INTERNAL REPORT 146

DEGRADATION OF ORGANIC COMPOUNDS IN FRESHWATER SEDIMENTS BY BACTERIA

Jack R. Matches and Marleen M. Wekell
University of Washington

INTRODUCTION

Bacteria of lake sediments as in all natural environments, occupy a key position in the economy of nature. As decomposers they are the principle regulators of biological and mineral cycles of the sediment environment. In both soil and water, bacteria mineralize or decompose a wide variety of substances, many of which cannot be broken down by any other organisms. Such biochemical activities make substantial and necessary contributions to cycles of energy, carbon, nitrogen, phosphorous, sulfur, and other elements. Bacteria are thus responsible both alone and in conjunction with fungi, for the mineralization of detritus and cycling of elements.

This report discusses the mineralization of glucose to CO_2 as a measure of the activity of bacterial populations found in lake sediments. These measurements give information on the numbers of bacteria present and their activity rates. This study will be correlated with other data being collected in the overall program to help explain the behavior of benthic environments in lakes. Further studies are under way to measure more directly the actual changes taking place in the environment.

MATERIALS AND METHODS

Sampling

Sediment samples of the fine sediment-water interface were obtained monthly in Lakes Washington, Sammamish, Chester Morse, and Findley. These sediment samples were collected aseptically by means of a suction device ("goo grabber"), transferred to sterile, screw-capped glass containers, and maintained on ice until analysis. All samples were analyzed within 10 hr of collection. Samples for the shore stations were obtained with a sterile plastic 10-ml pipet, attached to a sterile rubber bulb. The top 1-2 cm of sediment was aspirated and treated as described above. Samples collected for the isolation of cellulolytic bacteria in Lake Sammamish (shore-sunken forest station) were collected with the same device at a depth of 2 m by skin diving. The top 1-2 cm of sediment around sunken tree stumps was aspirated. Upon return to the laboratory, these latter samples were serially diluted, plated out on fresh cellulose agar plates, and incubated anaerobically and aerobically at the in situ temperature.

Sediment samples were obtained in each of the four lakes at a shore and deepwater station as shown in Figure 1. In addition, various other stations were occasionally sampled and are also shown in Figure 1. Station 1 at Findley Lake is sometimes difficult to locate in the winter months because of a thick ice cover. Consequently, some of the sediments collected from this site, although close to the area of maximum depth, of 27 m. were from lesser depth.

Temperature profiles (see Appendix) were measured with a TRI-R electronic thermometer.

Analysis

Dry weight of the sediment was determined by taking the mean of replicate samples (containing 5 ml of sediment/sample in tarred aluminum cups), dried at 90°C for 24 hr.

Glucose mineralization rates of the indigenous bacteria in lake sediments were measured by the method of Harrison, Wright, and Morita (1971). Uniformly labeled ^{14}C glucose (0.1 μm Ci), plus varying amounts of unlabeled glucose (1-10 $\mu g^{-1} g^{-1}$ dry wt sediment), were incubated with 5 ml of sediment (approximately 200 mg) in sterile 50-ml serum bottles for 10 min at the in situ temperature. The $^{14}CO_2$ was collected on a filter paper (Whatman no. 1) suspended over the reaction mixture with 0.2 ml of phenylethylamine and counted in Instagel in a Packard Tri-Carb scintillation counter. Corrections for quenching were made by running a series of similarly quenched ^{14}C glucose standards.

Bacterial Biomass

The bacterial biomass was estimated for sediment samples by the plate count method. The composition of the medium (D. Lighthart, personal communication) contained the following: yeast extract, 0.2 g; sodium caseinate, 0.5 g; peptone, 0.5 g; soluble starch, 0.5 g; glycerol 1.0 g; K_2HPO_4, 0.2 g; $MgSO_4$, 0.05 g; $FeCl_3$, trace; agar, 15 g; and distilled water, 1000 ml. The medium was sterilized at 121° for 15 min. All plates were incubated at the in situ temperature. Some anaerobic plate counts were also taken.

Numbers of bacteria capable of digesting chitin or cellulose were estimated by adding reprecipitated chitin (0.6%) or reprecipitated cellulose (0.1%) to the medium described above. Chitinoclastic or cellulolytic bacteria can be easily detected on these respective media by a clear zone or plaque which appears around the colonies capable of hydrolyzing either chitin or cellulose.

Preparation of Reprecipitated Chitin and Cellulose

Reprecipitated chitin was prepared according to the method of Chan (1970). Practical-grade chitin (P-2061, Eastman Organic Chemicals) was pulverized with an electric mill and sifted through a 0.1-mm screen. Fifty grams of this chitin was slowly added with stirring to 1000 ml of 18 N sulfuric acid prechilled to 1°-4°C in a 3000-ml Erlenmeyer flask. The viscous mixture was constantly mixed until the chitin was evenly dispersed. After an hour of constant stirring, the mixture became less viscous and could be stirred with a large Mag-Mix (Precision Scientific Co., Chicago) with the flask

kept in an ice bath throughout the operation. After 10 hr of hydrolysis at low temperature, the chitin was ready to be reprecipitated from solution. The hydrolysate was then filtered through several layers of spun glass wool into 15 ℓ of tap water in a 20 ℓ carboy. Shaking the carboy several times facilitated dilution and reprecipitation. Upon standing for a day, the reprecipitated chitin separated as a mass and floated to the top buoyed by air bubbles. By careful siphoning, the acid water layer could be removed from the bottom without disturbing the reprecipitated chitin layer. The chitin was then centrifuged at 1500 rpm for 15 min and neutralized with 10 n sodium hydroxide to pH 7.0. The chitin was placed in 6.4 cm, SSDC type cellophane tubing (Union Carbide, Chicago) and dialyzed in running tapwater for 48 hr. A glass marble placed in the tubing facilitated mixing. After dialysis, the tubings with chitin were hung to evaporate at room temperature with periodic inversion to prevent uneven drying. The chitin was ready to use when most of the water had evaporated and the chitin had begun to separate from the cellophane tubing. Chitin in excess of what was needed immediately was steamed at 100°C for 15 min and kept in glass jars, at 4°-8°C.

Reprecipitated cellulose was prepared by dissolving 5 g of cellulose powder (Whatman CF 11) in a solution of 100 ml of concentrated sulfuric acid and 60 ml of distilled water at 50°C. After 20 sec, 6 ℓ of cold tap water was added to precipitate the cellulose, and the mixture was allowed to stand for a few hours or overnight. The liquid was removed by siphon, and the cellulose was washed and centrifuged (5000 rpm) once, the pH was adjusted to 7.0 with sodium hydroxide, and the cellulose was rewashed with distilled water and centrifuged (5000 rpm) five times. The cellulose slurry was autoclaved at 121°C for 15 minutes, and the dry weight was determined.

Chemicals

Uniformly labeled ^{14}C glucose (250 mci/mm) was obtained from Amersham Searle (Arlington Heights, Ill.); ^{14}C-labeled cellulose from ICN Corporation (Irvine, Calif.); Instagel and phenylethylamine from Packard Instrument Company (Downers Grove, Ill.); glucose (unlabeled) from Merck Chemical Company (Rahway, N. J.): reagents for media from Difco Company (Detroit, Mich.). All other chemicals were of analytical or of reagent grade.

Expression of Results

All data are reported in terms of dry weight of the sediment. Glucose mineralization rates are in terms of turnover time or v_{max}. Glucose turnover times are reported in hours, or the time in hours necessary for the bacteria to mineralize glucose at the in situ concentration. The v_{max} is estimated as micrograms of glucose per gram per dry weight sediment per hour. Bacterial biomass is given as numbers per gram dry weight of the sediment or if estimated for water, numbers per milliliter. All temperatures are reported in degrees Celsius. All depth measurements are given in meters.

RESULTS

Glucose Mineralization Rates

Seasonal glucose mineralization rates of lake sediment bacteria were determined for shore and deepwater stations in each of the four lakes (Tables 1-4, Figures 2-9). In each lake sampled, with the exception of Lake Sammamish in the fall, the bacteria associated with the shore sediment were more numerous and manifested a consistently greater glucose mineralization rate, than those found in the deepwater stations. In all lakes, the glucose mineralization rate was greater in summer (lower turnover time, higher V_{max} value) and reached minimum levels in winter or early spring. The greatest seasonal variation was found in Findley Lake. This lake is at the highest elevation (1131 m) of the four lakes studied and is the only lake of the study group that completely freezes during the winter. The open surface period on Findley Lake lasts approximately four months between July and October. Highest glucose mineralization rates occurred in this lake at all stations sampled in July and August in 1972 and 1973. These rates declined steadily after the lake had frozen and reached lowest values by May 1973 toward the end of the frozen period. After the lake had cleared of ice in late June, the rates abruptly increased.

Glucose mineralization rates obtained for the other three lakes did not vary as much as did those for Findley Lake. Highest rates for Chester Morse Lake occurred in July-August and lowest in February-March. In Lake Washington, the highest and lowest rates were obtained in March-June and December-February, respectively. Lake Sammamish sediment samples from all stations exhibited the lowest rates of the lakes studied when compared on the basis of V_{max} values. The highest rates occurred in July-August, and lowest in April. Also in late fall, the sediment bacteria from station 1 (25 m) had a faster rate of glucose mineralization than those associated with the shore sediment. At present, this phenomenon has only been observed with Lake Sammamish sediments. In all of the other lakes the shore sediment bacteria exhibit a higher glucose mineralization rate than do those from sediment from the deeper stations.

Samples have been collected at various stations at Lakes Washington, Chester Morse, and Findley and analyzed. In some of these lakes significant differences occur in the glucose mineralization rate between stations. In deepwater stations in Lake Washington (Table 1, Exp. 23, 24, 35, and 56) highest rates have been found at station 1 (60 m) and at station 8 at the extreme north end of the lake. Lowest values have been obtained for the stations on the northeast side of the lake near the City of Kirkland.

In deepwater stations of Chester Morse Lake (Table 3, Exp. 25) lowest rates were found for station 1 (33 m) and the highest rate near the Cedar River inlet (station 3). No significant difference in rate was observed in this lake when samples from station 1 sediment were compared with sediment obtained 33 m to the southeast at approximately the same depth (Table 3, Exp. 39) and on the same day.

The site of maximum glucose mineralization rate in Findley Lake, Table 4, (Exp. 40, 43, 48, 55, and 58) has been shore station 2 near the outlet. Higher rates occurred once in shore station 3 in July. The lower lake has also been analyzed. Sediment from this lake (shore, near Findley

Creek) exhibited rates comparable or slightly higher than those observed for shore stations at Findley Lake.

Temperature

The rate of glucose mineralization by the sediment bacteria has been found to be directly related to the temperature for shore sediment for all of the lakes when compared on the basis of glucose turnover times (Figures 10-13) and V_{max} values. A similar temperature relation exists for bacteria associated with greater depths, but the correlation is not as good as for the shore samples (Figures 14-17). When glucose turnover times are plotted against temperature at both stations for each lake, it becomes apparent that each lake has its own unique temperature relationship. For example, at 5°C the glucose turnover time for shore samples for Findley Lake are 5 hr; for Chester Morse the 11 hr; Washington, 23 hr; and Sammamish, approximately 35 hr.

When Lake Washington shore sediment samples collected in February were analyzed at varying temperatures, a close correlation was evident when compared with samples collected seasonally and run at the differing in situ temperatures (Figure 10). These data indicate that populations of bacteria responsible for glucose mineralization are influenced greatly by prevailing temperatures in the winter. When the same experiment was conducted for sediment samples collected in June the correlation was not as great. There was no significant difference in bacterial biomass (10^8/g) between the two samples. During the warmer summer months other factors besides temperature are probably involved.

Sediment from Lake Washington Station 1 (60 m) was also analyzed at temperatures up to 26°C, a temperature never encountered at this depth. A different curve was obtained when compared to the seasonal samples incubated at the in situ temperature (Figure 18).

Effects on Data Outcome

A variety of parameters capable of affecting the data were investigated. These parameters included: storage of sample, sampling device used, and effect of environmental pollutants on the sediment bacteria glucose mineralization rate.

Storage of sample for a period of 24 hr to 1 week was shown to increase the mineralization rate (Table 1, Exp. 26; Table 3, Exp. 27). These data demonstrate the necessity for analysis immediately after sample collection.

The effect of sampling device was investigated for both shore and 33 m (Station 1) stations in Lake Chester Morse (Table 3, Exp. 37). Sediment samples were collected with the suction device and a coring device. The top 3 cm of the core sample and interstitial water were transferred by pouring to a sterile glass jar. The suction device was estimated to collect approximately the top 1-2 cm and interstitital water. Glucose turnover times were higher in both shore and 33 m samples obtained with the coring device when compared to those collected with the suction device.

Preliminary experiments are now underway to determine the effects of environmental substances on the glucose mineralization rate. In one experiment diesel oil 2 was found to decrease the rate of sediment bacteria glucose utilization in Lake Washington shore sediment.

Sediment bacteria

The aerobic bacteria associated with the shore sediment were found to range from 10^6 to 10^8 g^{-1} dry wt sediment in each of the four lakes. Highest counts were found in Lake Washington and lowest in Findley Lake. Anaerobic bacteria counts ranged from 10^5 to 10^7 in these samples and were the same or an order of magnitude lower than the corresponding aerobic plate counts. The bacteria associated with the deeper sediments ranged from 10^5 to 10^8 g^{-1} dry wt sediment and were the same or an order of magnitude lower when compared to shore samples collected the same day. Anaerobes range from 10^5 to 10^7 g^{-1} dry wt sediment. In all of the lakes, both shore and deepwater sediment bacteria exhibited a slight tendency to decrease in winter and increase in numbers in the summer. All of these data are given in Tables 1-4.

Chitin

Bacteria capable of digesting chitin have been found at all of the stations sampled in each of the four lakes (Table 5). There is a slight tendency for increased numbers of chitinoclasts in terms of percent of the total bacterial population and biomass in the winter months than in the summer. Initially, sediment samples were plated out on chitin agar and incubated both anaerobically and aerobically. No chitinoclastic bacteria were isolated from the anaerobic plates, with the exception of one facultative anaerobe from Findley Lake; therefore, serial dilutions of lake sediment are presetly incubated on chitin agar plates aerobically. All of the chitinoclastic bacteria tested have been found to be obligate aerobes with the exception noted above. Several of these organisms have been isolated in pure culture and are being maintained for future work.

It appeared from the data collected that the percentage and total biomass of chitinoclastic bacteria were higher in the shore areas than in the deeper sampling stations in each of the four lakes. Also, it appeared that in Lakes Chester Morse and Findley, the percentage of aerobic chitinoclastic bacteria was higher when compared to the lakes of lower altitude, Lakes Washington and Sammamish; however, the sediment bacteria of the latter two lakes have been found to be more rapid in action with zones appearing within one week of incubation, contrasted with periods up to one month for a noticeable clear zone to form on samples incubated from Lakes Chester Morse and Findley. This phenomenon may be because of temperature. The degree of chitinoclastic activity is directly related to the temperature (Chan 1970), and Lakes Washington and Sammamish usually exhibit warmer temperatures than the other two lakes.

Cellulose

Estimating sediment bacterial cellulose decomposition rates in the four lakes has proven to be more difficult than anticipated. No rates of

cellulose decomposition are yet available for any of the four lakes. At present work is progressing with the ^{14}C cellulose. Also filter paper in sealed plastic netting has been placed in the sediment of Findley Lake for the isolation of cellulolytic bacteria. Enrichment cultures, containing a sterile solution of mineral salts and sterile strips of cigarette papers, inoculated with sediment from Lakes Washington and Findley were begun in January 1973. As of September 1973, the paper in many of these cultures grew extremely thin when compared to controls, but no cellulolytic bacteria have been successfully subcultured from them.

Recently, cellulolytic bacteria were found in shore samples of Lakes Washington and Sammamish (Table 6). So far, all of these bacteria are obligate anaerobes, are extremely difficult to obtain and maintain in pure culture and comprise a small percentage (1%-3%) of the total anaerobes counted. As yet, no aerobic cellulolytic bacteria have been found.

SUMMARY

Sampling, sampling sites, analytical methods, and results have been described in the preceding pages. Glucose mineralization rates by the sediment bacteria were obtained for Lakes Washington, Sammamish, Chester Morse, and Findley. In each lake sampled, the bacteria associated with the shore sediment were more numerous and exhibited a higher rate of glucose mineralization than those found in the sediment of deep water stations. In all lakes, the glucose mineralization rate was greater in summer, decreased in fall, and attained a minimum value in winter-early spring. As of August 1973, data was collected for one year. Glucose mineralization rates found in 1973, were quite similar to those determined for the same time last year in these lakes.

The rate of glucose utilization by the sediment bacteria was found to be directly related to the temperature with the greatest correlation existing for the shore sediment samples. A variety of other factors were also found to affect the outcome of the data such as storage of the sample, and the sampling device used.

In many of the lakes, significant differences occur in the glucose mineralization rate at various stations sampled, although no difference occurred between samples obtained 33 m apart.

Aerobic and anaerobic bacterial plate counts are given. Aerobic and shore sediment counts tend to be an order of magnitude higher than those determined for the anaerobic and deeper water station counts respectively.

Bacteria capable of digesting chitin were found in all stations sampled in the four lakes. All of the chitinoclasts tested are obligate aerobes, with one exception. The total biomass and percentage of total aerobes counted tend to be higher for shore samples when compared to the deeper stations. There is a tendency for increased numbers of chitinoclasts in the winter months than in the summer. Highest numbers of chitinoclasts were found in the shore stations at Lakes Chester Morse and Findley; however, the organisms capable of degrading chitin in Lakes Washington and Sammamish are more rapid in action.

Cellulolytic bacteria have been found in Lakes Sammamish and Washington. These organisms are all obligate anaerobes.

All of the data described in this report are now in the computer data bank and in notebooks maintained by the authors. Future work will include a more detailed analysis of rates of chitin and cellulose decomposition in the four lakes; temperature and environmental effects on sediment bacterial degradation of cellulose, chitin, and other carbohydrates; and a continuation of the determination of glucose mineralization by lake sediment bacteria.

ACKNOWLEDGMENT

We express thanks to the Pacific Fisheries Technology Laboratory and its director, Dr. Maynard Steinberg, for the use of the Packard Tri-Carb scintillation counter.

REFERENCES

CHAN, JOHN G. 1970. The occurrence, taxonomy, and activity of chitinoclastic bacteria from sediment, water, and fauna of Puget Sound. Ph.D. thesis, Univ. Washington, Seattle. p. 66-68.

HARRISON, M. J., R. T. WRIGHT, and R. Y. MORITA. 1971. Method for measuring mineralization in lake sediments. Appl. Micro. 21. p. 698-702.

Table 1. Lake Washington

Exp. no.	Sample depth[a]	Date	Temp (°C)	Turnover time (hr)[b]	V_{max} (µg glu g^{-1} sed hr^{-1})[c]	Plate count[c] Aerobic	Plate count[c] Anaerobic
		1972					
3	shore	28 Jun	20.0	2.4	46	7.5×10^8	
	4 m			2.6	23	2.2×10^8	
9	shore	8 Sep	18.5	7.5 ± 4	1.03 ± 0.3	7.6×10^7	
	60 m (1)		8.0	7.5 ± 1	0.75 ± 0.1	6.65×10^6	
	H_2O		18.5	100.0		1.6×10^3	
15	shore	5 Nov	10.0	3.4 ± 0.2	8.3 ± 0.2	1.2×10^8	
	60 m (1)		7.0	17.8 ± 3.5	0.23 ± 0.1	3.6×10^6	
	H_2O		10.0			9.3×10^3	
		1973					
18	shore	23 Jan	6.0	17.7 ± 2.8	0.63 ± 0.12	9.7×10^7	
20	shore	18 Feb	3.0	15.0 ± 1.3	0.13 ± 0.1	1.7×10^8	
	shore		9.5	5.2 ± 0.8	0.41 ± 0.2	1.7×10^8	
	shore		19.0	3.4 ± 0.3	2.8 ± 0.8	1.7×10^8	
23	60 m (1)	6 Mar	6.5	2.3 ± 1.4	0.15 ± 0.04	6.3×10^6	8.75×10^5
24	14 m (3)	7 Mar	7.0	7.0 ± 0.2	9.2 ± 0.6	8.3×10^6	6.2×10^6
	45 m (5)		7.0	6.2 ± 3.7	0.21 ± 0.1	3.1×10^6	7.1×10^6
	8 m (7)		7.0	8.0 ± 2.8	0.35 ± 0.1	9.4×10^6	1.3×10^7
	30 m (4)		7.0	23.2 ± 6.0	0.41 ± 0.4	2.1×10^6	5.7×10^6
29	shore	10 Apr	10.2	3.2	2.6	1.5×10^8	
	shore	11 Apr	11.2	2.7 ± 0.1	7.2 ± 0.2	1.5×10^8	
	shore + oil	11 Apr	11.2	2.3 ± 0.1	1.2 ± 0.05	1.3×10^8	
32	shore	26 Apr	13.0	1.9 ± 0.2	9.3 ± 0.3	9.7×10^8	3.8×10

Table 1 (cont.)

Exp. no.	Sample depth[a]	Date	Temp (°C)	Turnover time (hr)[b]	V_{max} (µg glu g^{-1} sed hr^{-1})[c]	Plate count[c] Aerobic	Plate count[c] Anaerobic
35	(2)	10 May	7.0	32.0 ± 5.8	0.02 ± 0.02	1.2×10^7	
	(5)		7.0	9.8 ± 2.0	0.14 ± 0.08	1.4×10^7	
	(8)		13.0	7.2 ± 1.1	0.13 ± 0.09	9.6×10^6	
41	60 m (1)	19 Jun	7.6	7.1 ± 1.3	0.26 ± 0.06	2.4×10^7	
	60 m (1)		17.0	4.6 ± 1.1	0.45 ± 0.09	2.4×10^7	
42	shore	26 Jun	5.0	5.2 ± 0.5	0.88 ± 0.1	6.3×10^8	
	shore		11.0	3.6 ± 0.5	1.1 ± 0.1	6.3×10^8	
	shore		18.0	3.4 ± 0.2	3.31 ± 0.2	6.3×10^8	
49	60 m (1)	31 Jul	8.5	3.95 ± 1.3	0.53 ± 0.09	5.2×10^7	7.7×10^5
51	shore	20 Aug	19.0	5.5 ± 0.8	1.1 ± 0.4	1.7×10^8	
53	62 m (1)	23 Aug	8.5	14.8 ± 2.0	0.4 ± 0.1	9.2×10^6	
			12.5	5.0 ± 2.0	0.2 ± 0.08		
			26.0	4.7 ± 0.9	0.9 ± 0.1		
56	9 m (8)	25 Sep	17.5	6.0	2.0	4.4×10^7	
	21 m (2)		11.0	9.00	0.85	5.6×10^7	
	60 m (1)		8.2	5.0	0.17		
	51 m (5)		7.5	10.0	0.15		

[a]Numerals in parentheses indicate station numbers.

[b]Mean ± standard deviation.

[c]per gram dry weight sediment.

Table 2. Lake Sammamish.

Exp. no.	Sample depth	Date	Temp (°C)	Turnover time (hr)[a]	V_{max} (µg glu g^{-1} sed hr^{-1})	Plate count[b] Aerobic	Plate count[b] Anaerobic
		1972					
10	shore	20 Sep	15.0	11.5	0.6	8.4×10^7	
	25 m		8.0	6.1	1.0	1.9×10^7	
	H_2O		15.0			8.3×10^2	
16	shore	29 Nov	9.0	8.1 ± 1.3	0.85 ± 0.1	2.8×10^8	
	26 m		8.0	5.1 ± 0.5	0.89 ± 0.06	3.4×10^7	
		1973					
30	shore	13 Apr	11.2	8.3 ± 1.0	0.17 ± 0.6	5.4×10^6	
	26 m		7.0	19.5 ± 2.0	0.06 ± 0.08	3.8×10^6	
33	shore	27 Apr	10.0	12.2 ± 1.6	0.18 ± 0.1	2.3×10^7	
	27 m		7.0	18.2 ± 0.9	0.42 ± 0.1	1.4×10^7	
36	shore	11 May	16.0	13.4 ± 0.7	0.5 ± 0.1	2.1×10^7	
	30 m		7.0	17.6 ± 1.7	0.2 ± 0.07	1.3×10^7	
45	shore	13 Jul	25.0	8.5 ± 2.0	0.25 ± 0.2	1.0×10^7	2.12×10^6
	27 m		8.5	11.3 ± 0.7	0.96 ± 0.1	6.3×10^6	4.5×10^5
46	shore	17 Jul	26.0	5.3 ± 0.2	1.3 ± 0.1	1.6×10^7	
	27 m		7.2	15.0 ± 1.7	0.89 ± 0.1	1.3×10^7	
50	shore	2 Aug	24.0	5.3 ± 1.9	1.16 ± 1.1	3.2×10^7	
	25 m		7.6	10.6 ± 1.0	1.3 ± 0.2	1.2×10^7	
52	shore	21 Aug	20.5	7.6 ± 0.2	2.15 ± 0.2		3.1×10^6
	25 m		8.2	6.7 ± 0.6	0.81 ± 0.1		
57	shore	2 Oct	18.5	9.4	1.68	3.0×10^7	
	26 m		8.0	7.0	0.57		

[a]Mean ± standard deviation.

[b]Per gram dry weight sediment.

Table 3. Lake Chester Morse

Exp. no.	Sample depth[a]	Date	Temp (°C)	Turnover time (hr)[b]	V_{max} (µg glu g^{-1} sed hr^{-1})	Plate count[c] Aerobic	Plate count[c] Anaerobic
		1972					
7	shore	22 Aug	17.0	1.0 ± 0.7	4.09 ± 0.2	2.7×10^7	
	31 m (1)			10.0	0.92	1.2×10^5	
11	31 m (1)	27 Sep	7.0	8.12 ± 2.3	0.94 ± 0.1		
	H_2O		11.0			4.7×10^3	
21	shore	1973					
21	shore	28 Feb	3.1	6.7 ± 1.4	0.27 ± 0.1	2.1×10^7	
			7.7	1.8 ± 1.2	0.15 ± 0.07	2.1×10^7	
			25.8	6.0 ± 2.1	0.52 ± 0.41	2.1×10^7	
25	31 m (1)	8 Mar	2.2	11.6 ± 8	0.16 ± 0.06	3.4×10^8	3.5×10^5
	31 m (3)		2.2	2.3 ± 2.1	0.09 ± 0.09	2.6×10^7	2.7×10^5
	31 m (4)		2.2	8.9 ± 2.8	0.11 ± 0.1	2.6×10^6	2.0×10^5
26	shore	20 Mar	3.5	16.5 ± 1.8	5.4 ± 0.6	1.1×10^9	
	30 m (1)		3.5	51.0 ± 9.4	1.3 ± 4.6	1.6×10^6	
28	shore	3 Apr	3.6	33.2 ± 15.5	0.04 ± 0.02	3.07×10^6	
	32 m (1)		3.5	38.9 ± 10.7	0.11 ± 0.4	5.3×10^5	
31	32 m	18 Apr	5.0	6.29 ± 0.5	1.07 ± 0.23	6.1×10^6	6.0×10^5
34	shore	1 May	7.4	5.0 ± 0.6	1.5 ± 0.1	1.3×10^7	
	32 m (1)		4.6	9.2 ± 1.6	0.42 ± 0.1	1.3×10^8	
37	shore core	22 May	12.6	13.5 ± 0.7	0.47 ± 0.1	7.7×10^6	
			12.0	5.6 ± 0.7	0.66 ± 0.1	4.7×10^7	
	31 m core		5.5	18.8 ± 5.9	0.12 ± 0.1	2.6×10^6	
	31 m gg		5.5	8.5 ± 1.4	0.22 ± 0.05	6.1×10^6	
39 A	32 m	5 Jun	6.8	7.4 ± 0.4	0.68 ± 0.1	3.1×10^7	
39 B	32 m		6.8	8.8 ± 0.6	0.51 ± 0.06	2.3×10^7	

Table 3. Lake Chester Morse (cont.)

Exp. no.	Sample depth[a]	Date	Temp (°C)	Turnover time (hr)[b]	V_{max} (µg glu g^{-1} sed hr^{-1})	Plate count[c]	
						Aerobic	Anaerobic
		1973					
44	31 m	10 Jul	6.5	9.4 ± 1.0	0.55 ± 0.1	7.3×10^6	4.8×10^6
47	shore	24 Jul	18.4	1.9 ± 0.3	0.73 ± 0.1	2.1×10^7	
	33 m (1)		6.0	7.1 ± 2.8	0.10 ± 0.05		
54	shore	28 Aug	16.0	6.0 ± 0.8	4.4 ± 0.7		
	33 m (1)		7.0	11.4 ± 0.9	0.27 ± 0.06		
59	shore	6 Oct	8.5	5.0	1.0		
	33 m (1)		7.5	6.0	1.34		

[a]Numerals in parentheses indicate station numbers.

[b]Mean ± standard deviation.

[c]Per gram dry weight sediment.

Table 4. Findley Lake

Exp. no.	Sample depth[a]	Date	Temp (°C)	Turnover time (hr)[b]	V_{max} (µg glu g^{-1} sed hr^{-1})	Plate count[c] Aerobic	Plate count[c] Anaerobic
		1972					
4.5	shore (2)	7 Jul	10.0	2.0	30.0	9.4×10^7	
	27 m		4.0	10.0	2.0	3.4×10^5	
6	shore (2)	8 Aug	20.5	1.0 ± 0.1	8.7 ± 0.1	1.1×10^9	
	27 m		4.0	11.5 ± 2.5	0.75 ± 0.1	1.0×10^7	
8	shore (2)	30 Aug	19.0	1.9 ± 0.5	5.5 ± 0.3	2.4×10^7	
	27 m		4.5	7.9 ± 1.8	0.84 ± 0.1	1.1×10^6	
	H_2O		19.0	250.0		1.2×10^3	
12	shore (2)	12 Oct	7.0	4.0	6.5	3.3×10^6	
	20 m		5.0	6.7 ± 4.0	1.18 ± 0.1	1.6×10^5	
	H_2O		7.0			2.3×10^3	
13	shore (2)	1 Nov	5.0	3.0 ± 0.5	7.5 ± 0.09	3.9×10^6	
	27 m		5.0	35.8 ± 19.0	0.66 ± 0.3	7.6×10^5	
	H_2O		5.0			4.0×10^2	
13.4	27 m	7 Nov	5.0	45.8 ± 12.5	0.16 ± 0.3	7.6×10^5	
17	shore (2)	13 Dec	2.0	3.2 ± 2.4	0.34 ± 0.1	1.2×10^{10}	1.3×10^6
	15 m		2.0	30.3 ± 4.0	0.4 ± 0.1	1.3×10^9	4.2×10^7
	H_2O		2.0			5.2×10^4	40
		1973					
19	shore (2)	9 Feb	0.5	10.0	0.42 ± 0.1	2.3×10^6	5.0×10^5
	H O	9 Feb	0.5			1.5×10^4	1.4×10^3
22	12 m	5 Mar	3.5	46.0 ± 8.0	0.18 ± 0.3	6.7×10^7	1.5×10^5
27	23 m	27 Mar	4.0	32.0	0.09	2.9×10^6	3.3×110^6

Table 4. Findley Lake (cont.)

Exp. no.	Sample depth[a]	Date	Temp (°C)	Turnover time (hr)[b]	V_{max} (µg glu g^{-1} sed hr^{-1})	Plate count[c]	
						Aerobic	Anaerobic
		1973					
38	shore (2)	24 May	4.2	22.4 ± 2.0	0.27 ± 0.1	9.7×10^6	
	26 m		3.8	64.5 ± 20.0	0.08 ± 0.1	$2.7\text{-}\times 10^5$	
40	27 m	7 Jun	4.4	12.8 ± 2.0	0.3 ± 0.1	7.2×10^6	
	shore (4)		4.5	5.3 ± 0.3	8.6 ± 1.0	3.9×10^7	
	lower lake		5.0	6.2 ± 0.7	4.5 ± 0.2	1.5×10^8	
	shore (2)		5.0	2.0 ± 0.4	0.6 ± 0.1	1.8×10^7	
43	27 m	5 Jul	4.8	17.5 ± 3.0	0.38 ± 0.2	1.1×10^7	3.4×10^6
	lower lake		11.5	4.5 ± 0.3	0.68 ± 0.2	1.4×10^8	3.1×10^7
	shore (3)		13.0	3.9 ± 0.3	2.1 ± 0.1	1.1×10^8	4.5×10^7
	shore (2)		13.0	3.9 ± 0.3	1.7 ± 0.1	1.6×10^8	2.1×10^7
48	shore (3)	26 Jul	23.0	2.4 ± 0.1	4.4 ± 0.1	2.0×10^7	2.0×10^6
	shore (2)		23.0	2.17 ± 0.4	23.6 ± 0.4	2.4×10^7	1.1×10^5
	27 m		4.5	4.9 ± 2.0	0.34 ± 0.1	8.0×10^6	1.7×10^6
55	shore (2)	30 Aug	13.5	5.5 ± 0.2	9.8 ± 0.2	6.4×10^7	
	26 m		6.0	13.6 ± 0.9	0.37 ± 0.06	4.3×10^6	
	shore (3)		14.5	4.6 ± 0.2	14.2 ± 0.2	1.0×10^9	
	lower lake		13.5	3.7 ± 0.2	5.2 ± 0.1	3.8×10^9	
58	shore (2)	4 Oct	12.5	4.7	3.54	4.8×10^7	
	27 m		6.5	19.0	0.29		
	shore (3)		12.5	4.0	1.5	4.5×10^8	

[a]Numerals in parentheses indicate station numbers.

[b]Mean ± standard deviation.

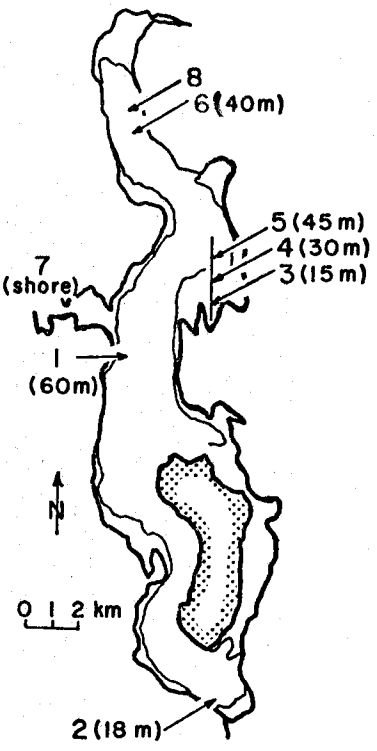
[c]Per gram dry weight sediment.

Table 5. Chitinoclastic bacteria.

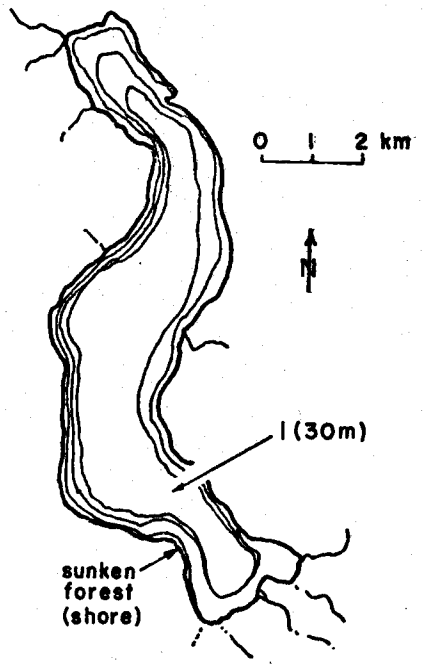
Exp. no.	Lake	Sample depth[a]	Date	Chitinoclasts of total aerobes counted (%)	Biomass chitinoclasts per gram dry wt. sediment
			1973		
20	Washington	shore	2/18/73	3.1	5.2×10^6
29		shore	4/10/73	2.7	3.7×10^6
42		shore	6/26/73	1.5	1.6×10^6
49		60 m (1)	7/31/73	1.2	6.7×10^5
51		shore	8/20/73	7.1	1.8×10^7
56		21 m (2)	9/25/73	7.8	8.0×10^5
			1973		
30	Sammamish	shore	4/13/73	2.6	1.5×10^5
50		shore	8/2/73	6.0	1.5×10^6
50		25 m (1)	8/2/73	1.5	2.0×10^5
52		shore	8/21/73	0.7	2.6×10^6
			1973		
21	Chester Morse	shore	2/28/73	80.0	1.7×10^7
28		shore	4/3/73	10.5	5.7×10^5
44		33 m (1)	7/10/73	14.5	9.5×10^5
			1972		
12	Findley	20 m (1)	10/12/72	1.8	3.9×10^5
12		shore (2)	10/12/72	2.4	2.2×10^6
13		H_2O (inshore)	11/1/72	4.4	20/ml
			1973		
19		H_2O (inshore)	2/9/73	10.5	1.3×10^3
22		12 m (1)	3/5/73	40.0	4.5×10^6
43		27 m (1)	7/5/73	1.2	5.9×10^4
43		lower lake	7/5/73	2.4	1.7×10^6
43		shore (3)	7/5/73	4.0	3.6×10^6
43		shore (2)	7/5/73	0.4	7.7×10^5
		shore (2)	8/16/73	3.1	3.5×10^6
		shore (3)	8/13/73	8.6	7.5×10^5
58		shore (3)	10/4/73	1.8	1.7×10^5

Table 6. Cellulolytic bacteria.

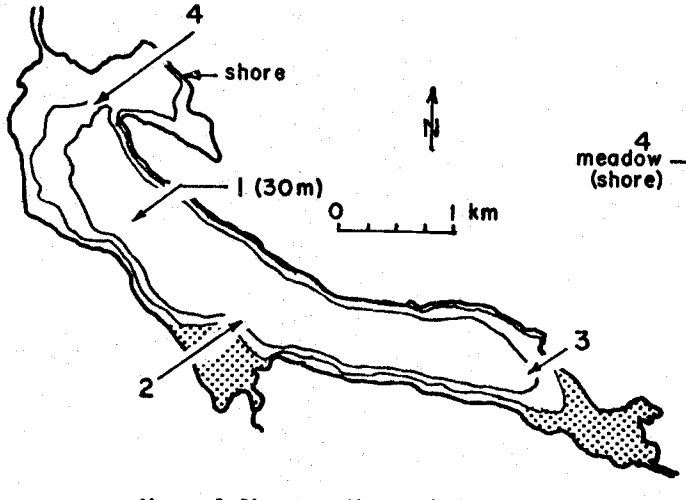
Exp. no.	Lake	Sample depth[a]	Date (1973)	Cellulolytic bacteria of total anaerobes counted (%)	Biomass cellulolytic bacteria per gram dry wt. sediment
32	Washington	shore	4/26/73	1.3	5.6×10^4
45	Sammamish	shore	7/13/63	2.5	3.1×10^3
45		27 m (1)	7/13/73	1.2	5.3×10^3
52		shore	8/21/73	2.4	8.6×10^4



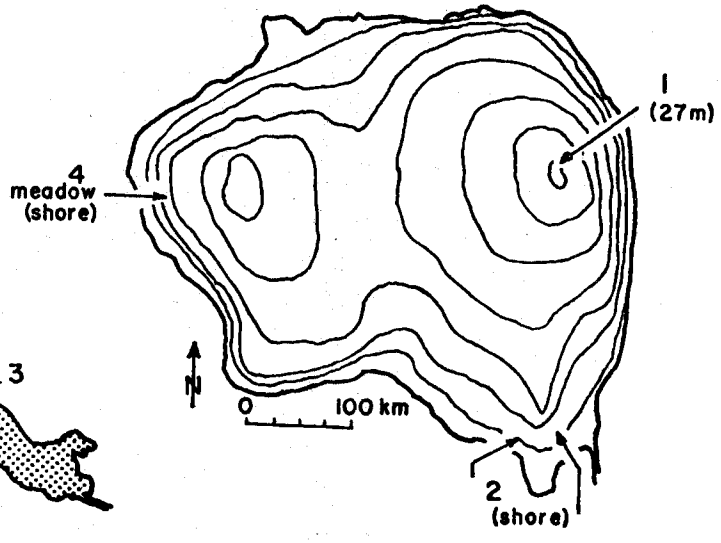
Map of Lake Washington



Map of Lake Sammamish



Map of Chester Morse Lake



Map of Findley Lake

Figure 1. Sampling stations with recorded depths at each of the four lakes within the Cedar River watershed.

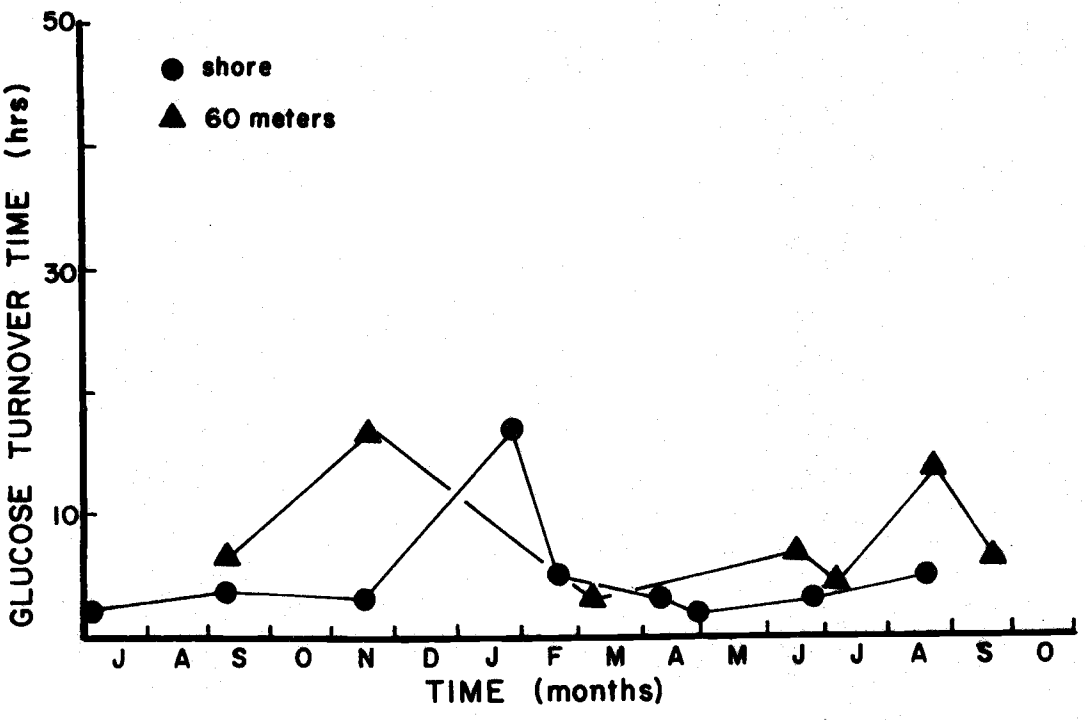


Figure 2. Seasonal glucose turnover time of sediments at two locations in Lake Washington during 1972 and 1973.

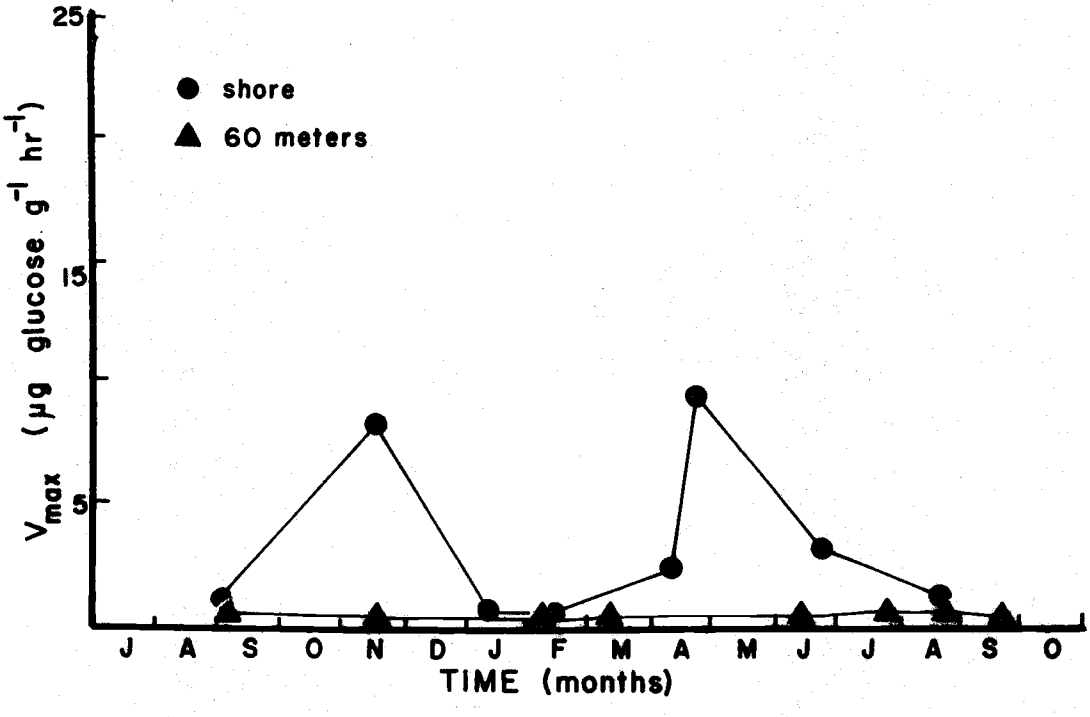


Figure 3. Seasonal V_{max} values of sediments at two locations in Lake Washington during 1972 and 1973.

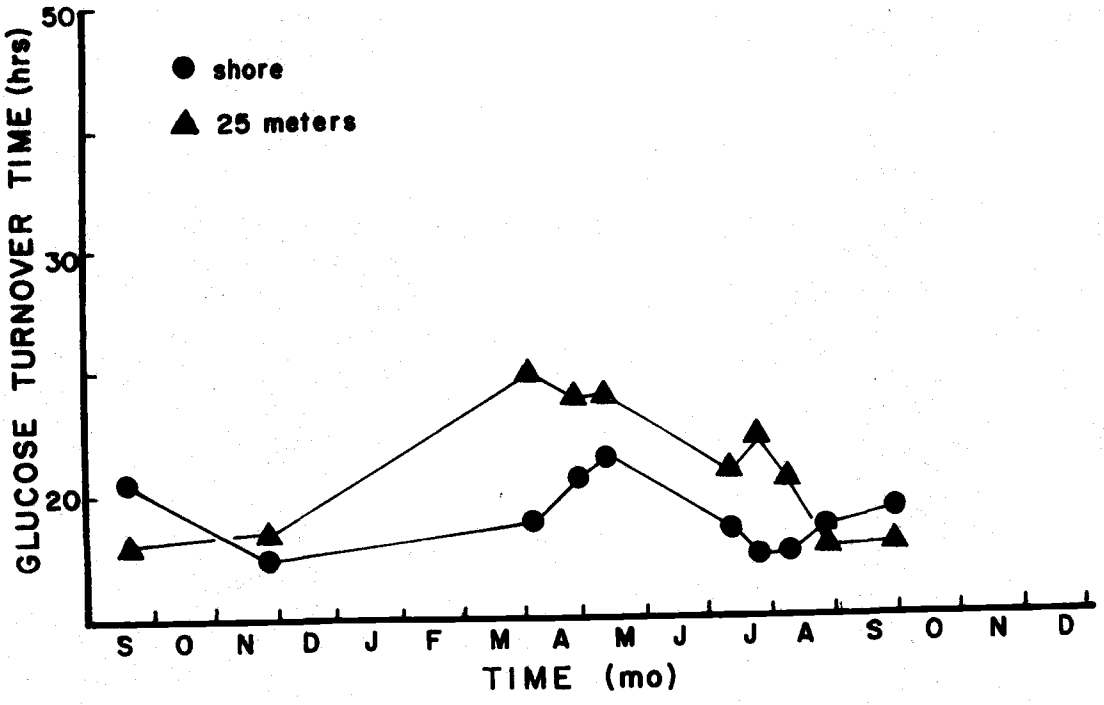


Figure 4. Seasonal glucose turnover time of sediments at two locations in Lake Sammamish during 1972 and 1973.

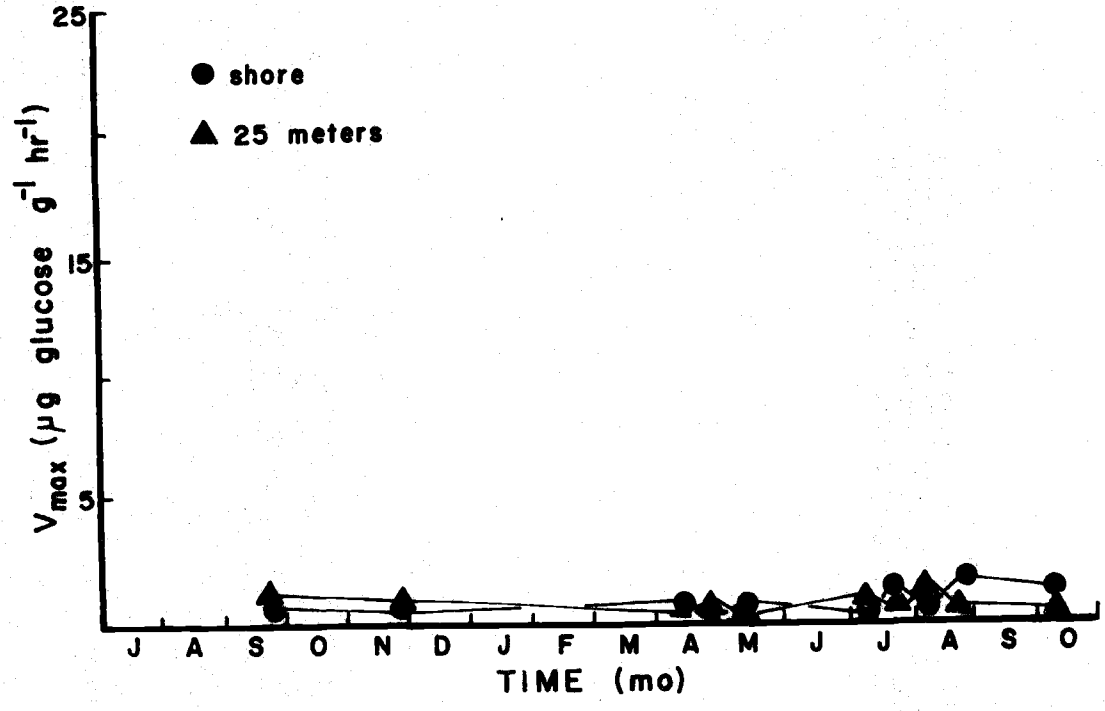


Figure 5. Seasonal V_{max} values of sediments at two locations in Lake Sammamish during 1972 and 1973.

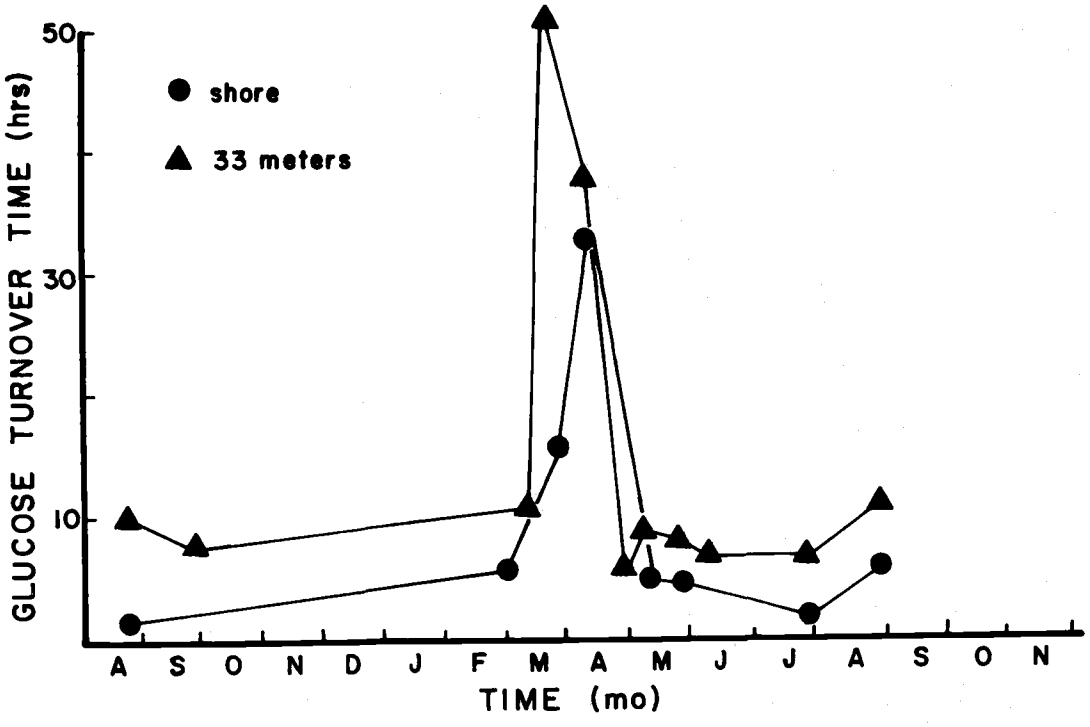


Figure 6. Seasonal glucose turnover time of sediments at two locations in Lake Chester Morse during 1972 and 1973.

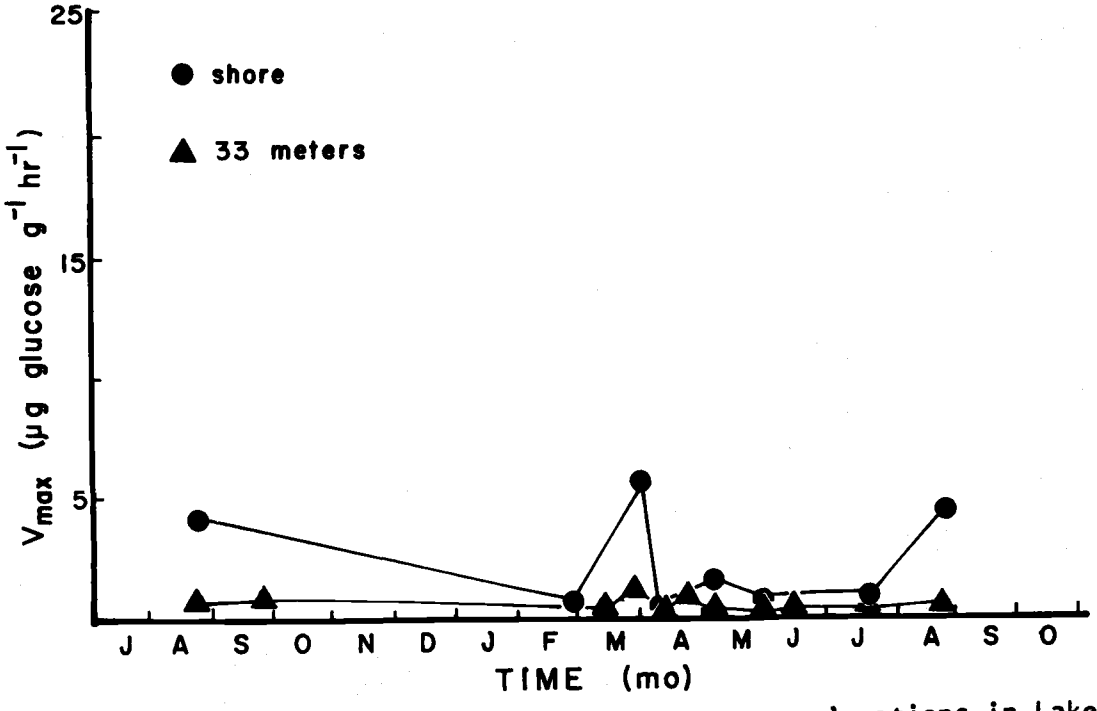


Figure 7. Seasonal V_{max} values of sediments at two locations in Lake Chester Morse in 1972 and 1973.

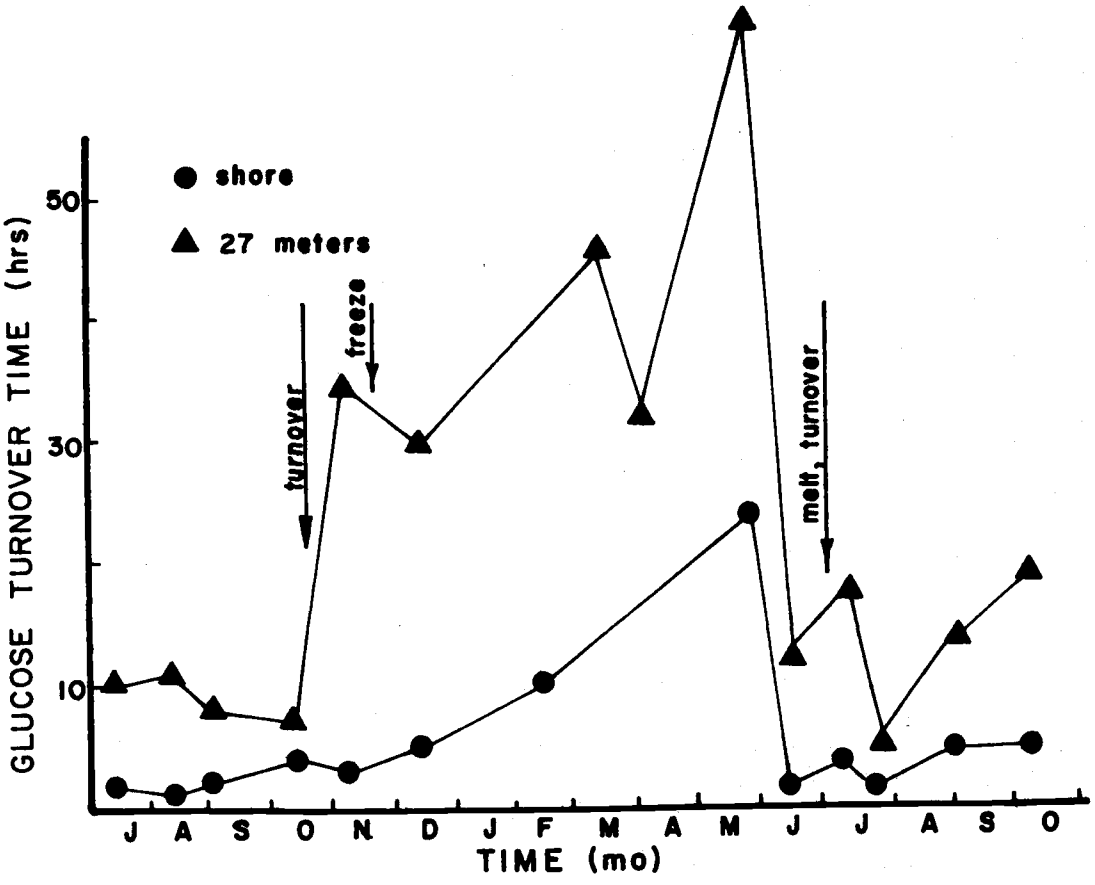


Figure 8. Seasonal glucose turnover time of sediments at two locations in Findley Lake during 1972 and 1973.

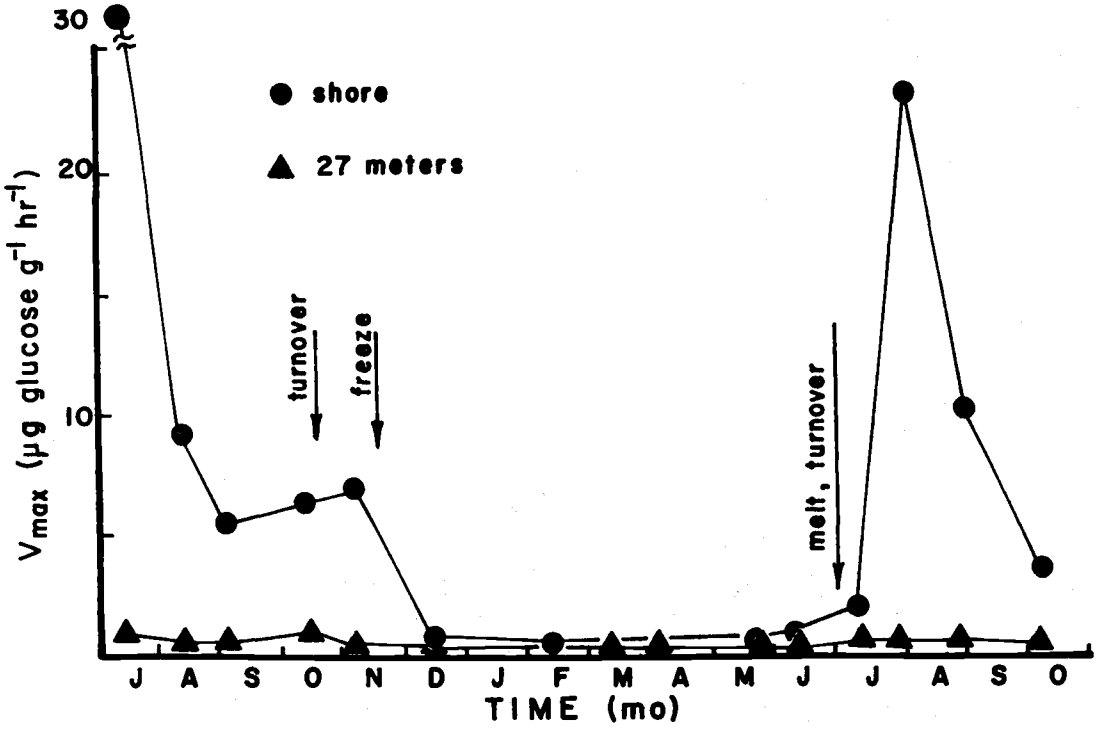


Figure 9. Seasonal V_{max} values of sediments at two locations in Findley Lake during 1972 and 1973.

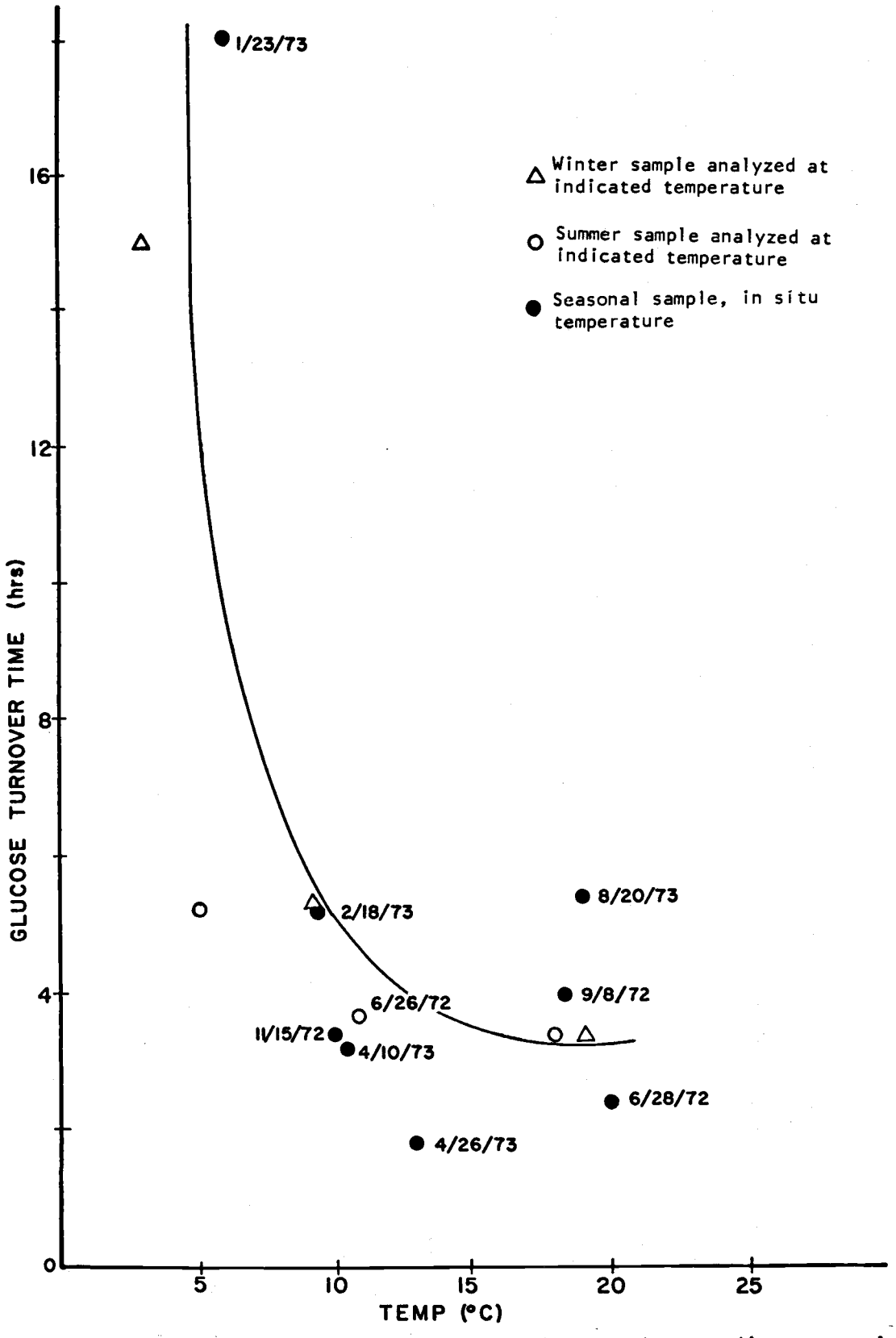


Figure 10. Glucose turnover time in Lake Washington shore sediment samples incubated at various temperatures. Seasonal sediment samples were incubated at the in situ temperatures.

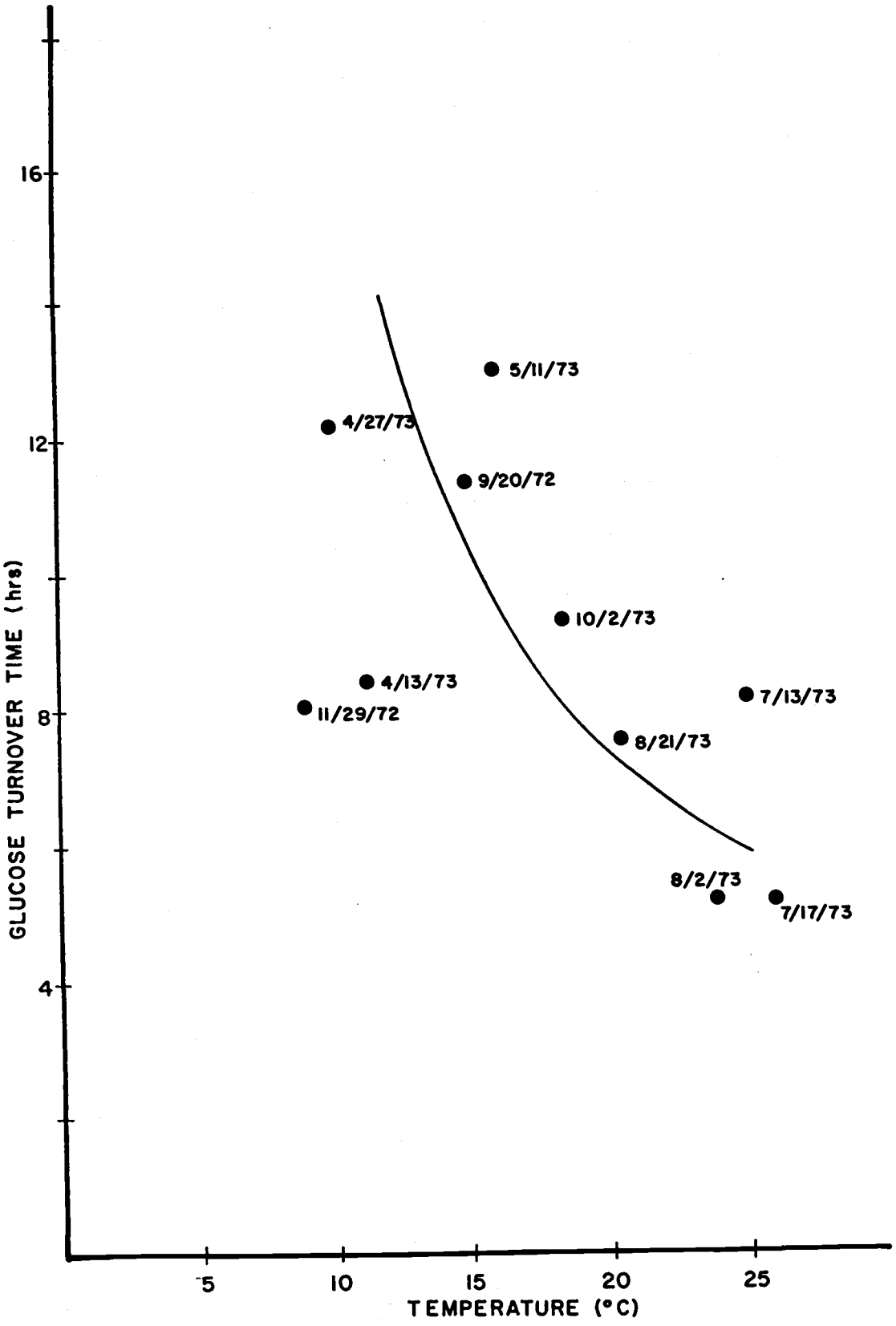


Figure 11. Glucose turnover time with respect to seasonal temperature in Lake Sammamish shore sediment.

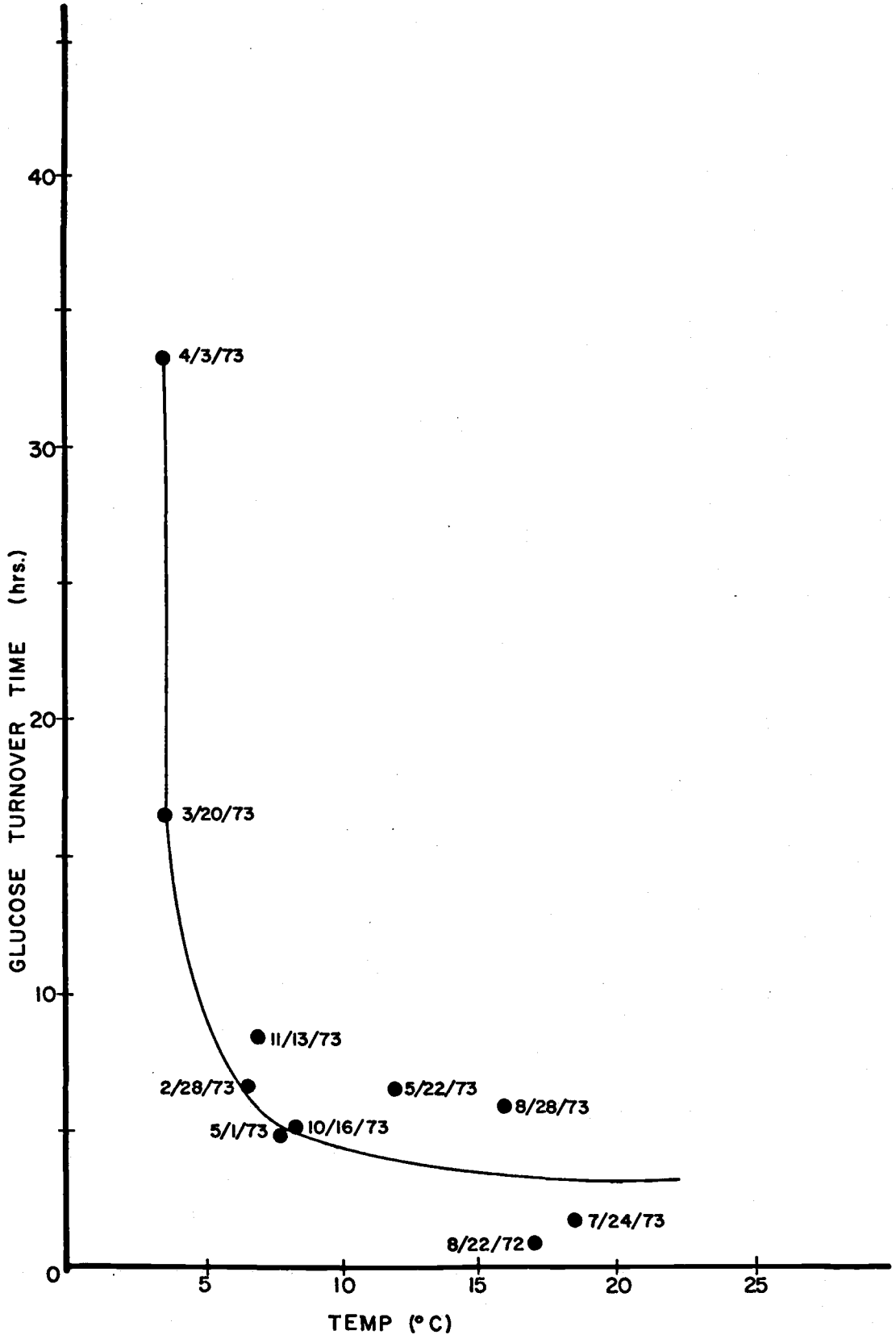


Figure 12. Glucose turnover time with respect to seasonal temperature in Lake Chester Morse shore sediment.

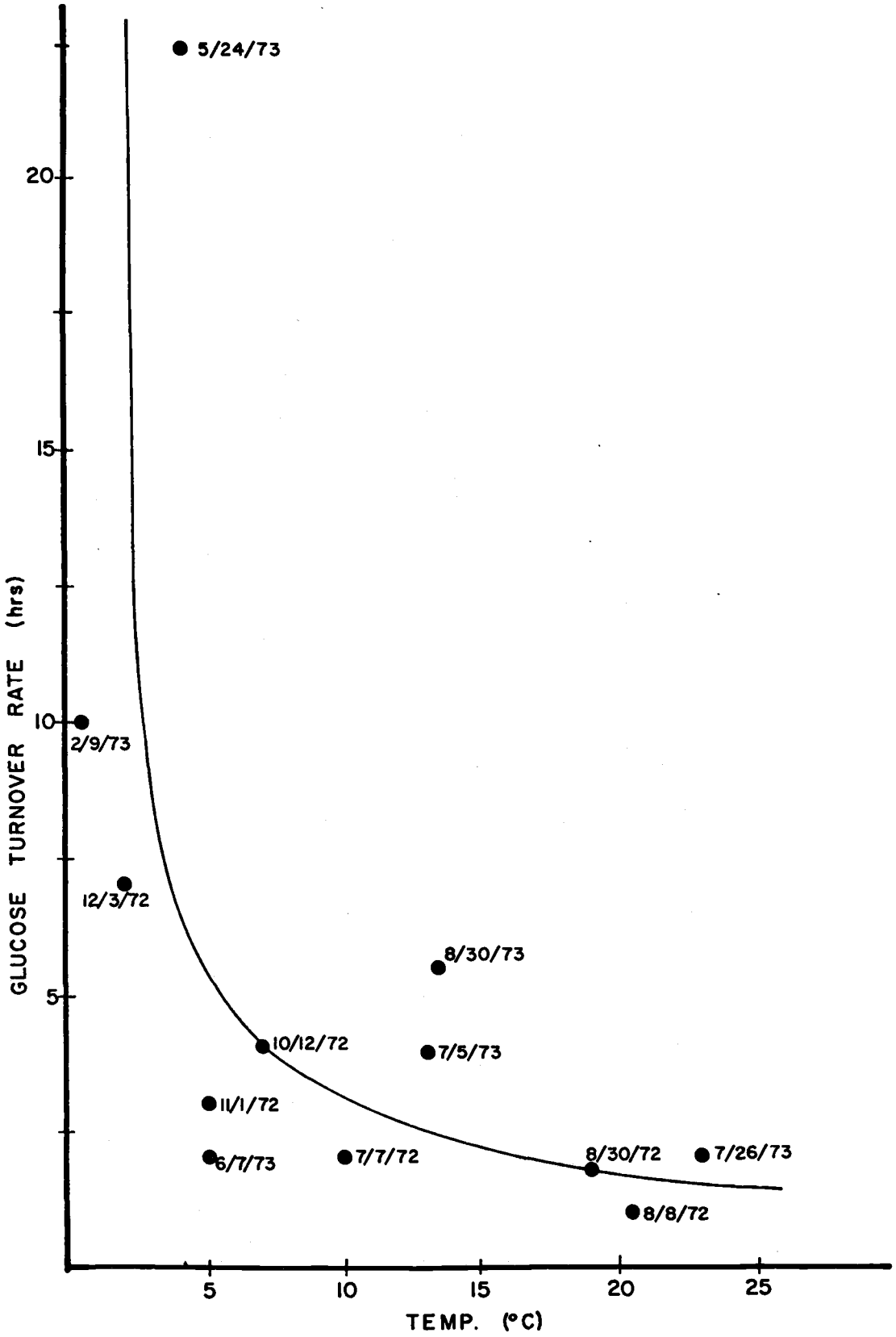


Figure 13. Glucose turnover time with respect to seasonal temperature in Findley Lake shore sediment.

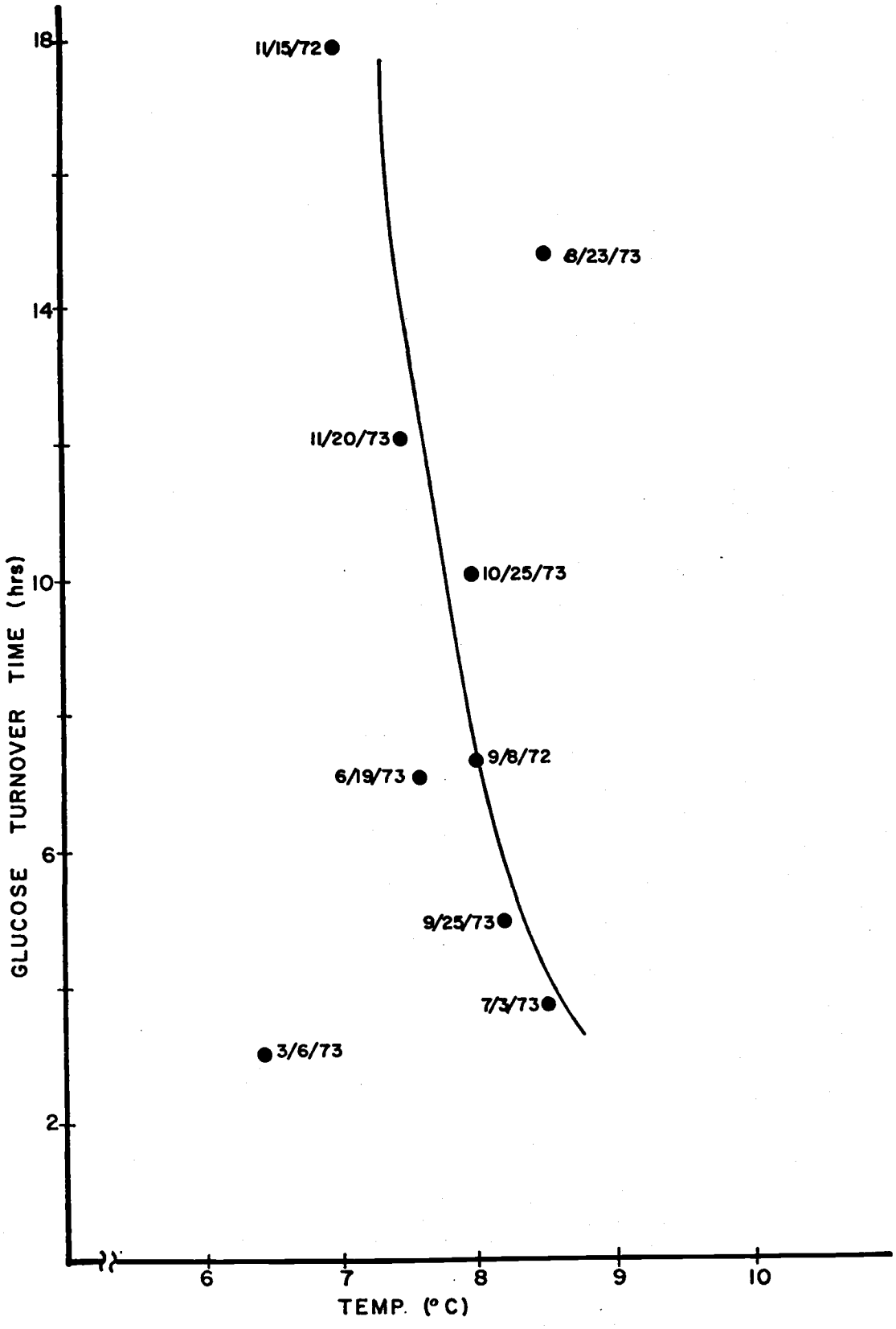


Figure 14. Glucose turnover time with respect to seasonal temperature in Lake Washington sediment samples from 60-m depth.

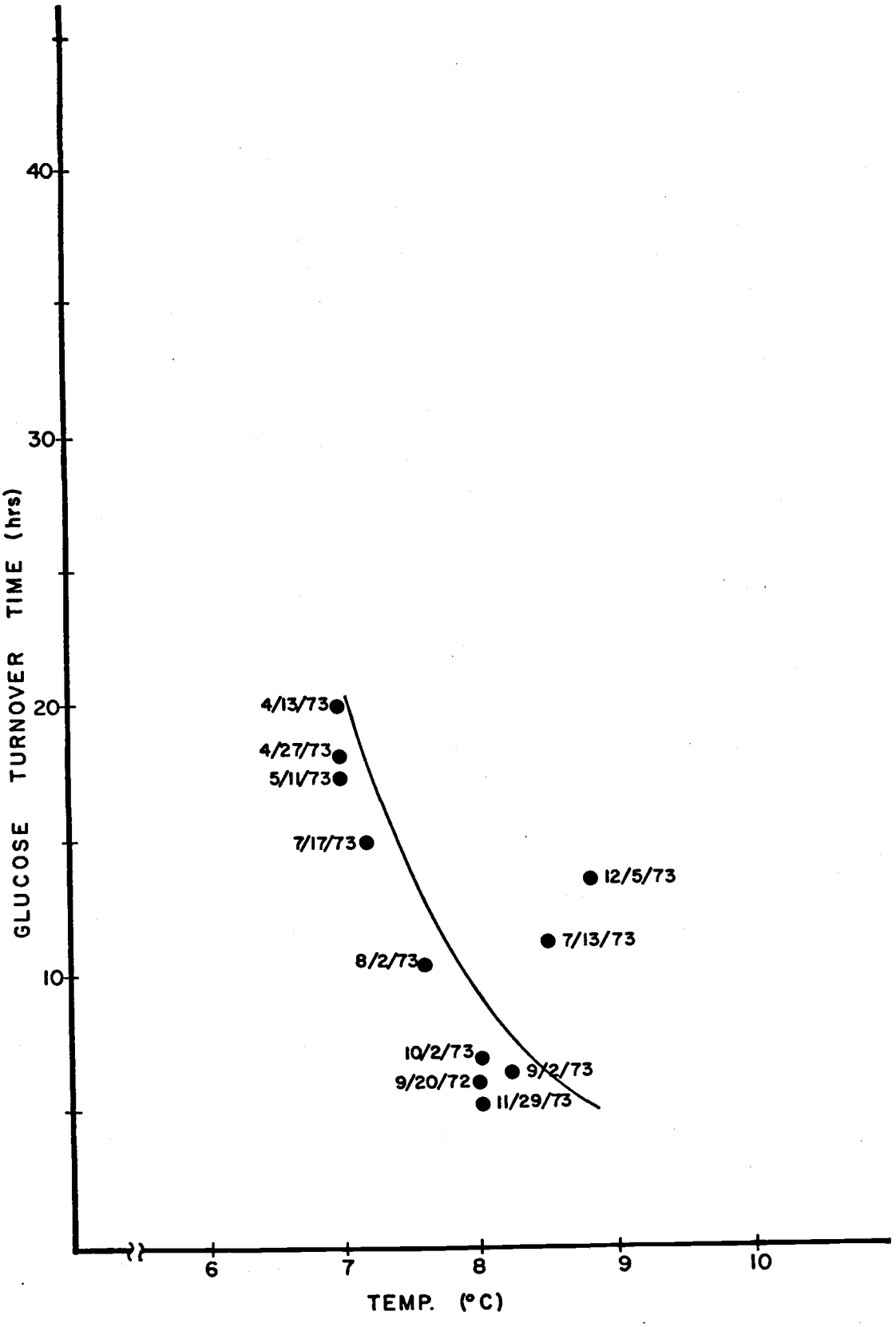


Figure 15. Glucose turnover time with respect to seasonal temperature in Lake Sammamish sediment samples from 25-m depth.

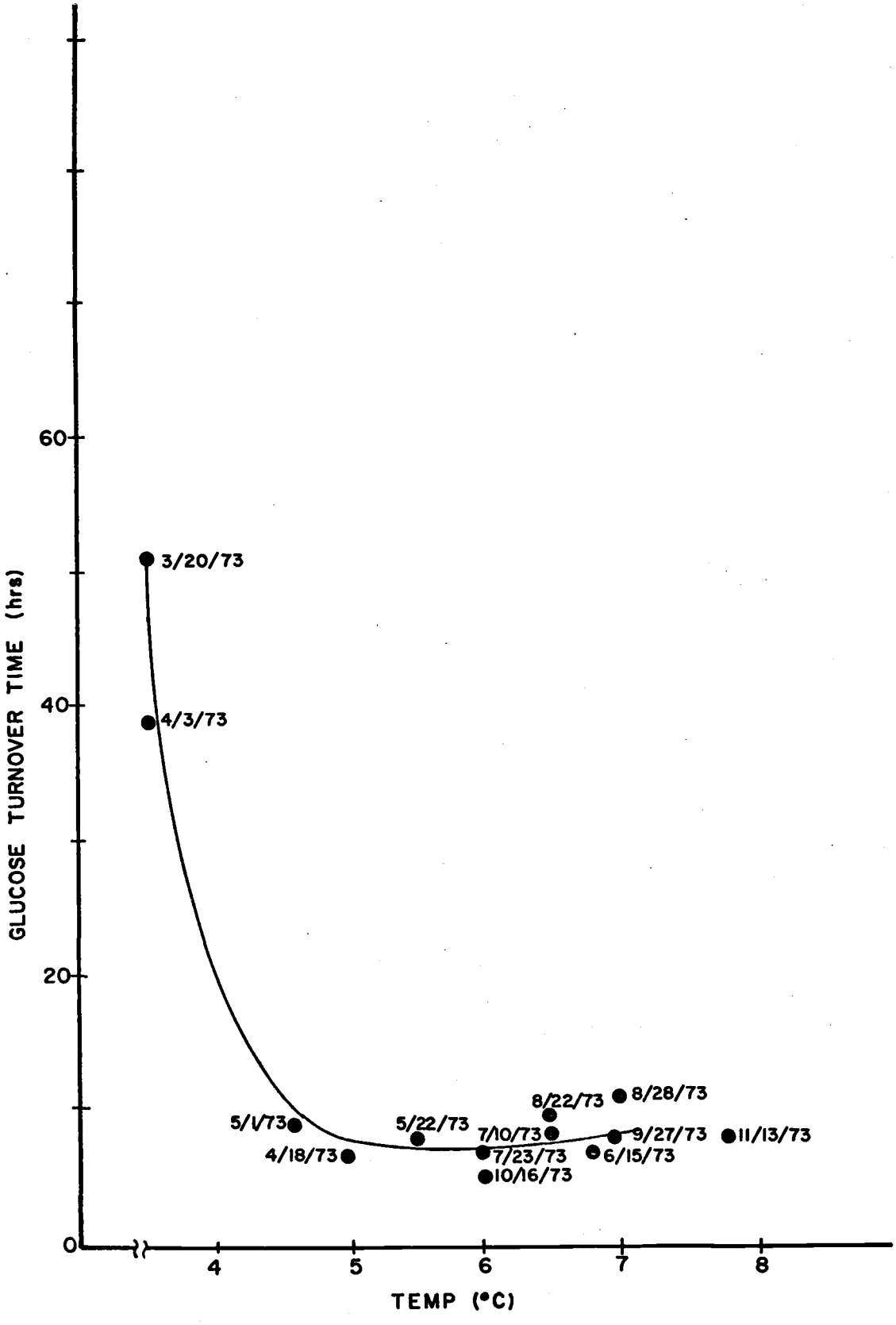


Figure 16. Glucose turnover time with respect to seasonal temperature in Lake Chester Morse sediment samples from 33-m depth.

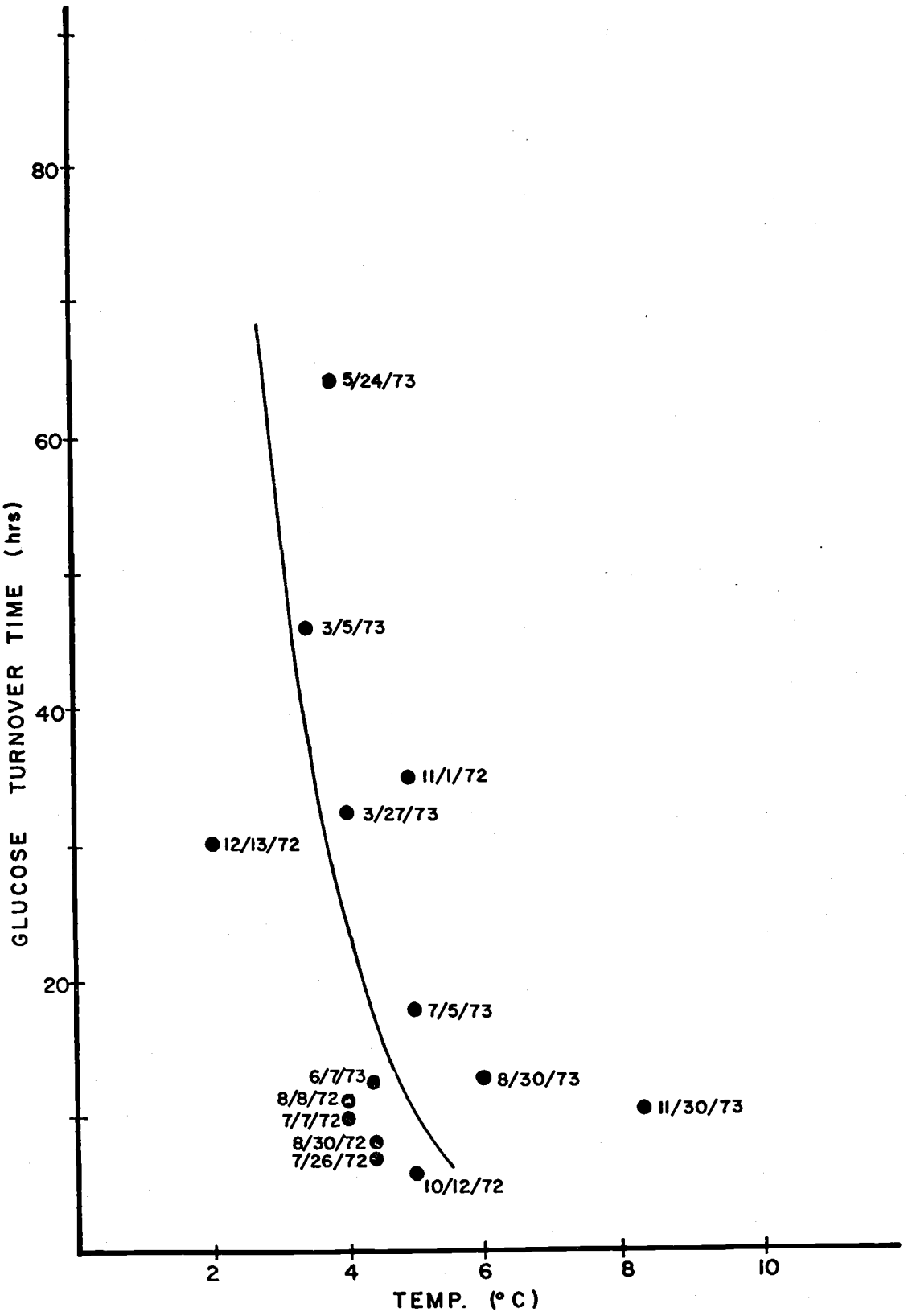


Figure 17. Glucose turnover time with respect to seasonal temperature in Findley Lake sediment samples from 27-m depth.

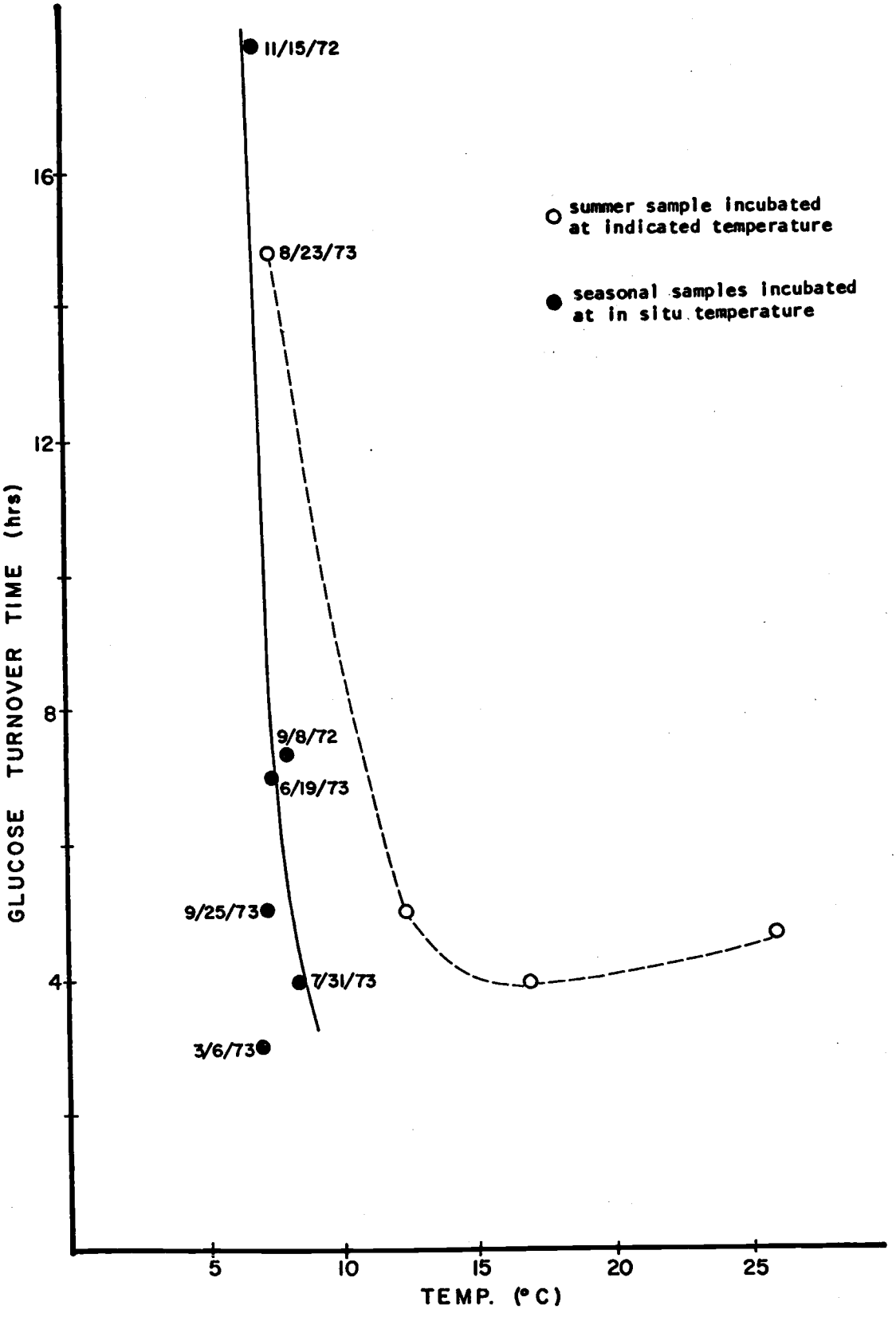


Figure 18. Glucose turnover time in Lake Washington sediment samples from 60-m depth and incubated at various temperatures. Seasonal sediment samples were incubated at the in situ temperature.

Table 1. Temperature profile in Lake Washington.[a]

Depth (m)	Date and experiment number						
	1973					1972	
	6 Mar 23	10 May 35	19 Jun 41	31 Jul 49	25 Sep 56	8 Sep 9	15 Nov 15
0	7.45	10.9	16.5	22.5	17.6	20.0	10.5
1	7.45		15.8		17.6	20.0	10.5
2	7.45	10.8	15.5	22.3		20.0	10.5
3	7.25		15.5		17.5	20.0	10.5
4	7.1		15.4	22.2		20.0	10.5
5	7.0	10.8	15.4		17.6	20.0	10.5
6	7.3			21.5		20.0	10.5
7			15.3			20.0	10.5
8	7.25			19.0		20.0	10.5
9		10.7				20.0	10.5
10	7.0	10.6	15.1	18.5	17.6	20.1	10.5
11		10.6				20.2	10.5
12	6.95	10.6		17.2		20.1	10.5
13		10.6	15.0		17.4	20.1	10.5
13.5						20.0	10.5
13.7						17.0	10.5
14		10.6		16.0		16.0	10.5
14.5						15.0	10.5
15	6.9	10.6			14.0	13.6	10.5
16		10.5	14.9	15.0		12.6	10.5
17	6.85	10.5	12.5		11.7	12.2	10.5
18		10.5	10.1	12.5		11.5	10.5
19	6.8	10.5	9.7			11.0	10.5
20	6.8	10.4	9.4	11.0	10.7	10.4	10.5
21	6.8	10.2	9.0			9.8	10.5
22	6.8	10.1	9.0	10.0		9.5	10.5
23	6.8	10.0	8.7			9.4	10.5
24	6.8	10.0	8.5	9.4		9.2	10.4
25	6.8	10.0			9.8		
26		9.9		9.2			
27		9.8	8.1			8.8	8.8
28		9.5		9.0			

Table I. Temperature profile in Lake Washington (cont.).[a]

Depth (m)	Date and experiment number						
	1973					1972	
	6 Mar 23	10 May 35	19 Jun 41	31 Jul 49	25 Sep 56	8 Sep 9	15 Nov 15
29		9.5					
30	6.75	9.5	8.0	8.8	9.1	8.4	8.0
31		9.3					
32		9.2		8.6			
33	6.75		7.8			8.3	8.8
34				8.5			
35	6.8				8.9		
36			7.9	8.5		8.1	7.7
37							
38				8.5			
39						8.0	7.5
40				8.4	8.6		
41							
42			7.5	8.4		8.0	7.4
43							
44				8.3			
45			7.4			7.8	7.5
46				8.2			
47							
48			7.3	8.2		7.8	7.4
49							
50				8.1	8.7		
51			7.2			7.8	7.4
52			7.2				
53			7.2				
54			7.2			7.6	7.3
55	6.8		7.2				
56			7.2		8.1		
57			7.2			7.6	7.2
58	6.85		7.2	8.1	8.1		
59			7.2				
60	6.9		7.2	8.0		7.6	7.0

[a]Temperatures in degrees Celsius.

Table 11 Temperature profile in Lake Sammamish[a]

Depth (m)	Date and experiment number									
	1973								1972	
	13 Apr 30	27 Apr 33	11 May 36	13 Jul 45	17 Jul 46	10 Aug 50	21 Aug 52	2 Oct 57	20 Sep 10	29 Nov 16
0	13.3	12.2	13.3	21.5	24.0	23.0	20.4	18.5	17.6	8.9
1	13.0	12.1	12.8	21.0	23.5	23.1	20.6	18.2	17.6	8.9
2	12.4	12.1	12.5		23.3	23.2	20.8	18.0	17.8	9.0
3	11.2	12.1	11.9		23.2	23.5	20.9	17.8	17.8	9.0
4	10.4	12.1	11.3	20.8	23.0	23.4	20.7	17.8	17.7	9.0
5	9.7	12.1	11.0	20.8	21.4	23.4	20.7	17.7	17.7	9.0
6	9.2	12.0	10.9	19.5	20.4	22.6	20.7	17.7	17.8	9.0
7	8.8	10.6		17.9	19.0	22.6	20.7	17.6	17.8	8.9
8	8.6	10.2	10.8	14.5	17.0	15.7	20.7	17.6	17.7	8.9
9	8.4	9.8	10.6	14.2	15.0	14.9	17.4	17.6	17.0	8.9
9.5									16.5	8.9
10	8.3	9.4	10.2	11.7	13.6	13.1	14.6	16.6	15.4	8.9
11		8.9	9.9	10.5	11.7	11.6	13.3	14.1	13.6	8.9
12	8.2	8.8	9.7	10.0	10.2	11.0	11.6	12.4	12.0	8.9
13	7.8	8.6	9.6	9.5	9.5	10.4	10.2	11.0	10.5	8.9
14	7.6	8.4	9.4	9.3	9.3	9.8	9.6	10.4	9.6	8.9
15	7.4	8.0	8.7	9.0	9.1	9.4	9.4	9.7	9.3	8.9
16	7.0	7.8	8.4	8.8	8.9	9.1	9.0	9.3	9.0	8.9
17	6.7	7.6		8.6	8.7	9.0	8.6	9.0	8.6	8.9
18	6.6			8.5	8.6	8.9	8.5	8.8	8.5	8.9
19				8.4	8.5	8.6	8.2	8.6	8.4	8.9
20	6.4	7.6	8.1	8.3	8.3	8.5	8.1	8.5	8.2	8.3
21	6.4	7.3	7.8	8.1	8.0	8.4	8.0	8.3	8.0	8.2
22	6.4	7.2	7.5	8.0	7.9	8.3	7.9		8.0	8.1
23	6.3	7.0	7.4			8.2	7.7		8.0	8.0
24			7.3	7.9		8.0	7.8	8.0	8.0	7.9
25					7.9	8.0	7.8		8.0	7.8
26	6.2	7.0				8.0		7.9		
27			6.9	7.9		7.9				

[a]Temperatures in degrees Celsius.

Table III. Temperature profile in Lake Chester Morse.[a]

Depth (m)	Date and experiment number										
	1973									1972	
	20 Mar 26	3 Apr 28	19 Apr 31	1 May 34	22 May 37	6 Jun 39	10 Jul 44	24 Jul 47	28 Aug 54	22 Aug 7	27 Sep 11
0	0	4.8	6.7	7.9	11.5	12.5	17.6	18.0	16.1	18.0	11.4
1		4.5	6.8	7.7	11.4	12.0	17.2	18.5	16.4	17.8	11.3
2	3.5	4.4	6.7	7.4			17.1	18.3	16.6	17.8	11.2
3		4.3		7.2	11.3	11.9	16.9		16.7	17.8	11.1
4				7.1	11.2		16.9	18.2	16.8	17.7	11.1
5			6.6	7.0			16.9	18.1	16.8	17.6	11.1
6		4.3	6.4	7.0		11.8	16.0		16.8	17.0	11.1
7			6.2		10.9	11.5	14.8	18.0	16.8	16.2	11.1
8	3.45		6.0	6.9	10.7		14.4	16.1	16.8	14.2	11.1
9		4.3	5.9		9.9		13.5	13.0	15.1	12.5	10.7
10	3.45			6.8		11.1	11.9	11.6	11.7	10.9	10.5
11			5.9		9.7	10.9	11.0	10.7	10.6	10.0	10.2
12		4.3		6.5	9.0	10.6	10.3	10.1	9.6	9.4	10.0
13			5.8		8.9	10.1	9.7	9.6	8.9	8.9	9.8
14				6.3	8.7	10.0	9.5	9.4	8.4	8.6	9.4
15		4.2	5.6		8.5	9.5	9.0	9.1	8.1	8.4	9.3
16				6.2	8.3	9.1	8.8	8.9	7.9	8.2	8.8
17			5.5		8.0	8.5	8.5	8.5	7.8	8.2	8.7
18		4.2		5.8	7.8	8.4	8.3	8.4	7.6	8.0	8.3
19			5.3			8.0	8.1	8.3	7.5	8.0	8.0
20				5.6	7.7	8.0	8.0	8.2	7.5	8.0	7.9
21		4.2	5.2			7.9	7.9	8.1	7.4	7.8	7.9
22				5.5	7.3	7.7		7.9	7.3	7.8	7.9
23			5.1			7.7		7.7	7.3	7.6	7.8
24		4.2	5.0	5.4	7.1	7.5	7.5	7.4	7.2	7.6	7.5
25						7.3					
26						7.2					
27		4.2	4.9	5.1	6.7	7.0	7.3		6.9	7.4	7.2
28	3.5					6.8					

Table III. Temperature profile in Lake Chester Morse (cont.).[a]

Depth (m)	Date and experiment number										
	1973									1972	
	20 Mar 26	3 Apr 28	19 Apr 31	1 May 34	22 May 37	6 Jun 39	10 Jul 44	24 Jul 47	28 Aug 54	22 Aug 7	27 Sep 11
29						6.8					
30	3.4	4.2	4.8	4.8	6.4	6.9	7.0	7.3	6.9	7.0	6.8
31									6.9		
32	3.4	4.2	4.8	4.7							
33					5.9		6.9				

[a]Temperatures in degrees Celsius.

Table IV. Temperature profile in Findley Lake[a]

Depth (m)	Date and experiment number										
	1973						1972				
	27 Mar 27	24 May 38	7 Jun 40	6 Jul 43	26 Jul 48	30 Aug 55	8 Aug 6	30 Aug 8	12 Oct 12	11 Nov 13	13 Dec 17
0	0.4	0.5	4.5	13.4	17.4	15.0	19.5	16.9	7.8	5	0
1	2.1	1.6			17.0	15.1	19.5	16.9	7.8	5	
2	3.3	3.0		13.2	16.5	15.2	18.5	16.6	7.8	5	
3	3.5	3.5		11.0	16.0	15.2	14.0	16.5	7.8	5	
4	3.7	3.5		9.5	15.5		12.2	16.2	7.8	5	
5	3.7	3.6		8.5	13.5		10.6	15.5	7.8	5	
6	3.8	3.7		8.2	12.6		9.5	13.7	7.8	5	
7		3.7	4.5	7.9	11.5		8.8	11.7	7.8	5	
8		3.8	4.2	7.4	10.2	15.0	7.7	10.5	7.8	5	
9		3.8	4.1	6.6	7.4	12.1	6.8	9.0	7.8	5	
10		3.8	4.0	6.2	7.3	11.0	6.0	7.9	7.8	5	
11			4.0	5.5		10.5	5.6	7.2	7.8	5	
12		3.9	4.05	5.5	6.5	9.5	5.2	6.5	7.8	5	
13			4.0	5.4	6.2	8.7	5.0	6.1	7.8	5	
14				5.2	6.8	8.3	4.8	5.8	7.5	5	
15		3.9			6.5	7.8	4.5	5.5	6.2	5	
16				5.0	6.4	7.5		5.3	5.9	5	
17		4.0			6.3	7.2		5.2	5.7	5	
18					6.0	6.9	4.2	5.0	5.5	5	
19					5.9	6.6		4.8	5.1	5	
20		4.0			5.6	6.4		4.6	5.0	5	
21					4.5	6.2	4.0	4.5	4.9	5	
22		4.0		4.9				4.5		5	
23					4.4	6.0		4.4		5	
24		4.0			4.2		4.0	4.4		5	
25					4.2					5	
26						5.9				5	
27				4.6	4.1	5.9	4.0	4.3		5	
28											

Not measured