FRESHWATER MICROBIOLOGY IN THE ANTARCTIC: II. MICROBIAL NUMBERS AND ACTIVITY IN NUTRIENT-ENRICHED HEYWOOD LAKE, SIGNY ISLAND

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ABSTRACT. Heywood Lake (Signy Island, South Orkney Islands) is a shallow, coastal water body subject to considerable enrichment from nearby seal wallows. This has resulted in substantial phytoplankton development and the high turbidity of the water has restricted benthic vegetation to the shallow areas of the lake. During 1976–78, fluctuations in numbers and activity of the planktonic bacteria were monitored using direct microscopic counts and plate counts of total heterotrophs, exo-enzyme producing bacteria and yeasts. These revealed consistently low numbers in winter, but a significant increase in counts at the onset of spring-thaw, coinciding with rapid development of the algal population. With the disappearance of ice cover in January both algal and bacterial numbers decreased to a lower summer level. In contrast, maximum photosynthetic and heterotrophic activity was measured during summer open-water, when optimum light conditions and maximum temperatures occurred. Photosynthetic production was far higher than in comparable Arctic systems and an extremely high maximum assimilation efficiency of 10 mg C h^{-1} mg^{-1} (chlorophyll-*a*) was recorded. Heterotrophic uptake of ^{14}C-glucose and ^{14}C-acetate revealed low assimilation rates comparable with similar Arctic lakes. Turnover times of 39 h or less, in summer, contrasted markedly with times in excess of 500 h (suggesting little activity) measured under winter ice cover. A significant correlation was found ($P<0.001$) when acetate V_{max} data was regressed on direct microscopic count data, giving a V_{max} per average bacterial cell of 4.17×10^{-11} µg acetate h^{-1} $cell^{-1}$. Algal production was severely reduced in bottom-water samples as the turbid water column limited light penetration. Bacterial production changed little with depth and was comparable with algal production near the bottom. On an annual basis, bacterial biomass production was estimated as 12–80 g C m^{-2}, compared with algal production of 172 g C m^{-2}.

HEYWOOD (1967) has described the lakes on Signy Island and established that they can be conveniently divided into shallow coastal lakes dominated by phytoplankton and clear upland lakes dominated by benthic vegetation, e.g. Moss Lake (see Priddle, 1980). Most of the early limnological work at Signy Island has centred on shallow, coastal Heywood Lake (Heywood, 1967, 1970*a, b*; Light, 1977; Light and others, 1981; Weller, 1977). Light (1977) reported that during the period 1970–75, Heywood Lake was apparently becoming more eutrophic due to increasing numbers of seals in and around the lake. This system was therefore adopted to compare microbial numbers and activity with those in ultra-oligotrophic Moss Lake (Ellis-Evans, 1981; hereafter referred to as Paper I).

THE STUDY AREA

Heywood Lake, a paternoster type basin (Type 28a of Hutchinson, 1957) situated in Three Lakes Valley (see fig. 1, Paper I) on the east coast of Signy Island (lat. 60°45′S, long. 45°38′W), has an area of 45 050 m^2 and consists of two basins each with a steep-sided trough surrounded by an extensive shallow shelf (Fig. 1). The deeper North Basin has a maximum depth of 6.4 m. Morainic material and moss banks drained by several small streams cover a catchment area of 41 hectares. The lake is frozen to a depth of 1 m for 8–10 months of the year and lies 4 m above sea level and 200 m from the sea. During the brief ice-free period, elephant seals, *Mirounga leonina,* and fur seals, *Arctocephalus gazella,* haul out around the lake and often swim in both basins. As a result of enrichment from seal excreta, Heywood Lake is now mesotrophic in comparison with other Signy Island lakes.

MATERIALS AND METHODS

Samples were taken in Heywood Lake (Fig. 1) at monthly intervals during summer and winter, and fortnightly during spring, weather permitting. The sampling site was situated 2–3 m from the lake's deepest point where the lake monitoring programme samples were obtained. Samples were obtained as described in Paper I at 1.5 m below the surface of the lake and 1 m

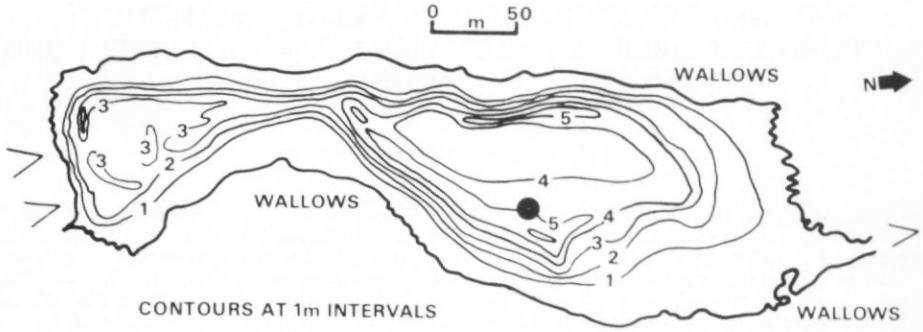

Fig. 1. Heywood Lake morphometry. Arrows indicate direction of flow of the main streams. Bathymetric contours are at 1-m intervals. ● Sampling station.

above the mud surface. All samples were returned to the Station within 1.5 h, and immediately stored at +3°C. All further manipulations were undertaken at this temperature to minimize temperature shock. Treatment of samples is described fully in Paper I.

Results

Physico-chemical and phytoplankton data

The seasonal physico-chemical data revealed a large variation in the concentration of certain variables such as chlorophyll-*a*, phosphate and oxygen (Fig. 2) but the harshness of the environment was evident in the low annual temperature range (0.2–5.3°C). Light reaching the water surface was also potentially limiting as a result of the ice/snow cover in winter and the extreme cloudiness of the Signy Island area. The ice/snow layer, itself a function of the consistently low air temperatures, was possibly the single most important feature of the lake's physico-chemical seasonality. Formation of the ice and subsequent snow cover reduced light levels, resulting in the decline of photosynthesis (Fig. 3) and sedimentation of the phytoplankton. Respiration by both water column and sediment organisms depleted dissolved oxygen (Fig. 2) resulting in anaerobic conditions at the bottom of the lake where phosphate levels increased markedly. The spring melt started in September/October and was characterized by several freeze–thaw cycles before a steady melt began. Chlorophyll-*a* levels increased almost simultaneously as did temperature and oxygen, whilst ortho-phosphate quickly declined. As the ice cover melted around the lake margin and areas of open water appeared, wind mixing became increasingly significant. With the disappearance of the ice, wind mixing of the whole water column occurred and all physico-chemical stratification disappeared. Although chlorophyll-*a* levels dropped sharply to a summ[illegible] level at the onset of the open-water period, carbon fixation was markedly increased (Fig. 3) [illegible] response to the high light levels and the long day length. The decrease in chlorophyll-*a* may have reflected grazing as zooplankton numbers were high at this time. By autumn (March–April), light levels and day length had decreased significantly, as had temperature (Fig. 2), and algal activity steadily declined (Fig. 3) to winter values as the ice cover reformed.

Although the physico-chemical parameters of the lake followed similar patterns each year, considerable variation occurred between years in the amplitude of these patterns (Fig. 2). Much of the variation can be explained by annual climatic differences. Thus, the differences between maximum chlorophyll-*a* levels in Heywood Lake during the spring periods (October–December) of 1976 (40 μg l^{-1}) and 1977 (21 μg l^{-1}) can be attributed at least in part to increased sunshine (292 h in October–December 1976 against 222 h in October–December 1977) and the formation in spring 1977 of an opaque layer of refrozen snow resulting from a particularly large number of freeze–thaws.

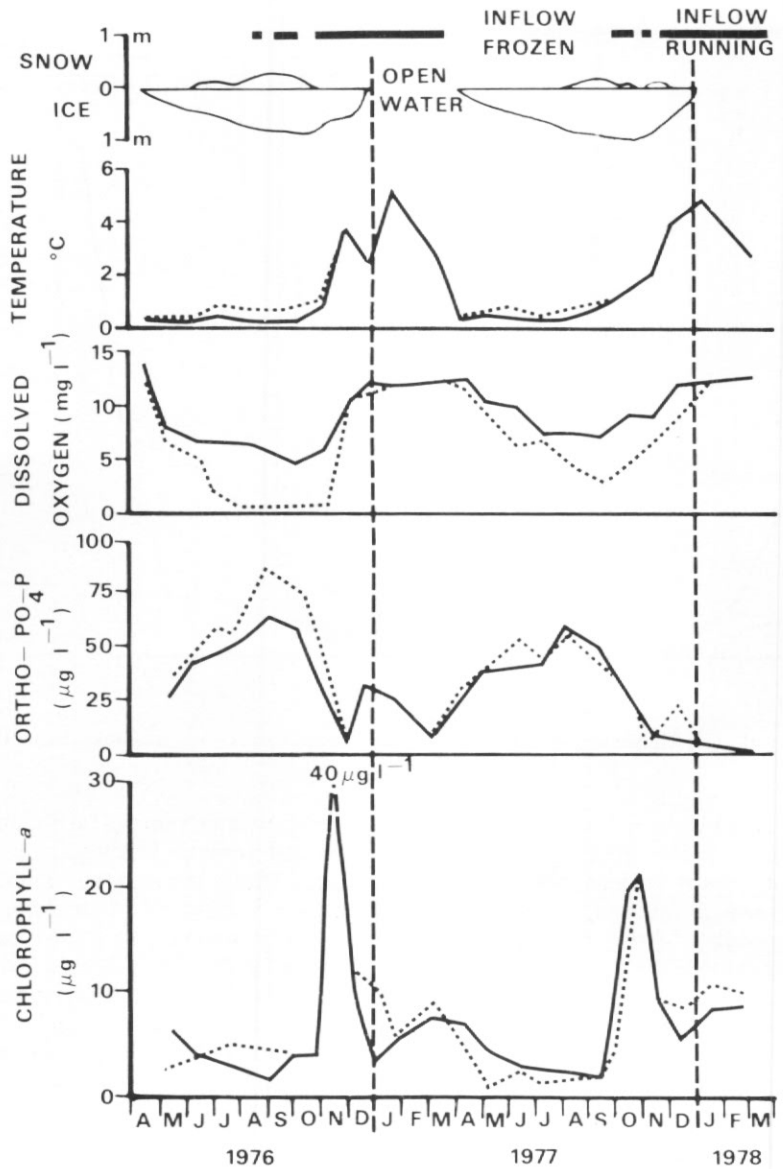

Fig. 2. Seasonal physico-chemical characteristics of Heywood Lake and variations in algal biomass as estimated by chlorophyll-*a*. Data presented for top-water (———) and bottom-water (– – – –) samples.

Bacterial population counts

Seasonal fluctuations in the bacterial populations (Fig. 4) followed an apparently similar pattern to the chlorophyll-*a* data (Fig. 2), numbers being low in winter, but increasing rapidly to give maximum counts in spring. Bacterial counts then declined to a lower summer level, possibly reflecting grazing or a response to changes in the nutrient regime, before returning to winter minimum counts. Differences between years are illustrated by the direct microscope counts (Fig. 4a) which gave a higher maximum spring value (17.07 (S.E. = 1.55) $\times$ 10^8 cells l^{-1}) in 1976

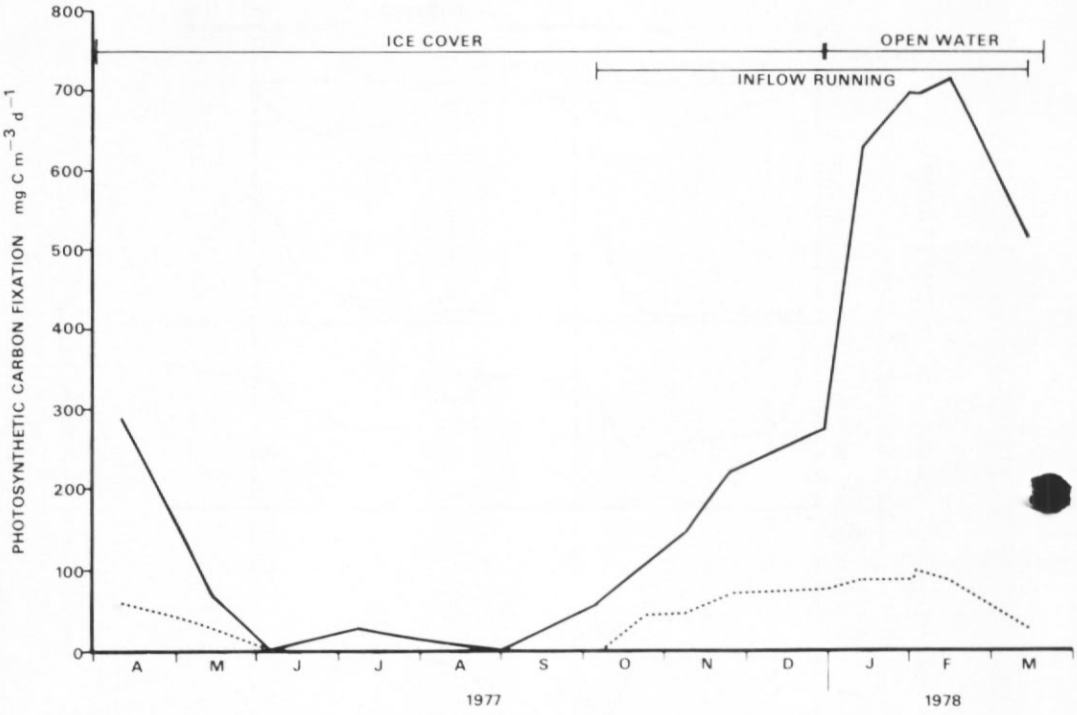

Fig. 3. Photosynthetic CO_2 fixation by top-water (———) and bottom-water (– – – –) samples during 1977–78.

than in 1977 (10.22 (S.E. = 1.89) × 10^8 cells l^{-1}) for surface water samples. Although bacterial counts (both direct and viable) from top- and bottom-water samples followed similar seasonal patterns, variation was evident, notably in direct counts. Direct microscopic counts (Fig. 4a) increased from winter values approximately simultaneously with the chlorophyll-*a* increase and just after the opening of the inflow (Fig. 2). Viable bacteria numbers (Fig. 4b), expressed as colony forming units (CFU) l^{-1}, began to increase soon after the direct counts in both spring periods, possibly reflecting a lag phase. Comparison of direct counts, and viable counts of bacteria in Heywood Lake using Spearman's ranked correlation coefficient (Siegel, 1956) gave r_S values of 0.78 ($P<0.001$, $n = 24$) for top-water samples and 0.48 ($P<0.05$, $n = 24$) for bottom-water samples, indicating a high correlation between rankings.

Attached bacteria

On four occasions during 1977–78, a study was made of bacteria attached to particles in the water column. Water samples were homogenized as described in Paper I. A parallel set of samples was prepared by vortex mixing volumes of lake water for 10 s. Direct microscope counts were then made on both sets using the method outlined above. The counts of non-homogenized samples were subtracted from the homogenized samples to give percentage attached bacteria. Replication revealed considerable variation in counts from non-homogenized samples, but the final data nevertheless gave some indication of changes in numbers of attached cells (Table I). The results suggest that few bacteria were attached in winter. For the rest of the year, however, a significant percentage of the total microbial population was attached, mainly to algal cells. No attempt was made in this study to separate bacteria quantitatively on the basis of the particle to which they were attached.

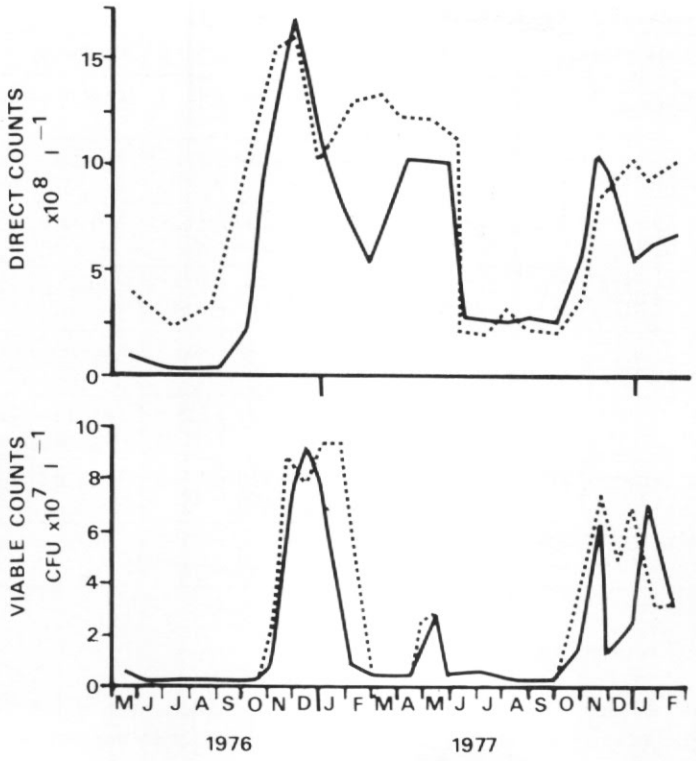

Fig. 4. Direct microscopic counts and viable aerobic plate counts of bacteria. Top-water counts (———) and bottom-water counts (– – – –).

Generation times

On the same four occasions in 1977–78, population doubling rates were calculated for the Heywood Lake water column. The results showed that doubling times were fastest in late spring/early summer (79 h), considerably slower in autumn (178 h) and winter (201 h) before accelerating again during early spring (102 h). These rates correlated well with the seasonal temperature pattern for Heywood Lake (Fig. 2).

Spring population changes

During both spring melt periods, a CPS plate count study was made of changes in bacterial numbers in the inflow, shelf and trough regions of the lake (Table II). Initial winter counts were highest in the lake shelf area and revealed a higher percentage of pigmented bacteria than was

TABLE I. PERCENTAGE BACTERIA ATTACHED TO LARGER PARTICLES IN THE LAKE WATER COLUMN

Period study	*Winter (August)*	*Spring (October)*	*Summer (January)*	*Autumn (April)*
% Attached Bacteria – Top water	8 ± 1.8*	33 ± 4.6	23 ± 6.8	32 ± 4.5
% Attached Bacteria – Bottom water	12 ± 4.1	18 ± 4.0	29 ± 6.3	43 ± 11.1

* Results expressed as mean of three values ± 1 S.E.

TABLE II. SPRING INCREASE IN MICROBIAL NUMBERS IN HEYWOOD LAKE WATER SAMPLES

Date	*Inflow station*	*Shelf station*	*Trough station*	*Comments*
14/10/76	B Frozen Y	5 760 ± 720 0	438 ± 82 0	Lake in winter condition.
31/10/76	B 420 ± 66 Y 200	8 270 ± 897 0	6 040 ± 321 100	First thaw. Soil profile still frozen.
17/11/76	B 25 790 ± 3 225 Y 3 900 ± 100	26 000 ± 4 825 2 700 ± 500	25 200 ± 1 628 1 200 ± 200	Top 2 cm soil profile thawed.
29/11/76	B 20 000 ± 4 062 Y 2 200 ± 300	79 800 ± 4 335 1 700 ± 100	74 400 ± 10 677 1 600 ± 200	>10 cm soil profile thawed.
31/8/77	B Frozen Y	3 304 ± 169 200	795 ± 105 0	Lake in winter condition.
5/10/77	B 515 ± 80 Y 0	3 415 ± 729 100	2 142 ± 328 0	First thaw. Soil profile still frozen.
20/10/77	B 19 080 ± 4 775 Y 5 400 ± 700	8 400 ± 491 3 100 ± 600	13 950 ± 3 372 1 000 ± 200	Top 1 cm soil profile thawed.
8/11/77	B 26 800 ± 5 680 Y 2 700 ± 500	61 000 ± 8 056 1 800 ± 200	66 500 ± 4 752 2 100 ± 400	>10 cm soil profile thawed.

B Bacteria viable counts
Y Yeast viable counts
CRU $^{-1}$ (Means of triplicate plates ± 1 S.E.)

found in trough samples. The higher winter counts coincided with minor freeze–thaw cycles preceding spring. These cycles caused partial melting of the ice in the shelf area, possibly giving rise to increased temperatures or oxygen concentrations and even some algal development. At the time of the major spring thaw, the inflow contained meltwater with a very low bacterial count. Examination of the trough sample revealed enhanced colony counts similar in number and appearance to those of the shelf area CPS plates. This suggests that the shelf provided the initial contribution to the spring increase in trough population numbers. As the moss banks of the Heywood Lake catchment area thawed to a depth of about 2 cm, microbial counts in both the inflow and lake increased sharply revealing a population at both lake stations similar in size and composition to that on the inflow sample plates.

Further evidence of the role of the inflow at this time was obtained from plate counts of yeasts (Table II). Although both yeasts and fungi (probably of terrestrial origin) are found in relatively large numbers in the lake mud, they are virtually absent from the water column of all Signy Island lakes. Yeasts occur in highest numbers in the top few centimetres of the surrounding *Drepanocladus* sp. moss bank profile and decrease with depth (Ellis-Evans, unpubl. data). As the top layers of the moss thawed, a significant increase in yeast counts was detected in the inflow, declining steadily on a transect from the inflow point to the centre of each basin. Preliminary studies suggested that the yeasts isolated from the inflow and trough samples were the same species. A depth profile of Heywood Lake revealed that although their numbers decreased, similar yeast types were found throughout the trough station water column consistent with a certain amount of vertical mixing under ice cover by the inflow of this lake. As the lower levels of the moss bank thawed, bacterial numbers in the inflow stabilized and yeast numbers decreased significantly (Table II). Increasing algal activity (Fig. 3) under ice (in response to higher light levels) coupled with the first significant rise in temperature (Fig. 2), seem the most likely explanation for the four-fold increase in bacterial counts detected in the lake at this time (Table II). As areas of open water appeared, wind mixing increased, virtually eliminating horizontal and vertical variations of microbial populations in the lake.

In 1976, this three-stage process culminating in a spring population maximum occurred during the period 31 October–3 December. However, the unusual climatic conditions of 1977 resulted in the process being initiated earlier (5 October–8 November). The higher monthly mean air temperature (−5.4°C) in September 1977 compared with that of 1976 (−8.1°C) could explain this early start to the spring increase. But the decline in viable counts during November 1977 and later recovery in open-water conditions, partly attributable to a large number of freeze–thaw cycles in late November, resulted in a more confused picture during this second spring.

Exo-enzyme-producing bacteria

Exo-enzyme-producing bacteria represented a significant proportion of the heterotrophic bacteria population in Heywood Lake. In comparison with amylase and protease producers, lipase-producing bacteria appeared to be relatively unimportant in the water column, rarely exceeding 6% of the total heterotrophic count on CPS (range 0–14%). Discussion has therefore been restricted to the two major groups.

Viable counts of enzyme producers in the lake (Fig. 5a) appeared to decline in February and were consistently low through to October, each group rarely exceeding 5×10^6 CFU l^{-1} throughout the water column. With the onset of spring melt, numbers quickly increased in early November. Maximum numbers were recorded in January ($(20–26) \times 10^6$ CFU l^{-1}) before counts returned to winter levels in February/March. In general, amylase- and protease-producer numbers both followed similar patterns and preliminary identification suggested that the same five or six bacterial groups were involved in both processes in Heywood Lake. When represented as a percentage of the total viable heterotrophs on CPS plates (Fig. 5b), a rather different pattern emerged. The amylase- and protease-producers each represented 40–75% (mean 55%) of the total counts during February–April, but this proportion decreased to about 15% in May as total viable counts briefly increased (Fig. 4b). As these counts declined again to winter levels, both major enzyme producers became more significant (range 35–65%) in June before slowly decreasing through the winter to 10–25% of the total viable counts. In spring, amylase- and protease-producers became increasingly important in the surface water layers (30–60%) peaking in late December (50–60%). However, in bottom-water samples, the two major groups were most significant in January (58–72%) before stabilizing at both depths in February.

Heterotrophic uptake of glucose and acetate

Regular measurements of heterotrophic potential (V_{max}) of the lake microbial populations were undertaken during 1977–78, using uniformly labelled ^{14}C-acetate and ^{14}C-glucose. However, less than half the kinetic data following Lineweaver–Burk transformation and analysis by least-squares linear regression proved statistically significant at $P<0.10$ and only about 10% were significant at $P<0.05$. Nevertheless, 88% of the plots were considered sufficiently clear to permit an eye fit and all kinetic data for labelled acetate uptake were derived by this means. Thirty-four sets of ^{14}C-glucose kinetic data, after transformation, gave only two regression lines significant at $P<0.05$ and a further ten gave negative regression lines. Nine plots were eventually fitted by eye giving a range of uptake rates of 8–39 ng glucose l^{-1} h^{-1} for the lake.

Due to the paucity of glucose V_{max} data, interpretation of seasonal patterns was restricted. However, sufficient data were available to interpret the seasonal changes in acetate V_{max} (Fig. 6) which appeared to have a similar pattern. Uptake of labelled acetate was highest in late spring/early summer when maximum bacterial counts and temperatures occurred, declined in late summer and then peaked briefly in autumn before returning to low winter levels. Winter V_{max} (range: 19–50 ng l^{-1} h^{-1}), were comparable for both top- and bottom-water samples, but in summer, top-water values (peaking at 101 ng l^{-1} h^{-1}) generally exceeded those of the bottom

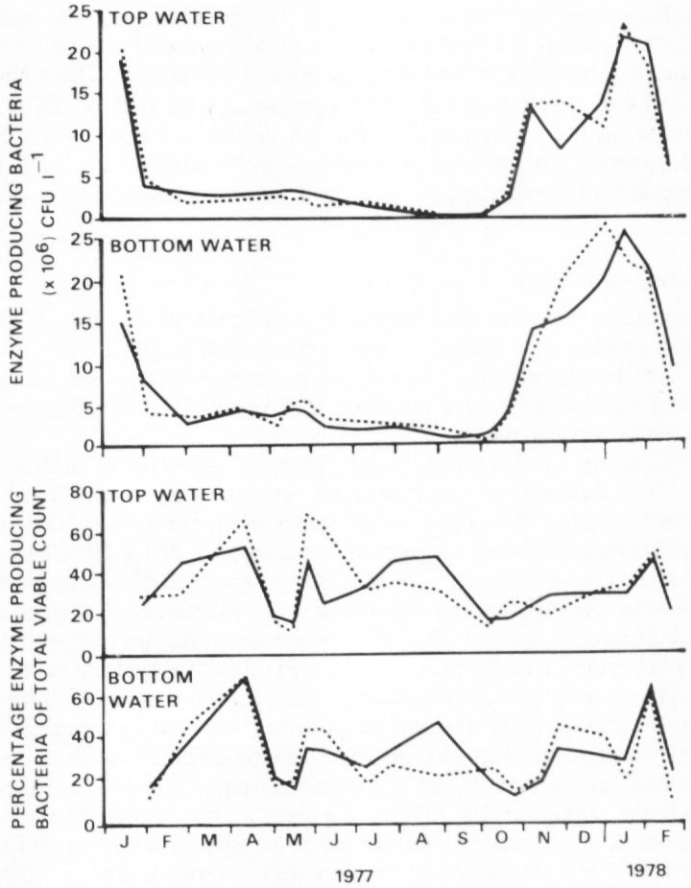

Fig. 5. (a) Viable plate counts of exo-enzyme-producing bacteria from both sampling depths. Protease producers (————) and amylase producers (– – – –).
(b) Exo-enzyme-producing bacteria expressed as a percentage of total viable counts. Protease producers (————) and amylase producers (– – – –).

water. However, whereas V_{max} was still increasing in bottom samples as late as March, values for the top water were declining in February. It was also interesting to note that organic solute uptake measurements at the shelf site (36 ng l^{-1} h^{-1}) were almost twice as high as the trough and top water values in late winter (31 August 1979). After the melt had started, differences between the two sites were minimal.

Turnover times (T_t) serve as an indication of a population's physiological state. Heywood Lake turnover times for glucose were as low as 39 h in December–February and a maximum value of 601 h was measured in winter (Table III) indicative of a highly active population for much of the year despite consistently low temperatures. Acetate turnover times were very similar with a range of 27–512 h for the full seasonal data. The expression, $K_t + S_n$ gives an indication of the maximum natural substrate concentration. Values for both glucose (1.0–4.9 µg l^{-1}) and acetate (1.3–15.7 µg l^{-1}) data showed a trend towards lower values in summer and suggested that levels of both solutes remained relatively low throughout the year (Table III). Respiration rates were calculated on several occasions but rarely conformed to Michaelis–Menten kinetics, apparently as a result of manipulation errors and the low ^{14}C counts. Glucose respiration data

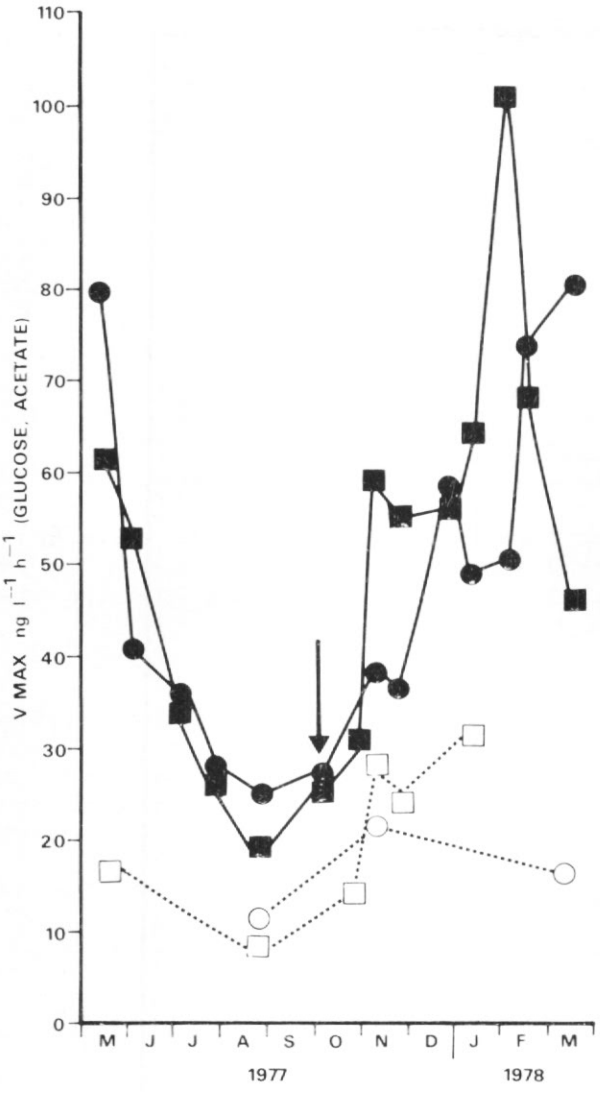

Fig. 6. Seasonal patterns of heterotrophic uptake rates (V_{max}) of ^{14}C-glucose and ^{14}C-acetate in Heywood Lake. Acetate uptake: top water ■ and bottom water ●. Glucose uptake: top water □ and bottom water ○. Arrow indicates date on which inflow started running.

gave only four recognizable plots and a range of 21–33% of total assimilation was found. Values for acetate respiration ranged from 19–45% for the six acceptable plots. There was no significant difference in the rate of uptake of labelled glucose and acetate between light and dark bottles from either sample depth throughout the year. This implies that photoheterotrophy was either not occurring in this lake or proceeding at a rate which was not detected.

Glucose and acetate uptake rates per cell were calculated for Heywood Lake water column regressing the kinetic data for acetate and glucose (y) on direct and viable counts (x). The limited glucose uptake data did not conform to a linear model. The acetate kinetic data correlated

TABLE III. TURNOVER TIMES AND $K_t + S_n$ VALUES FOR HEYWOOD LAKE WATER SAMPLES

	Date	Top water		Bottom water	
		T_t (h)	K_t+S_n (µg l^{-1})	T_t (h)	K_t+S_n (µg l^{-1})
Lake in winter condition	15/5/77	84(172)	5.5(3.1)	29	2.7
	7/6/77	195	9.8	295	12.5
	7/7/77	430	15.0	402	14.6
	27/7/77	470	12.3	469	14.1
	31/8/77	512(601)	9.7(4.9)	503(463)	12.6(5.1)
Spring melt in progress	5/10/77	491	13.2	488	13.5
	20/10/77	444(234)	14.0(3.4)	397	13.7
	8/11/77	134 (51)	9.2(2.8)	310(102)	11.8(2.4)
	25/11/77	154 (54)	8.5(1.3)	418	15.7
	28/12/77	126	7.0	39	1.3
Open-water period	13/1/78	60 (39)	3.9(1.0)	160	8.0
	1/2/78	27	2.5	181	9.0
	3/2/78	36	2.7	110	6.2
	16/2/78	72	5.2	42	3.6
	11/3/78	232	10.5	84(145)	5.4(2.5)

T_t and (K_t+S_n) values for glucose uptake in brackets. All other values are for acetate uptake.

significantly with direct counts ($r = 0.67$, $P<0.001$, $n = 30$) and the regression equation gave a V_{max} per cell value (slope of the line) of 4.17×10^{-11} µg acetate h^{-1} cell^{-1}. Such high significance suggests that uptake rates per cell, at least for acetate, varied little throughout the year. No correlation was found with plate counts even at $P<0.10$.

Production values

Bacterial production (Table IV) was comparable with algal production in winter, but fell below peak algal activity (639 mg C m^{-3} d^{-1}) measured at a depth of 1.5 m under open-water conditions (Fig. 3). Self-shading drastically reduced the activity of algae in bottom-water samples and the bacterial component was therefore always significant at this depth. Both algal and bacterial production curves followed essentially the same pattern with the lowest activity in winter and the

TABLE IV. BACTERIAL PRODUCTION BY ^{14}C-UPTAKE AND DIRECT COUNT METHODS

Date	*Bacterial production* (mg C m^{-3} d^{-1})			
	$^{14}CO_2$-*uptake derived*		*Direct count derived*	
	Top water	*Bottom water*	*Top water*	*Bottom water*
A 12/4	186	182	19	22
A 3/5	127	179	19	20
W 15/5	127	114	18	18
W 7/6	85	79	4	4
W 7/7	49	55	4	4
W 27/7	44	77	4	5
W 31/8	39	71	2	4
W 5/10	68	105	4	4
SP 20/10	101	130	18	14
SP 8/11	187	221	34	28
SP 25/11	149	207	32	59
SP 28/12	178	250	26	38
S 13/1	236	263	31	42
S 1/2	245	254	—	—
S 3/2	227	249	—	—
S 15/2	251	274	36	42
A 14/3	169	297	20	22

For derivation of bacterial production values, see text.
A Autumn, W Winter, SP Spring, S Summer.

highest in summer. Annual data for bacterial production (Table V) yielded markedly different values of 12 and 80 g C m^{-2} y^{-1} for the two methods employed. Bacterial dark fixation comprised about 73% of total dark fixation in winter and about 45% in summer implying significant dark fixation by algae during the latter period. On an annual basis, bacteria fixed 4.8 g C m^{-2} whilst the algal population dark fixed 5.6 g C m^{-2}. Photosynthetic production was estimated at 139 g C m^{-2} y^{-1} for 1977–78, significantly higher than heterotrophic bacteria production, but only 52% of bacterial decomposition based on $^{14}CO_2$-uptake data. No seasonal pattern was discernible for extracellular release of DOC from algal populations in Heywood Lake (range 9–19% of total C fixed). However, algae in bottom-water samples consistently released a higher percentage (mean 18.62 ± 1.60 S.E., $n = 6$) of total carbon fixed than the top-water samples (mean 12.72 ± 1.37 S.E., $n = 6$). This extracellular component (*c.* 28 g C m^{-2} y^{-1}) constituted a significant proportion (*c.* 16%) of total annual algal production which was then 173 g C m^{-2} y^{-1} for the whole lake.

TABLE V. ANNUAL DATA FOR ASSIMILATION AND DECOMPOSITION OF ORGANIC CARBON IN THE WATER COLUMN

Particulate algal production	139.0
Extracellular algal production	28.0 (16% of total)
Algal dark fixation	5.6
Total algal production (A)	172.6
Heterotrophic CO_2-fixation (H)	4.8
Total carbon fixed (P)	177.4 (A+H)
Bacterial biomass production (1)	12.4
Bacterial biomass production (2)	80.0 (H×16.66)
Bacterial decomposition (D)	268.8 (H×56)
Total allochthonous C input	unknown
Losses to outflow	unknown

All values expressed as g C m^{-2} y^{-1}
(1) by direct microscopical examination
(2) by $^{14}CO_2$-uptake method

Dissolved organic carbon

Total dissolved organic compounds (TDOC) were present in significant amounts all year, but the highest values were recorded in winter (9.3 mg l^{-1}) at the top of the water column. This almost certainly reflects the concentration effect of "freeze out" as ice cover developed, since bottom-water samples at the time were markedly lower (4.5 mg l^{-1}). As the spring melt developed, TDOC values decreased to 3.3 mg l^{-1} in the top-water sample (possibly reflecting to some extent dilution by the inflow) and 3.6 mg l^{-1} at the bottom of the lake. When wind mixing began in January a concentration of 2.7 mg l^{-1} was found throughout the water column. These levels were maintained up to the time of ice formation (April–May).

DISCUSSION

The sudden changes in physico-chemical and biological parameters associated with the spring melt period (October to early December) were a striking feature of the data presented here. The interaction of two outside agencies: increasing air temperatures and high total light conditions appear to have been the trigger for a sequence of events that continued into February before the converse situation returned the lake to winter conditions. Light and others (1981) suggested that the rapid increase in algal biomass associated with the under-ice chlorophyll-*a* peak in Heywood Lake was indicative of a population that had been active for some time, possibly in the shelf region. This agrees with the finding in the present study of an active bacterial population in the shelf region before the spring melt. Absorption of solar radiation by the dark algal felt in this

shallow area may have caused local melting of the overlying ice in early spring. Increased water temperature and light levels coupled with the proximity of the shelf sediment would then have permitted early development of the algal and bacterial community. The higher air temperatures of September/October removed the snow layer from Heywood Lake and melt-water entering the lake probably transported algae (and bacteria) from the shelf to the trough region (Light and others, 1981). Kalff and Welch (1974) demonstrated that it is the snow layer that cuts out most of the incident light entering an ice covered lake (see also Albrecht, 1964; Maeda and Ichimura, 1973). Snow cover also alters the spectral composition of the transmitted radiation (Curl and others, 1972; Richardson and Salisbury, 1977; Adams, 1978). Thus, the removal of snow created the necessary light conditions for development of the algal inoculum throughout the lake. That these low light conditions were not optimal can be inferred from the high chlorophyll-*a* content cell^{-1} associated with low ^{14}C fixation rates reported by Light and others (1981). Similar low fixation rates were recorded in the present study for the spring melt period.

A close seasonal relationship between bacterial direct counts and chlorophyll-*a* was shown in this study. The lower bacterial maximum in 1977 compared with 1976 coincided with a smaller chlorophyll-*a* peak. Light (1977) presented evidence of increasing eutrophication in Heywood Lake between 1969–74. This was based on increasing chlorophyll-*a* and orthophosphate levels far higher than those measured in 1976–78, and suggests that bacterial numbers may also have been much higher in Heywood Lake in the recent past. Bacterial direct counts followed a similar pattern in Meretta Lake in the High Arctic (Morgan and Kalff, 1972) which is comparable in many respects with Heywood Lake (see Schindler and others, 1974; Heywood, 1968). However, Meretta Lake receives sewage from Resolute Base, which enters the lake in large quantities during the spring melt (Schindler and others, 1974). This resulted in a spring bacterial direct count maximum four times higher (Morgan and Kalff, 1972) than the equivalent in Heywood Lake. The influence of the sewage output of Resolute Base was clearly far less in summer (probably due to reduced inflow rates) as numbers returned to levels similar to those in Heywood Lake. Thus, the amplitude of response of Heywood Lake bacteria and algae during the spring melt is a function of the nutrient status as well as air temperatures and light levels. Schindler and others (1974) suggested that removal of the sewage input to Meretta Lake would quickly result in self-purification, and this would appear to have already occurred in Heywood Lake. However, the apparent reversal of eutrophication may in fact reflect a lower nutrient level due to the reduced incidence in recent years of elephant seals which create large wallow areas around the lake. In contrast, fur seal numbers have increased markedly since 1975 but do not create wallows and so contribute relatively far less to nutrient enrichment of Heywood Lake.

The high proportion of enzyme producers in the viable counts were a feature of the Heywood Lake results and this has also been found in other Signy Island lakes (Paper I). Jones (1971) reported much lower mean values for amylolytic and proteolytic (but not lipolytic) bacteria from two English lakes (Table VI). This could be a reflection of greater diversity in a less rigorous environment. But Table VI also shows that whereas enzyme producers as a percentage of viable counts in the present study increased with trophic status, the reverse occurs in the English lakes. The significance of this finding is not immediately apparent and requires further study.

The sharp decrease in late spring chlorophyll-*a* appeared to coincide with the development of viable bacteria maxima, particularly of exo-enzyme producers. Light and others (1981) concluded that chlorophyll-*a* decrease was linked to a decline in the spring algal population and development of the summer open-water population of cryptophytes with lower chlorophyll-*a* content per cell. The presence of large numbers of decaying algae from the spring population provides a potentially large source of nutrients to exo-enzyme producers and heterotrophs generally. This follows Drabkova's (1965) suggestion that peaks of bacterial numbers were a response to decaying rather than viable algae. Jones (1971) reported that exo-enzyme-producing bacteria maxima closely followed the algal chlorophyll peaks and in a later paper (Jones, 1976) showed high colonization of senescing (but not of healthy) algal cells by bacteria. The present

TABLE VI. MEAN PERCENTAGE COMPOSITION OF VIABLE BACTERIA REPRESENTED BY ENZYME-PRODUCING BACTERIA

Lake		*Protease producers*	*Amylase producers*	*Lipase producers*
Polar				
Amos Lake	(E)	40.0	47.8	1.8
Heywood lake	(M)	32.3	31.8	1.9
Moss Lake	(O)	15.5	16.6	1.7
Temperate				
Esthwaite Water	(E)	7.0	6.6	3.4
Windermere North Basin	(O)	9.0	13.7	3.6

Temperate lake data from Jones (1971)
Amos lake data (Ellis-Evans, unpubl.).
Heywood Lake data (this study).
Moss Lake data (Paper I).
E Eutrophic, M Mesotrophic, O Oligotrophic

study found that the highest percentage of attached bacteria recorded in Heywood Lake were associated with decreases in chlorophyll-*a* in spring and autumn. Several laboratory experiments to measure cell-free enzyme activity in Heywood Lake, using methods outlined by Jones (1971) revealed no measurable activity. However, enzyme activity in water samples containing bacterial cells was readily detectable at lake temperatures. This suggests that enzyme attack on macromolecules occurred at the bacterial cell envelope and that perhaps attached bacteria were the source of these enzymes. Another point to emerge from these latter enzyme assays was that there was no appreciable lag before enzyme activity was detectable. This suggests that the bacterial enzyme operons were activated and thus the plate counts may not merely reflect the potential for exo-enzyme production in the natural population. Further evidence of the relationship between decaying algae and viable bacteria counts was obtained from direct count (DC): viable count (VC) ratios. These were in the range 10–1 000 for Heywood Lake, whilst Jones (1976) reported a more restricted range of 100–1 000 for some English lakes. The smallest Heywood Lake ratios coincided with the decline of the algal population in spring and autumn and the largest values were calculated from the winter data. In late spring, the water column was well mixed and a low DC : VC ratio was recorded at the same time throughout the profile, whilst in autumn, the onset of ice formation induced stratification so the bottom-water ratios apparently decreased after the top-water ratios as decaying algae settled out.

The kinetic approach to studies of uptake of organic solutes by natural bacterial populations has been applied to many different aquatic environments, but certain environments exist where the results do not fit saturation kinetics and these tend to involve oligotrophic systems and winter conditions (Vaccaro and Jannasch, 1967; Hamilton and Preslan, 1970). The problems associated with the kinetic approach have been discussed by Hobbie (1971), Wright (1973) and Hobbie and Rublee (1977). The annual cycle of acetate uptake in Heywood Lake was similar to that found for both glucose and acetate in Lake Erken, Sweden (Hobbie, 1971) and for glucose in Meretta Lake (Morgan and Kalff, 1972). Data for both these lakes showed two uptake maxima, occurring in late spring and late summer/autumn. The first peak coincides with that found in Heywood Lake. However, practical problems prevented uptake measurements for this lake in April and May, 1977, so that the second peak, suggested both by the cycles in the above lakes and by the bacteria count data in the present study, could not be confirmed.

Evidence has been presented to suggest a close link between organic solute uptake rates and trophic status as indicated by primary production (Morgan and Kalff, 1972, fig. 3). But in lakes where sediments or pollutants are dominant influences on water column activity, Hobbie and Rublee (1977, fig. 14.7) demonstrated that the direct relationship can become confused. Heywood Lake, however, was not apparently strongly influenced by such factors and the suggested relationship categorized the lake as "low mesotrophic". Similarly, turnover times

appear to be related to the trophic status of the lake systems. Turnover times of 500 h or more indicate virtually no bacterial activity, but a value of 39 h or less as found in the summer water column of Heywood Lake suggested a considerable turnover of substrate in open-water conditions, whilst Meretta Lake had a range of 5–175 h and was clearly active throughout the year (Morgan and Kalff, 1972).

No correlation was found between acetate V_{max} and viable counts but a significant correlation was found between acetate V_{max} and direct counts. Ramsay (1978) reported a weak but significant correlation between V_{max} and direct counts in New Zealand oligotrophic and mesotrophic lake samples, but not for seawater sampled near industrial outfalls. No relationship could be demonstrated in Meretta Lake by Morgan and Kalff (1972) and this was attributed to changing levels of organic input (mainly sewage) resulting in markedly different uptake rates per cell. Also, no correlation was found between acetate or glucose uptake and direct counts in a small eutrophic lake (Amos Lake) on Signy Island which received large nutrient inputs at intervals from both seal wallows and bird colonies (unpubl. data). Stanley and Staley (1977) reported up to twenty-fold variations in acetate uptake and related this to differences in cell morphology of bacterial groups. Although Morgan and Kalff (1972) did not report much variation in the morphology of Meretta Lake bacterial populations ($1–2 \times 0.5$ μm rods) significant variation was found by Spencer and Ramsay (1978) between stations on the Ri[illegible] Waikato, New Zealand where V_{max} cell^{-1} varied greatly. It is perhaps significant that whilst Heywood Lake bacteria were of relatively constant morphology ($0.7–1.4 \times 0.5$ μm) considerable variation was found in the Amos Lake population, ($0.5–7.0 \times 0.5–1.0$ μm) especially in summer. On the basis of such information it would appear that the stability of the V_{max} cell^{-1} value describes the degree of influence exerted by allochthonous sources of enrichment and is an indication of the increasing instability associated with eutrophication. The range of V_{max} cell^{-1} in Heywood Lake was $(3.6–19.0) \times 10^{-11}$ μg h^{-1} cell^{-1} ($n = 30$) for acetate. For comparison, a range of $(4–37) \times 10^{-11}$ μg h^{-1} cell^{-1} ($n = 15$) was found in Amos Lake.

Photo-heterotrophy was not demonstrated in the present study, although this may have been due to the relatively crude method of assessment used. McKinley and Wetzel (1979) using ^{3}H-compounds calculated that photo-heterotrophic assimilation was only a very small proportion of carbon uptake by lake plankton communities. Light and others (1981) considered that Heywood Lake phytoplankton "switched off" during the winter period by depressing respiration rates (assisted by the low temperatures) or encystment. From the admittedly limited evidence presented here, this still appears the most likely explanation. In summer, the comparatively low temperatures may well have restricted potential bacterial production since optimum temperatures for lake isolates appear to be about 15°C. Algal production was not affected to the same extent and could take advantage of high light levels and significant nutrient input due to a mixing depth greater than the photic zone.

Annual algal production for Heywood Lake during 1977–78 was only about 75% of the m[illegible] values calculated for the lake during 1969–72 (Light and others, 1981). However, this value w[illegible] over ten times higher than the value reported by Kalff and Welch (1974) for enriched Meretta Lake. Light and others (1981) gave comparative production rates for a large number of Arctic, alpine and Antarctic lakes. The data suggested that some Antarctic lakes are markedly more productive than lakes in other cold regions, often in spite of a short growing season. These Antarctic lakes tended to conform to the pattern for highly productive lakes suggested by Uhlmann (1978). When assimilation numbers (a measure of assimilation efficiency) were calculated for Heywood Lake phytoplankton during January 1971, a maximum value of 10.5 mg C h^{-1} mg^{-1} (chlorophyll-*a*) emerged (Light and others, 1981). In the present study, a remarkably similar maximum value of 10 mg C h^{-1} mg^{-1} (chlorophyll-*a*) was calculated from short-term incubations (5 h) for January 1978. These assimilation numbers are comparable with values obtained for Ethiopian soda lakes (Talling and others, 1973) where high temperatures, large reserves of carbon dioxide and surplus dissolved inorganic phosphate exist, contrasting

sharply with the low temperatures and comparatively low nutrient status of Heywood Lake. Both values for this lake were at the upper end of the 1–10 mg C h^{-1} mg^{-1} (chlorophyll-*a*) range proposed by Talling (1975) as typical of fresh-water phytoplankton. A markedly lower assimilation value of 3.0 mg C h^{-1} mg^{-1} (chorophyll-*a*) was calculated for oligotrophic Moss Lake during January 1978, whereas Kalff and Welch (1974) reported that ultra-oligotrophic Char Lake and nearby enriched Meretta Lake gave remarkably similar assimilation numbers. Both Talling (1966) and Megard (1972) suggested that limitation of nutrients acts primarily on chlorophyll concentrations and has little bearing on assimilation efficiency. The markedly higher assimilation efficiency found in Heywood Lake compared with Moss Lake could reflect differences in species composition, but the light regime of these two lakes also merits further study.

Bacterial production estimated from measurements of dark CO_2 fixation using filtered lake-water samples has been applied extensively to lake and reservoir studies in East European countries (Overbeck, 1972; Kuznetzov, 1977). However, in recent years, evidence has emerged suggesting that use of the 6% constant may generate substantial overestimates (Jordan and Likens, 1980). Data presented here and in Paper I clearly support this suggestion. Anderson and Dokulil (1977) applied the $^{14}CO_2$ technique to some Canadian lakes and found that bacterial production averaged 3.8 times phytoplankton production. Kuznetzov (1968) reported that bacterial production in the Rybinsk reservoir equalled algal production over the growing season. Hobbie (1971) attributed such high bacterial contributions to the large input of organic material from the surrounding catchment areas. However, visual evidence indicates that the greater part remains undegraded and is lost through sedimentation or via the outflow. Consistently low temperatures probably restrict microbial response to the large organic input. Although bacterial production was significantly less than algal production, the values were nevertheless high when compared with values published elsewhere (Kuznetzov, 1977; Anderson and Dokulil, 1977). It would appear that the high assimilation efficiency of Heywood Lake algae could explain the observed discrepancy between algal and bacterial assimilation rates reported here when compared with other similar systems.

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