down to 4.9°C at 21 meters. An inversion took place and the temperature, as measured on 1 November 1972, was 5.0°C from the surface to a depth of 27 meters. The temperature profile obtained on 27 September 1972 at Lake Chester Morse is shown in Figure 7. The temperature varied from a high of 11.4°C at the surface down to 6.8°C at 30 meters.

In conclusion, it is interesting to note from the data that the bacteria associated with the sediments collected from Lake Sammamish behave quite differently from those tested in sediments collected from the other three lakes. At the present time it is not known why the glucose turnover times do not show the same relationship to temperature. Also, shore samples do not always exhibit a faster rate of glucose mineralization than benthic samples. Clearly more data are needed for this lake and more extensive sampling is planned for the 1973 sampling year, especially before and after the benthic sediment becomes anaerobic in the summer. More sampling is planned for the other three lakes in 1973 to complete the annual profile of glucose rates of mineralization.

Preliminary studies are being carried out with glucose, but more complex molecules such as cellobiose, cellulose, and chitin will be stressed to a greater degree in the future. Glucose was used in these studies because it is a good small representative substrate with a rapid turnover. It is also the end product of cellulose degradation by way of cellobiose. The role of glucose turnover is very rapid in the aquatic environment and, as a result, the glucose level always remains very low in the environment. Bacteria able to utilize chitin have been isolated from the freshwater environment. These bacteria are important from the standpoint of insect chitin mineralization. Other substrates such as cellobiose and cellulose which are probably more important but are also more difficult to work with will be stressed more in the future. Temperature profiles have been taken at the four lakes and these will be continued until data are a ailable for the entire year.

Studies on oxygen uptake are well underway at the present time. The methodology is being studied and samples are bing run under different conditions. We should have the methods working in the very near future so that we can begin to accumulate the data that are necessary for the overall Biome program.

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Table 1.

FINDLEY LAKE

Exp #	Sample	Date	Temp °C	Turnover Time (hr)	V max µgm glu/ hr/gm sed	Plate* Count (Aerobic)	Dry wt gm/5 ml
4, 5	Shore	7/7/72	10	2.0	30.0	9.4×10^7	.042
	27 M		4	10.0	2.0	3.4×10^5	.102
6	Shore	8/8/72	20.5	1.2	11.2	1.07×10^9	.240
	27 M		4.0	15.0	0.8	1.0×10^7	.176
8	Shore	8/30/72	19.0	2.0	5.0	2.38×10^7	.037
	27 M		5.0	11.0	1.1	1.1×10^6	.026
	H_2O		19.0	250.0	1.1×10^{-3}	1.18×10^3	
12	Shore	10/12/72	7.0	4.0	6.5	3.25×10^6	.042
	20 M		5.0	7.0	1.2	1.57×10^5	.105
	H_2O					2.27×10^3	
13, 14	Shore	11/1/72	5.0	2.8	5.5	3.94×10^6	.064
	27 M		5.0	28.0	.44	7.6×10^5	.073
	H_2O					4.0×10^2	
17	Shore	12/13/72	1.0	7.0	.45	1.16×10^{10}	.04
	15 M		ND^{40}	24.0	.25	1.34×10^9	.173
	H_2O		0.0			5.2×10^4	
17	Shore	12/13/72	1.0			(Anaerobic)	
	15 M		ND^{-20}			1.34×10^6	
	H_2O		0.0			4.15×10^7	
						40	
19	Shore	2/9/73	0.5	10.0	0.4		.021

* Per gram dry wt sediment or per ml H_2O

Table 2.

Exp. No.	Sample	Date	Temp °C	Turnover Time (hr)	V_{max} µg glu/ hr/gm sed	Plate* count (Aerobic)	Dry wt. gm/5 ml
LAKE WASHINGTON							
3	Shore	6/28/72	20.0	2.4	46	7.5×10^8	.0476
	4 M		20.0	2.6	23	2.2×10^8	.0325
9	Shore	9/8/72	18.5	3.0	1.0	7.6×10^7	.256
	60 M		8.0	7.0	0.87	6.6×10^6	.089
	H_2O		18.5	100.0	6.7×10^3	1.6×10^3	
15	Shore	11/15/72	10.0	3.3	7.7	1.28×10^8	.262
	60 M		7.0	15.3	0.2	3.6×10^6	.11
	H_2O					9.3×10^3	
18	Shore	1/23/73	6.0	10.0	0.48	8.1×10^6 (Anaerobic)	.371
18	Shore	1/23/73	6.0			9.8×10^7	.371
20	Shore	2/18/73	9.5	5.0	0.43		.482
CHESTER MORSE							
7	Shore	8/22/72	17.0	1.0	3.9	2.7×10^7	.452
	31 M		6.5	10.0	0.92	1.21×10^5	.189
11	Shore	9/27/72	11.4	ND	ND	ND	ND
	31 M		7.0	7.0	0.9	ND	.35
	H_2O					4.7×10^3	
LAKE SAMMAMISH							
10	Shore	9/20/72	15.5	11.5	0.6	8.35×10^7	.6
	25 M		8.0	6.1	1.0	1.85×10^7	.135
	H_2O					8.3×10^2	
16	Shore	11/29/72	9.0	4.8	0.75	2.6×10^8	.13
	25 M		7.8	7.5	0.91	3.4×10^7	.13
	H_2O						

* Per gram dry wt. sediment or per ml H_2O

Table 3

Lake Chester Morse T° Profile 1972

Depth (meters)	8/22/72 Exp. 7 T°C	9/27/72 Exp. 11 T°C
Surface	18.0	11.4
1	17.8	11.3
2	17.8	11.2
3	17.8	11.1
4	17.7	11.1
5	17.6	11.1
6	17.0	11.1
7	16.2	11.1
8	14.2	11.1
9	12.5	10.7
10	10.9	10.5
11	10.0	10.2
12	9.4	10.0
13	8.9	9.8
14	8.6	9.4
15	8.4	9.3
16	8.2	8.8
17	8.2	8.7
18	8.0	8.3
19	8.0	8.0
20	8.0	7.9
21	7.8	7.9
22	7.8	7.9
23	7.6	7.8
24	7.6	7.5
27	7.4	7.2
30	7.0	6.8

Table 3, Cont'd.

Findley Lake T° Profile 1972

Depth (meters)	8/8/72 Exp. 6 T°C	8/30/72 Exp. 8 T°C	10/12/72 Exp. 12 T°C	11/1/72 Exp. 13 T°C	12/13/72 Exp. 17 T°C
Surface	19.5	16.9	7.8	5	0
1	19.5	16.9	7.8	5	
2	18.5	16.6	7.8	5	
3	14.0	16.5	7.8	5	
4	12.2	16.2	7.8	5	
5	10.6	15.5	7.8	5	Not Measured
6	9.5	13.7	7.8	5	
7	8.8	11.7	7.8	5	
8	7.7	10.5	7.8	5	
9	6.8	9.0	7.8	5	
10	6.0	7.9	7.8	5	
11	5.6	7.2	7.8	5	
12	5.2	6.5	7.8	5	
13	5.0	6.1	7.8	5	
14	4.8	5.8	7.5	5	
15	4.5	5.5	6.2	5	
16		5.3	5.9	5	
17		5.2	5.7	5	
18	4.2	5.0	5.5	5	
19		4.8	5.1	5	
20		4.6	5.0	5	
21	4.0	4.5	4.9	5	
22		4.5		5	
23		4.4		5	
24	4.0	4.4		5	
25				5	
26				5	
27	4.0	4.3		5	

Table 3, Cont'd.

Lake Washington T° Profile 1972

Depth (meters)	9/8/72 Exp. 9 T°C	11/15/72 Exp. 15 T°C
Surface	20.0	10.5
1	20.0	10.5
2	20.0	10.5
3	20.0	10.5
4	20.0	10.5
5	20.0	10.5
6	20.0	10.5
7	20.0	10.5
8	20.0	10.5
9	20.0	10.5
10	20.1	10.5
11	20.2	10.5
12	20.1	10.5
13	20.1	10.5
13.5	20.0	10.5
13.7	17.0	10.5
14	16.0	10.5
14.5	15.0	10.5
15	13.6	10.5
16	12.6	10.5
17	12.2	10.5
18	11.5	10.5
19	11.0	10.5
20	10.4	10.5
21	9.8	10.5
22	9.5	10.5
23	9.4	10.5
24	9.2	10.4
27	8.8	8.8

Table 3, Cont'd.

Lake Washington T° Profile 1972, Cont'd.

Depth (meters)	9/8/72 Exp. 9 T°C	11/15/72 Exp. 15 T°C
30	8.4	8.0
33	8.3	8.8
36	8.1	7.7
39	8.0	7.5
42	8.0	7.4
45	7.8	7.5
48	7.8	7.4
51	7.8	7.4
54	7.6	7.3
57	7.6	7.2
60	7.6	7.0

Table 3, Cont'd.

Lake Sammamish T° Profile 1972

Depth (meters)	9/20/72 Exp. 10 T°C	11/29/72 Exp. 16 T°C
Surface	17.6	8.9
1	17.6	8.9
2	17.8	9.0
3	17.8	9.0
4	17.7	9.0
5	17.7	9.0
6	17.8	9.0
7	17.8	8.9
8	17.7	8.9
9	17.0	8.9
9.5	16.5	8.9
10	15.4	8.9
11	13.6	8.9
12	12.0	8.9
13	10.5	8.9
14	9.6	8.9
15	9.3	8.9
16	9.0	8.9
17	8.6	8.9
18	8.5	8.9
19	8.4	8.9
20	8.2	8.3
21	8.0	8.2
22	8.0	8.1
23	8.0	8.0
24	8.0	7.9
25	8.0	7.8

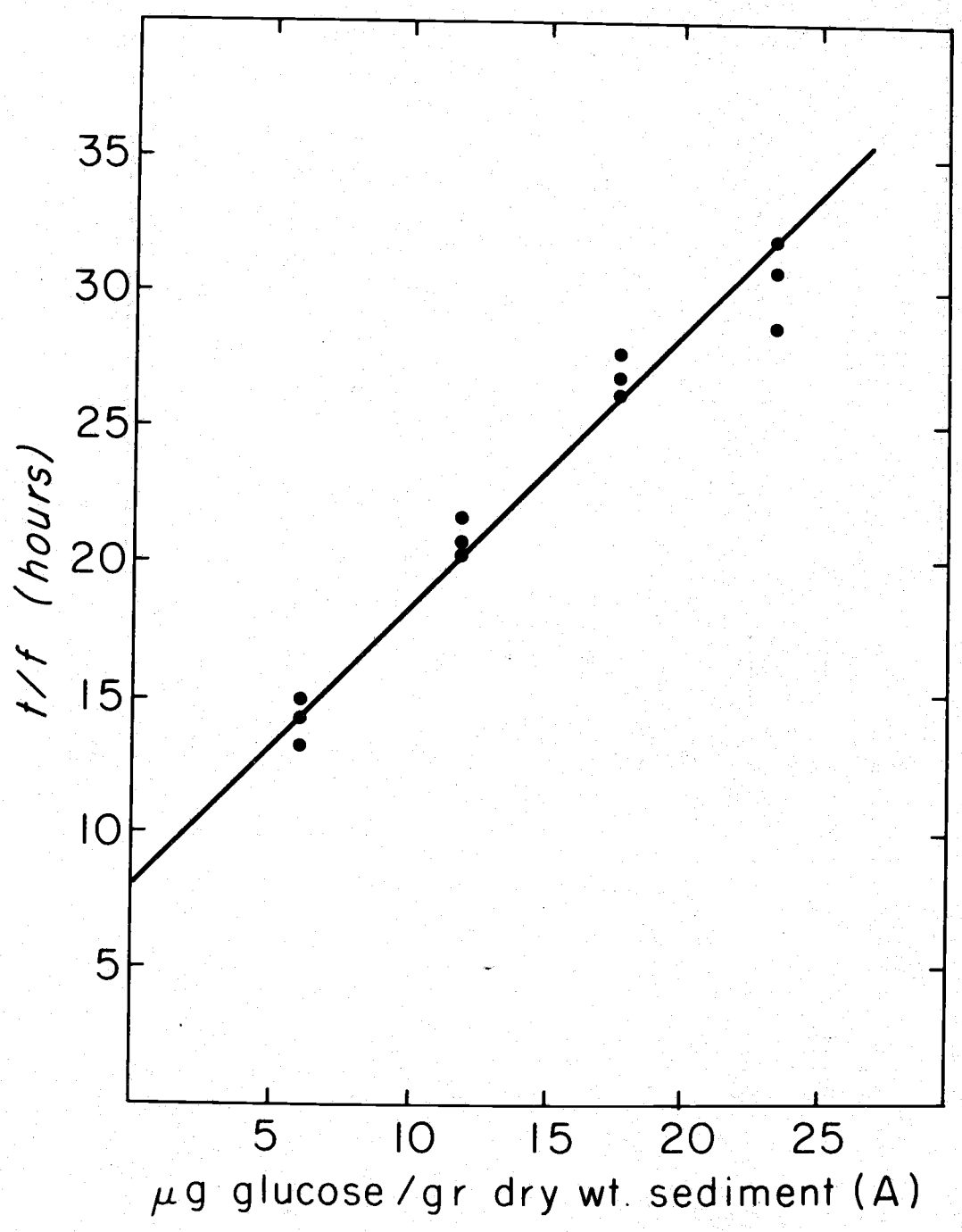

Figure 1. Typical plot of incubation time in hours/fraction of available substrate mineralized vs. the concentration of added substrate.

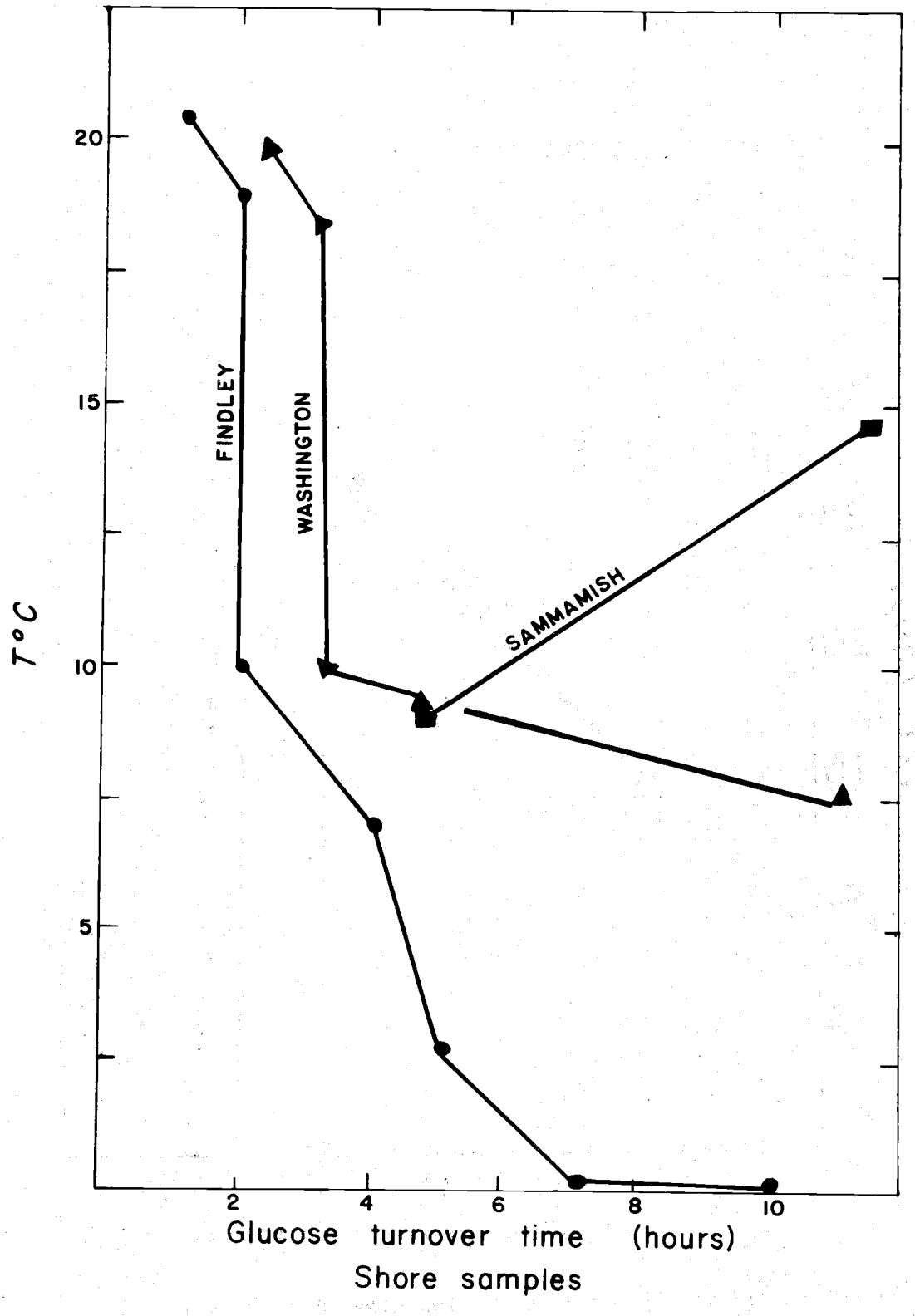

Figure 2. Results from shore sediment samples.

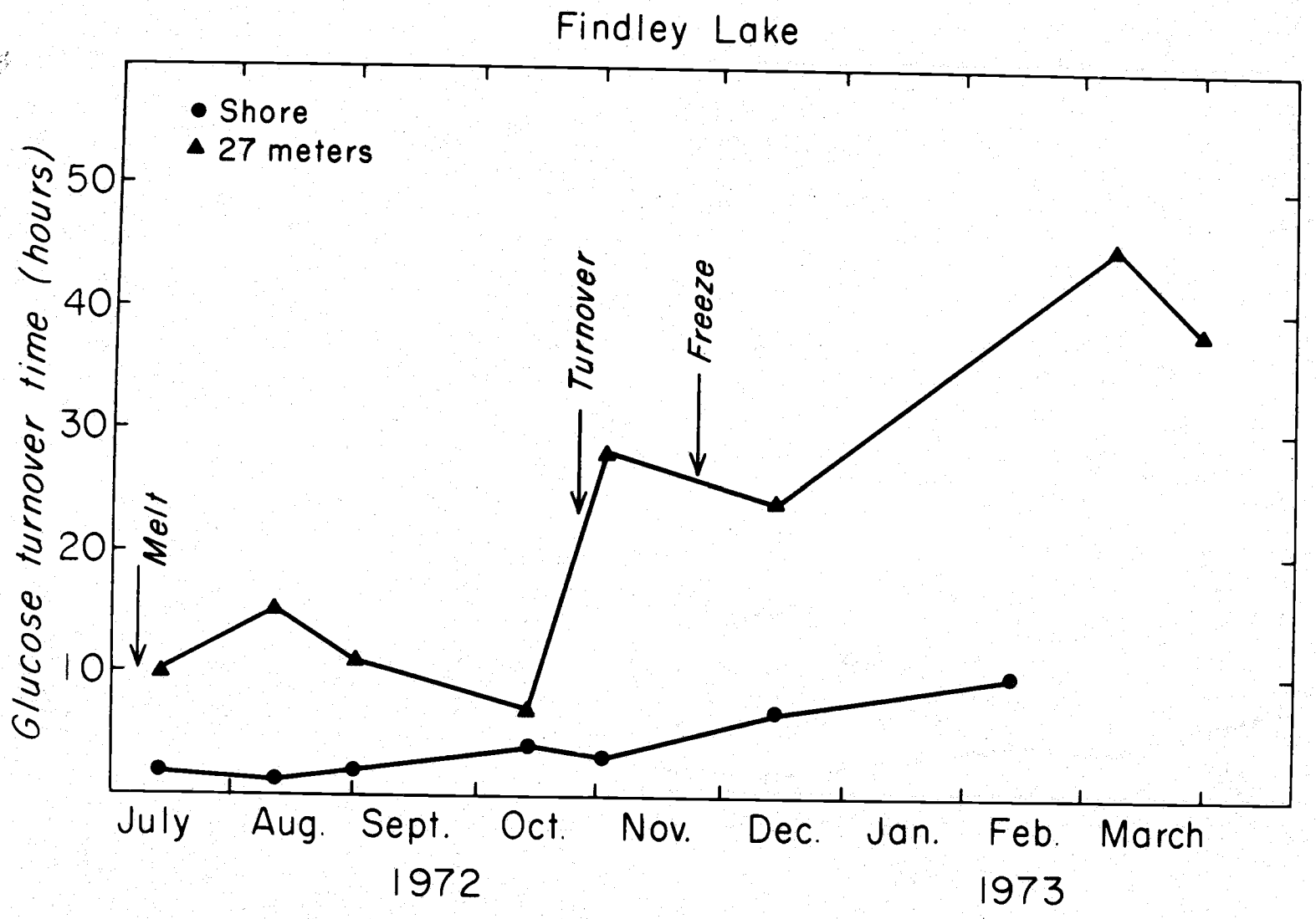

Figure 3. Seasonal variation in glucose utilization for Findley Lake.

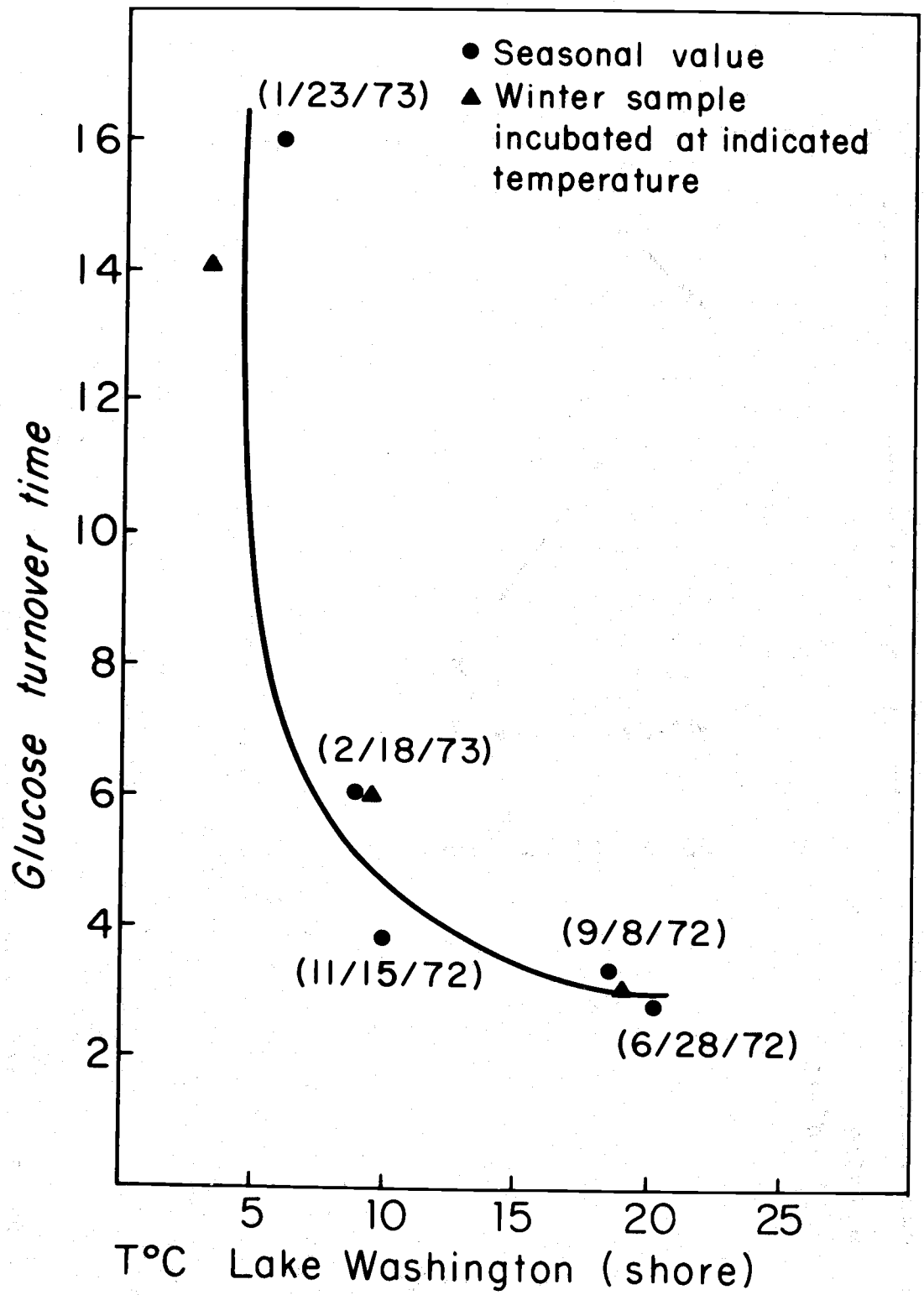

Figure 4. Differences in glucose turnover time in Lake Washington.

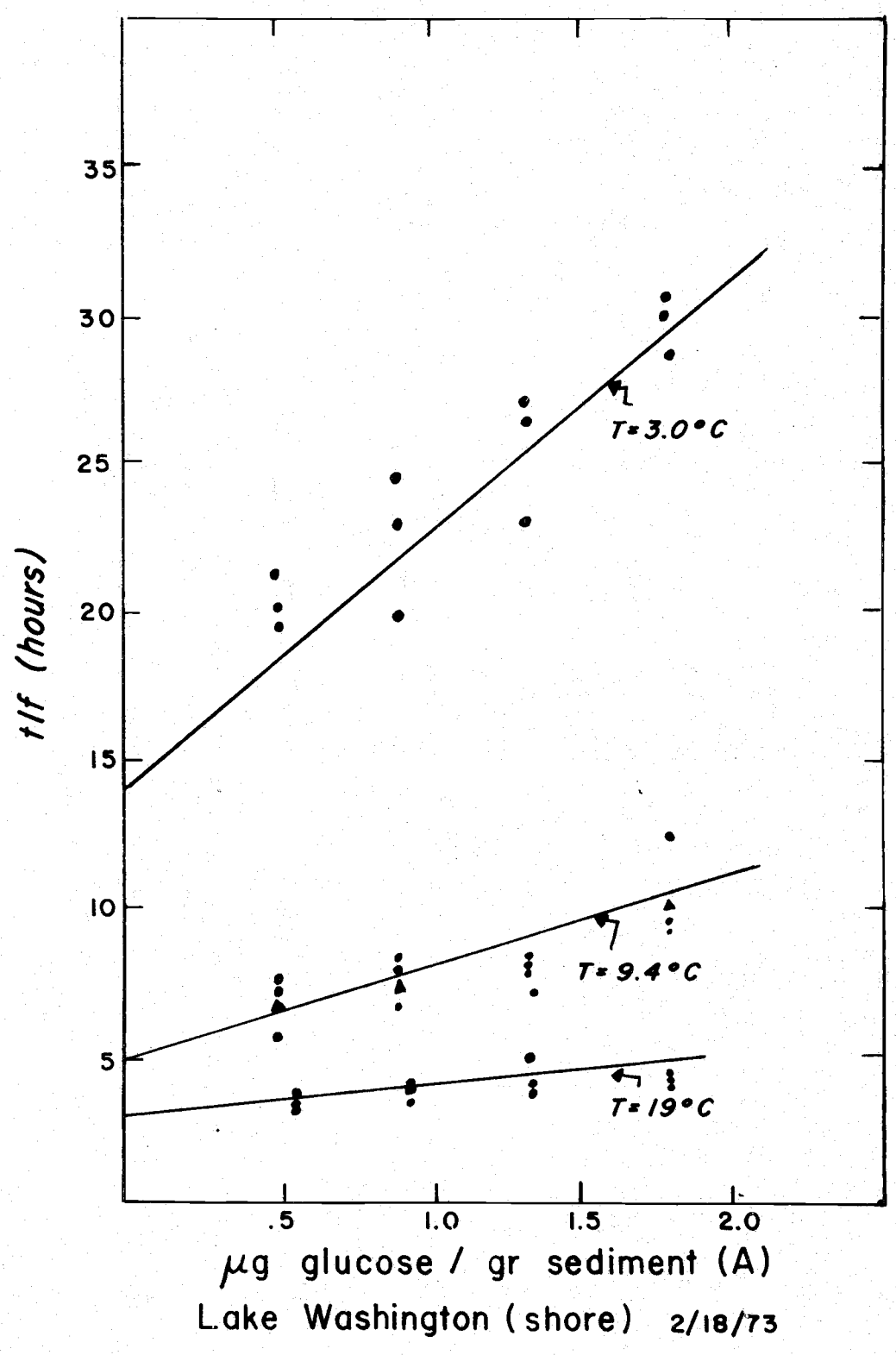

Figure 5. Plot of glucose turnover time at three temperatures for the winter sample.

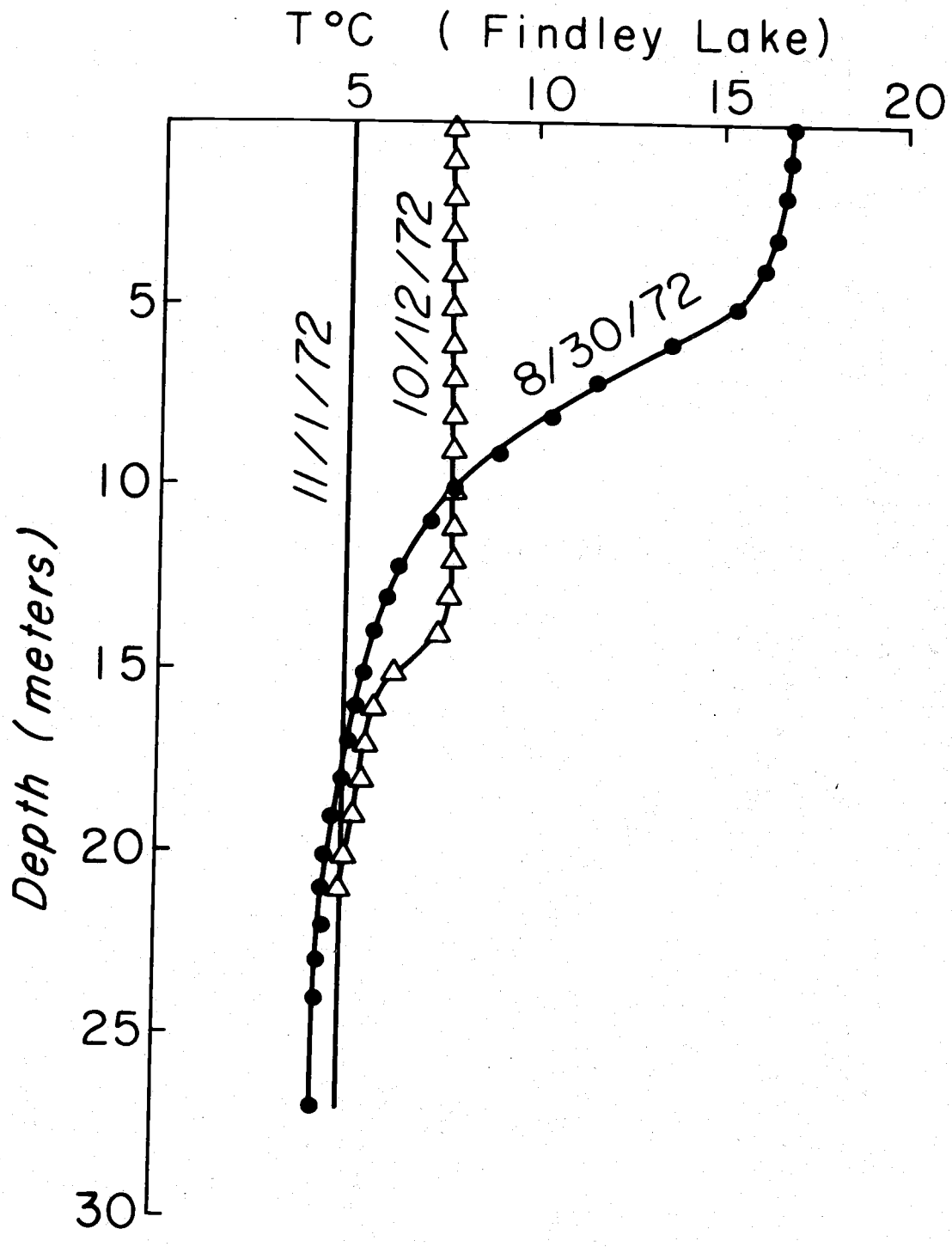

Figure 6. Temperature profiles in Findley Lake.