A STUDY OF MICROORGANISMS FOUND IN THE RED RIVER

by

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ABSTRACT

A nutritional distribution study was carried out on the bacteria of the Red and Assiniboine Rivers. The results showed a great variation in the levels of the different nutritional groups. Microorganisms utilizing as their minimal medium, a medium containing glucose, amino acids and basal salts, were generally the most numerous. River waters at sampling stations within metropolitan Winnipeg had a high percentage of such organisms in winter; this percentage fell during the spring and summer. The percentage increased again during the fall, rising towards the values obtained during the winter. River waters at sampling stations outside metropolitan Winnipeg, on the other hand, had a low percentage of such organisms in winter; this percentage rose during the spring, summer and fall. At all sampling stations, the general pattern of change in the percentage of microorganisms utilizing a medium containing glucose, yeast extract and basal salts as their minimal medium showed that this percentage was higher in the winter than during the rest of the year. The population curve of microorganisms utilizing a minimal medium of glucose and basal salts showed that these organisms were more numerous in the

spring; they then increased greatly during the summer and decreased in the fall. Population changes in the groups of microorganisms utilizing the remaining types of minimal media did not appear to be seasonal.

When the different nutritional types were taken separately but averaged over all sampling stations at all depths are compared throughout the seasons a clear pattern emerged. Those microorganisms utilizing the simplest medium as their minimal medium were found to be most numerous in the fall, declining through the winter and spring and then increasing toward the fall value during the summer. Those microorganisms which utilized the more complex media as their minimal media were found to be most numerous in the winter and spring and declined in the summer and fall.

Temperature, pH, total bacterial counts and faecal coliform counts were also determined. The faecal coliform count showed an increase through the summer and decreased again in the fall at all the sampling stations except at the station furthest downstream. At this sampling station the faecal coliform count reached a maximum in the winter, decreased during the summer, and increased again in the fall.

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ABBREVIATIONS

Type 1 microorganisms - microorganisms utilizing medium 1 as their minimal medium.

Type 2 microorganisms - microorganisms utilizing medium 2 as their minimal medium.

Type 3 microorganisms - microorganisms utilizing medium 3 as their minimal medium.

Type 4 microorganisms - microorganisms utilizing medium 4 as their minimal medium.

Type 5 microorganisms - microorganisms utilizing medium 5 as their minimal medium.

Type 6 microorganisms - microorganisms utilizing medium 6 as their minimal medium.

Type 7 microorganisms - microorganisms utilizing medium 7 as their minimal medium.

INTRODUCTION

The Red River has its source in the northern United States, forming the boundary between the states of North Dakota and Minnesota. The river flows north, crossing the international boundary into the province of Manitoba, Canada. From here the Red River is a winding, slow moving river, passing through metropolitan Winnipeg, where it is joined by the Assiniboine River, which flows west from the into the city from the Saskatchewan prairies. The joint river continues its northward flow as the Red River and empties into Lake Winnipeg.

Between the Canada-U.S. boundary and Winnipeg there are several small towns and villages in which the predominant industry is agriculture. Winnipeg, a city of 500,000, is a railroad centre associated with several primary agricultural processing plants in central Winnipeg, meat packing plants in the St. Boniface district of east Winnipeg and a sugar refinery in the Fort Garry district of south Winnipeg. There are also some oil refineries in the city, as well as a large percentage of the province's secondary manufacturing. Although the amount of wastes these industries contribute to the river has been greatly reduced in recent years as a result of government action, contamination of the river from various

sources, agricultural and industrial, remains considerable and as a consequence the Red River can be catagorized only as "badly polluted". Moreover agriculture and industry are not the sole source of pollution; in the older areas of Winnipeg storm sewers and sanitary sewers were not, and are not separated. When rainfall is any more than light, the sewers cannot handle the combination of rain water and sewage, so the excess is dumped directly into the river. Another major source of raw sewage is that which is added directly to the river by several municipalities where sewage treatment plants are not available (9).

The climatic conditions in this area are extreme. The average temperature for the last thirty years is thirty-six degrees Fahrenheit, with temperatures of minus forty and plus ninety being common. The precipitation averaged only twenty inches per year in the last thirty years. Most of the precipitation leaves the Red River basin in the spring causing varying degrees of flooding along the river each year.

With ice covering the river for at least five months, and in the absence of rapids or falls, other than the locks at Lockport, north of Winnipeg, re-aeration of the river is kept to a minimum. The river in this area has a clay base, thus producing murky waters. Because of the low oxygen

content and murky waters few game fish can be found; moreover, the high sewage content of the water makes bathing hazardous.

With these facts in mind the provincial government became interested in improving the situation. Funds were made available for basic research on the Red River and as a result work contained in this thesis was initiated. Sampling stations were selected upstream and downstream from metropolitan Winnipeg, as well as at three points in the metropolitan area. One of these stations was selected downstream from the junction of the Red and Assiniboine Rivers. One was selected upstream on the Red River and one was selected on the Assiniboine River upstream from this junction. Temperature, pH, total microbial counts, faecal coliforms and seasonal changes in nutritional distribution were to be monitored for one full year.

HISTORICAL

Water pollution has always been an ecological problem. Since man is part of the natural ecological system, he does not stand alone and anything he does is likely to reflect on himself; the destruction of fish by water pollution is an excellent example of man's eliminating his own food supply. Water pollution is also a political problem; the main effort in the direction of solving it has been local self-education, but since the flow of polluted waters does not respect municipal, provincial or international boundaries, political cooperation is necessary. Moreover, the esthetic value of water has to be considered on a political level.

For hundreds of years, man has been dumping his sewage and other wastes into streets, rivers, lakes or whatever was convenient. As populations became more concentrated, epidemics became more common. It was not until 1832, when Edwin Chadwick was appointed Assistant Commissioner to enquire into England's poor laws, that any steps were taken in public health. With cholera sweeping continental Europe in 1848, public pressure forced the passage of the first Public Health Act. This act was largely ineffective, and London was not included in its

jurisdiction. In 1849, John Simon, the London Health Commissioner, reported to the Commissioners of Sewers that typhus had become epidemic in many quarters of the city. He associated the causative agent with detritus which contaminated the drinking water supply (Fig. 1). This was the first association of the causative agent of the disease with water (2, 4).

In Canada, early public health measures were under the jurisdiction of the Dominion Government but they were not, however, directed towards sanitation but towards the prevention of the distribution of spoiled agricultural produce; thus in 1872 these measures were the concern of the Department of Agriculture and it was not until 1919 that the Department of Health was created. In 1923 regulations were adopted for the supervision of water on vessels navigating the Great Lakes and other inland waters, as well as on other forms of transportation.

Most of the authority in the public health field was assigned to the provinces at Confederation and has remained with them to the present time. In 1873 Ontario passed its first Public Health Act, and in 1890 the first public health laboratory was set up in Toronto, with branches developing later. In 1912 a sanitary engineer was placed at the head of the provincial health department. At this time public

water supplies and sewage removal became supervised, resulting in a marked drop in typhoid deaths.

A transition period followed; water-borne disease was now under some form of control and emphasis was being placed on the examination of water used for industrial, agricultural and recreational purposes. The industrial usage and disposal of water helped the public health workers to attack the problem of water pollution on a wide front.

Today the federal government has taken a more active interest in the problem. Provincial governments have placed water supervisors under separate administrative control. With the financial backing from these two levels of government, many municipalities have been able to build water and sewage treatment plants. This helped to stop, or at least lower the levels of, water pollution in the inland waters (2).

The agents of water pollution have become as diverse as the activities of man himself. Water has almost become the universal dispersive agent, and thus the types of pollution vary as the types of uses that water is put to. The result has been that more and more emphasis has been placed on basic research to further resolve the nature of this complex problem.

In many respects, the microbiology of waste water

could be termed the "microbiology of heterogeneous populations". In the past the major interest in this field has been centered on removing, killing or controlling specific species, or specific groups, of microorganisms in waste waters. The challenging aspect to researchers in this field is the necessity of uncovering facts of predictive value for natural populations rather than for a single pure culture (11).

At the present time there are many reports in the literature (5, 6) which deal with tests which at best are only indicator tests. BOD, COD, total bacterial counts and faecal coliform counts are tests of this type. BOD is the biochemical oxygen demand; and may be defined as the quantity of oxygen required during the stabilization of decomposable organic matter and oxidizable inorganic matter by aerobic biological action. COD is chemical oxygen demand; and may be defined as the oxygen equivalent of that portion of the organic matter in the sample that is susceptible to oxidation by a strong chemical oxidation. Both the BOD and COD give varying results depending on the temperature and the type of organic material (1). Total counts and faecal coliform counts are both tests that vary with temperature of the water, type of organic material added to the river, and the predominant type of microorganisms

present in the river at the sampling point, thus the method of enumeration will greatly influence the total count. If during the summer the majority of the organic breakdown is caused by aerobic microorganisms, there will be a certain amount of correlation between the BOD and the total count. If, however, during the winter, or in a river which is anaerobic, there would be little if any correlation between the BOD and the total count. If there is a shift in the type of microorganisms present in the river during the various seasons there may also be a shift in the nutritional requirements of these microorganisms. Thus, in one season there may be a complete breakdown of the organic materials to CO_2 and water while during another season the breakdown may stop at organic acids or alcohols. This would change the oxygen requirements of the river and would not show up in the BOD, COD, total counts or faecal coliform tests. If is therefore suggested that a more accurate way is needed to assay the biological activity in the river or lake being studied.

It was evident that there were very little data available about the fluctuations of microorganisms during seasonal changes in a river. The Red River was used as a sample river because it is conveniently located and is known to carry a certain amount of pollution. Moreover, the

temperature of the river and the amount of pollution it carries are known to vary considerably during the seasons and if as a result of these changes there were changes in the microbial flora, there might also be a change in the biochemical or nutritional requirements of these micro-organisms. To study a possible change in the biochemical or nutritional requirement, the Lochhead and Chase method (10) of classifying the members of a mixed bacterial population according to their minimal nutritional requirements was used. This method, which is commonly referred to as "nutritional distribution", has been used to divide microorganisms occuring in the soil into seven different groups, based on their ability to utilize one of the seven different media for their minimal requirements.

The work in this thesis is an attempt to obtain an overall picture of microbial patterns in the Red river throughout the seasons based, in the first place, on the Lochhead-Chase "nutritional distribution".

MATERIALS AND METHODS

Sampling stations were located along the Red and Assiniboine Rivers as shown in Fig. 2. Sampling station A was located near Morris off the bridge on provincial highway 23. Sampling station B was located off the old Elm Park Bridge, just upstream from the new Osborne Bridge. Sampling station C was located off the railroad bridge just downstream from the St. James Bridge. Sampling station D was located off the Louise Bridge on Higgins Avenue. Sampling station E was located off the bridge on the road between Selkirk and East Selkirk.

Samples were taken on the dates shown in Table 1. Samples were taken midway between the banks; or, in cases where the river was too shallow, from the middle of the main channel. Three samples were taken at each sampling station: from the top few inches, half-way to the bottom and from the bottom of the river. Temperature, total count and faecal coliform count were determined in all samples and pH was determined for all samples taken after May 29, 1966. Four sets of samples were taken for nutritional distribution studies on the dates shown in Table 1. The temperatures of the samples were taken immediately as obtained.

Total counts were determined by plating dilutions of 10^1, 10^2, 10^3, and 10^4 of each sample on soil yeast extract agar, without glucose, at pH 6.8. These plates were incubated at 28° C for one week and counted.

Faecal coliforms counts were performed as outlined in recommended procedures for the bacteriological examination of sea-water and shell fish (3), and modified according to the method of Geldreich (7).

For the nutritional distribution studies, stock cultures were picked at random from those dilutions which, when plated on soil-yeast extract without glucose, yielded isolated colonies. One hundred and fifty stock cultures from each level of each sampling station for each sample taken and were picked and stored on soil-yeast extract agar slants. When the stocks were required for further studies, five ml of soil-yeast extract was added aseptically to each slant. These slants were incubated at 28° C for two days. Stock cultures were Gram stained and examined microscopically, and those stock cultures that were obviously contaminated were discarded. Each of the apparently pure stock cultures was used to inoculate tubes of the seven different media of Lochhead and Chase (10), which will be described in detail later. The tubes were incubated at 28° C for one week. After this time, the amount of growth was assessed visually

and recorded on an arbitrary scale from zero (no growth) to three (heaviest growth). Tubes showing no growth were discarded. Where a tube showed any growth at all, a loopful of the culture was used as an inoculum for a tube containing the same type of medium. These tubes were then incubated at 28° C for a week. The tubes were examined, and those showing growth were transferred into the same medium and incubated for another week at 28° C. The growth was recorded at this point as being characteristic of the medium and microorganism.

The medium which was the least complex and supported growth throughout the entire operation for each of the isolates tested of each sampling level was tabulated. The results for each medium were expressed as the number of cultures which showed their minimal growth requirements in each medium times one hundred and divided by the total number of cultures tested for that sampling level. The results for each medium were thus expressed as the percentage of the total number of bacteria present which found their minimal growth requirements in that medium. Thus if ten percent grew in medium 1, fifteen percent in medium 2 and thirty percent in medium 3 the percentage figures for media 1, 2 and 3 would be ten, five and fifteen respectively.

The seven different media used for the nutritional distribution studies were dispensed in five ml samples and sterilized for 20 minutes at 15 p.s.i. pressure. The compositions of the media are listed below:

Medium 1 (Basal medium) glucose, 1.0 gm; K_2HPO_4, 1.0 gm; KNO_3, 0.5 gm; $MgSO_4$, 0.2 gm; $CaCl_2$, 0.1 gm; NaCl, 0.1 gm; $FeCl_3$, 0.01 gm; distilled water, 1 liter. The medium was heated to 100° C, filtered and adjusted to pH 6.8.

Medium 2 (Amino acid medium) Medium 1 plus 0.05 gm per liter each of cysteine, alanine, proline, asparagine, glutamic acid, aspartic acid, arginine, leucine, glycine, and lysine.

Medium 3 (Growth factor medium) Medium 1 plus cysteine, 0.05 gm; thiamine, 100 gm; biotin, 0.1 gm; pyridoxin, 200 gm; pantothenic acid, 100 gm; nicotinic acid, 100 gm; riboflavin, 200 gm; and inositol, 0.05 gm per liter.

Medium 4 (Amino acid and growth factor medium) Medium 1 plus amino acids as in Medium 2 and growth factors as in Medium 3.

Medium 5 (Yeast extract medium) Medium 1 plus yeast extract, 1.0 gm per liter.

Medium 6 (Soil extract medium) seven hundred and fifty

ml of Medium 1 plus two hundred and fifty ml soil extract, prepared by adding 1 kgm of soil to 1 liter of water, heating for thirty minutes at 121° C, filtering and making the filtrate up to one liter.

Medium 7 (Soil-yeast extract medium) Medium 6 plus yeast extract, 1.0 gm per liter.

RESULTS

General Survey

The temperature, pH, and total bacterial counts for the top, middle and bottom levels of the river at each sampling point are shown in Figures 3 to 7. The maximum temperature did not vary appreciably from point to point and reached between 25.5 and 28.0° C on the sampling date July 10, 1966. From November to the early part of May, ice covered the entire river system. The temperature at this time was 0° C.

The pH was found to vary between 7.8 and 8.5 and apparently bore no correlation with temperature at any point at any time. The fact that the pH is appreciably above neutrality is probably due to the influence of the clay base over which the river flowed.

With one or two minor exceptions, there was a reasonable correlation at all sampling points between the total counts recorded for top, middle and bottom samples; e.g., when the top count was high, the bottom count was high; when the top count was low, the bottom count was low. The total counts at Morris (Fig. 3) fluctuated between 2×10^3 and 7×10^4 microorganisms per ml. There was one exception;

the bottom count on May 17, 1966 was 1.3×10^5 microorganisms per ml. This rise in count was probably due to the parallel rise in temperature which allowed the microorganisms to proliferate at the expense of the organic materials deposited on the bottom during the winter.

The total counts at Elm Park Bridge (Fig. 4) were approximately 1.0×10^5 microorganisms per ml on February 1, 1966 and gradually decreased to 2×10^4 by the end of August. The average count then rose to 1.4×10^5 microorganisms per ml in early September. The count then decreased to 2.3×10^4 microorganisms per ml.

The total counts at the St. James Bridge (Fig. 5) on the Assiniboine River began at an average of 1.7×10^3 microorganisms per ml in February and then rose in the spring to an average of 1.7×10^5 microorganisms per ml. The total count then dropped to 3.7×10^4 microorganisms per ml throughout the remainder of the study. The higher count in the spring was probably due to the spring discharge from the Charleswood lagoons which serve the West End of metropolitan Winnipeg.

The total counts at the Louise Bridge on Higgins Avenue (Fig. 6) fluctuated between 2×10^3 microorganisms per ml and 6.4×10^4 microorganisms per ml for most of

the year. A slight rise was noted in the latter part of July. There was, however, a marked rise in September to an average of 1.8×10^5 microorganisms per ml. This rise in count was probably due either to the work on the mouth of the floodway, south of Winnipeg, or to the effluent discharge of the sugar refinery in Fort Garry.

The total counts at Selkirk (Fig. 7), a small community some 45 miles downstream from metropolitan Winnipeg, began at an average count of 1.1×10^5 microorganisms per ml in the spring and gradually decreased to 4×10^3 microorganisms per ml by early August. In early September the average total count rose to 1.7×10^5 microorganisms per ml and then decreased rapidly to the winter range of 2 to 4.2×10^4 microorganisms per ml.

In summary, all sampling stations except Higgins exhibited a higher total count in the early spring than in the summer or winter, while Elm Park, Higgins and Selkirk exhibited a marked increase in total counts in early September.

Faecal coliform counts at Morris (Fig. 8) were at a maximum in the middle of July, while the faecal coliform counts at Elm Park (Fig. 9) showed a dip in the early spring and then increased to a maximum in early August. The faecal coliform counts at St. James (Fig. 10) reached

a maximum in early June. Although the faecal coliform count was generally in the region of 10^3 per 100 ml the count at Higgins reached more than 240,000 per ml in early August. At Selkirk (Fig. 12) a surprising change took place; the faecal coliform count dropped during the summer. It reached a minimum in late July which was a hundred fold decrease from the winter level.

Faecal coliform counts at the three different sampling levels of each sampling station did not follow any specific pattern. No one level gave counts consistently higher than any other level.

When the average faecal coliform counts for each sampling station were plotted (Fig. 13) an interesting pattern developed. The faecal coliform levels in the Red River started from a minimum at Morris, increasing through each of the following stations: Elm Park, Higgins and Selkirk in the winter. During the summer the faecal coliform counts at Selkirk decreased while the counts at Higgins increased. The coliform counts on the Assiniboine River (St. James station) were in general lower than those on the Red River (all sampling stations other than Morris, which is 40 miles upstream from metropolitan Winnipeg).

Nutritional Distribution Studies

Comparison of the results obtained from the seven "nutritional" media for each level of each sampling station are shown in Figures 14 to 20. For easier interpretation, the results were replotted in several ways. These were: (a) variation of nutritional distribution with depth at one sampling station; (b) variation with seasons at one sampling level of one sampling station; (c) variation with location along the river at the same sampling level. The number of isolates tested at each sampling level are tabulated in Table II.

Variations with Depth

The changes in nutritional distribution with depth at Morris (Fig. 21 and 22) were found to be very small during the winter and fall. In the spring there was a marked decrease in the levels of type 5 microorganisms with increasing depth and a corresponding increase in the levels of type 2 microorganisms. In the summer an increase was noted in the percentage in type 1 microorganisms with increasing depth and a similar decrease in the percentage of type 2 microorganisms.

No significant change in the nutritional distribution with depth was noted at Elm Park (Fig. 22) during the

winter. During the fall of 1965 (Fig. 22) there was a decrease in the percentage of type 5 microorganisms with increasing depth. The percentage of type 1 microorganisms fluctuated inversely with the percentage of type 2 microorganisms. During the spring (Fig. 23) the percentage of type 4 microorganisms fluctuated inversely with the percentage of type 2 microorganisms with increasing depth. The percentage of type 1 microorganisms fluctuated inversely with the percentage of type 5 microorganisms. During the fall of 1966 (Fig. 23) the percentage of type 1 microorganisms fluctuated inversely as the percentage of type 2 microorganisms with increasing depth.

No significant change in the nutritional distribution with depth was found during the summer at the St. James Bridge (Fig. 24). During the winter (Fig. 24) the percentage of type 2 microorganisms decreased markedly with increasing depth. There were small increases in the percentages of types 1, 3 and 6 microorganisms which, in total, were enough to compensate for the marked decrease in the percentage of type 2 microorganisms. During the spring (Fig. 24) the percentage of type 2 microorganisms increased markedly with depth while the percentage of type 5 microorganisms decreased markedly. During the fall (Fig. 25) the percentage of type 5 microorganisms increased

with increasing depth, while the percentage of type 1 microorganisms decreased. The percentage of type 3 microorganisms fluctuated with increasing depth.

At Higgins there was no significant change in the nutritional distribution with depth during the summer (Fig. 26). During the winter (Fig. 25) the percentage of type 2 microorganisms fluctuated inversely with the sum of the percentages of types 1, 4 and 5 microorganisms. During the spring (Fig. 25) the percentage of type 2 microorganisms fluctuated inversely as the percentage of type 5 microorganisms with increasing depth. During the fall (Fig. 26) the percentages of types 1, 2 and 4 microorganisms varied in such a manner that any increase in the percentage of one type of microorganism was compensated for by the total decrease of the other two types.

At Selkirk there was no significant change in the nutritional distribution with depth during the spring (Fig. 27). During the winter (Fig. 26) there was a decrease in the percentage of type 2 microorganisms with increasing depth. The percentage of type 5 microorganisms fluctuated inversely with the sum of the percentages of types 1, 3, 4, and 6 microorganisms. During the summer (Fig. 27) the percentage of type 1 microorganism fluctuated

inversely with the percentage of type 2 microorganisms. During the fall (Fig. 27) the percentage of type 1 microorganisms increased with increasing depth, while the percentage of type 2 microorganisms decreased.

Variations with Seasons

The changes in nutritional distribution in the top level at Morris through the seasons (Fig. 28) showed a marked decline in the percentage of type 5 microorganisms, starting from the winter through to the fall. There was at the same time a marked increase in the percentage of type 2 microorganisms. Types 1 and 4 microorganisms showed considerable fluctuation. Types 3 and 6 microorganisms accounted for only a small percentage of the total in the winter and tapered off to almost zero during the other seasons.

The changes in nutritional distribution in the middle level of the river at Morris through the seasons (Fig. 28) showed a slow, but steady increase in the percentage of type 2 microorganisms from the winter through to the summer. Then there was a sharp increase in this percentage to the fall. The percentage of type 1 microorganisms fluctuated far more in the middle level of the river than at the top. The fluctuation that occurred in the middle level of the river at Morris was as much as

fifty percent of the total number of microorganisms present. The percentage of type 5 microorganisms remained steady during the winter and spring and then dropped to near zero for the summer and fall. The percentage of type 3 microorganisms showed a steady decrease from the winter to the fall. Type 4 microorganisms showed an increase during the winter, but for the other seasons their numbers remained near zero.

The changes in nutritional distribution at the bottom level of the river at Morris through the seasons (Fig. 29) showed a decrease in the percentage of type 5 microorganisms from the winter to the summer and through to the fall. The percentage of type 3 and 6 microorganisms declined from their winter value to near zero for the rest of the seasons. The percentage of type 4 microorganisms showed only a slight increase in the spring, and remained near zero for the other seasons. The percentage of type 1 microorganisms fluctuated inversely with the percentage of type 2 microorganisms. Both these percentages varied greatly. The sum of the percentages of types 1 and 2 microorganisms showed a steady increase from the winter through to the fall.

The changes in nutritional distribution near the top level of the river at Elm Park through the seasons (Fig. 29)

appeared to be part of a repeating pattern. The samples from the fall of 1966 were taken later in the year than the samples from the fall of 1965. The percentage of type 2 microorganisms had a peak in the winter and declined to a low in the summer and then increased during the fall of 1966. The percentage of type 1 microorganisms started from a high in the fall of 1965 and then gradually decreased to zero in the spring. This percentage increased again to the values of the previous fall by the fall. The percentage of type 5 microorganisms was at a low of zero in the winter and increased to form a peak in the summer; the percentage then decreased during the fall. The percentage of type 4 microorganisms was near zero for all the seasons, except for the spring when it showed a significant peak. The percentage of the remaining types of microorganisms showed only minor fluctuations.

The nutritional distribution in the middle level of the river at Elm Park through the seasons (Fig. 30) showed only minor fluctuations in the percentages of all the types of microorganisms except types 1 and 2. The percentage of type 1 microorganisms showed a peak in the fall of 1965, dropping to zero in the winter and spring. This percentage rose towards the level of the previous fall during the summer and fall. The percentage of type 2 microorganisms

varied inversely as the percentage of type 1 microorganisms; forming a low in the fall and a peak in the winter with a steady decline through the remaining seasons.

The nutritional distribution at the bottom level of the river at Elm Park through the seasons (Fig. 30) showed greater fluctuations than at the other two levels for this sampling station. The percentage of type 1 microorganisms was at high in the fall of 1965, dropped to a low of near zero during the winter and then increased steadily through to the fall of 1966. The percentage of type 2 microorganisms showed a declining oscillation starting from a high in the fall of 1965. The percentage of type 5 microorganisms was negligible except during the spring and summer when values significantly above zero were obtained. The percentage of type 4 microorganisms showed a peak in the spring, but was negligible during the other seasons. The percentages of the remaining types of microorganisms showed only minor fluctuations.

The nutritional distribution near the top level of the river at St.James (Fig. 31) through the seasons indicated significant changes in the percentage of three types of microorganisms. The percentage of type 5 microorganisms was at a low in the winter, rose to a peak in the spring and then gradually decreased to the winter

level through the summer and fall. The percentage of type 1 microorganisms was almost zero through the winter and spring but rose to a high level for the summer and fall. The percentage of type 2 microorganisms showed a declining oscillation starting from a high in the winter.

The nutritional distribution in the middle level of the river at St. James (Fig. 31) (through the seasons) showed that a greater number of different types of microorganisms were present. The percentage of type 1 microorganisms was near zero in the winter and spring; then rose to a significant level for the summer and fall. The percentage of type 2 microorganisms started from a high in the winter and then showed a declining oscillation through the remaining seasons. The percentage of type 3 microorganisms showed a slight rise in the fall after remaining near zero throughout most of the year. The percentage of type 4 microorganisms rose to a slight peak in the spring and then gradually tapered off during the remaining seasons. The percentage of type 5 microorganisms rose to a peak in the spring and then gradually declined through the rest of the year. The percentage of type 6 microorganisms showed a slight peak in the spring, but remained near zero during the remainder of the year while the percentage of type 7 microorganisms remained near zero throughout the year.

The nutritional distribution at the bottom of the river at St. James (Fig. 32) through the seasons showed only a few changes compared with the nutritional distribution in the middle of the river at the same sampling station. The percentage of type 1 microorganisms declined from the winter to a low in the spring and then increased towards a maximum in the fall. The percentage of type 2 microorganisms oscillated throughout the year. The percentage of type 3 microorganisms showed a slight peak in the winter and remained near zero for the remainder of the year. The percentage of type 4 microorganisms was near zero throughout the year, except in the summer when a small peak was apparent. The percentage of type 5 declined from the winter value to near zero for the spring and summer and then in the fall. The percentage of types 6 and 7 microorganisms remained near zero during the year.

The nutritional distribution near the top level of the river at Higgins (Fig. 32) through the seasons showed only slight variations in the percentages of all types of microorganisms except for types 1 and 2. The percentage of type 1 microorganisms started from a low in the winter, rose to a peak in the summer and then declined to the winter value in the fall. The percentage of type 2 microorganisms started from a peak in the winter, declined through the

spring to a low in the summer, and increased again to the winter value in the fall.

The nutritional distribution in the middle level of the river at Higgins (Fig. 33) through the seasons showed the great deal more variation than the top level at the same sampling station. In the spring the percentage of type 1 microorganisms dropped to zero from the winter value, formed a peak in the summer, and then declined to the winter value in the fall. The percentage of type 2 microorganisms dropped to a low in the spring from the winter value and then rose through the summer to form a peak in the fall. The percentage of type 5 microorganisms started from a low winter value to form a peak in the spring and dropped to near zero during the summer and fall. The percentages of the remaining types of microorganisms fluctuated near zero throughout the year.

The nutritional distribution at the bottom level of the river at Higgins (Fig. 33) through the seasons showed the same type of nutritional distribution as was found in the middle level at the same sampling station. The percentage of type 1 microorganisms remained near zero through the year, except during the summer when a large peak was formed. The percentage of type 2 microorganism declined from a winter high to a low in the summer, then

approached the winter value in the fall. The percentage of type 4 microorganisms showed a minor peak in the spring and a major peak in the fall but remained near zero in the winter and in the summer. The percentage of type 5 microorganisms remained near zero throughout the year, except during the spring when a large peak was formed. The percentages of the remaining types of microorganisms showed only minor fluctuations near zero throughout the year.

The nutritional distribution near the top level of the river at Selkirk (Fig. 34) through the seasons showed great variations. The percentage of type 1 microorganisms remained near zero through the winter and spring, formed a large peak during the summer, and then declined somewhat in the fall. The percentage of type 2 microorganisms remained steady at a significant level from the winter to the summer, and formed a large peak in the fall. The percentage of type 5 microorganisms started from a high winter value and declined steadily throughout the year to near zero in the fall. The percentage of type 4 microorganisms rose slightly from the winter value to form a peak in the spring but dropped to near zero in the summer and fall. The remaining types of microorganisms fluctuated near zero throughout the year.

The nutritional distribution in the middle level of the river at Selkirk (Fig. 34) through the seasons showed wide fluctuations. The percentage of type 1 microorganisms declined from the winter value to a low near zero in the spring, formed a large peak in the summer, and declined in the fall. The percentage of type 2 microorganisms rose gradually from the low winter value through the year to a large peak in the fall. The percentages of both types 3 and 4 microorganisms remained near zero throughout the year, except in the spring when a small peak was formed. The percentage of type 5 microorganisms dropped from a high winter value through the spring to summer and fall values which were near zero. The percentages of the remaining types of microorganisms remained near zero throughout the year.

The nutritional distribution at the bottom of the river at Selkirk (Fig. 35) through the seasons was of an unusual type compared to the other levels of the same sampling station, as well as those of the other sampling stations. The percentage of type 1 microorganisms remained near zero through the winter and spring, rose to a large peak in the summer and then declined in the fall. The percentage of type 2 microorganisms rose from a low of near zero in the winter and increased throughout the year

to a high in the fall. The percentage of type 4 microorganisms fluctuated near zero throughout the year except in the spring when a peak was formed. The percentage of type 5 microorganisms declined from a high in the winter through the spring to a low of near zero in the summer and fall. The percentage of type 6 microorganisms formed a peak in the winter, and fluctuated near zero for the remainder of the year. The percentages of the remaining types of microorganisms had values which remained near zero throughout the year.

Variations Along the River

The variation in nutritional distribution at the same sampling level at different sampling stations along the river in the winter (Fig. 36 and 37) showed that the percentage of type 2 microorganisms started from a low value at Morris and increased when the river entered metropolitan Winnipeg. This percentage decreased to the same range as it had been at Morris when the river reached Selkirk. The percentage of type 5 microorganisms started at values of 45, 20 and 20 at the top, middle and bottom sampling levels at Morris. These values remained near zero at all the other sampling stations downstream until the river reached Selkirk. Here this percentage increased to

27, 54 and 33 for the top, middle and bottom respectively. At the bottom sampling level in St. James (Fig. 37) there was a slight increase in the percentage of type 5 microorganisms, to a value of 18. The percentage of type 1 microorganisms started at Morris with values of 19, 33 and 35 for the top, middle and bottom respectively. This percentage fluctuated between zero and 15 at all other sampling stations.

The variation in nutritional distribution at the same sampling level at different sampling stations along the river in the spring (Fig. 37 and 38) showed a marked change from the pattern that appeared in the winter. The percentage of type 2 microorganisms increased compared to that in the winter. At the top sampling level (Fig. 37) this percentage had values of 50 at Morris and Elm Park. This percentage then fluctuated to 30, 60, and 29 at St. James, Higgins and Selkirk. At the middle sampling level (Fig. 38) the percentage of type 2 microorganisms had values of 45, 63, 38, 28, and 25 at Morris, Elm Park, St. James, Higgins and Selkirk respectively; thus forming a peak at Elm Park. At the bottom sampling level (Fig. 38) this same percentage had values of 67, 46, 25, 37 and 34 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. No definite trend could be detected in the

percentage change of type 2 microorganisms as the river flowed north through Winnipeg.

The overall percentage change of type 5 microorganisms was higher in the spring than in the winter. At the top sampling level (Fig. 37) this percentage had values of 25, 14, 54, 15 and 24 at Morris, Elm Park, St. James, Higgins and Selkirk respectively, thus forming a peak at St. James. At the middle sampling level (Fig. 38) this percentage had values of 21, 10, 34, 54 and 24 at Morris, Elm Park, St. James, Higgins and Selkirk respectively, thus forming a peak at Higgins. At the bottom sampling level (Fig. 38) this percentage had values of 11, 25, 9, 42 and 21 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. At this sampling level two peaks were formed: a minor one at Elm Park and a major one at Higgins.

The overall percentage of type 4 microorganisms was higher in the spring than in the winter. At the top sampling level in the spring (Fig. 37) this percentage had values of 14, 24, 2, 6 and 18 at Morris, Elm Park, St. James, Higgins and Selkirk respectively, thus forming a peak at Elm Park. At the middle sampling level (Fig. 38) this percentage had values of 21, 10, 16, 6 and 25 at Morris, Elm Park, St. James, Higgins and Selkirk respectively, forming peaks at Morris and Selkirk. At the

bottom sampling level (Fig. 38) this percentage had values of 8, 14, 8, 10 and 23 at Morris, Elm Park, St. James, Higgins and Selkirk respectively, thus forming a peak at Selkirk.

The percentage of the remaining types of micro-organisms at all three sampling levels in the spring (Fig. 37 and 38) fluctuated between values of zero and 10.

The variation in nutritional distribution at the same sampling level at different sampling stations during the summer (Fig. 39 and 40) showed a great increase in the percentage of type 1 microorganisms compared with the spring values. At the top sampling level (Fig. 39) this percentage had values of 27, 8, 20, 59 and 58 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. At the middle sampling level (Fig. 39) this percentage had values of 56, 34, 23, 46, and 64 at Morris, Elm Park, St. James, Higgins and Selkirk. At the bottom sampling level (Fig. 40) this percentage had values of 51, 12, 25, 51 and 51 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. This percentage formed a similar pattern at all three sampling levels: a high value at Morris which decreased at Elm Park and St. James and then increased, to at least the Morris values, at Higgins and Selkirk.

The overall percentage of type 2 microorganisms

obtained in the summer (Fig. 39 and 40) was similar to that obtained in the spring (Fig. 37 and 38). At the top sampling level (Fig. 39) this percentage had values of 56, 38, 52, 37 and 31 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. At the middle sampling level this percentage had values of 39, 51, 46, 37 and 30 at Morris, Elm Park, St. James, Higgins, and Selkirk respectively. At the bottom sampling level (Fig. 40) this percentage had values of 38, 60, 49, 37 and 43 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. This percentage seemed to develop a slight pattern. This pattern seemed to indicate a gradual decrease in the value of this percentage as the distance downstream from Morris increased.

The percentage of type 5 microorganisms was greater than 10 once at each sampling level during the summer (Fig. 39 and 40). At the top sampling level (Fig. 39) this peak occurred at Elm Park, reaching a value of 45. At the middle sampling level (Fig. 39) this peak occurred at St. James, reaching a value of 16. At the bottom sampling level (Fig. 40) this peak occurred at Elm Park, reaching a value of 19. The reason for this type of pattern was not discovered.

The percentages of the remaining types of

microorganisms at all three sampling levels during the summer (Fig. 39 and 40) generally fluctuated between zero and 10.

The nutritional distribution at the same sampling level at different sampling stations along the river in the fall (Fig. 40 and 41) generally showed that the changes in the percentages of types 1 and 2 microorganisms predominate. At the top sampling level (Fig. 40) the percentage of type 1 microorganisms had values of 18, 15, 45, 12 and 31 at Morris, Elm Park, St. James, Higgins and Selkirk respectively, forming a peak at St. James. At the middle sampling level (Fig. 41) this percentage had values of 12, 36, 32, 33 and 33 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. At the bottom sampling level (Fig. 41) this percentage had values of 13, 24, 28, 3 and 25 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. No general pattern seemed to evolve using this percentage during the fall.

The overall percentage of type 2 microorganisms was higher than the type 1 microorganisms during the fall. At the top sampling level (Fig. 40) this percentage had values of 77, 65, 56, 82 and 63 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. At the middle sampling level (Fig. 41) this percentage had values of 86,

49, 44, 68 and 58 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. At the bottom sampling level (Fig. 41) this percentage had values of 85, 57, 55, 69 and 71 at Morris, Elm Park, St. James, Higgins and Selkirk respectively. The general pattern that evolved using this percentage indicated a high value at Morris, which decreased at Elm Park and St. James. This percentage then increased to the Morris value at Higgins and Selkirk.

The percentage of the remaining types of microorganisms generally fluctuated between the values of zero and 10 at all three sampling levels during the fall (Fig. 40 and 41); there were two exceptions. One was the percentage of type 3 microorganisms which had a value of 17 at the middle sampling level of St. James (Fig. 41). The other exception was the percentage of type 4 microorganisms which had a value of 23 at the bottom sampling level of Higgins (Fig. 41).

DISCUSSION

In discussing the results of this thesis the first question to be answered is: Does any pattern in numbers, faecal coliforms, nutritional groups of microorganisms with respect to depth emerge when the results are examined?

As far as the number of microorganisms with respect to depth is concerned, the answer appears to be no. For instance the total counts from the top sampling level were not consistently higher than the total counts from either the middle or bottom levels at any sampling station. This is probably due to the fact that the river is fairly shallow and in flowing from Morris to Selkirk the water of the river must have been subjected to a good deal of turbulence resulting in a migration of what was originally the top layer to the bottom and visa versa; since the flow of microorganisms must follow that of the water, this lack of differentiation with depth is perhaps not unexpected. In a deep river it is possible that the relative turbulence might not be so great and as a consequence the lack of differentiation between layers found in the present study might not be so marked.

On the other hand the total number of microorganisms present in the river did show some variation with seasons. For instance all the sampling stations except Higgins exhibited a higher total count in the spring than in the winter or the summer. There are several possible reasons for this: (a) the increase in temperature in the spring allowed the microorganisms to proliferate more rapidly in the same environment than they had been able to do during the winter; (b) organic matter added to the river in the winter probably tended to accumulate because of the low metabolic activity of the microorganisms during this period; in the spring the accumulated reserve became available to the microorganisms which consequently proliferated at its expense, thus augmenting the simple effect of the rise in temperature noted above; (c) the disappearance of ice from the river surface allowed better aeration of the water and thus encouraged microbial proliferation. This "spring flush" soon dissipated itself and as a consequence the total counts in summer were not very different from total counts in winter. There was a curious, perhaps anomalous, rise in total counts in early September at the Elm Park station and stations downstream from this. This rise may have been due to one or both of two possible causes: (a) the

addition of nutrients as effluent discharge from the sugar refinery in the Fort Garry district of south Winnipeg, which had just started operation at this time, promoted microbial growth; (b) the construction of the Winnipeg floodway, leading from the Red River at a point upstream from the Elm Park sampling station, caused a disturbance of the river banks thus increasing the amount of soil in the river, and as a consequence increasing the number of microorganisms added to the river.

Turning now to faecal coliforms, it is clear that all the sampling stations except Selkirk showed higher values in summer than at any other season. At Selkirk the faecal coliform counts were higher in the winter, declined to a low in the summer and then increased again in the fall. The generally higher summer counts, within the confines of metropolitan Winnipeg, are probably due to the fact that the growth of coliforms tends to be highly temperature-dependent and thus the change in their numbers merely shows the pattern that would be expected. However, the very fact that their proliferation rate is high automatically means that they must tend to exhaust very quickly the supplies of available substrates; as a consequence by the time the river reaches Selkirk, 25 miles downstream from Winnipeg, with no significant addition of

substrates to the river water between the two points, the coliform count has dropped markedly. During the winter substrates available for coliforms are being added to the river water all the time in the metropolitan area but are not being metabolized as rapidly; the coliform count thus tends to remain at the same value for a more extended stretch of the river, and to peak at a point much further downstream, i.e., Selkirk.

An examination of the nutritional distribution figures at any one sampling station has failed to reveal any very marked or consistent pattern with respect to either depth or to season. At Elm Park, type 1 micro-organisms fluctuated with depth during the summer; on the other hand at Selkirk during the summer there was a similar but not identical fluctuation with respect to depth, but the percentage of these microorganisms was higher than at Elm Park. During the winter type 1 micro-organisms increased according to depth at Elm Park and decreased at Selkirk. These results are merely typical and examination of figures 21 to 27 has shown a similar lack of correlation between the distribution of any one type of microorganisms with respect to depth between any one sampling station and any other. This lack of correlation probably reflects the effect of turbulence

in a fairly shallow river; as yet unpublished results from this laboratory (Halvorson and Ishaque) have shown that in the comparatively unturbulent waters of sewage lagoons the distribution of nutritional groups varies markedly with depth that the samples are taken. It is possible that in a deep river a pattern similar to that of the sewage lagoons would have emerged.

When the different nutritional types taken separately but averaged over all sampling stations at all depths are compared throughout the seasons (Table III) a very clear pattern emerges. In the summer the two least exacting types (types 1 and 2) microorganisms are the most common. The incidence of type 1 microorganisms falls steadily from its high summer value to its low winter value. The incidence of the next most exacting type of microorganisms (type 2) rises temporarily in the fall and then drops through the winter to the spring to its lowest value. On the contrary the next three types of microorganisms (types 3,4, and 5) show low incidence value during the summer and fall but these values tend to rise markedly during the winter and spring. This distributional pattern with respect to seasons is clear but difficult to clarify. A tentative explanation is that the simpler substrates are used up more rapidly by types 1 and 2 microorganisms during

the summer leaving in excess of slightly more complex substrates in the fall. These substrates are used up by type 2 microorganisms, a process reflected in the rise of type 2 microorganisms in the fall. During the winter it appears that the more exacting microorganisms living on the more complex substrates seem to have a relative advantage over their less exacting competitors. The incidence of the more exacting microorganisms therefore rises relatively as the spring approaches reaching a maximum coinciding with a minimum of types 1 and 2. With the advent of summer the cycle repeats itself again.

Perhaps also important in this context is the work of Jannasch (8) in which it is shown that if microorganisms X (because it utilizes substrates more rapidly) predominates over microorganism Y in a rich medium, it does not follow that the same predominance will be evident in a poor medium. It may be that microorganism Y uses the substrate less rapidly but more efficiently. It is beyond the scope of the present work however to decide whether such considerations would explain the seasonal variations in the exacting and non-exacting microorganisms in the Red River.

A more detailed analysis of the data on which Table III is based is shown in Table IV. It will be seen

that the winter-spring decline in the non-exacting microorganisms begins at Selkirk and then progressively proceeds in an upstream direction.

On the basis of the figures obtained in this work, it is interesting to calculate the possible "biochemical activity" taking place each day in the Red River within the city limits.

The minimum flow rate of the Red River (August) is 4"/second; the maximum rate (April) is 60"/second. Taking an average of 39" (1 metre)/second, the flow rate is 3600 x 24 metres/day = 86 Km/day, i.e., the river water is renewed three times per day within the city limits (30 Km of the river).

According to "Biosynthesis in E. coli" (12) E. coli can produce 1135 µM CO_2/gm cells/100 sec

i.e., 1135 x 12 µg C/gm cells/100 sec
14 mgm C/gm cells/100 sec
14 x 3600/100 mgm C/gm cells/hr
500 mgm C/gm cells/hr

or approximately oxidize 1 gm of organic material/gm cells /hr.

The Red River is approximately 10,000 cm wide, 500 cm deep and 30 Km long in metropolitan Winnipeg. Thus the volume of this part of the river is:

$10,000 \times 500 \times 30 \times 1000 \times 100$ cc

or 1.5×10^{13} cc

It contains approximately 10^5 microorganisms/cc; thus the total number of microorganisms in the metropolitan Red River is:

$1.5 \times 10^{13} \times 10^5$

or 1.5×10^{18}

But 10^{13} E. coli weigh one gm, thus the weight of microorganisms in the metropolitan Red River is:

$1.5 \times 10^{18}/10^{13}$

or 1.5×10^5 gm

Since one gm of E. coli can oxidize approximately one gm of organic material per hr, this section of the river could oxidize under ideal conditions about 1.5×10^5 gm of organic material per hr or 3.6 tons daily, i.e., 10.8 tons daily allowing for the renewal of the river water (see above).

In southern Winnipeg alone 5,000,000 gallons of sewage are added daily directly into the Red River. The solid organic content of this sewage averages between 160 and 200 ppm. This means that 4 to 5 tons of solid organic materials are added to the Red River every day which is half the amount that can be theoretically handled by the river. This amount does not account for any of the

dissolved organic material or any raw sewage that has to be dumped into the Red River because of the inability of the sewage treatment plant to handle the volume of rain water and sewage any time it rains.

However, the figures for E. coli were obtained under ideal laboratory conditions of temperature and aeration; moreover E. coli is an active, fast-growing bacterium. Under river conditions it is very doubtful whether the total bacterial population would show a biochemical activity that would exceed 10% of the "ideal" E. coli activity. This means that there will always be an excess of waste materials in the river within the confines of the city limits. There is no solution to this problem other than a much more widespread and intensive treatment of sewage waste before the sewage is piped into the river.

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APPENDIX

TECHNICAL DATA

TABLE I

Dates of sampling and determination of total counts, faecal coliforms, temperature and pH

Date	Station	Nutritional Distribution	Total Count	Faecal Coliform Count	Temperature	pH
25-10-65	Elm Park	*	*	*	*	
10-1-66	Morris	*	*	*	*	
	Selkirk	*	*	*	*	
1-2-66	Elm Park	*	*	*	*	
	St. James	*	*	*	*	
	Higgins	*	*	*	*	
17-5-66	All Stations	*	*	*	*	
29-5-66	" "		*	*	*	*
12-6-66	" "		*	*	*	*
26-6-66	" "		*	*	*	*
10-7-66	" "		*	*	*	*
24-7-66	" "		*	*	*	*
7-8-66	" "	*	*	*	*	*
22-8-66	" "		*	*	*	*

TABLE I (continued)

Date	Station	Nutritional Distribution	Total Count	Faecal Coliform Count	Temperature	pH
5-9-66	All Stations		*	*	*	*
18-9-66	" "		*	*	*	*
17-10-66	" "		*	*	*	*
2-11-66	Elm Park	*	*	*	*	*
	St. James	*	*	*	*	*
	Higgins	*	*	*	*	*
3-11-66	Morris	*	*	*	*	*
	Selkirk	*	*	*	*	*

* operation performed

TABLE II

Number of isolates used for the nutritional distribution study

Sampling Station		Fall '65	Winter	Spring	Summer	Fall
Morris	Top	-	74	104	103	109
"	Middle	-	53	105	117	96
"	Bottom	-	90	108	110	98
Elm Park	Top	39	103	110	104	117
" "	Middle	34	92	116	120	112
" "	Bottom	25	12	91	110	103
St. James	Top	-	68	114	107	110
" "	Middle	-	83	116	117	82
" "	Bottom	-	59	109	114	117
Higgins	Top	-	80	113	95	92
"	Middle	-	68	112	118	111
"	Bottom	-	73	110	120	90
Selkirk	Top	-	78	117	99	108
"	Middle	-	24	117	118	120
"	Bottom	-	84	120	100	89
Total number of isolates tested			1041	1662	1652	1554

GRAND TOTAL 5909

TABLE III

The percentage of nutritional types taken separately but averaged over all sampling stations at all depths compared throughout the seasons.

Type of nutritional group*	Summer	Fall	Winter	Spring
1	39	24	13	4
2	44	65	53	46
3	1	3	7	3
4	3	4	4	15
5	9	3	17	25
Total	96	99	94	93

*Nutritional groups 6 and 7 were not included as they were quantitatively unimportant.

TABLE IV

The percentage of nutritional types taken in two groups, one made up of types 1 and 2 the other made up of the remaining types, at each sampling station compared through the seasons.

Season	Winter		Spring		Summer		Fall	
Groups of nutritional types	1 & 2	others	1 & 2	others	1 & 2	others	1 & 2	others
Sampling Station								
Morris	46	54	63	27	90	10	96	4
Elm Park	83	17	54	46	69	31	82	18
St. James	75	25	50	50	68	22	83	17
Higgins	83	17	44	56	94	6	86	14
Selkirk	30	70	30	70	63	27	92	8

FIGURE 1. London drinking water, exhibiting the living and dead organic matter, as supplied by the Lambeth Company in 1847 (magnification 200X) from the Age of Paradox, page 338.

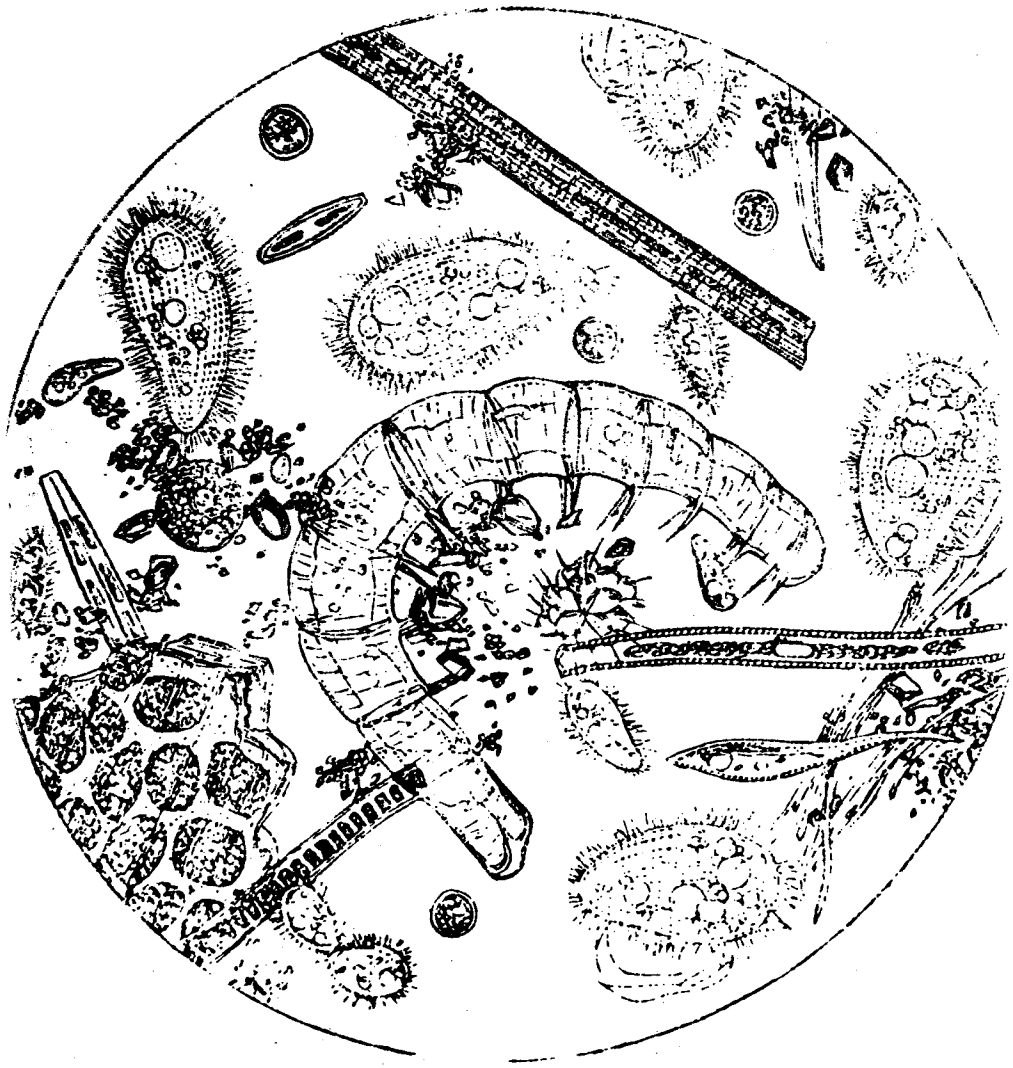
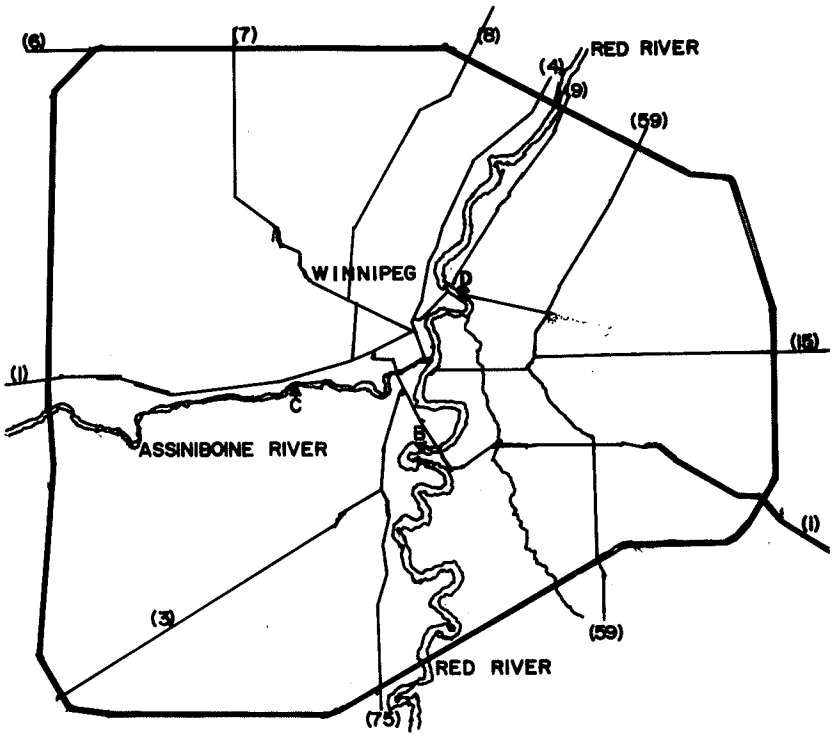


FIGURE 2. A map showing the sampling locations on the Red and Assiniboine Rivers.



A MORRIS
B ELM PARK
C St. JAMES
D HIGGINS
E SELKIRK
( ) HIGHWAY NUMBER

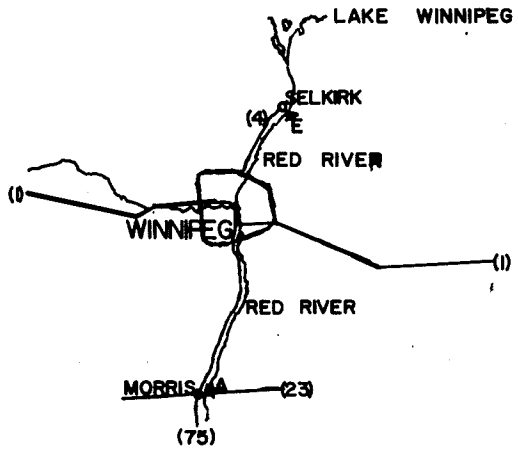


FIGURE 3. Total count, temperature and pH data obtained at Morris.

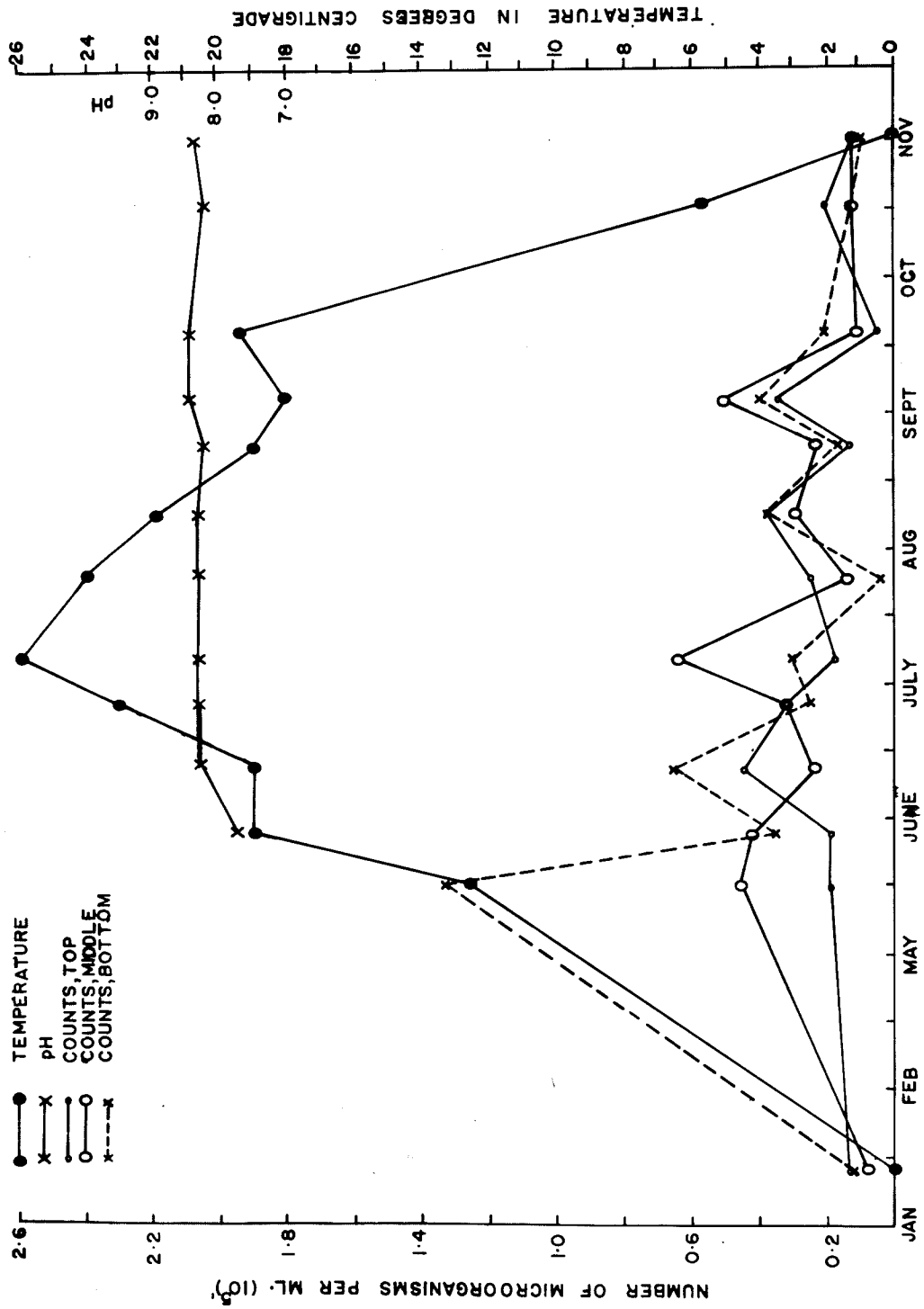

TEMPERATURE IN DEGREES CENTIGRADE
pH
NUMBER OF MICROORGANISMS PER ML. (10⁵)
TEMPERATURE
pH
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 4. Total count, temperature and pH data obtained at Elm Park.

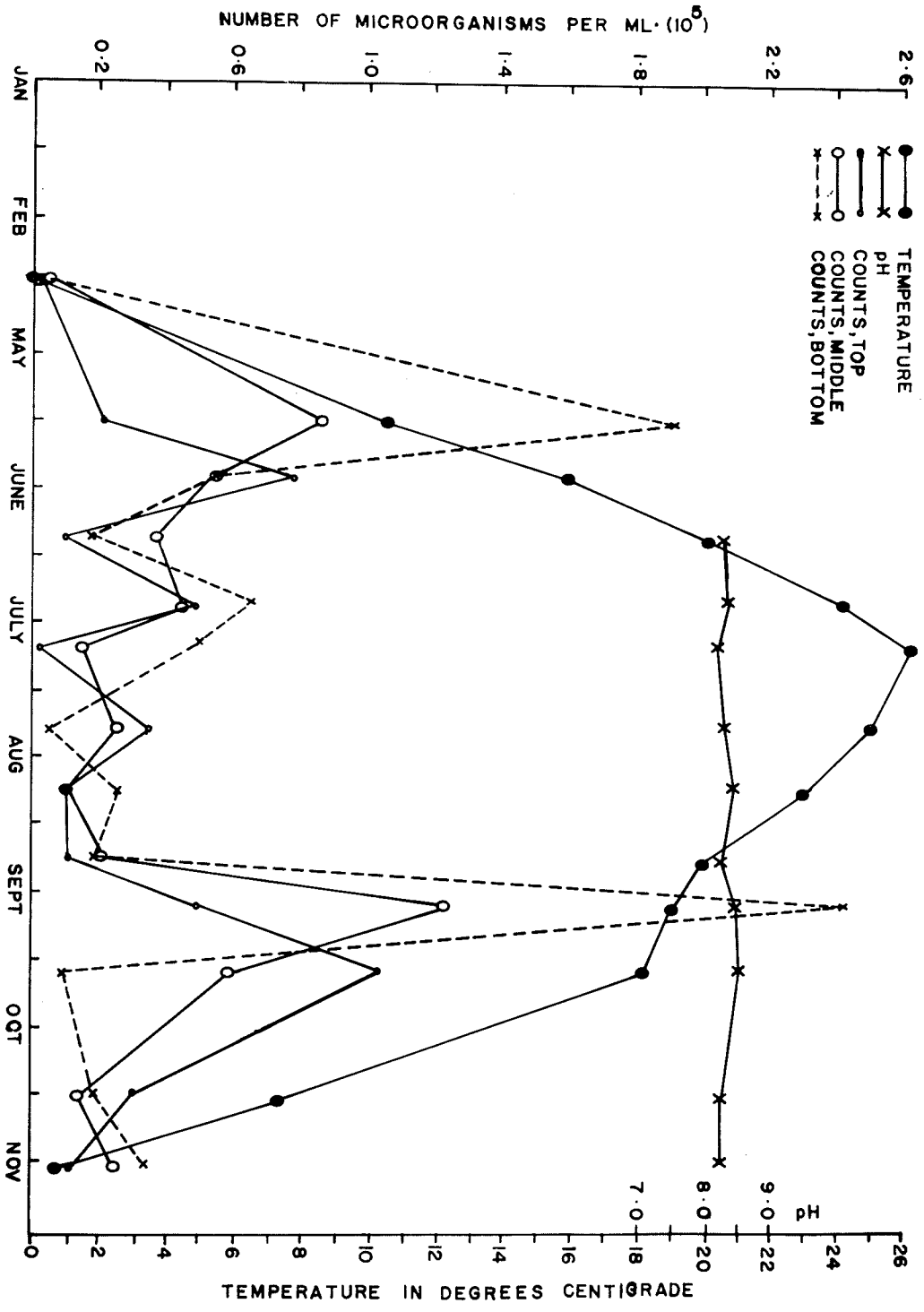

NUMBER OF MICROORGANISMS PER ML·(10⁵)
0·2
0·6
1·0
1·4
1·8
2·2
2·6
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
TEMPERATURE
pH
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
7·0
8·0
9·0
pH
0
2
4
6
8
10
12
14
16
18
20
22
24
26
TEMPERATURE IN DEGREES CENTIGRADE

FIGURE 5. Total count, temperature and pH data obtained at St. James.

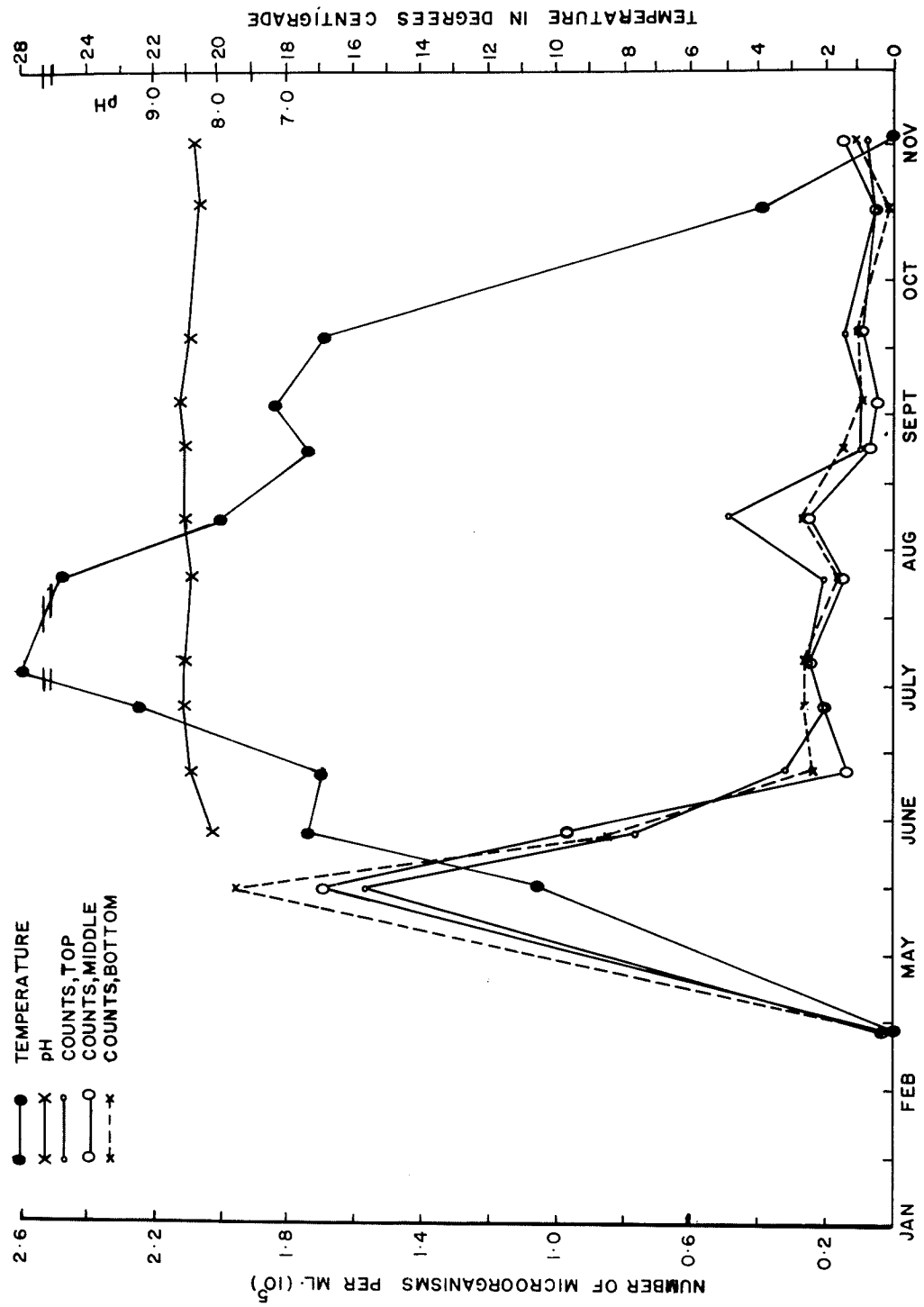

TEMPERATURE IN DEGREES CENTIGRADE
pH
NUMBER OF MICROORGANISMS PER ML. (10^5)
TEMPERATURE
pH
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 6. Total count, temperature and pH data obtained at Higgins.

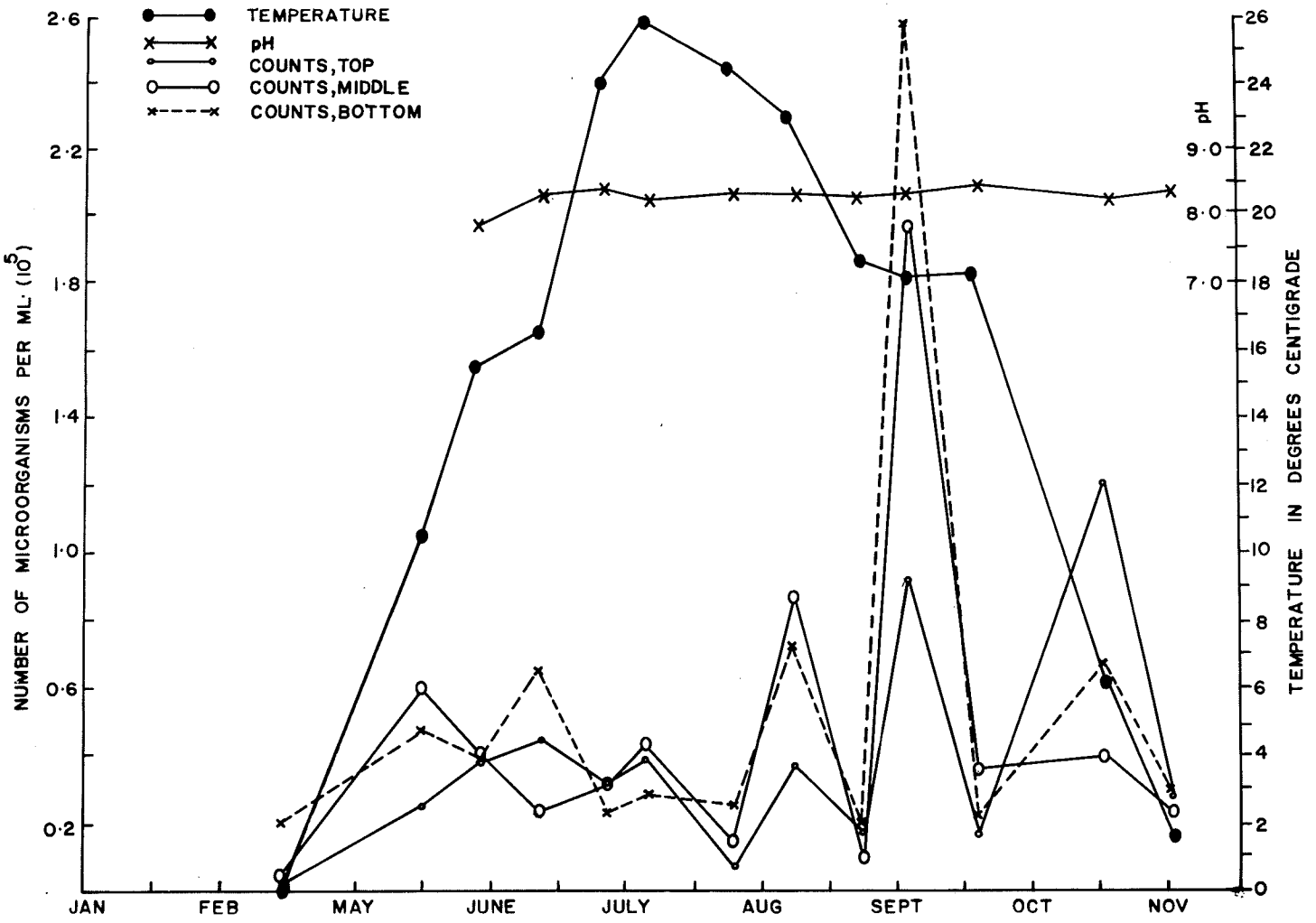

TEMPERATURE
pH
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
NUMBER OF MICROORGANISMS PER ML. (10^5)
2·6
2·2
1·8
1·4
1·0
0·6
0·2
pH
9·0
8·0
7·0
TEMPERATURE IN DEGREES CENTIGRADE
26
24
22
20
18
16
14
12
10
8
6
4
2
0
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 7. Total count, temperature and pH data obtained at Selkirk.

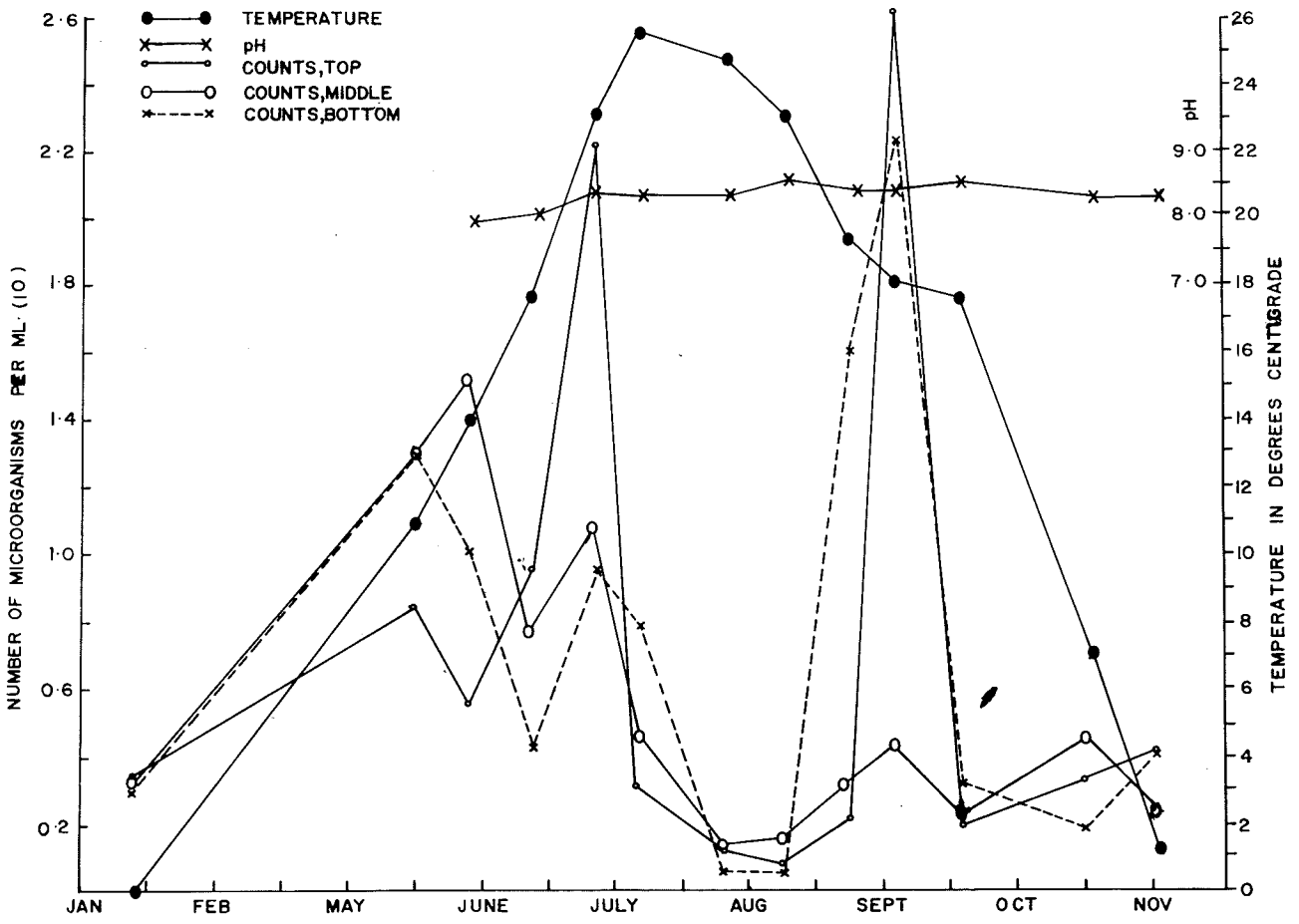

TEMPERATURE
pH
COUNTS,TOP
COUNTS,MIDDLE
COUNTS,BOTTOM
NUMBER OF MICROORGANISMS PER ML. (10)
TEMPERATURE IN DEGREES CENTIGRADE
pH
2·6
2·2
1·8
1·4
1·0
0·6
0·2
26
24
22
20
18
16
14
12
10
8
6
4
2
0
9·0
8·0
7·0
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 8. Temperature and faecal coliform data obtained at Morris.

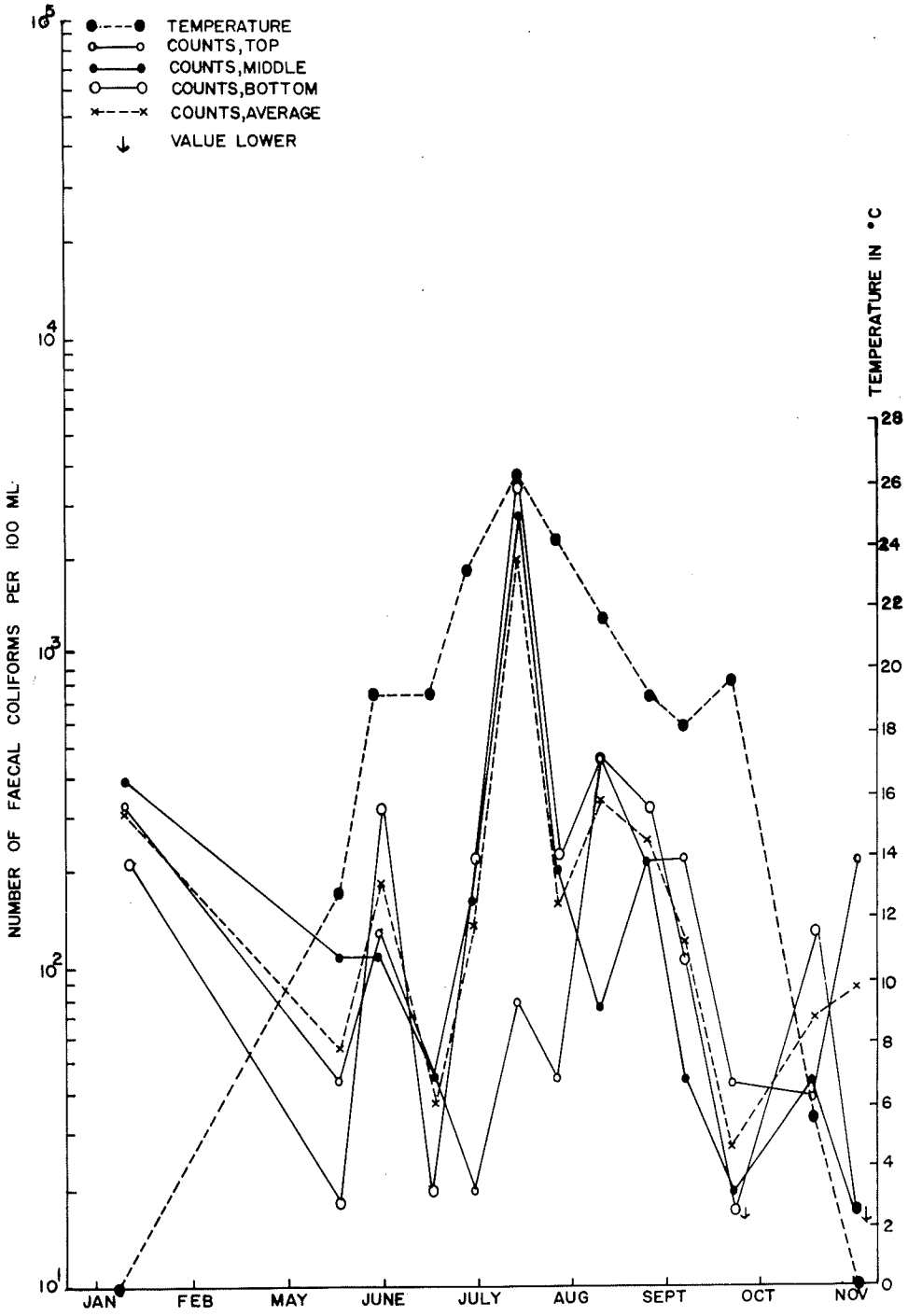

TEMPERATURE
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
COUNTS, AVERAGE
VALUE LOWER
NUMBER OF FAECAL COLIFORMS PER 100 ML
TEMPERATURE IN °C
10^5
10^4
10^3
10^2
10^1
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 9. Temperature and faecal coliform data obtained at Elm Park.

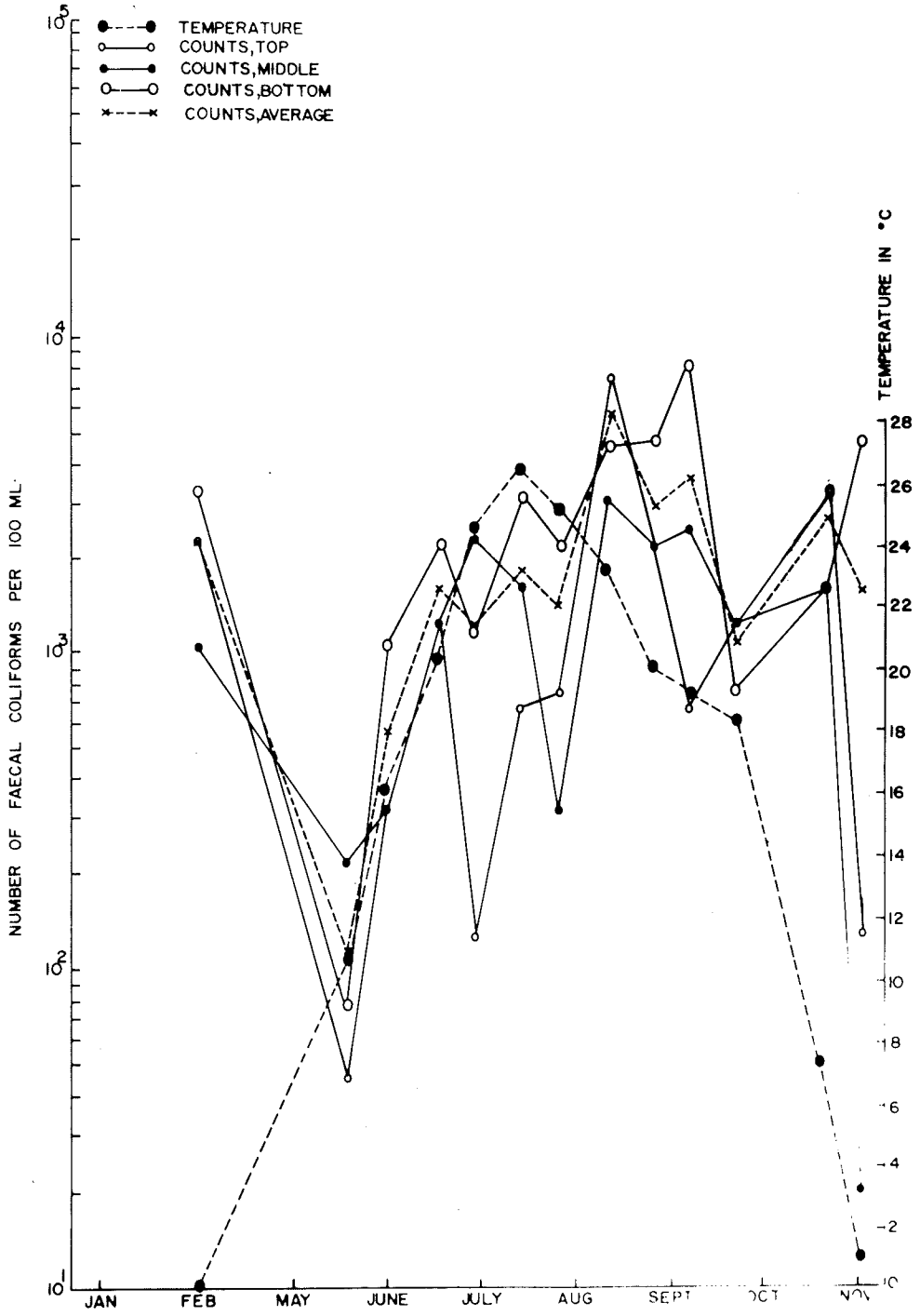

TEMPERATURE
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
COUNTS, AVERAGE
NUMBER OF FAECAL COLIFORMS PER 100 ML
TEMPERATURE IN °C
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT

FIGURE 10. Temperature and faecal coliform data obtained at St. James.

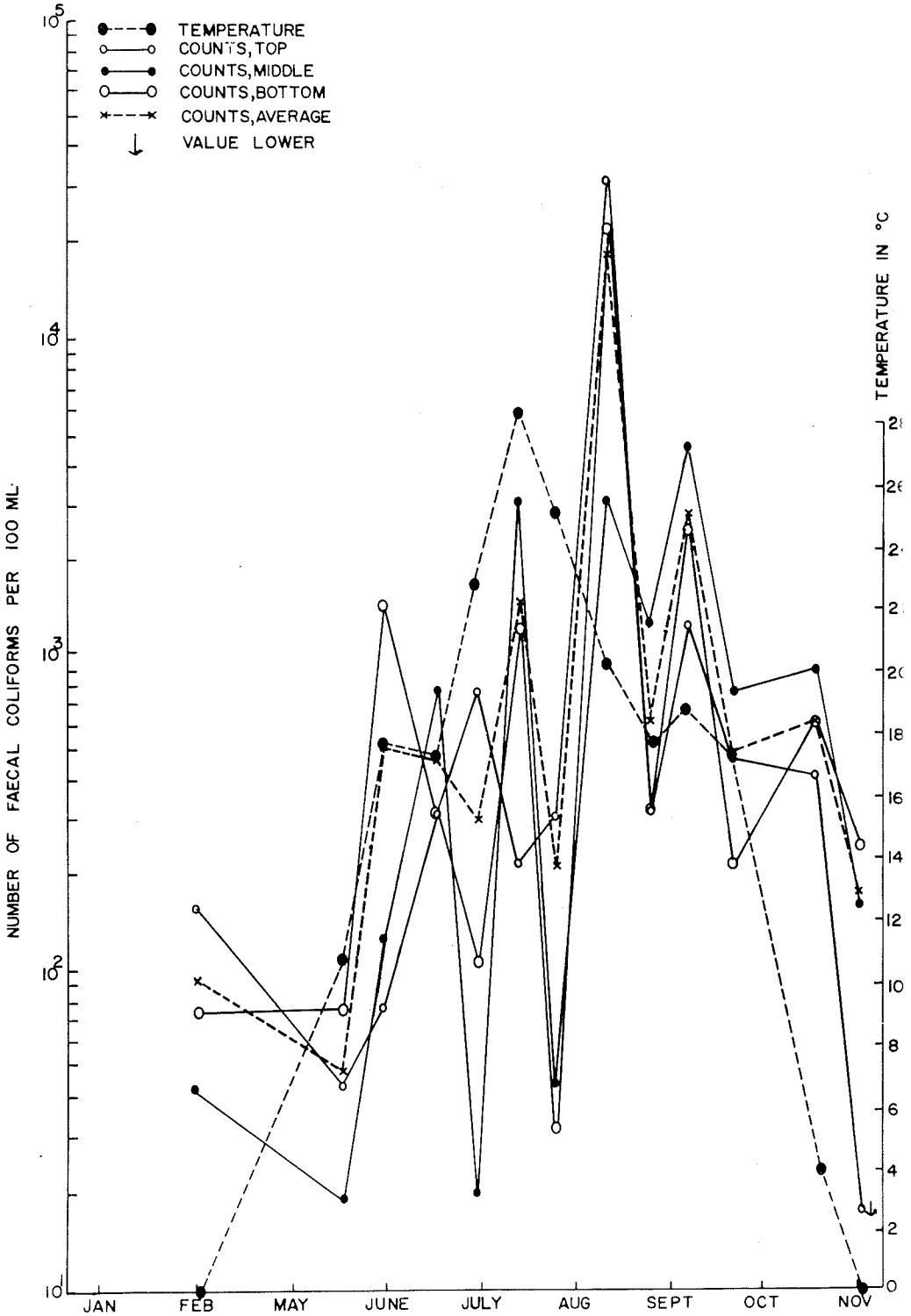

TEMPERATURE
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
COUNTS, AVERAGE
VALUE LOWER
NUMBER OF FAECAL COLIFORMS PER 100 ML
TEMPERATURE IN °C
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 11. Temperature and faecal coliform data obtained at Higgins.

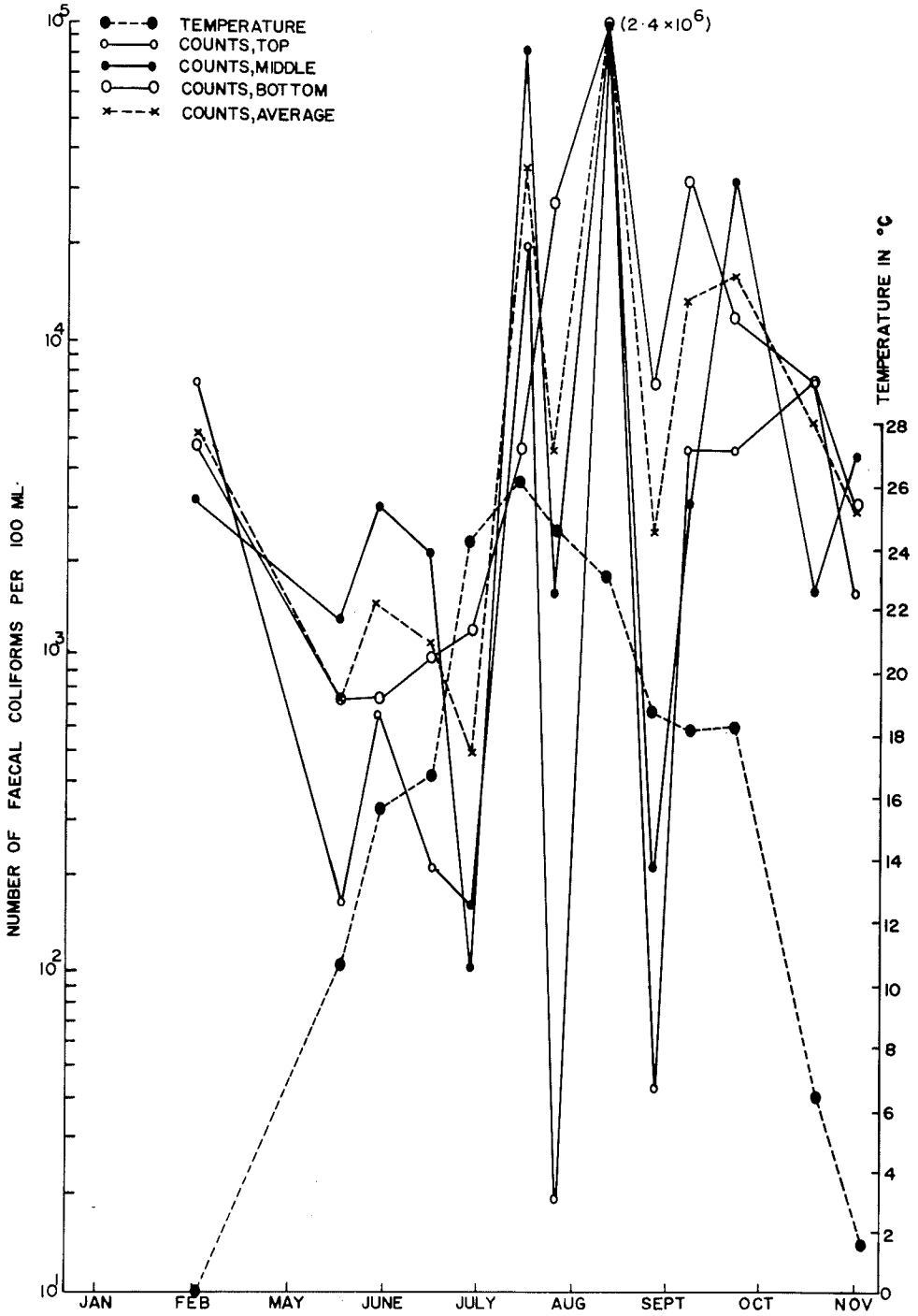

TEMPERATURE
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
COUNTS, AVERAGE
(2·4 ×10⁶)
NUMBER OF FAECAL COLIFORMS PER 100 ML
TEMPERATURE IN °C
10⁵
10⁴
10³
10²
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 12. Temperature and faecal coliform data obtained at Selkirk.

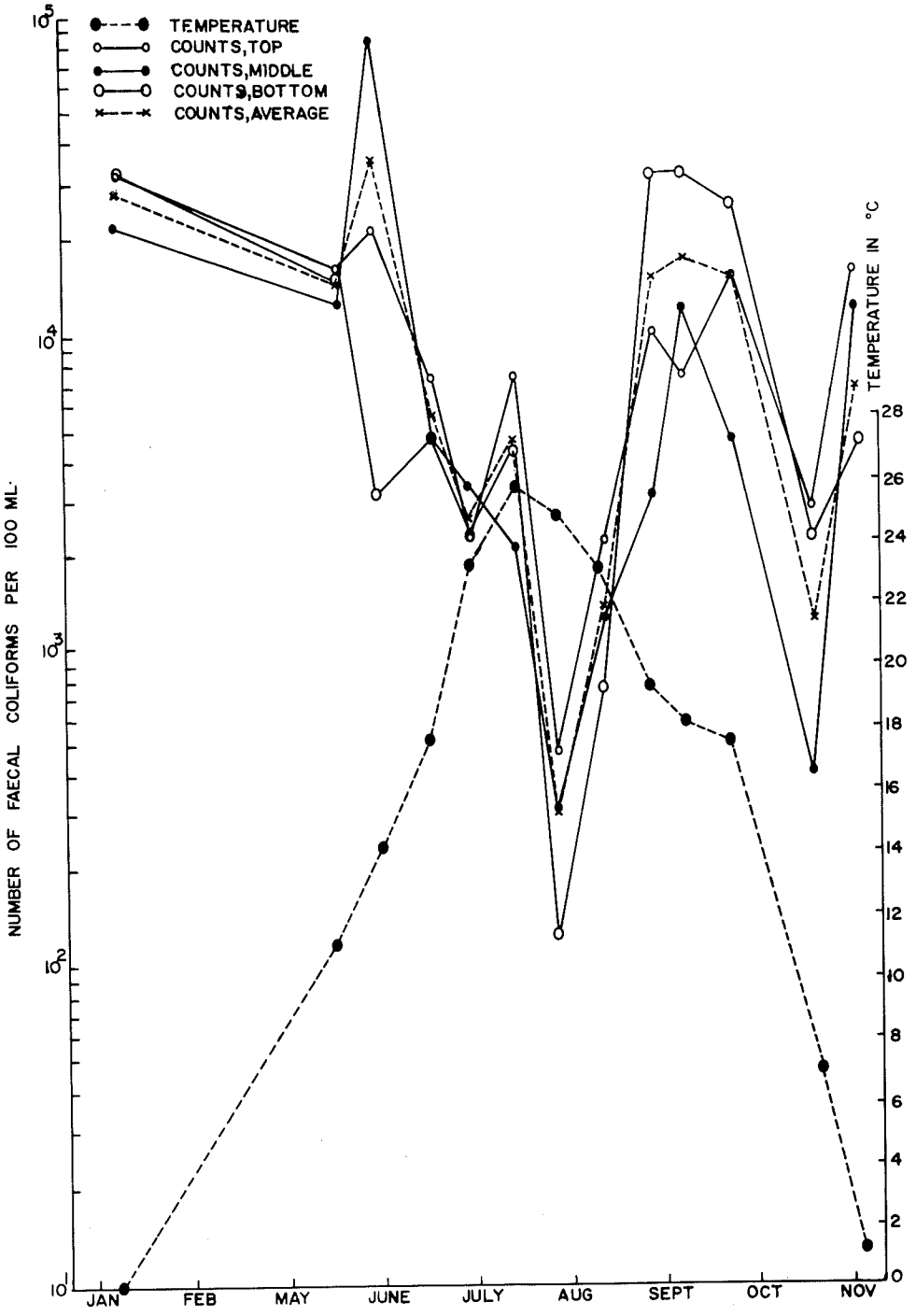

TEMPERATURE
COUNTS, TOP
COUNTS, MIDDLE
COUNTS, BOTTOM
COUNTS, AVERAGE
NUMBER OF FAECAL COLIFORMS PER 100 ML.
TEMPERATURE IN °C
10⁵
10⁴
10³
10²
10¹
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 13. Average faecal coliform data.

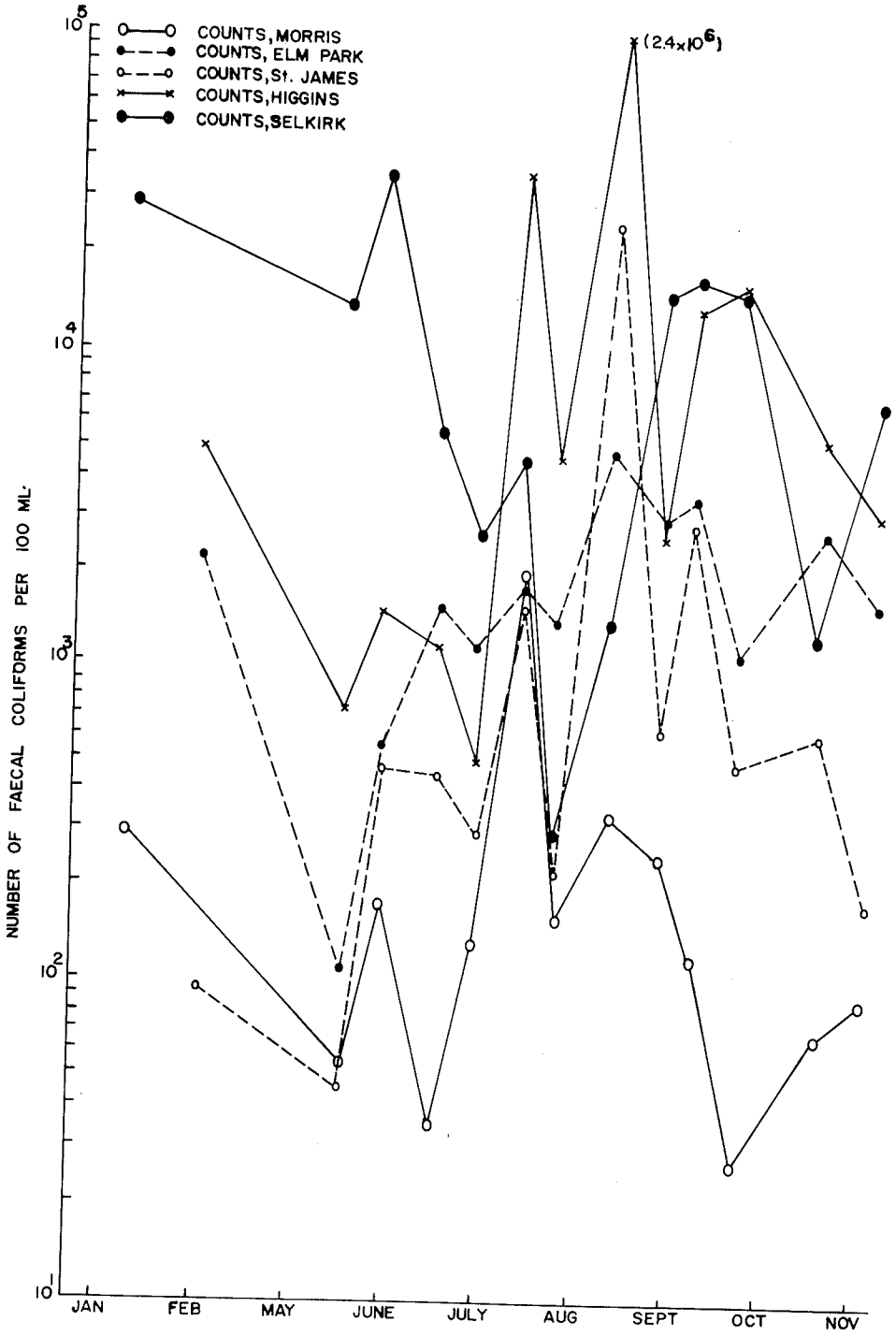

COUNTS, MORRIS
COUNTS, ELM PARK
COUNTS, St. JAMES
COUNTS, HIGGINS
COUNTS, SELKIRK
(2.4x10^6)
NUMBER OF FAECAL COLIFORMS PER 100 ML.
10^5
10^4
10^3
10^2
10^1
JAN
FEB
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV

FIGURE 14. Nutritional distribution data from the top, middle and bottom sampling levels at Elm Park, Fall 1965; Morris, Winter; Elm Park, Winter.

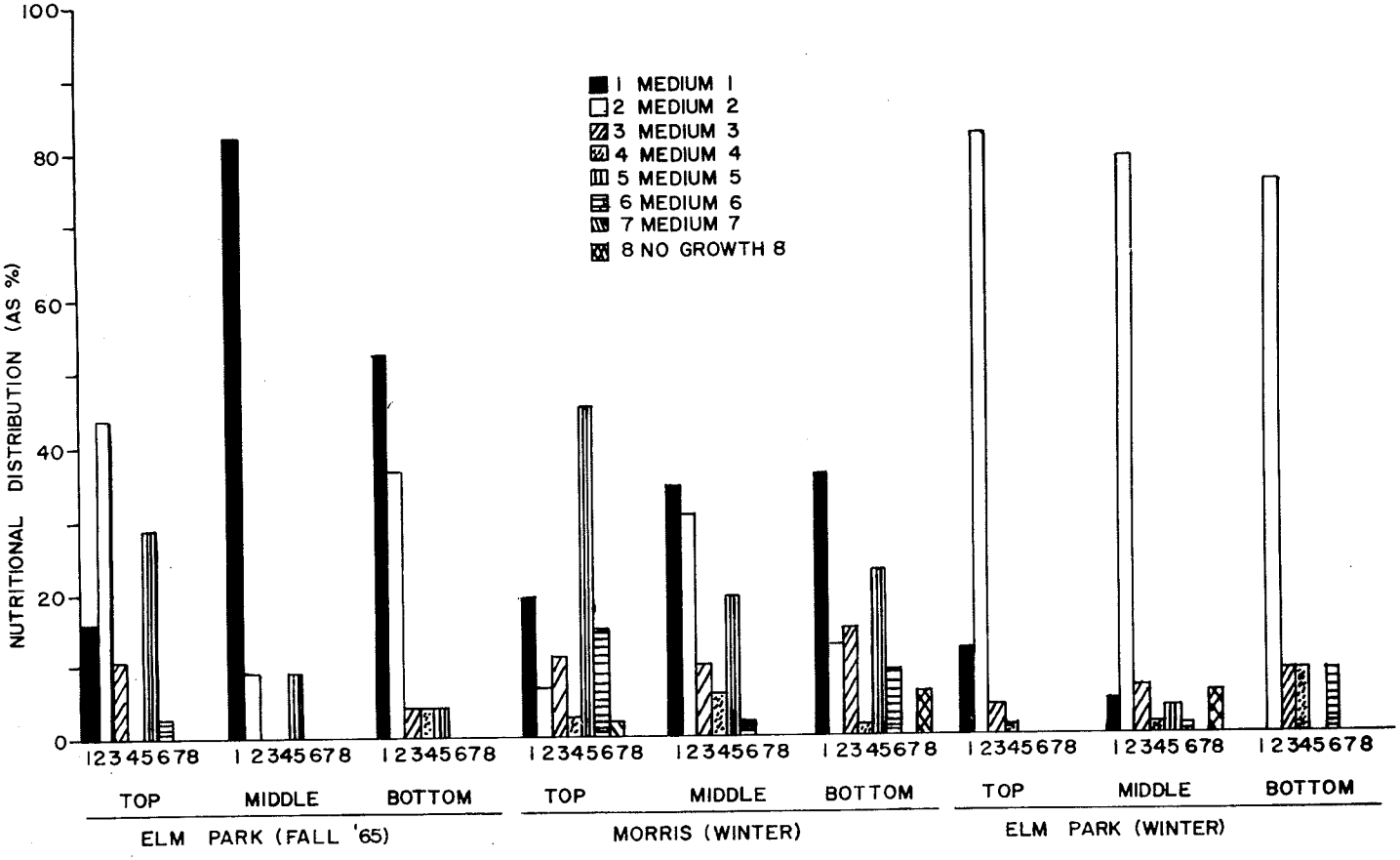

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
12345678
TOP
MIDDLE
BOTTOM
ELM PARK (FALL '65)
MORRIS (WINTER)
ELM PARK (WINTER)

FIGURE 15. Nutritional distribution data from the top, middle and bottom sampling levels at St. James, Winter; Higgins, Winter; Selkirk, Winter.

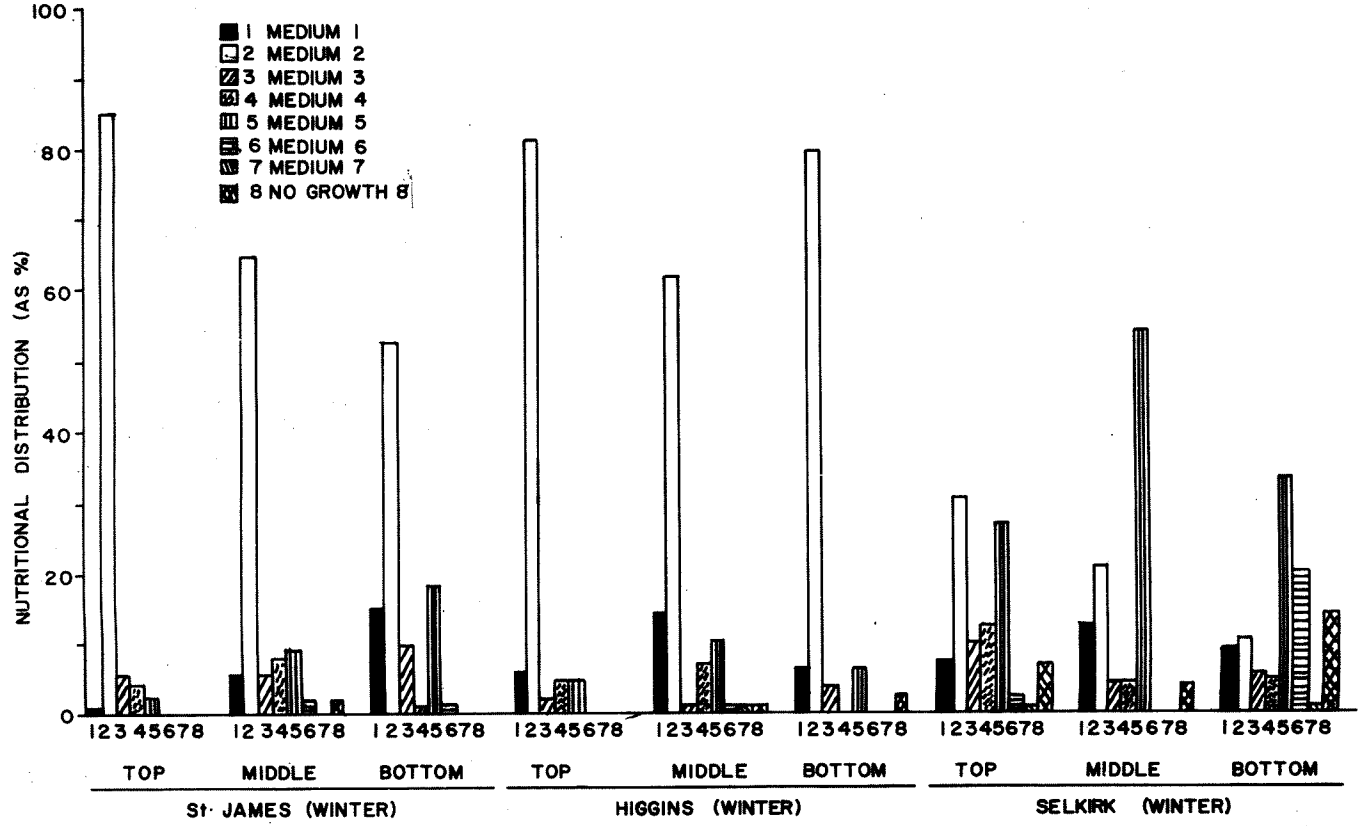

1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
12345678
TOP
MIDDLE
BOTTOM
St. JAMES (WINTER)
HIGGINS (WINTER)
SELKIRK (WINTER)

FIGURE 16. Nutritional distribution data from the top, middle and bottom sampling levels at Morris, Spring; Elm Park, Spring; St. James, Spring.

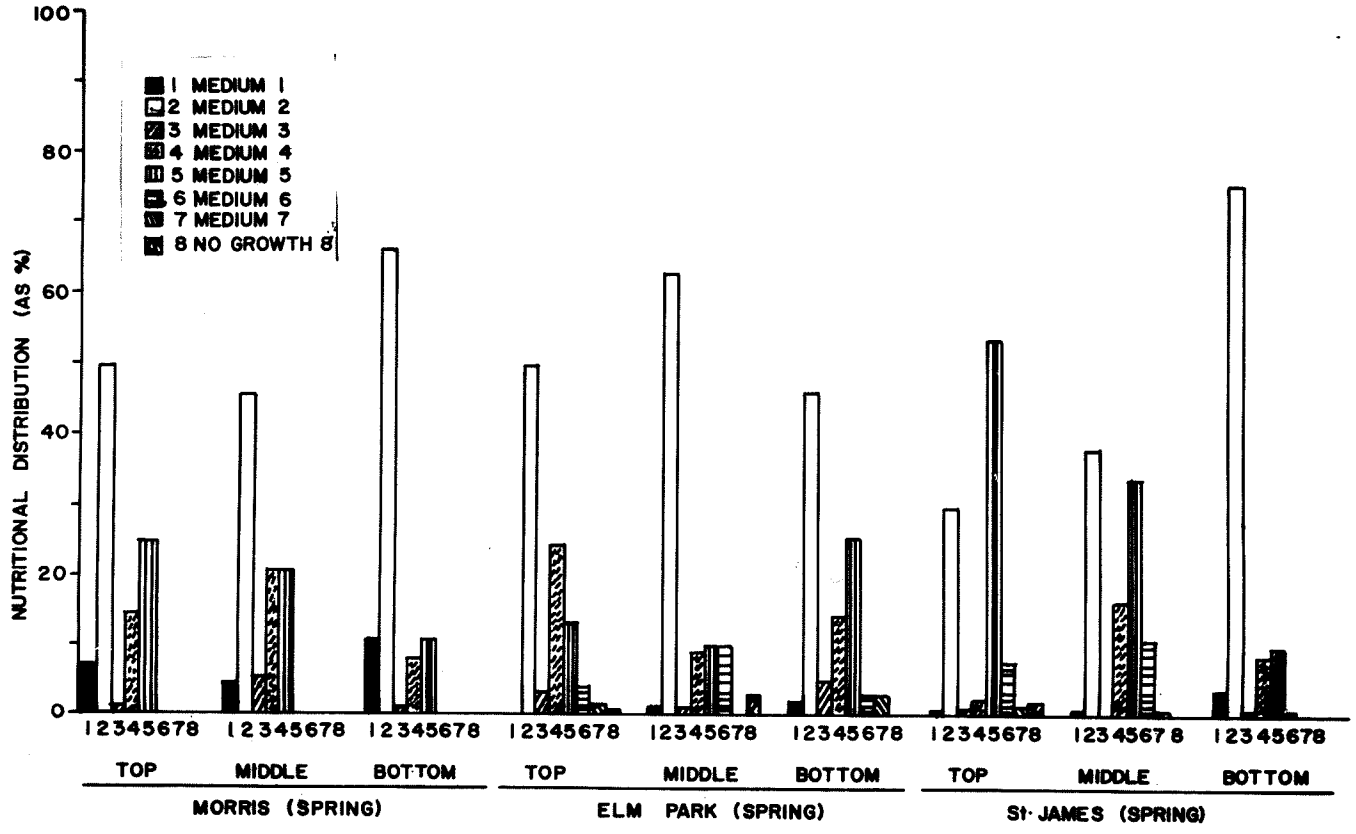

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
12345678
TOP
MIDDLE
BOTTOM
MORRIS (SPRING)
ELM PARK (SPRING)
St. JAMES (SPRING)

FIGURE 17. Nutritional distribution data from the top, middle and bottom sampling levels at Higgins, Spring; Selkirk, Spring; Morris, Summer.

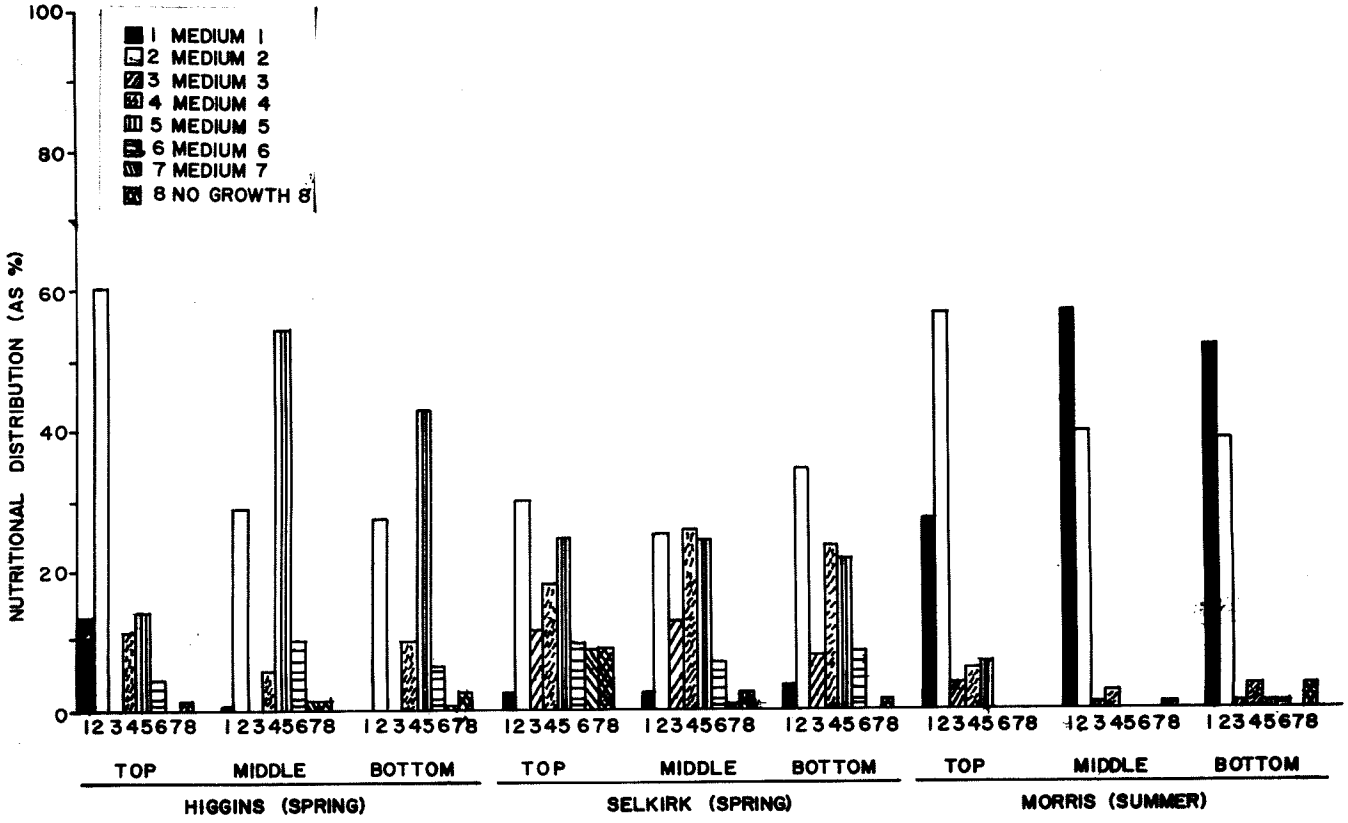

1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
12345678
TOP
MIDDLE
BOTTOM
HIGGINS (SPRING)
SELKIRK (SPRING)
MORRIS (SUMMER)

FIGURE 18. Nutritional distribution data from the top, middle and bottom sampling levels at Elm Park, Summer; St. James, Summer; Higgins, Summer.

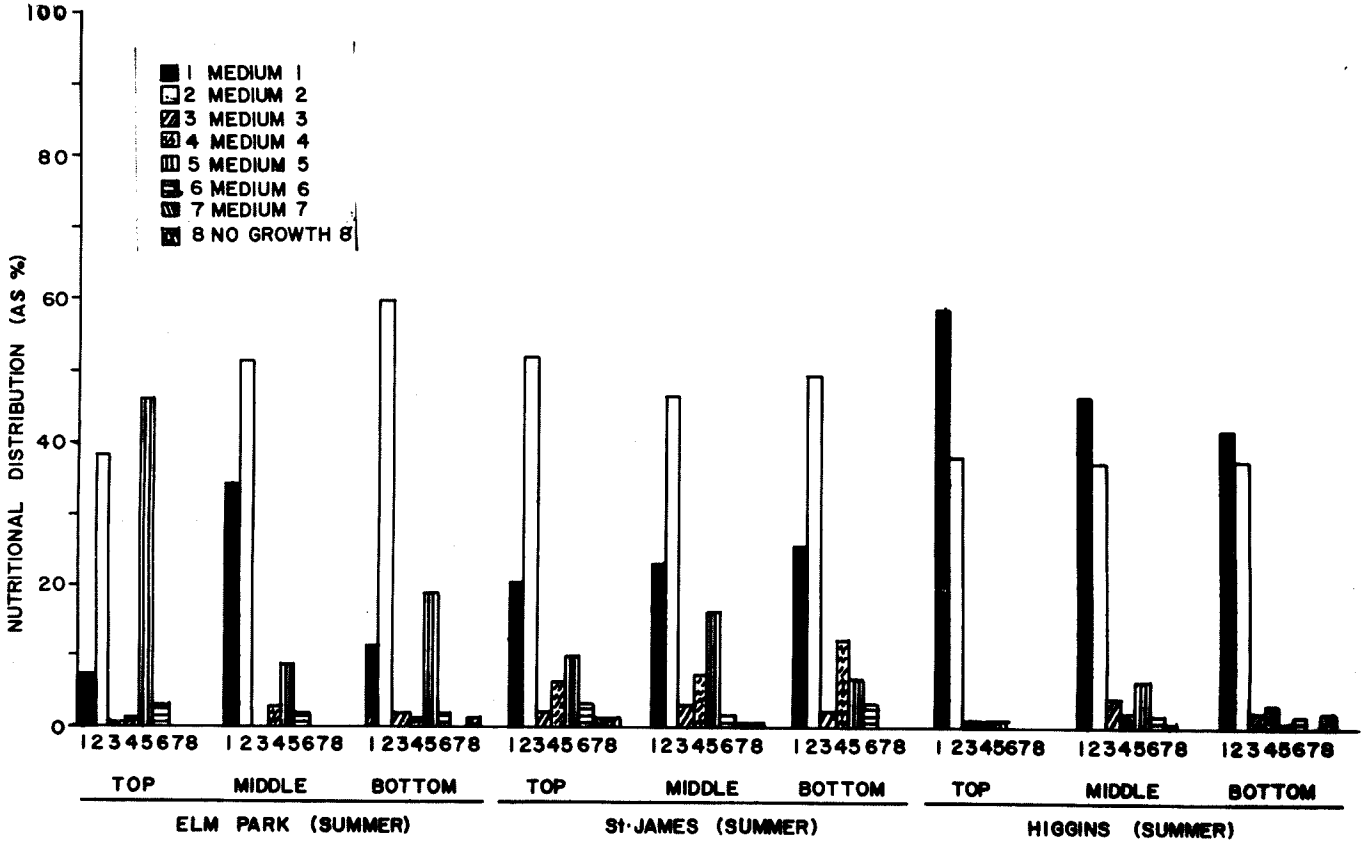

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
12345678 12345678 12345678 12345678 12345678 12345678 12345678 12345678 12345678
TOP MIDDLE BOTTOM TOP MIDDLE BOTTOM TOP MIDDLE BOTTOM
ELM PARK (SUMMER)
St·JAMES (SUMMER)
HIGGINS (SUMMER)

FIGURE 19. Nutritional distribution data from the top, middle and bottom sampling levels at Selkirk, Summer; Morris, Fall; Elm Park, Fall.

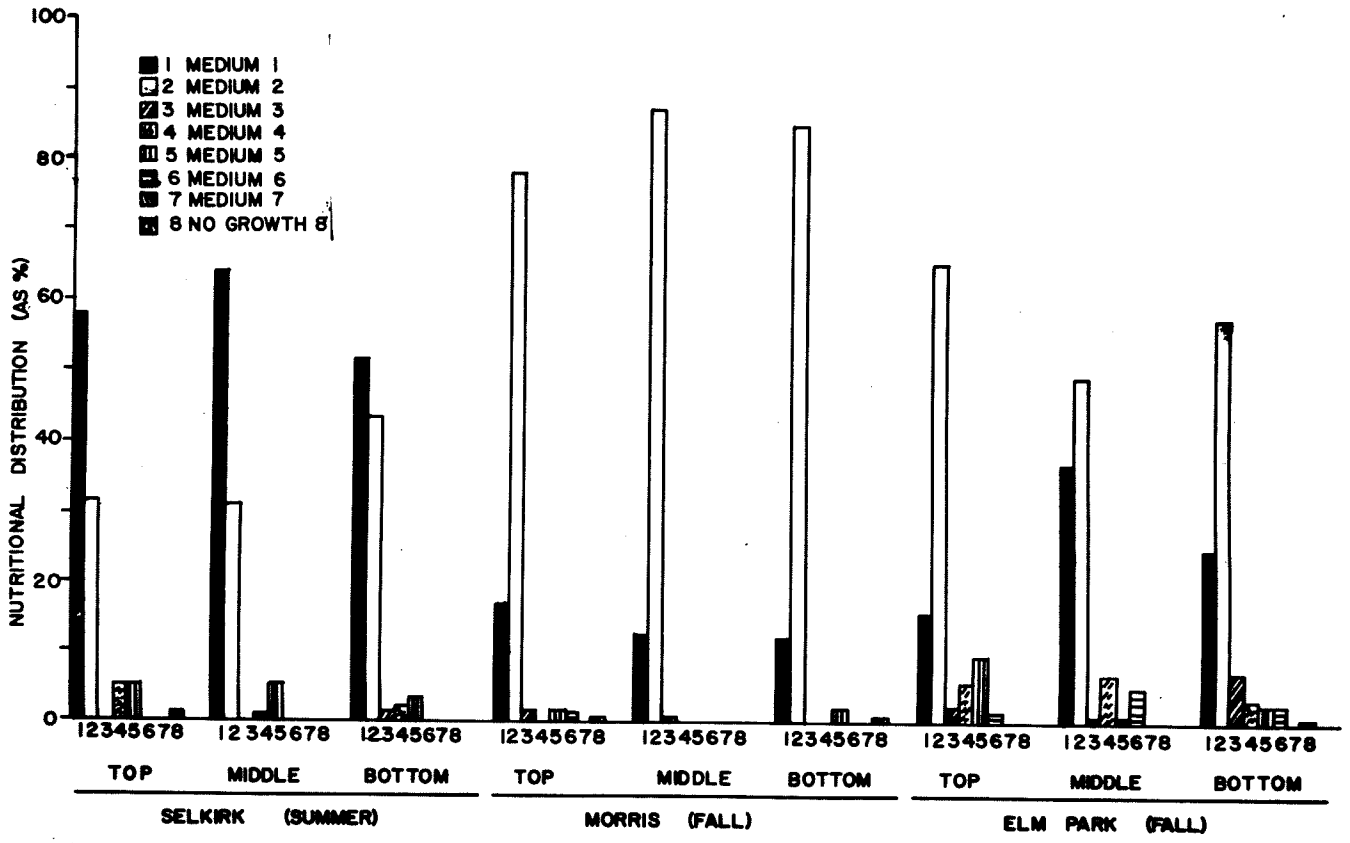

1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
12345678
TOP
MIDDLE
BOTTOM
SELKIRK (SUMMER)
MORRIS (FALL)
ELM PARK (FALL)

FIGURE 20. Nutritional distribution data from the top, middle and bottom sampling levels at St. James, Fall; Higgins, Fall; Selkirk, Fall.

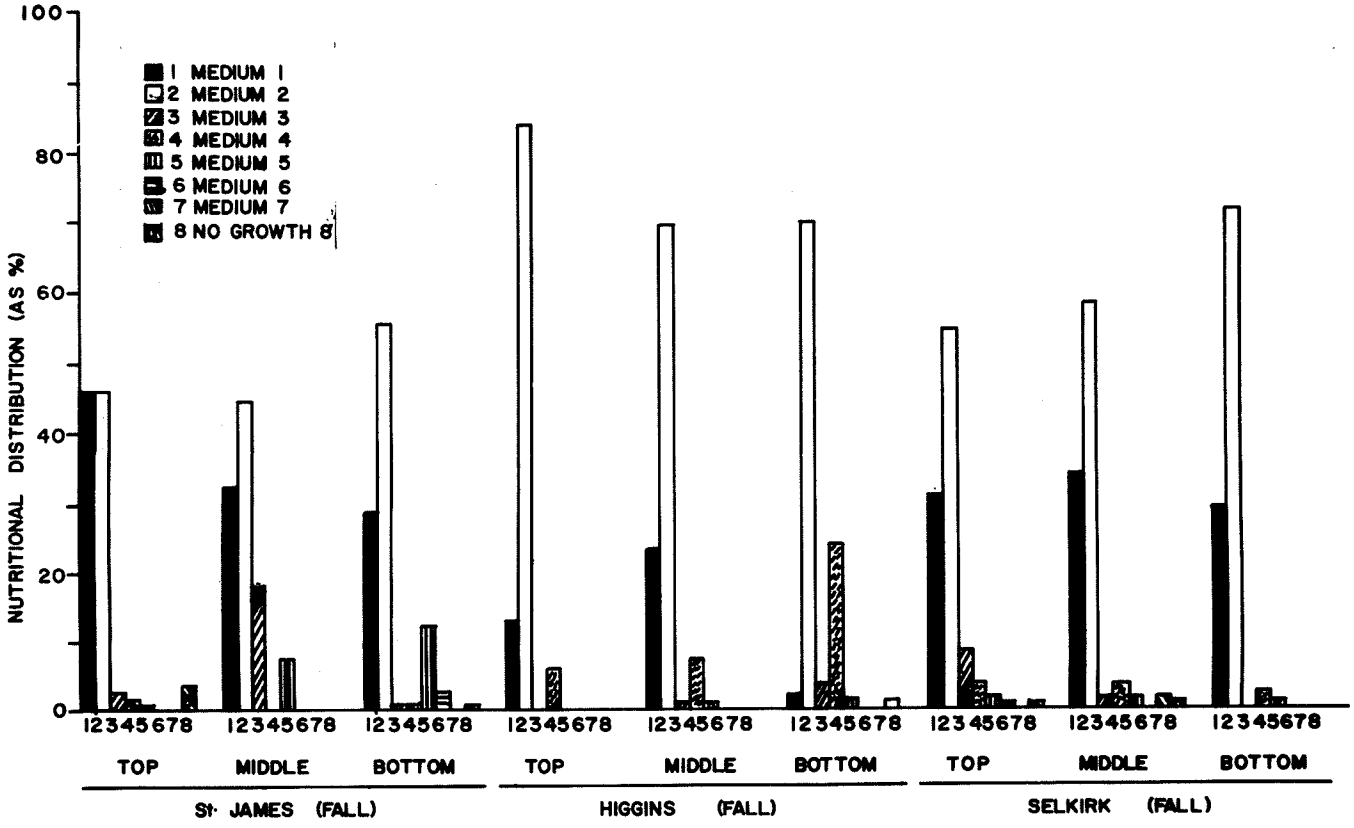

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
1 MEDIUM 1
2 MEDIUM 2
3 MEDIUM 3
4 MEDIUM 4
5 MEDIUM 5
6 MEDIUM 6
7 MEDIUM 7
8 NO GROWTH 8
12345678
TOP
MIDDLE
BOTTOM
St. JAMES (FALL)
HIGGINS (FALL)
SELKIRK (FALL)

FIGURE 21. Variation in nutritional distribution with increasing depth at Morris, Winter; Morris, Spring; Morris, Summer.

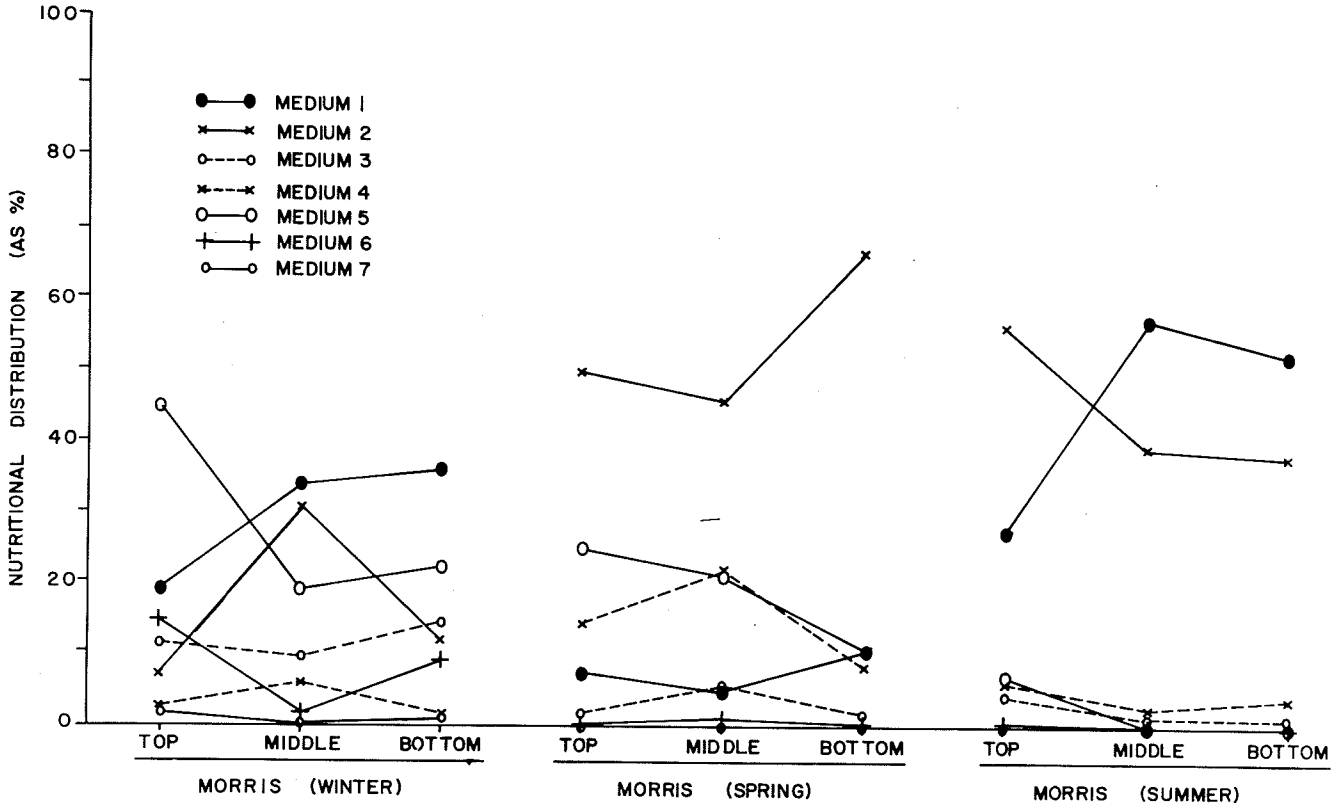

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
TOP
MIDDLE
BOTTOM
MORRIS (WINTER)
TOP
MIDDLE
BOTTOM
MORRIS (SPRING)
TOP
MIDDLE
BOTTOM
MORRIS (SUMMER)

FIGURE 22. Variation in nutritional distribution with increasing depth at Morris, Fall; Elm Park, Fall 1965; Elm Park, Winter.

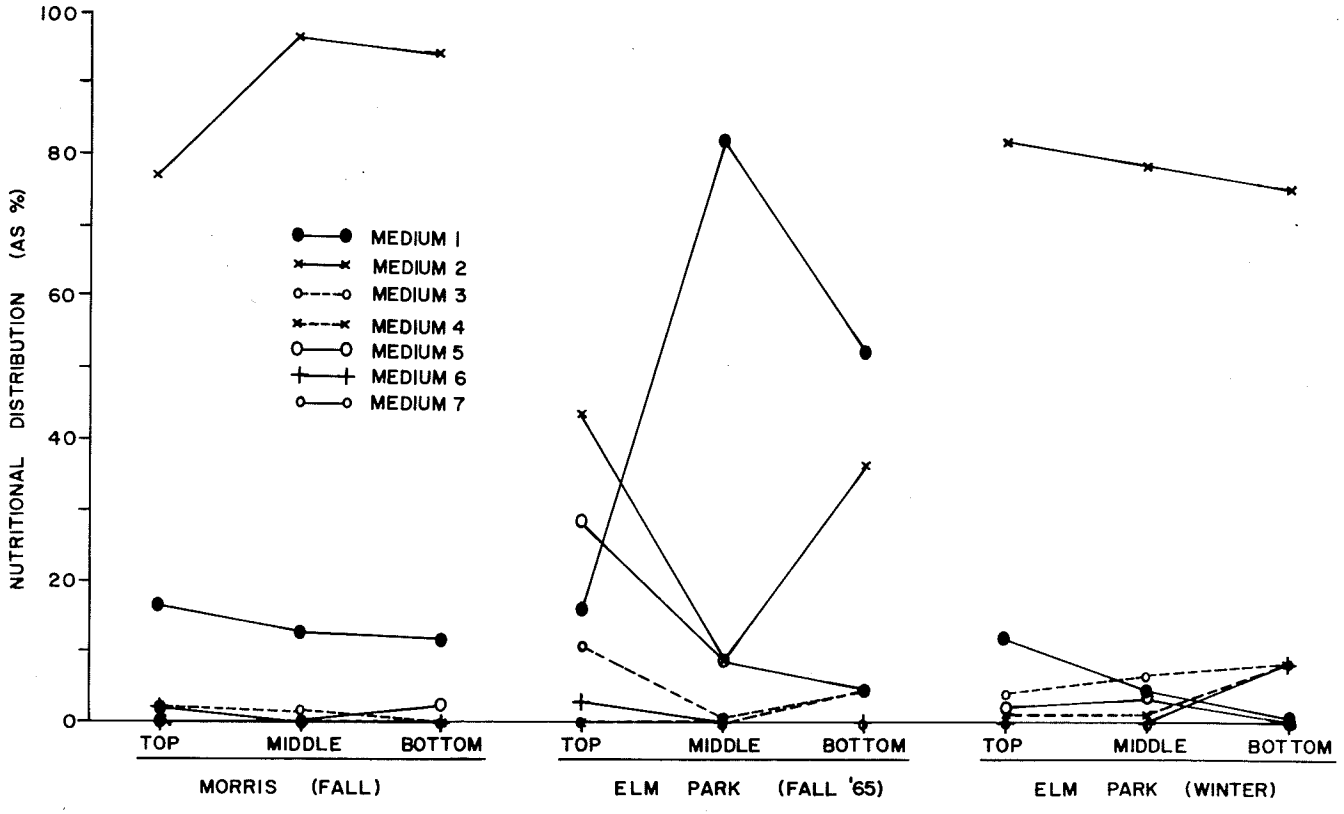

NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
TOP
MIDDLE
BOTTOM
MORRIS (FALL)
TOP
MIDDLE
BOTTOM
ELM PARK (FALL '65)
TOP
MIDDLE
BOTTOM
ELM PARK (WINTER)

FIGURE 23. Variation in nutritional distribution with increasing depth at Elm Park, Spring; Elm Park, Summer; Elm Park, Fall 1966.

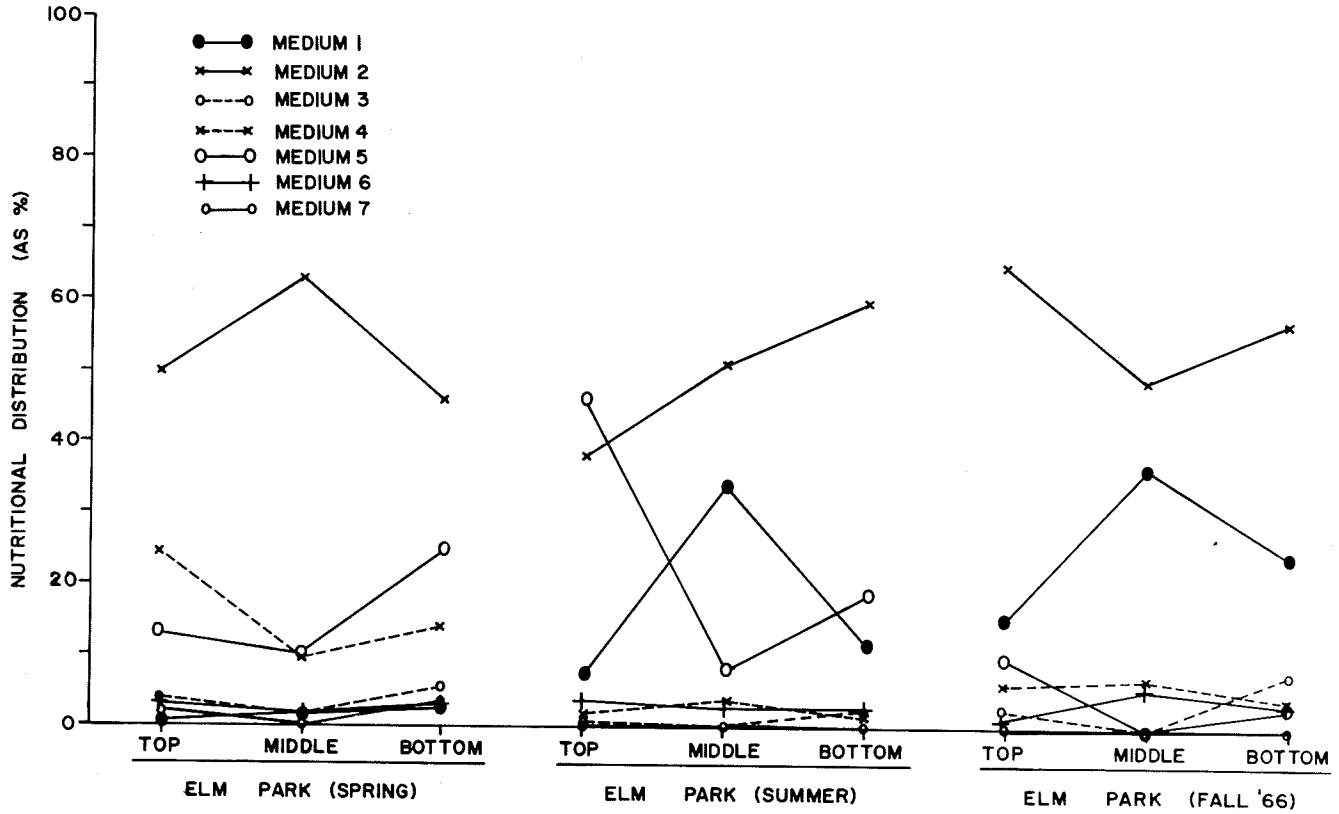

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
TOP
MIDDLE
BOTTOM
ELM PARK (SPRING)
TOP
MIDDLE
BOTTOM
ELM PARK (SUMMER)
TOP
MIDDLE
BOTTOM
ELM PARK (FALL '66)

FIGURE 24. Variation in nutritional distribution with increasing depth at St. James, Winter; St. James, Spring; St. James, Summer.

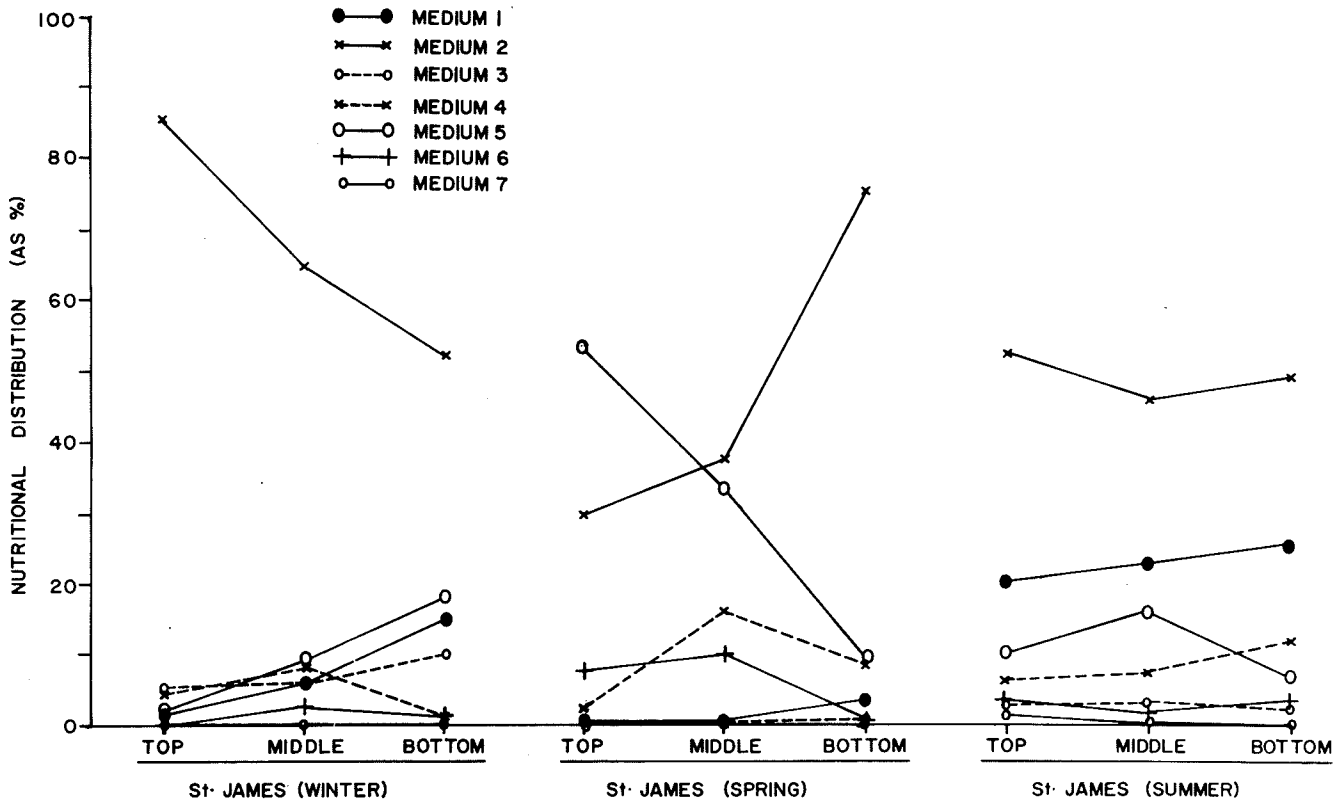

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
TOP
MIDDLE
BOTTOM
St· JAMES (WINTER)
St· JAMES (SPRING)
St· JAMES (SUMMER)

FIGURE 25. Variation in nutritional distribution with increasing depth at St. James, Fall; Higgins, Winter; Higgins, Spring.

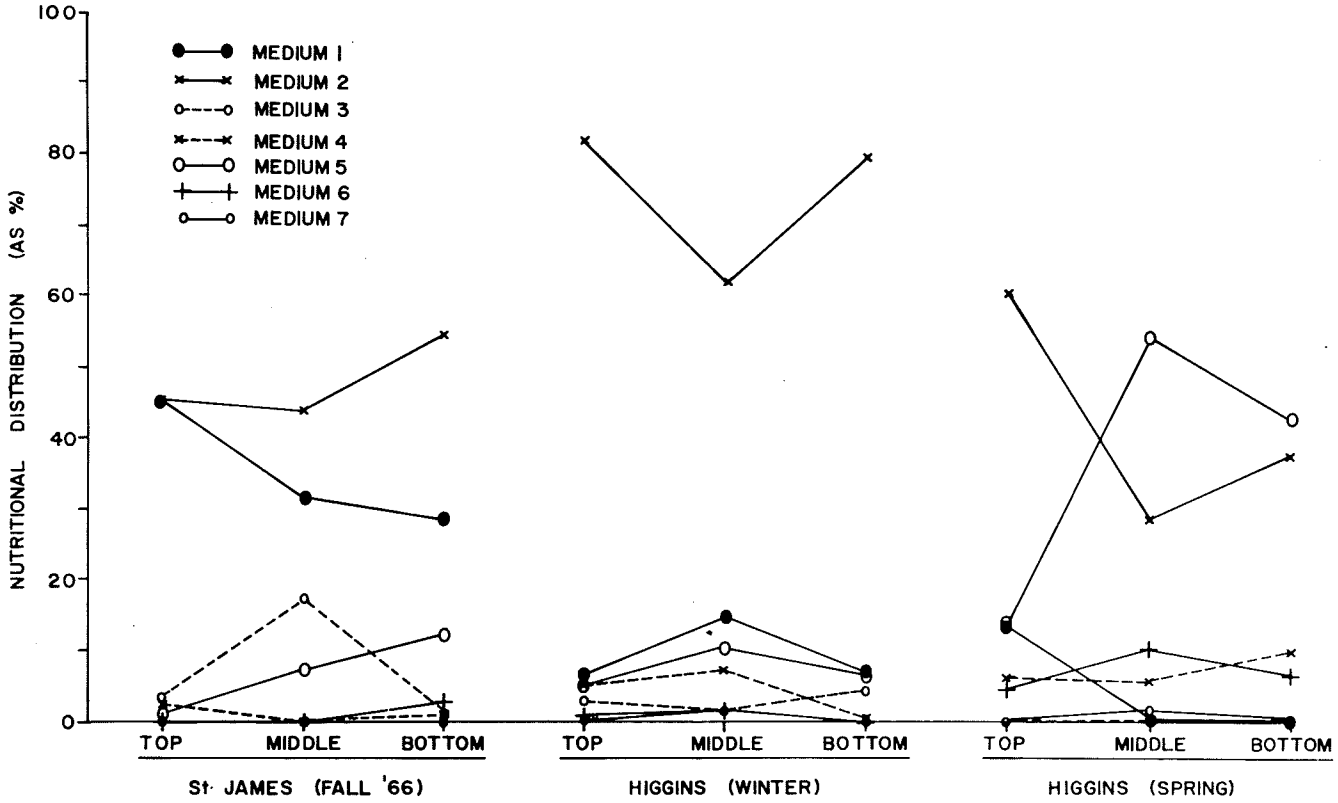

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
TOP
MIDDLE
BOTTOM
St. JAMES (FALL '66)
HIGGINS (WINTER)
HIGGINS (SPRING)

FIGURE 26. Variation in nutritional distribution with increasing depth at Higgins, Summer; Higgins, Fall; Selkirk, Winter.

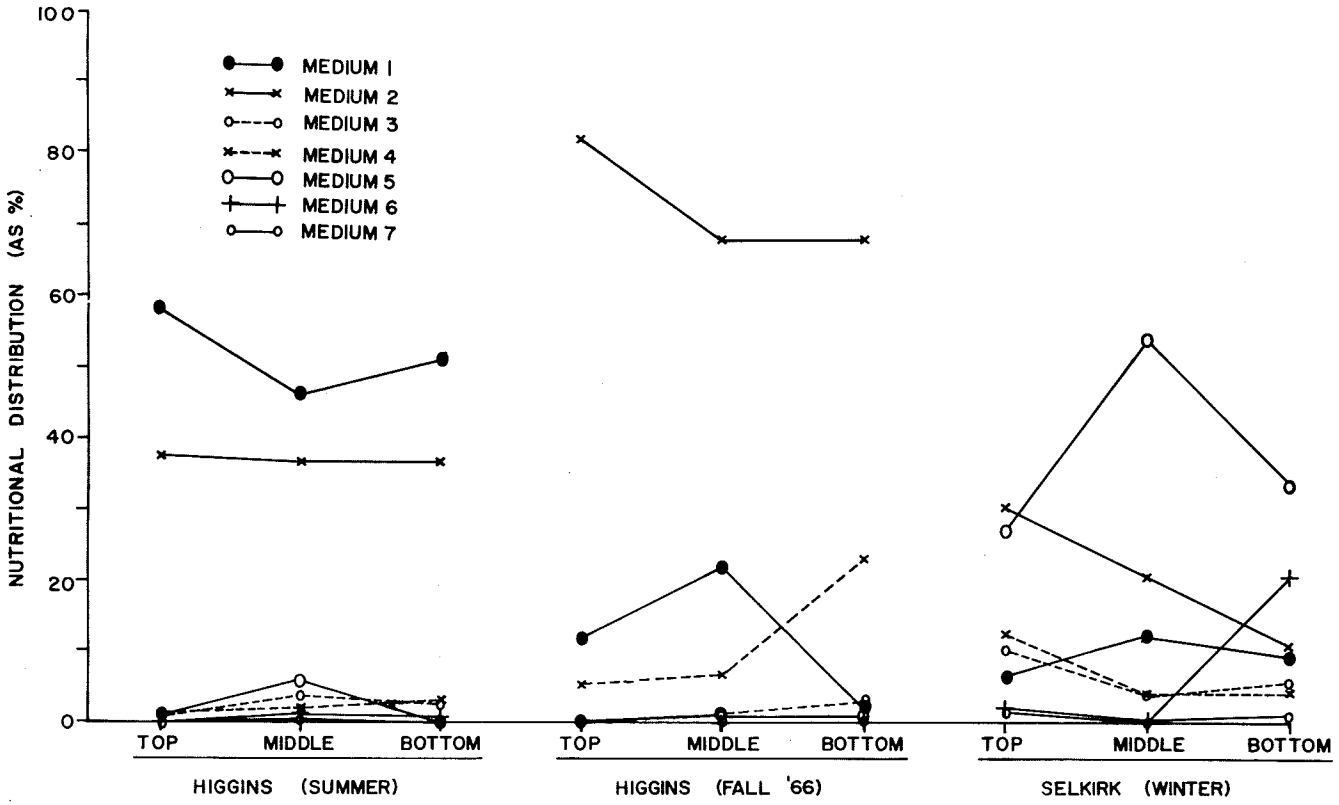

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
TOP
MIDDLE
BOTTOM
HIGGINS (SUMMER)
TOP
MIDDLE
BOTTOM
HIGGINS (FALL '66)
TOP
MIDDLE
BOTTOM
SELKIRK (WINTER)

FIGURE 27. Variation in nutritional distribution with increasing depth at Selkirk, Spring; Selkirk, Summer; Selkirk, Fall.

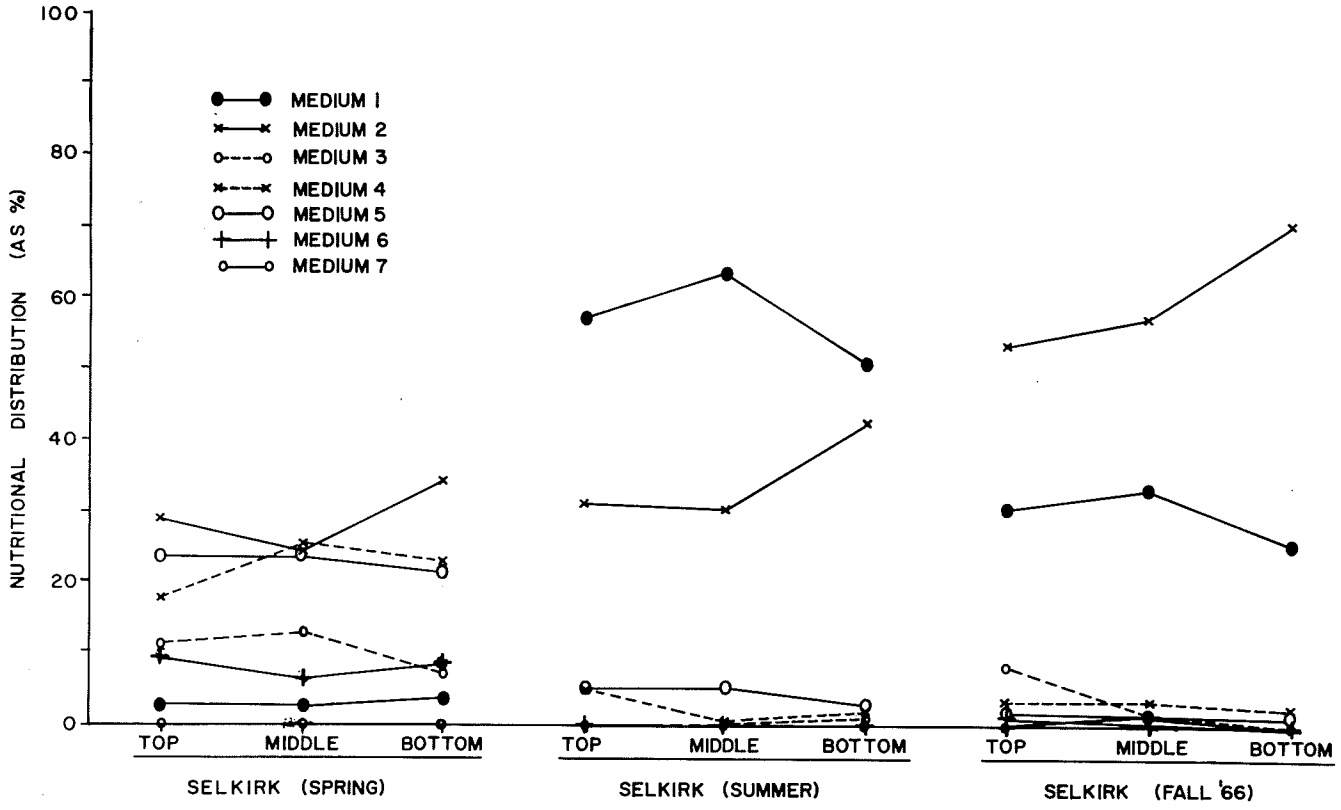

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
TOP
MIDDLE
BOTTOM
SELKIRK (SPRING)
TOP
MIDDLE
BOTTOM
SELKIRK (SUMMER)
TOP
MIDDLE
BOTTOM
SELKIRK (FALL '66)

FIGURE 28. Variation in nutritional distribution at one sampling level with seasonal changes at Morris, top; Morris, middle.

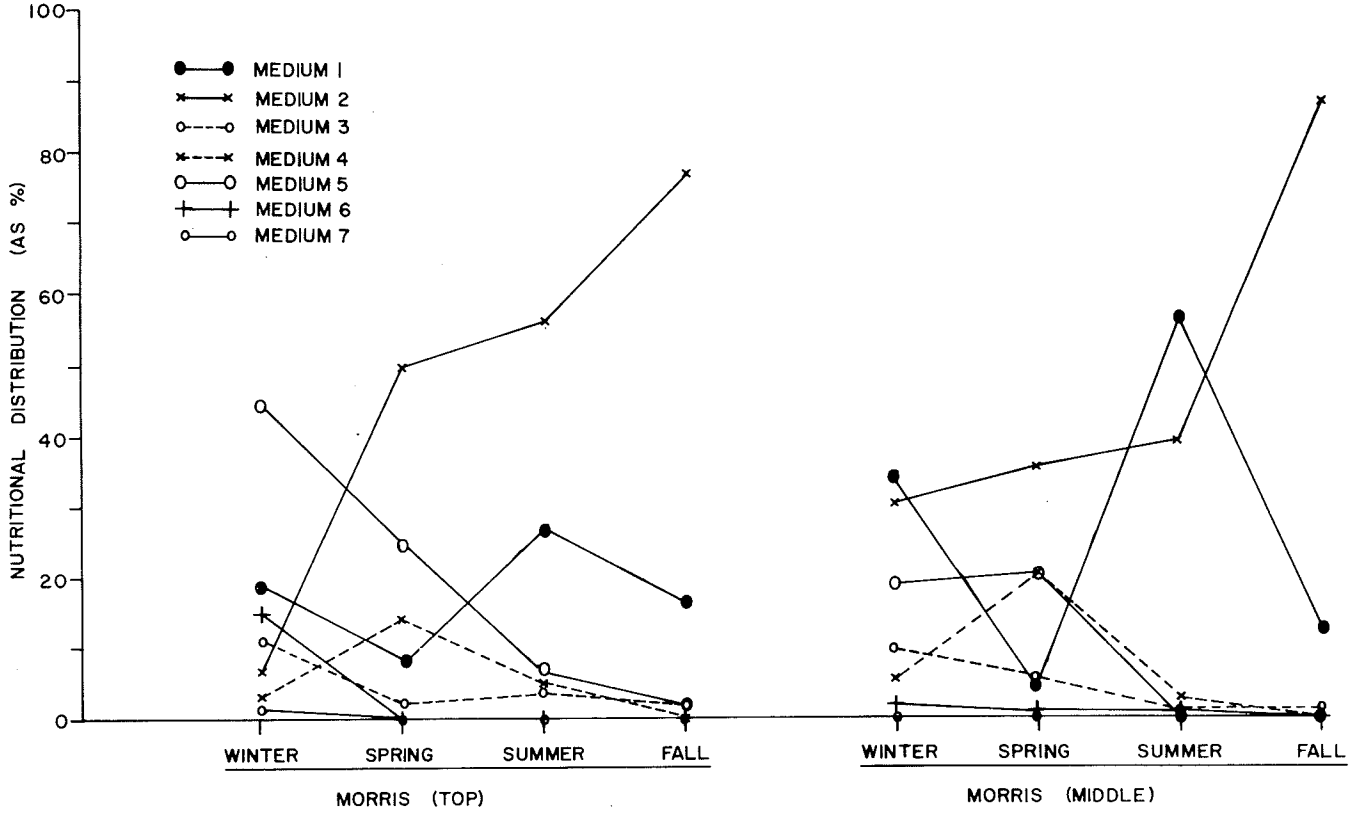

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
WINTER
SPRING
SUMMER
FALL
MORRIS (TOP)
WINTER
SPRING
SUMMER
FALL
MORRIS (MIDDLE)

FIGURE 29. Variation in nutritional distribution at one sampling level with seasonal changes at Morris, bottom; Elm Park, top.

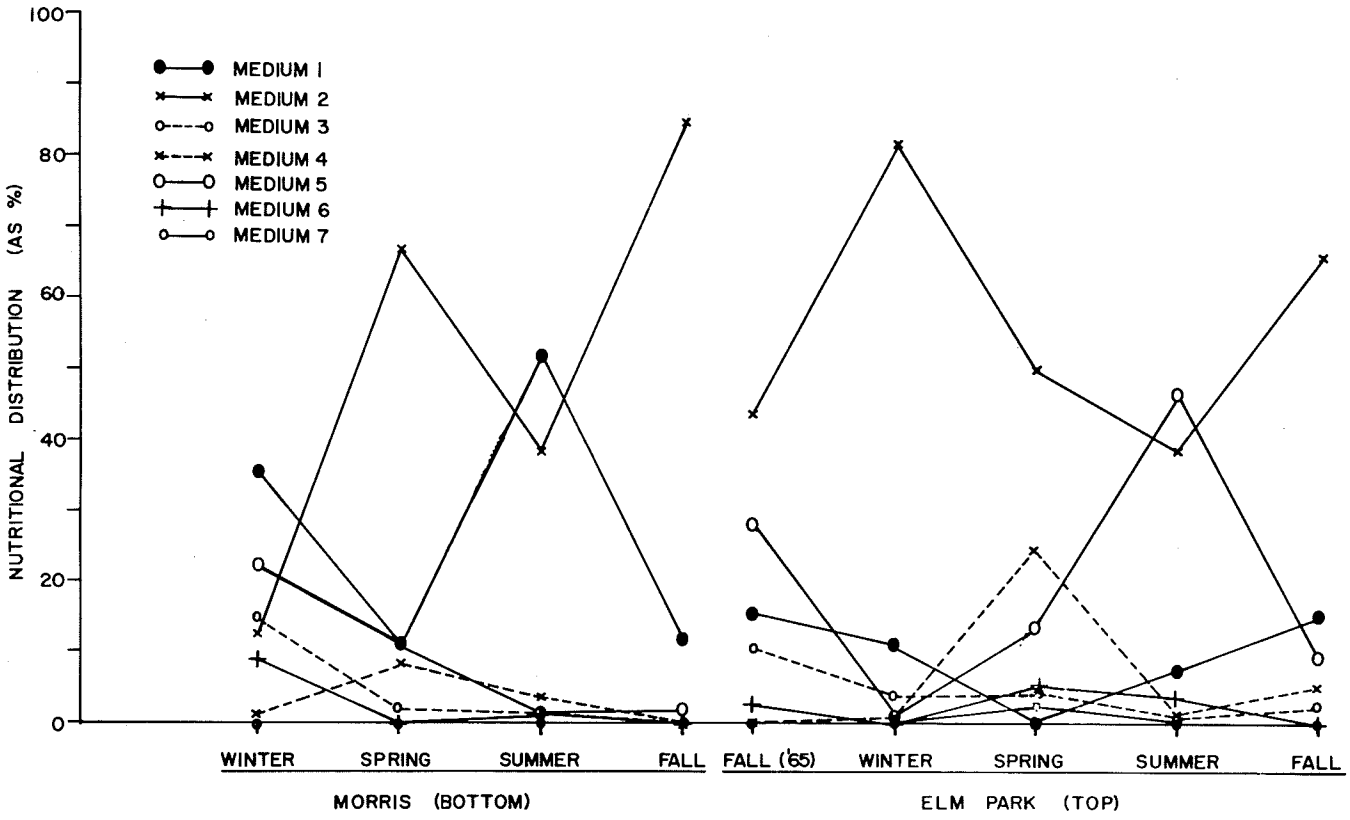

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
WINTER
SPRING
SUMMER
FALL
FALL ('65)
WINTER
SPRING
SUMMER
FALL
MORRIS (BOTTOM)
ELM PARK (TOP)

FIGURE 30. Variation in nutritional distribution at one sampling level with seasonal changes at Elm Park, middle; Elm Park, bottom.

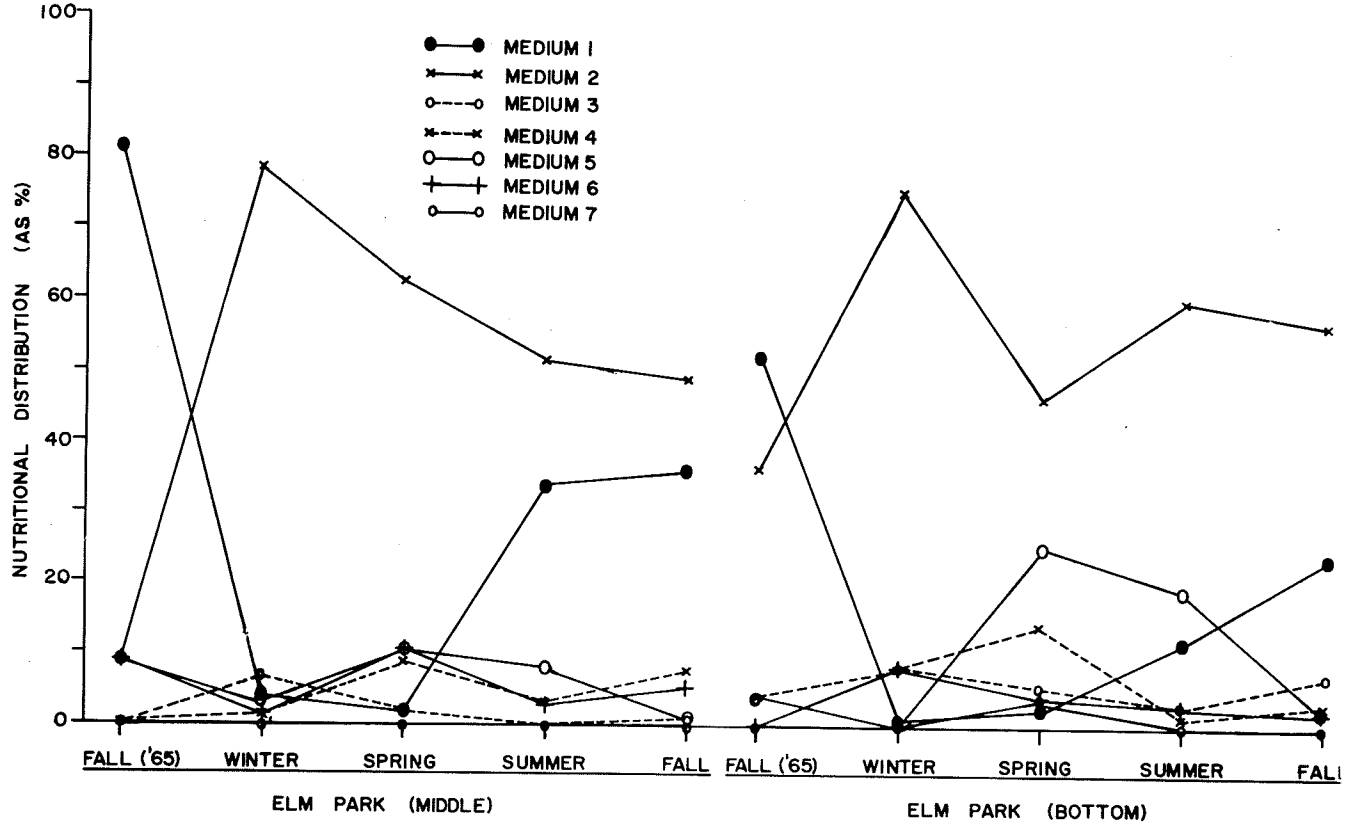

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
FALL ('65)
WINTER
SPRING
SUMMER
FALL
ELM PARK (MIDDLE)
FALL ('65)
WINTER
SPRING
SUMMER
FALL
ELM PARK (BOTTOM)

FIGURE 31. Variation in nutritional distribution at one sampling level with seasonal changes at St. James, top; St. James, middle.

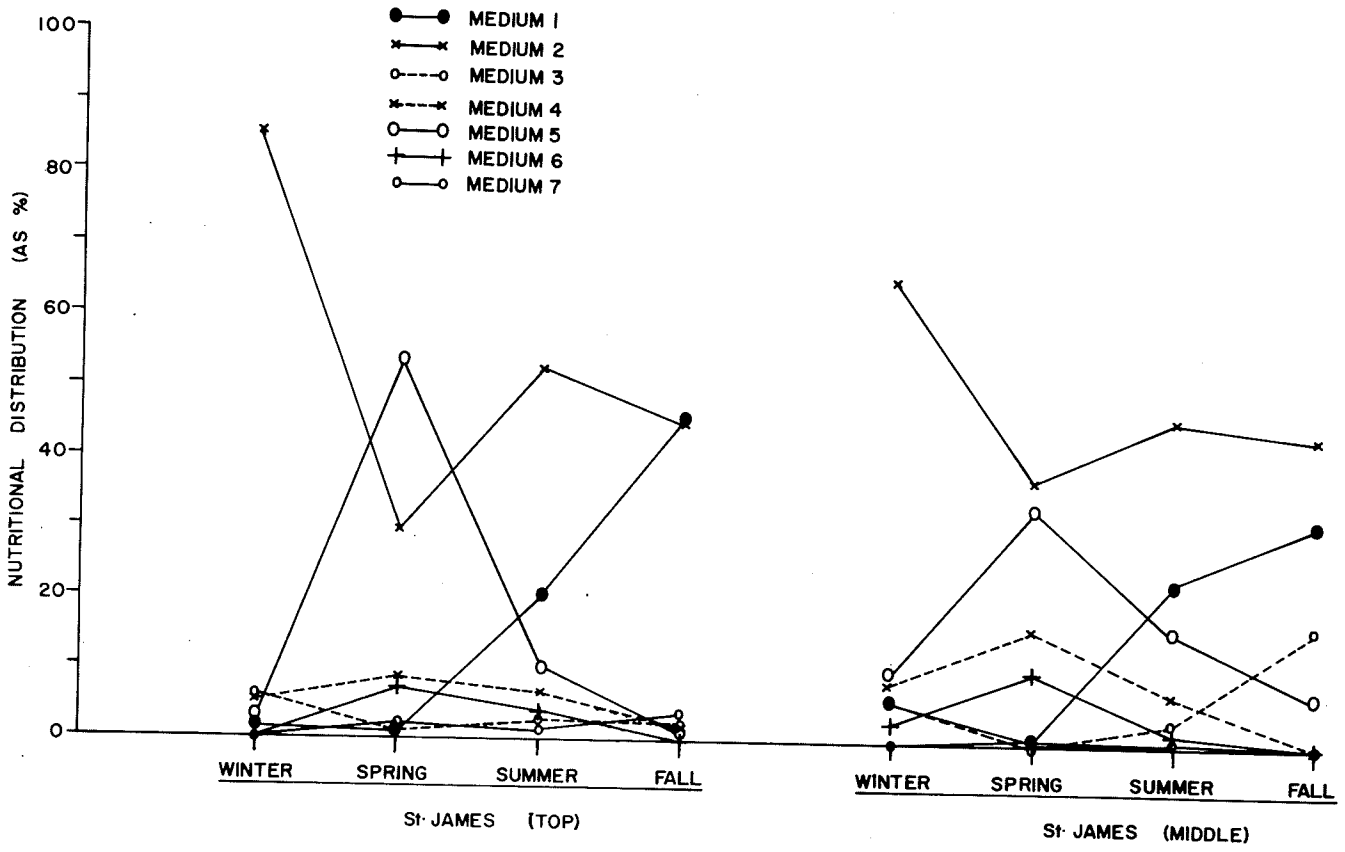

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
WINTER
SPRING
SUMMER
FALL
St. JAMES (TOP)
WINTER
SPRING
SUMMER
FALL
St. JAMES (MIDDLE)

FIGURE 32. Variation in nutritional distribution at one sampling level with seasonal changes at St. James, bottom; Higgins, top.

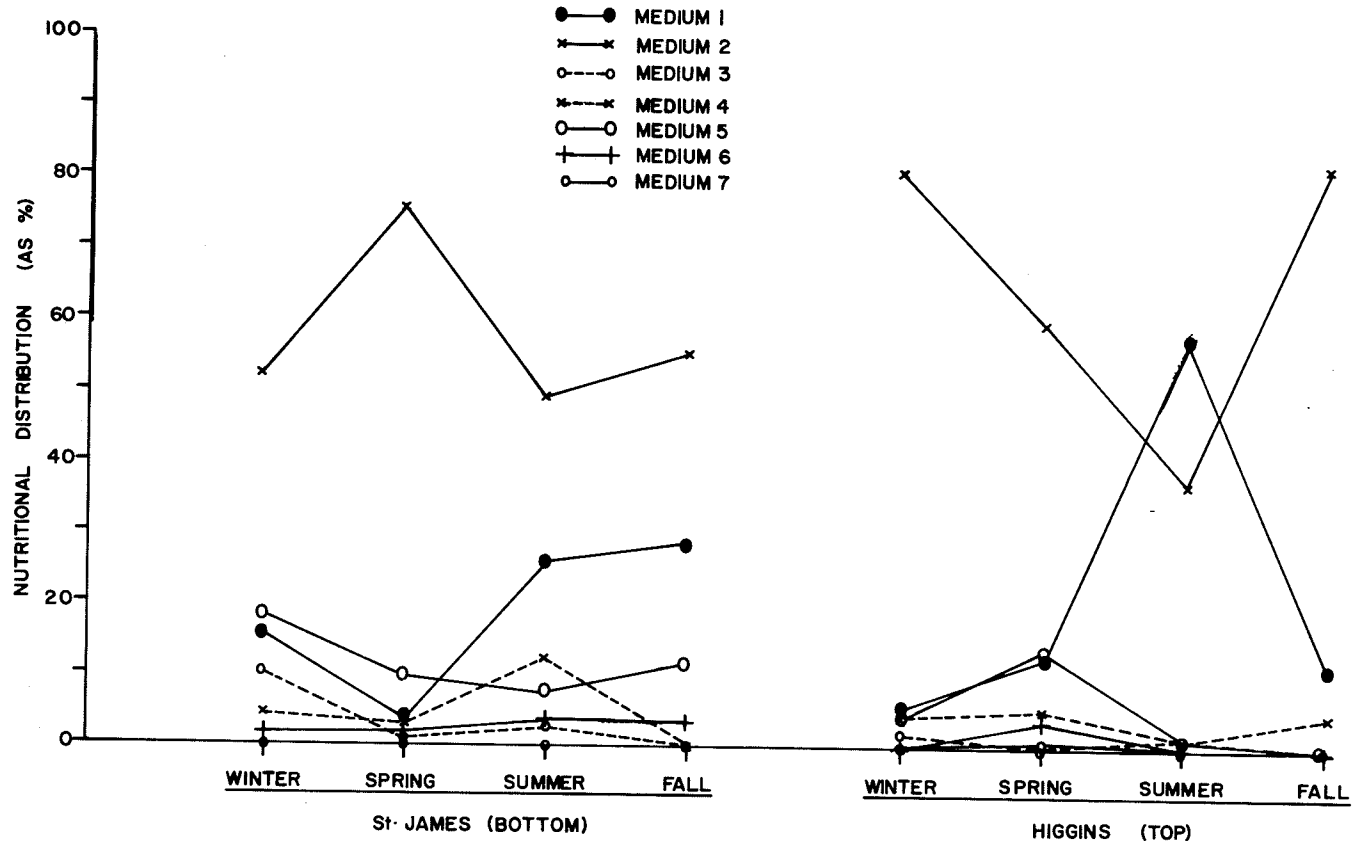

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
WINTER
SPRING
SUMMER
FALL
St. JAMES (BOTTOM)
WINTER
SPRING
SUMMER
FALL
HIGGINS (TOP)

FIGURE 33. Variation in nutritional distribution at one sampling level with seasonal changes at Higgins, middle; Higgins, bottom.

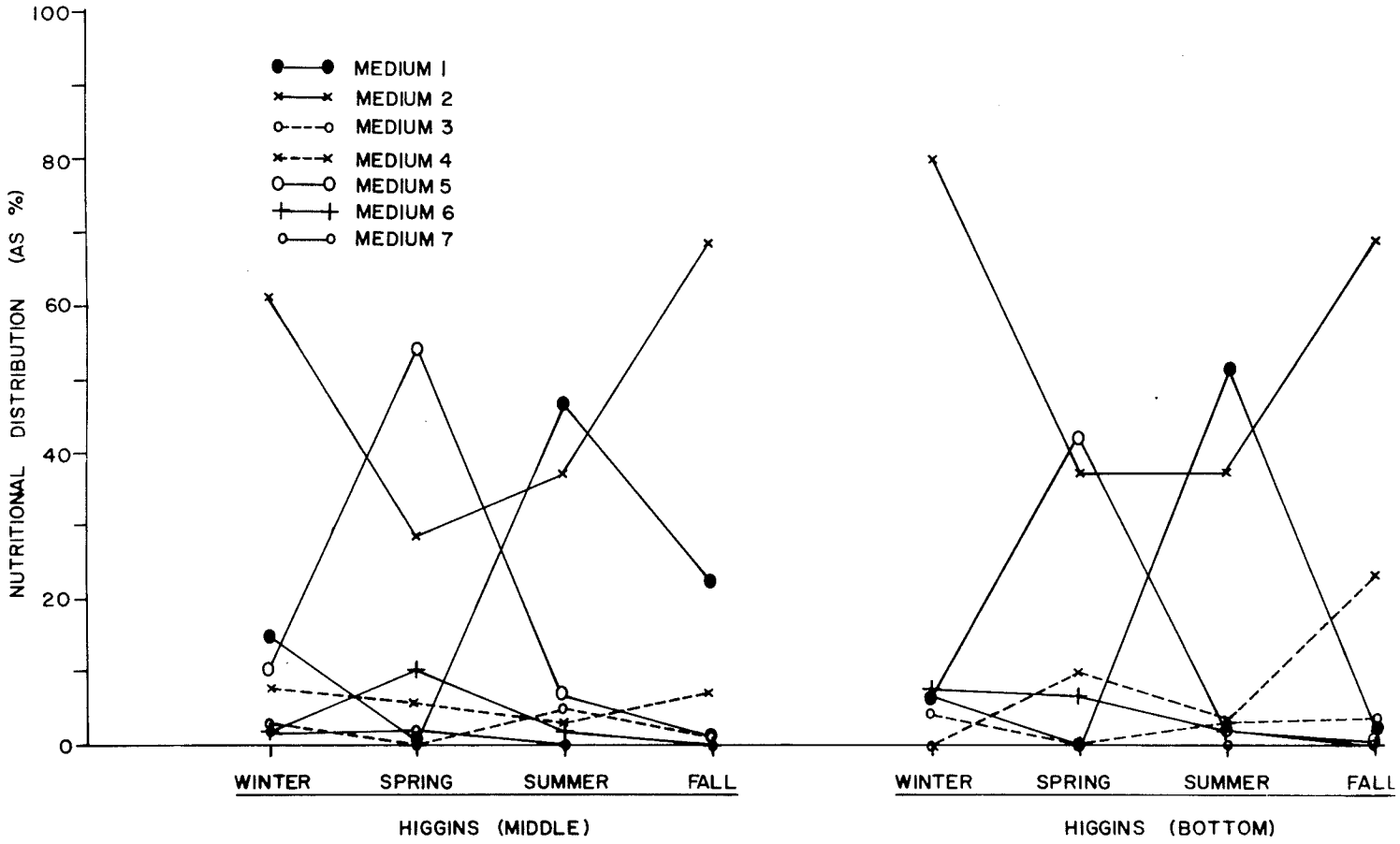

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
WINTER
SPRING
SUMMER
FALL
HIGGINS (MIDDLE)
WINTER
SPRING
SUMMER
FALL
HIGGINS (BOTTOM)

FIGURE 34. Variation in nutritional distribution at one sampling level with seasonal changes at Selkirk, top; Selkirk, middle.

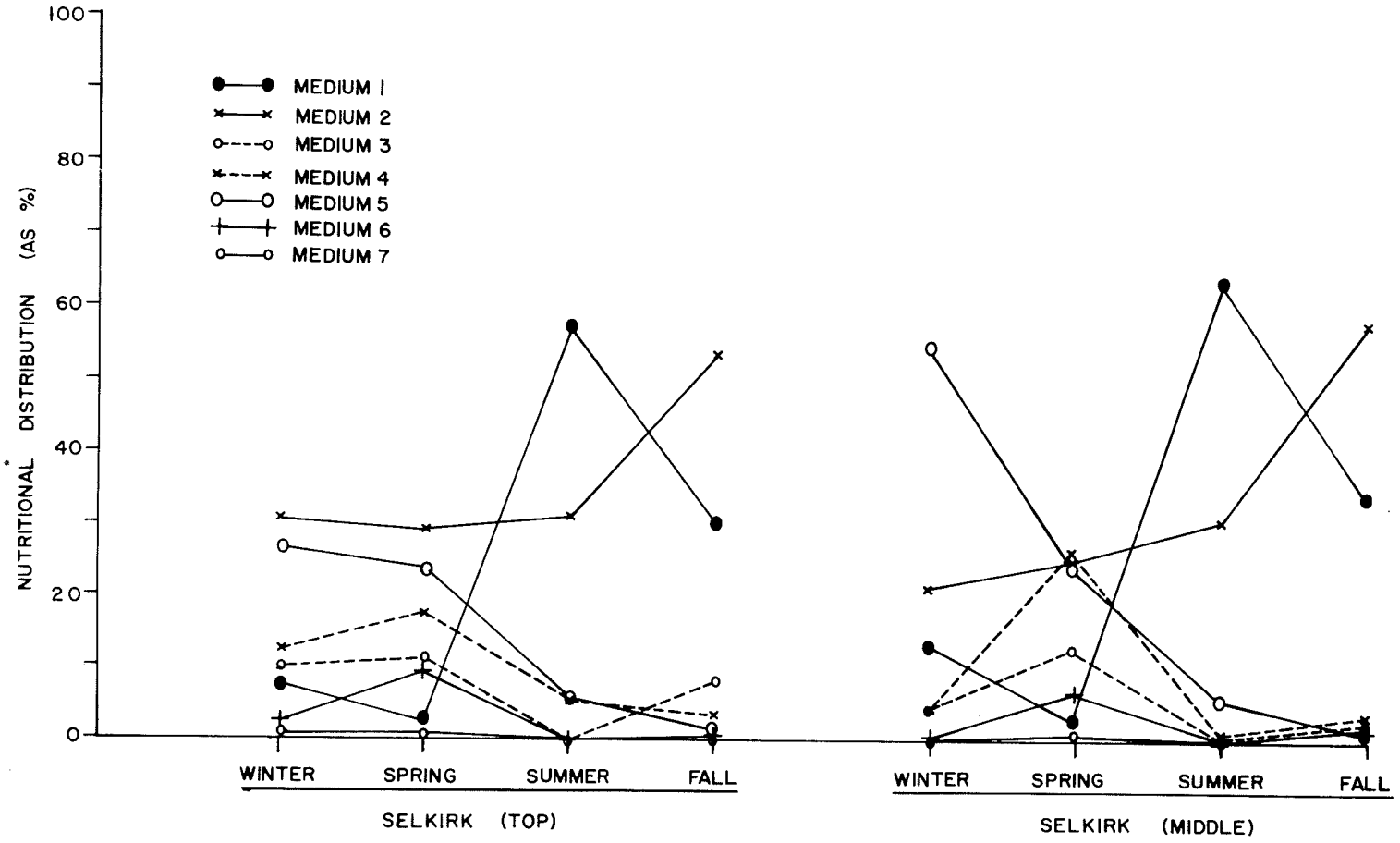

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
WINTER
SPRING
SUMMER
FALL
SELKIRK (TOP)
WINTER
SPRING
SUMMER
FALL
SELKIRK (MIDDLE)

FIGURE 35. Variation in nutritional distribution at one sampling level with seasonal changes at Selkirk, bottom.

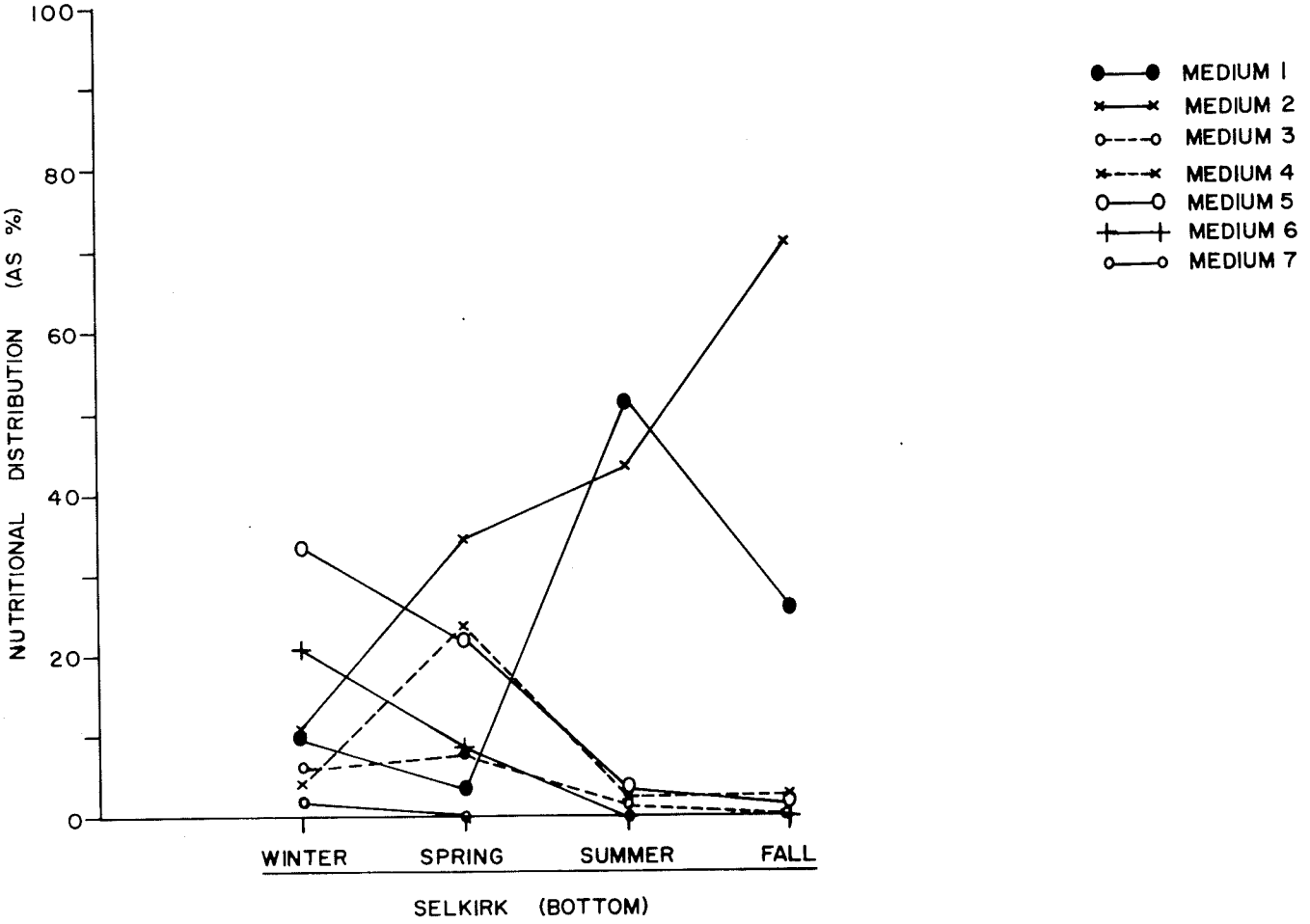

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
WINTER
SPRING
SUMMER
FALL
SELKIRK (BOTTOM)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7

FIGURE 36. Variation in nutritional distribution at one sampling level with the location along the river at top, winter; middle, winter.

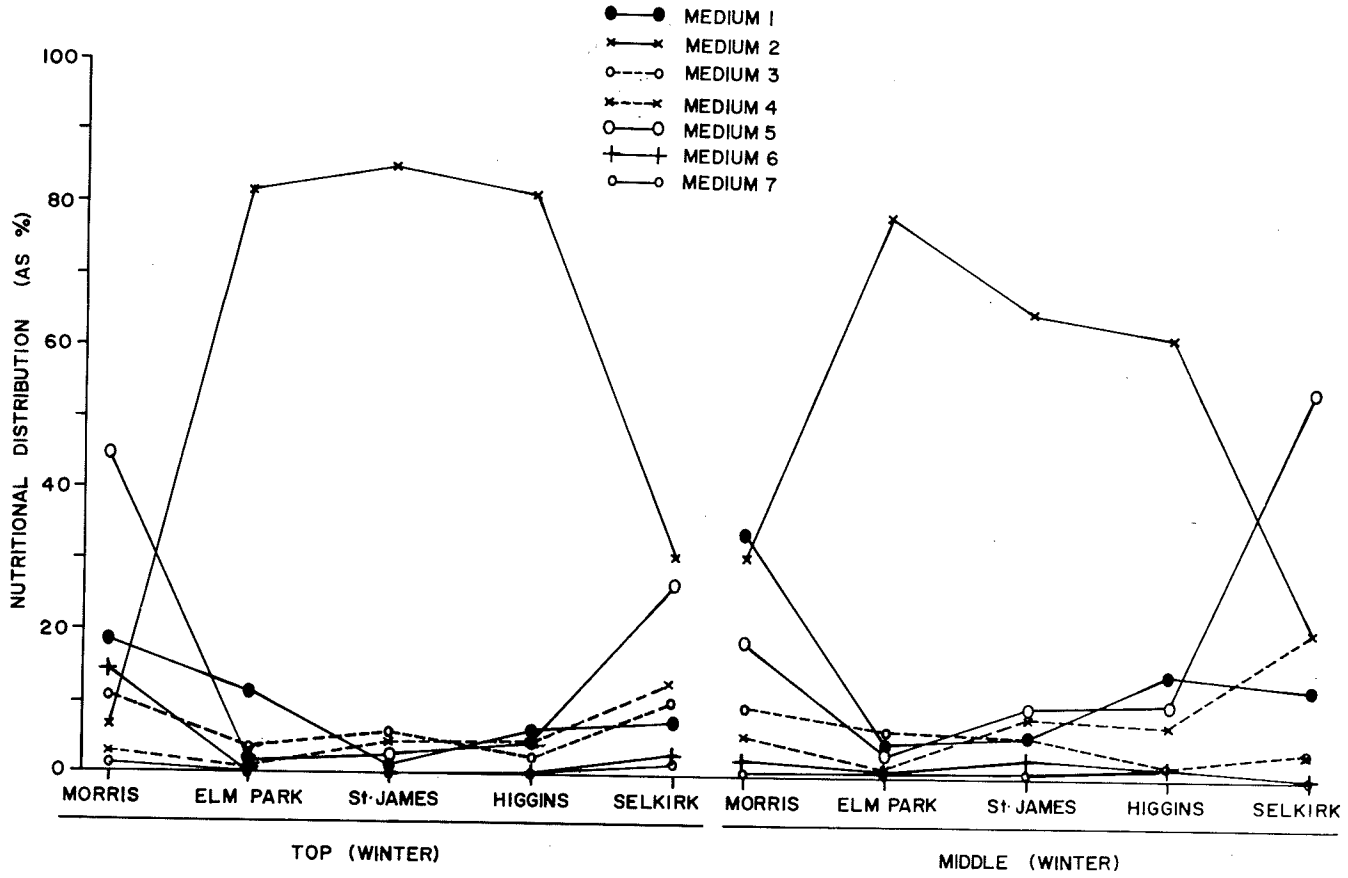

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
MORRIS
ELM PARK
St·JAMES
HIGGINS
SELKIRK
TOP (WINTER)
MORRIS
ELM PARK
St·JAMES
HIGGINS
SELKIRK
MIDDLE (WINTER)

FIGURE 37. Variation in nutritional distribution at one sampling level with the location along the river at bottom, winter; top, spring.

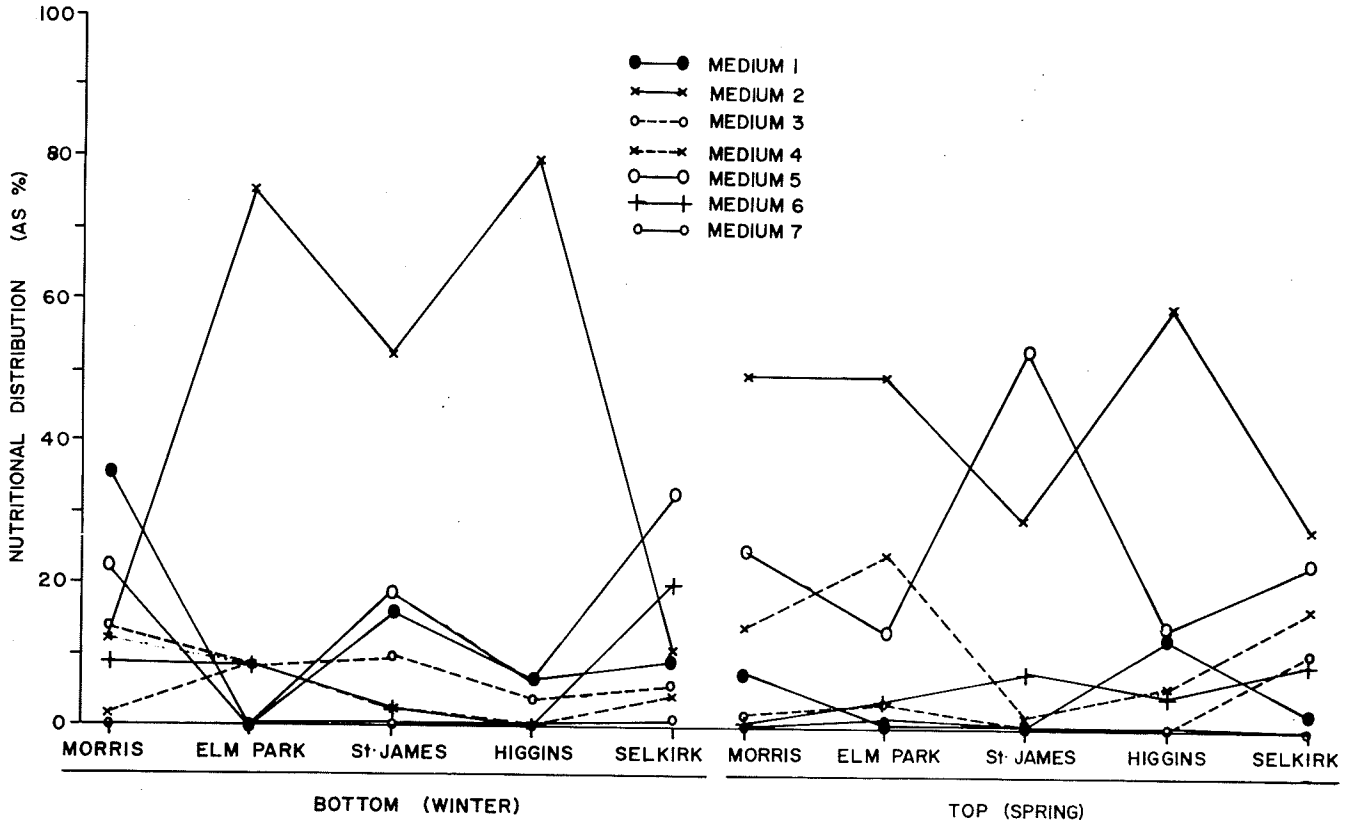

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
MORRIS
ELM PARK
St·JAMES
HIGGINS
SELKIRK
MORRIS
ELM PARK
St· JAMES
HIGGINS
SELKIRK
BOTTOM (WINTER)
TOP (SPRING)

FIGURE 38. Variation in nutritional distribution at one sampling level with the location along the river at middle, Spring; bottom, Spring.

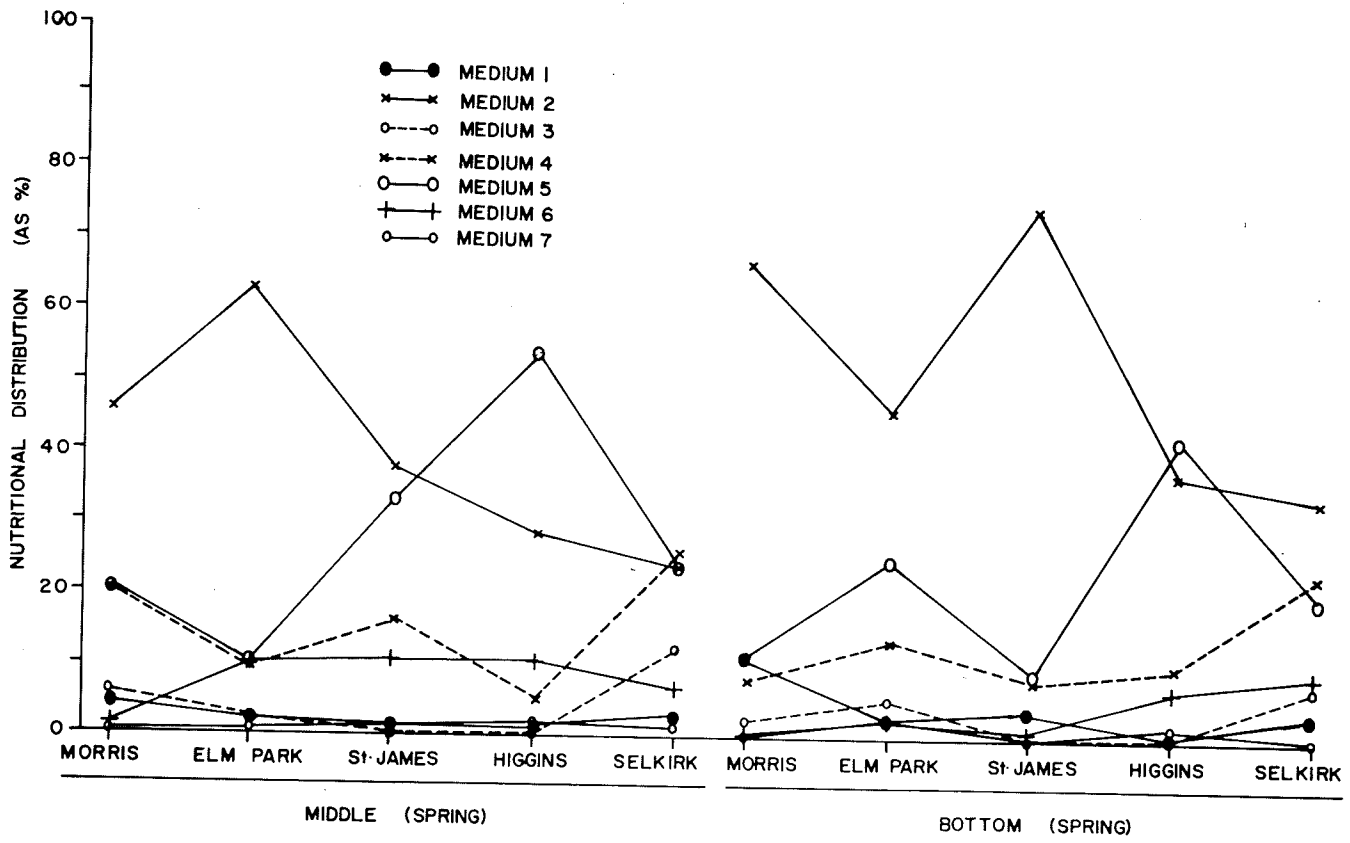

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
MIDDLE (SPRING)
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
BOTTOM (SPRING)

FIGURE 39. Variation in nutritional distribution at one sampling level with the location along the river at top, Summer; middle, Summer.

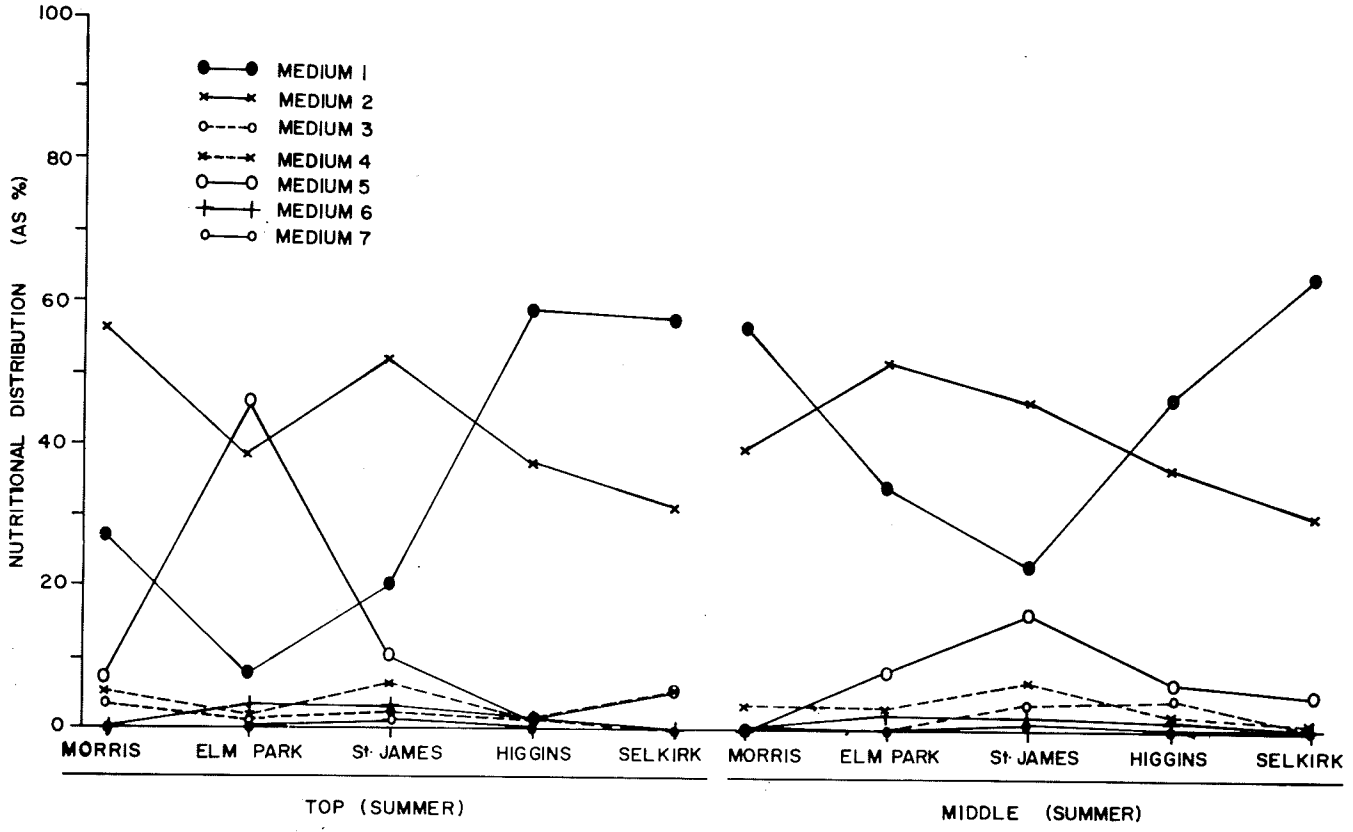

MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
TOP (SUMMER)
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
MIDDLE (SUMMER)

FIGURE 40. Variation in nutritional distribution at one sampling level with the location along the river at bottom, Summer; top, Fall.

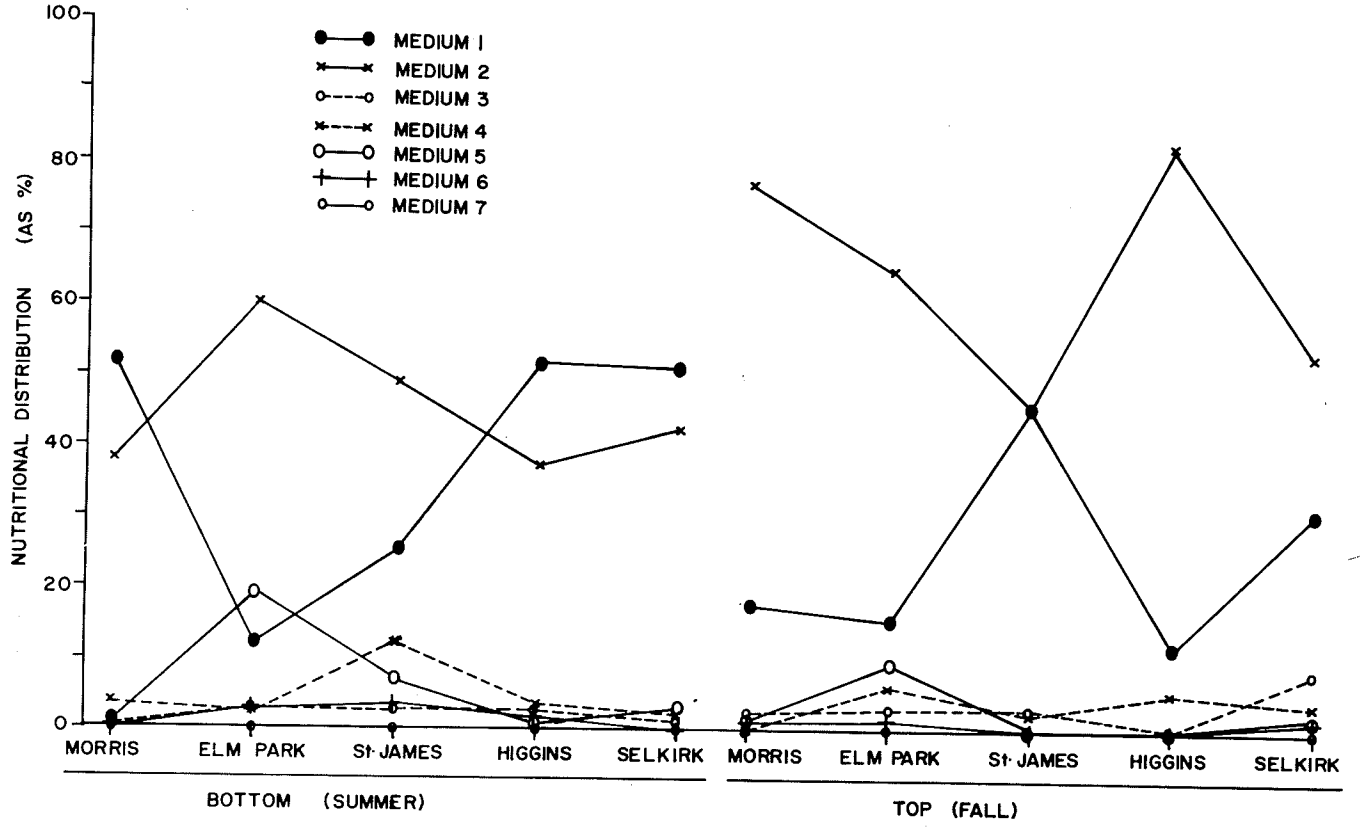
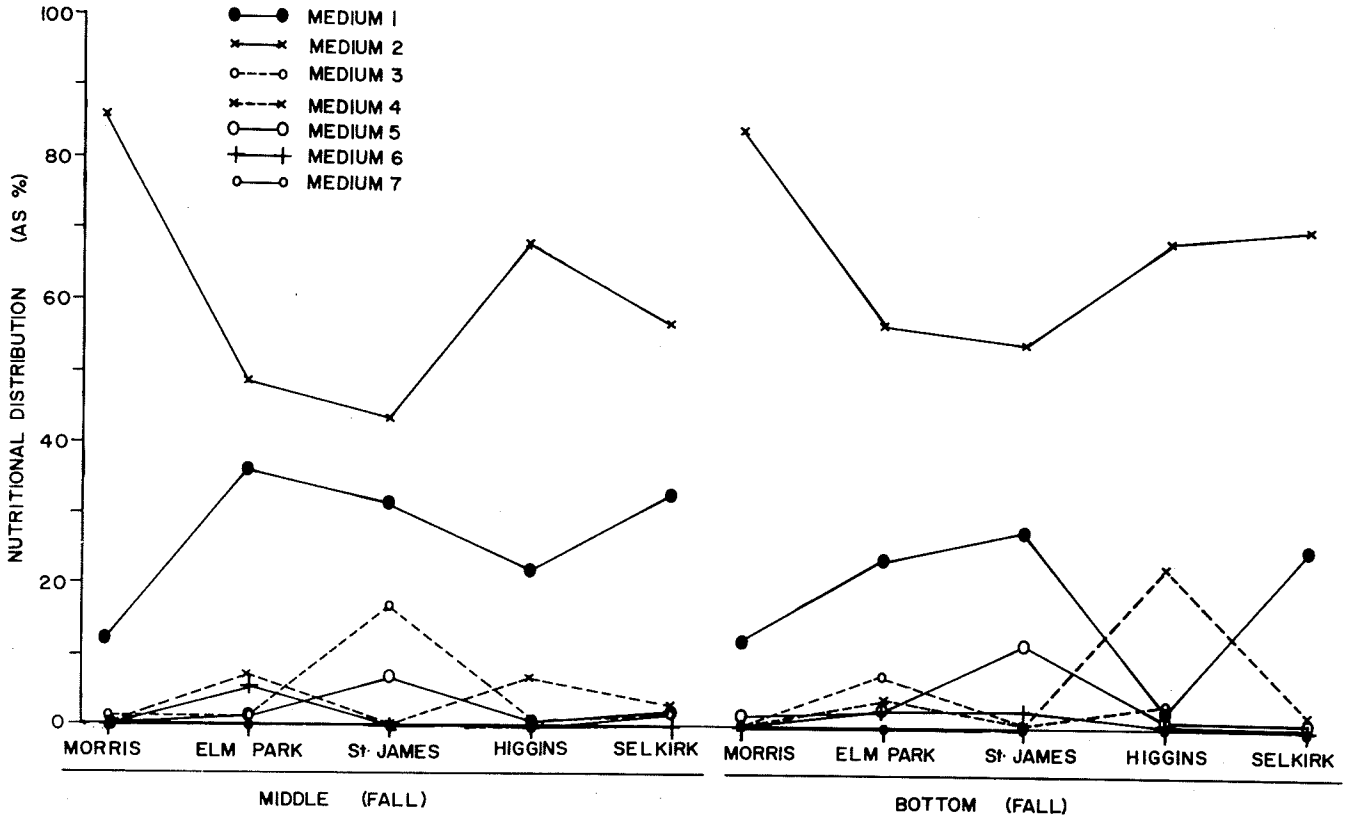

100
80
60
40
20
0
NUTRITIONAL DISTRIBUTION (AS %)
MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
BOTTOM (SUMMER)
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
TOP (FALL)

FIGURE 41. Variation in nutritional distribution at one sampling level with the location along the river at middle, Fall; bottom, Fall.


MEDIUM 1
MEDIUM 2
MEDIUM 3
MEDIUM 4
MEDIUM 5
MEDIUM 6
MEDIUM 7
NUTRITIONAL DISTRIBUTION (AS %)
100
80
60
40
20
0
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
MIDDLE (FALL)
MORRIS
ELM PARK
St. JAMES
HIGGINS
SELKIRK
BOTTOM (FALL)