MICRONUTRIENTS AND PHYTOPLANKTON BIOMASS

IN FRESHWATER RESERVOIRS

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Summary

Of the factors determining the abundance and species composition of the phytoplankton in freshwater lakes and reservoirs, the micronutrients are among the least studied despite their potential importance. An investigation was therefore initiated, with the aim of making a contribution to our existing knowledge of micronutrients in freshwater and their importance in phytoplankton ecology.

To this end three reservoirs in the Charnwood Forest area of Leicestershire, England were sampled at regular intervals over a period of two years and chlorophyll, phaeophytin and selected micronutrient concentrations measured in the samples. The micronutrients selected for study were the inorganic metals iron, manganese, copper, zinc and cobalt, and the organic vitamins B_{12}, thiamine and biotin. Three fractions of each metal (soluble, complexed and particulate metal) were measured in samples by atomic absorption spectroscopy. Dissolved vitamin B_{12} in samples was bioassayed with three different organisms (Euglena gracilis Klebs, Lactobacillus leichmanii ATCC 7830 and Ochromonas malhamensis Pringsheim). Particulate vitamin B_{12} was also routinely bioassayed with E. gracilis Through one year dissolved vitamin B_{12} was differentiated into soluble and complexed or bound forms by the ultrafiltration of samples taken monthly.

Thiamine and biotin were also looked for, but both vitamins were always undetectable by the bioassay organisms used - Lactobacillus viridescens ATCC 12706 and Monochrysis lutheri Droop for thiamine and Lactobacillus plantarium ATCC 8014 for biotin.

A survey of the micronutrient concentrations entering the reservoirs during a twelve month period was also made. The dissolved vitamin B_{12} and total metal concentrations in samples taken at monthly intervals were measured by the above methods.

A limited number of sediment samples were also taken from the reservoirs and their extractable vitamin B_{12} and metal micronutrient concentrations determined.

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C O N T E N T S

SECTION 1

INTRODUCTION

1.1. INTRODUCTION

The inorganic elements and organic nutrients which, together with a source of light, should enable most algae to grow were listed by Hutchinson (1967) as follows:- carbon, nitrogen, phosphorus, silica, calcium, magnesium, sodium, potassium, sulphur, iron, manganese, zinc, copper, cobalt, molybdenum, boron, vanadium, vitamin B_{12}, thiamine and biotin. The last eleven nutrients listed are more generally known as the micronutrients because they are needed, and are generally present in comparatively 'micro' quantities in natural waters.

The discovery of the importance of micronutrients in algal nutrition was made following improvements in the purity of chemical reagents and the replacement of soil extracts in algal culture media by mixtures of trace metals, vitamins and chelating agents (Provasoli & Pintner 1953).

With the advent of sensitive analytical techniques capable of detecting and measuring the low levels of these micronutrients in the environment, interest in the possible ecological significance of micronutrients in natural waters was stimulated. In a review of algal nutrition and eutrophication, Provasoli (1970) predicted that interesting correlations between micronutrients and phytoplankton growth were to be expected in the expanding literature on the subject and called attention to the need for data. Indeed, according to Lee (1970 a), one of the micronutrients, iron, was in the literature already the next most frequently suggested limiting nutrient after nitrogen, phosphorus and silica.

The importance of micronutrients in limiting the primary productivity

of some aquatic ecosystems is now well established (Goldman 1972). Much of the work from which Goldman (1972) drew this conclusion was based on the results of enrichment bioassay techniques*. This type of approach is commonly criticised as not duplicating the lake environment with regard to light and temperature (Goldman 1961), and because of 'bottle effects' such as the changes in the relative abundance of phytoplankton species which occur with time (Wetzel 1975). These criticisms have been largely met by the in situ incubation of nutrient enriched sample bottles for periods that can be made very brief by using ^{14}C uptake as a measure of the growth response of the phytoplankton (Goldman 1961). However, other criticisms, such as the reduction in water movement and the vast increase in the ratio of surface area to volume that the method brings about (which may promote such effects as increased bacterial growth - Provasoli 1963, and adsorption of nutrients, such as iron onto the walls of culture vessels - Lewin & Chen 1971) remain valid. Some workers, for example Lund (1972,1975), are therefore using large experimental tubes in lakes to study the effects of nutrient enrichments on the natural phytoplankton population under more natural conditions. Others, for example Goldman (1965,1972) and Schelske, Hooper & Haertl (1962) have enriched entire lakes with nutrients in order to identify nutrient limiting factors.

*In enrichment bioassay techniques a nutrient is considered limiting if (in situ) incubation of a water sample with the added nutrient shows a significant increase in total photosynthesis by the natural population in the sample as compared with a control.

The need for caution in the interpretation of enrichment experiments was emphasised by Goldman (1965) and Wetzel (1965). Dugdale (1967) suggested that indirect effects, such as possible changes in the ratio of divalent to monovalent cations, might confuse the interpretation of enrichment experiments.

These criticisms, together with the practical difficulties of the approach, constitute the rationale for choosing the more traditional approach of determining limiting nutrients in aquatic ecosystems; that is, to monitor the nutrient concentration in the water and to look for correlations with the size of the phytoplankton population.

The approach adopted is not, however, without its own problems. These problems largely determined the methods described in section 2 to estimate the micronutrient concentrations in the reservoirs studied. There are in fact two basic problems with this type of approach which need to be realised:-

i) effective availability of micronutrients

ii) species variability with respect to micronutrient requirements.

1. Availability

This point may be illustrated using iron as an example.

The chemical estimation of iron in natural waters is not difficult. Unfortunately, the concentration measured by such methods may not be the same as the concentration available to the phytoplankton. In Lake J,

Minnesota, for example, Shapiro (1969) concluded that only 30% of the soluble iron present was actually available to Microcystis aeruginosa. The bioassay of available iron would be an attractive solution to this problem but for the variation between algal species in the forms of iron available to them (as discussed further below). This variation led Shapiro (1967 b) to conclude that "the problem of determining 'available' iron seems therefore to be insoluble unless a method is elaborated separately for each alga".

Accepting Shapiro's (1967 b) conclusion the decision was made that in this investigation the bioassay of available iron would be impractical and that a chemical method would have to be chosen.

Lund (1965) suggested that the availability of iron to algae in natural waters is dependant on that water's organic matter content. Several workers (Goldman 1972; Sakamoto 1971; Schelske, Hooper & Haertl 1962; Wetzel 1965; Wetzel 1966) have demonstrated the stimulatory effect of organic chelators of metals (such as ethylenediaminetetraacetic acid-EDTA; hydroxyethylenediaminetetraacetic acid-HEDTA; and nitrilotriacetic acid-NTA) on photosynethetic carbon uptake when added alone or together with iron to natural waters. All of these workers concluded that the effect of the chelators alone was related to their bringing the iron already present into a form more readily available to algae.

In natural waters, the ability of the yellow organic acids or humic compounds to complex metals, and particularly their ability to hold large amounts of iron in apparent solution, have been known for a long time (Martin, Doig & Pierce 1971; Shapiro 1967 a and b). Other

compounds which may be present in natural waters with chelating properties include extracellular polypeptides (Fogg & Westlake 1955), iron specific chelators or 'siderochromes' (Gonye & Carpenter 1974), and 'ectocrines' (Harvey 1937).

The chemical estimation of organically complexed or chelated iron would therefore seem a suitable way of measuring available iron.

It is possible (but unknown) that similar comments may apply to other micronutrients. In the case of vitamin B_{12} for example, it is possible that a fraction of the vitamin in natural waters is protein-bound. Such bound vitamin B_{12} is unavailable to all the algae tested (Provasoli & Carlucci 1974), but may be included in measurements of the vitamin in natural waters by methods which include autoclaving (Droop 1962; Daisley 1969; Ohwada & Taga 1973). Such methods may therefore overestimate the concentration of vitamin B_{12} available to auxotrophic algae. The separate assessment of bound and free vitamin B_{12} thus becomes a necessary part of any investigation into the ecological significance of the vitamin, as was suggested by Daisley (1970).

2. Species Variability

This point may be illustrated using the vitamin micronutrients.

Species variation in the vitamin requirements of algae is diverse to the extent that not all algae require vitamins. Of the 388 algal species tested and listed by Provasoli & Carlucci (1974) only 52% require vitamin B_{12}, thiamine or biotin, either alone or in combination. Auxotrophic algae are found in all algal groups (except the Xanthophyceae

where the small number of species tested may not be representative). However, the incidence of auxotrophy in algal groups reveals differences; see Table 1. The variability of vitamin requirements both within and between algal groups is further complicated by the variability, even among isolates of the same species, first noted by Lewin & Lewin (1960) and reviewed in Provasoli & Carlucci (1974).

In the case of vitamin B_{12} further variation between algal species is apparent. This variability relates to the existence of vitamin B_{12} analogues. These analogues or congeners of vitamin B_{12} differ from each other in their lack of, or variation in, the nucleotide in the side chain of the cobalamin molecule. Organisms, including algae, which require cobalamins fall into three patterns of increasingly narrow specificity for these congeners. Table 2 shows the specificity patterns of the commonly used bioassay organisms. Provasoli & Carlucci (1974) listed 74 algal species whose specificity patterns have been determined. 49% have an Ochromonas malhamensis type response (to the same few vitamin B_{12} analogues that are utilised by higher animals), 20% have a Lactobacillus leichmanii type (i.e. intermediate) response and 31% an Escherichia coli type response (a wide range of analogues are effective as a source of the vitamin).

Interest in the vitamin B_{12} specificity of the algae was stimulated by the finding that many marine bacteria produce and release cobalamins into the sea, but only a minority produce 'true' vitamin B_{12}, that is the analogue with a dimethylbenzimidazole nucleotide side chain. Thus algae with a narrow specificity may be at an ecological disadvantage in natural waters and the vitamin B_{12} concentration measured using one bioassay organism may not represent the vitamin B_{12} concentration that

TABLE 1

Summary of Vitamin Requirements - (modified from Provasoli and Carlucci 1974)

Algal Group	Vitamin B_{12}	Thiamine	Biotin	Vitamin B_{12} Thiamine	Vitamin B_{12} Biotin	Thiamine Biotin	Vitamin B_{12} Thiamine Biotin	Autotrophs	Auxotrophs
Bacillariophyceae	35	5	0	8	0	0	0	23	48
Chlorophyceae	38	13	0	10	0	0	0	106	61
Chrysophyceae and Haptophyceae	2	8	0	12	2	1	2	1	27
Cryptophyceae	2	2	0	2	0	0	1	0	7
Cyanophyceae	8	0	0	0	0	0	0	35	8
Dinophyceae	17	0	1	1	1	0	5	2	25
Euglenophyceae	3	1	0	10	0	0	1	1	15
Phaephyceae	1	0	0	0	0	0	0	9	1
Rhodophyceae	11	0	0	0	0	0	0	1	11
Xanthophyceae	0	0	0	0	0	0	0	7	0
Totals	172	82	14					185	203

TABLE 2

The Specificity of Commonly used Bioassay Organisms toward analogues of Vitamin B_{12} (modified from Belser 1963)

Vitamin B_{12} Congener	Microbiological Activity			
	E. coli	L. leichmanii	E. gracilis	O. malhamensis (mammalian)
'True' Vitamin B_{12}	+++	+++	+++	+++
Pseudovitamin B_{12}	+	++	++	-
Factor A	++	++	++	-
Factor B	+	-	-	-
Factor C_1	+	+	+	-
Factor C_2	+	+	+	-
Factor D	-	-	-	-
Factor E	-	-		-
Factor F	++			+
Factor G		++		-
Factor H	++	++		++
Factor I	++	++		++

+++ denotes fully active
++ denotes activity of the order of 50% that of cyanocobalamin
+ denotes activity of the order of 10% that of cyanocobalamin
- denotes activity of less than 1% that of cyanocobalamin

is available to each and every alga present. Many workers, for example Provasoli & Carlucci (1974), have therefore suggested that assays for vitamin B_{12} in natural waters should be made with at least two bioassay organisms.

Similar comments may apply to other micronutrients. Inorganic cobalt is not required by all algae (O'Kelley 1974); the requirement is commonly related to the need for vitamin B_{12} of which cobalt is a constituent (Lund 1965). Holm-Hansen, Gerloff & Skoog (1954) found that blue green algae, which do not require vitamin B_{12}, nevertheless grew faster when the vitamin was supplied as a source of cobalt than when given inorganic cobalt.

In the case of iron, species variability is illustrated by a comparison of what forms of the metal are available to different algae. Thus Harvey (1937) concluded that iron in the form of colloidal or larger particles of ferric hydroxide or of ferric phosphate could support the growth of the marine diatoms he studied. Goldberg (1952) reported that Asterionella japonica Cleve can utilise only particulate and/or colloidal iron, and that organically complexed ferric iron (as the citrate, ascorbate or humate) is not available for uptake. The success of Chu's No. 10 medium (Chu 1942) for the culture of many freshwater algae, in which iron added as ferric chloride probably exists as ferric hydroxide or phosphate, also suggests that these forms are available to some algae. However some algae cannot grow in Chu's No. 10 medium unless the iron is chelated (Shapiro 1967 b). Shapiro (1967 b) compared the growth of several algae in Chu No. 10 medium with the iron supplied in one of three forms:- ferric chloride, ferric citrate and

ferric EDTA. The iron of the three sources was equally available to only one of the six species of algae tested. Even among species of the same group, the varied effectiveness of the iron source was marked.

Clearly the chemical differentiation of organically complexed or chelated iron may not provide a straightforward measure of the fraction available to all algae.

Of the inorganic micronutrients listed on page 2, iron, manganese, copper, zinc and molybdenum are considered to be required by all algae and not replaceable, even in part, by other elements (O'Kelley 1974). With the exception of molybdenum, only these elements, together with the vitamins B_{12}, thiamine and biotin, and cobalt (which is a component of the B_{12} molecule) were studied in this investigation. Molybdenum was omitted because no suitable lamp was obtainable for the atomic absorption spectrophotometer.

The methods described in section 2 were designed to provide estimates, not only of total concentrations of the micronutrients in the reservoirs studied, but also information about the biologically available concentrations of the micronutrients. Thus it was considered necessary to measure three fractions of each metal:- soluble inorganic, soluble organic and particulate fractions, and to assay vitamin B_{12} with organisms representing different analogue specificity patterns of the algae.

1.2. INTRODUCTION TO THE RESERVOIRS

The three impounding reservoirs investigated are all on the fringe of the Charnwood Forest in Leicestershire, England. Figure 1 shows the position of the study area in relation to the rest of England, and Figure 2 is a more detailed map of the study area showing the relative positions of the reservoirs, the extent of their catchment areas, the courses of feeder streams and the centres of population within the catchment areas.

The reservoirs and their catchment areas lie within an area of approximately 100 km^2 described by Longitude west 1^o 9' - 20' and Latitude 52^o 39' - 44'. The centre of this area is about 10 km north-west of the City of Leicester.

The reservoirs were all constructed in the latter half of the 19th century by building earth-filled dams across valley floors. Some physical parameters of the reservoirs and their catchment areas are tabulated in Table 3. Many of the parameters reflect the small surface area and shallowness of the reservoirs. As in any kind of dammed lake the deepest water is somewhat away from the centre of the reservoirs and towards the dam.

The reservoirs derive their water from springs and streams draining the catchment areas and supply the City of Leicester and parts of Leicestershire with potable water. For water treatment purposes, Cropston and Swithland reservoirs are operated as a pair. Water is pumped from Swithland into Cropston reservoir and abstracted from Cropston reservoir for treatment before going into public supply.

Figure: 1

Map of England Showing Relative Position of Study Area.

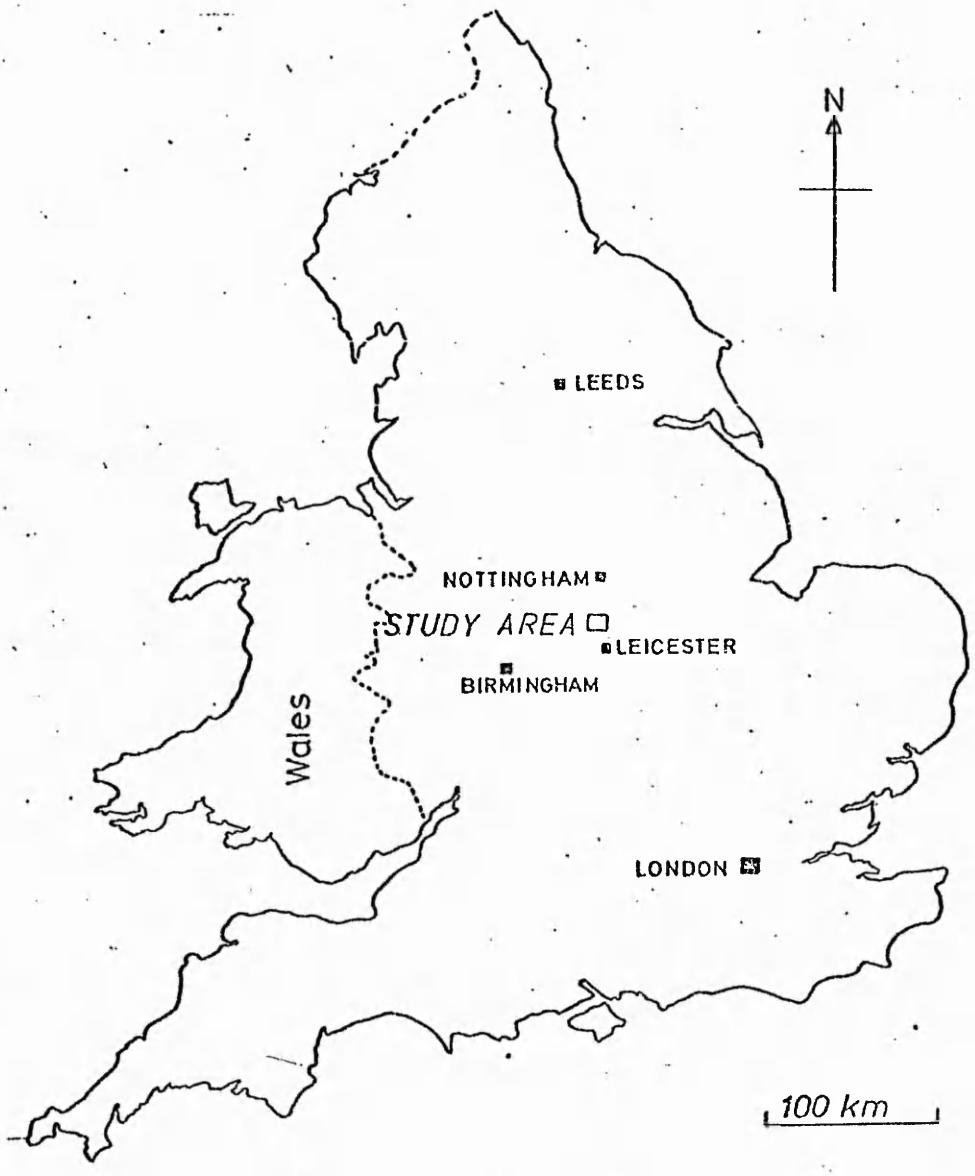


Figure: 2

Map of Study Area.

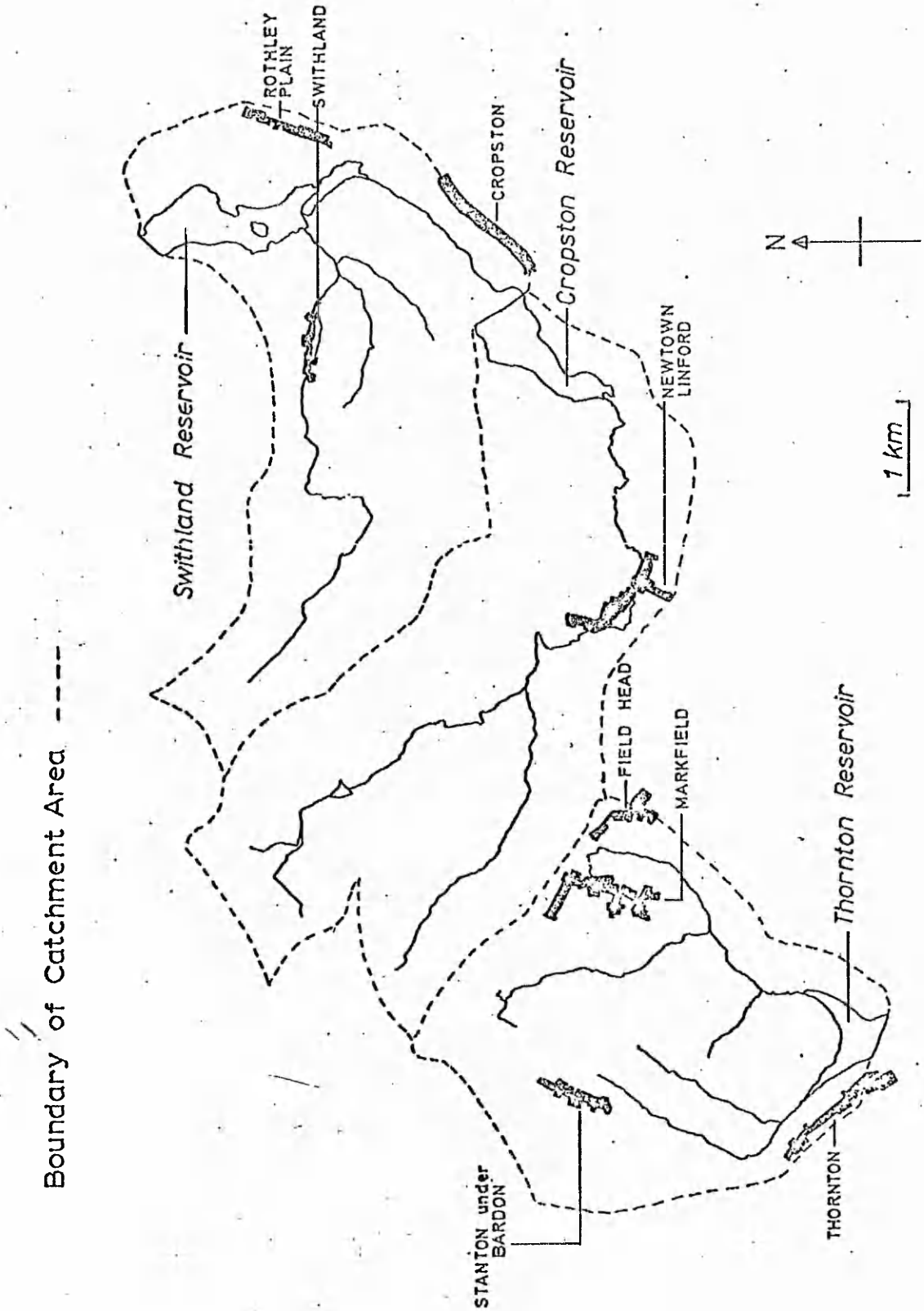


TABLE 3

Physical Parameters of the Reservoirs Investigated

	Reservoir		
	Cropston	Swithland	Thornton
National Grid Reference	SK 551 111	SK 557 148	SK 473 074
Altitude* (m)	69	58	123
Surface Area* (HA)	61	74	31
Length (km)	1.58	1.93	1.46
Mean breadth (km)	0.38	0.38	0.21
Volume* (m^3)	2.53×10^6	2.23×10^6	1.47×10^6
Maximum depth* (m)	9.8	6.7	10.7
Mean depth* (m)	4.2	3.0	4.8
Length of shore line* (km)	4.2	6.0	3.8
Development of shore line	1.52	1.97	1.93
Development of volume	1.28	1.35	1.34
Relative depth	1.11%	0.69%	1.70%
Insulosity	-	3.17%	-
Catchment Area (HA)	1780	1420	1160
Mean rainfall over catchment area (cm/year)	72.1	70.4	69.6
Retention time* (years)	0.6		0.6

* Estimated from, or at top water level

Geologically the study area is a complex mixture of Triassic Keuper marl (with sandstone and bands of gypsum) partially overlaid by large areas of glacial boulder clay (with isolated areas of under and over-lying sand and gravel) and over-lying Pre-Cambrian hornstones and grits (Charnwood rocks). Charnwood rocks also form several isolated areas of surface rock, including two large expanses, parts of which fall within the catchment areas of Cropston and Swithland reservoirs. Intrusions of igneous and syenite and granite also occur in the area. With the exception of Thornton reservoir, post glacial alluvium occurs along the beds of the streams feeding the reservoirs.

1.2.1. Cropston Reservoir

The formation of Cropston reservoir is illustrated in Figure 3. Some physical parameters of Cropston reservoir and catchment area may be found in Table 3. Some chemical characteristics of the reservoir water are tabulated in Table 4.

Cropston reservoir has the largest volume and catchment area of the three reservoirs investigated. With the exception of sulphate and nitrate concentrations, the chemical parameters of Cropston reservoir (Table 4) are the lowest in magnitude of the three reservoirs.

Water flows into the reservoir from two sources:-

a) Bradgate Brook (River Lin) and its tributaries, which drain the

Figure. 3

Map of Cropston Reservoir.

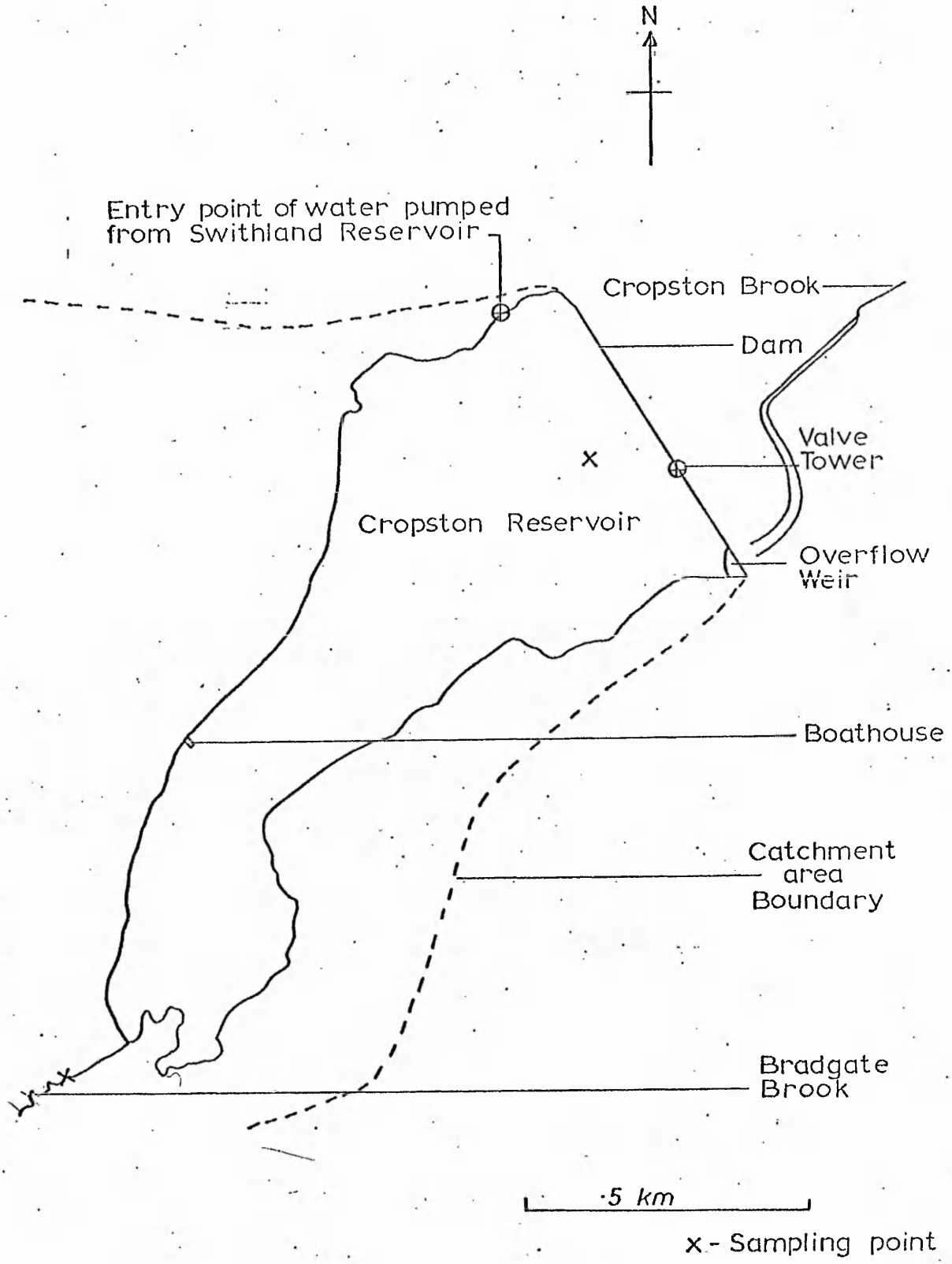


TABLE 4

Chemical Parameters of the Water in the Reservoirs Investigated

(Means of the Years 1972, 1973, 1974)+

	Reservoir		
	Cropston	Swithland	Thornton
pH	8.07	8.30	8.24
Electrical conductivity (μmhos. cm^3)	419	461	584
Calcium (mg/l)	52	60	60
Magnesium (mg/l)	22	26	26
Sodium (mg/l)	11	16	32
Potassium* (mg/l)	3	3	5
Carbonate and Biocarbonate (mg/l)	67	72	81
Sulphate	84	93	82
Chloride (as Cl^-) (mg/l)	34	36	60
Orthophosphate (mg/l)	0.061	0.267	0.175
Nitrate (mg/l)	1.38	1.05	1.45
Silica (mg/l)	3.60	6.27	4.62

+ Data collected by Severn-Trent Water Authority

* Mean of only 3 measurements

catchment area, enters the southern end of the reservoir. There are no consented or known discharges to Cropston reservoir via Bradgate Brook, but land drainage from farm areas may enter the brook and reservoir. It is remotely possible that some seepage into the brook occurs from a storm sewage overflow which receives land drainage (Clayfield 1974, personal communication).

b) Swithland reservoir by pumping. This source is intermittent and enters the reservoir near the dam on the northern shore of the reservoir (see Figure 3).

Water is abstracted from the reservoir for treatment via a valve tower in the dam of the reservoir (see Figure 3). There are two draw-off (abstraction) levels at 7.1 m and 12.9 m.

The reservoir is not used for any recreational purpose, but it is used as a resting and feeding area by many wildfowl in Autumn and Winter (Robinson 1973, personal communication). Cropston reservoir is particularly favourable to surface feeders because of the large areas of shallow water and mud margins at low water levels. Maximum numbers occur between November and February, the most common species being Mallard, Teal, Wigeon, Tufted duck, Pochard, Shoveller and Great Crested Grebe. In addition there is a small passage of Golden Eye and Goosander in the winter and early spring and waders and terns in spring and autumn.

The western shoreline and horizon development of Cropston reservoir form part of Bradgate Park, whilst the eastern shoreline and horizon development is mainly pastureland. The reservoir's catchment area of

1780 Ha includes most of Bradgate Park. The remainder is a mixture of woodland and pastureland and includes the village of Newtown Linford, population 1046*.

1.2.2. Swithland Reservoir

The formation of Swithland reservoir is illustrated in Figure 4. Some physical parameters of the reservoir and catchment area are tabulated in Table 3 and some chemical characteristics of the reservoir water in Table 4.

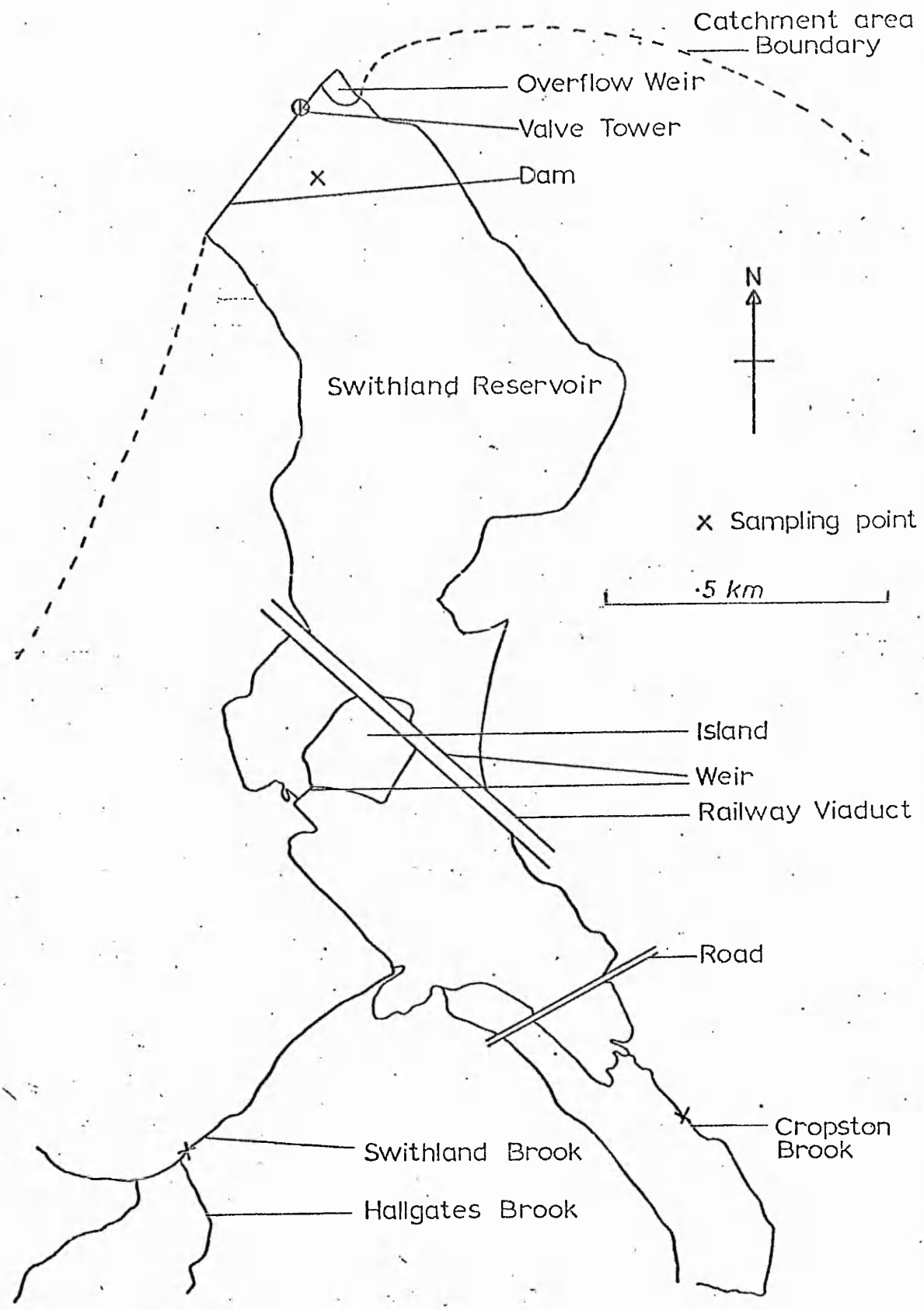
Swithland reservoir is divided into two sub-basins by an island of igneous granite, with weirs either side. The northern basin has a surface area of 56 Ha and the southern basin a surface area of 16 Ha. Swithland reservoir has the largest surface area of the three reservoirs investigated, but not the largest volume. Swithland reservoir is in fact the shallowest of the three reservoirs.

Swithland reservoir has the highest mean pH, sulphate, orthophosphate and silica concentrations of the three reservoirs (Table 4).

* Population figures are taken from the 1971 Census Small Area Statistics obtained from the Office of Population Censuses and Surveys, Titchfield, Hants. PO15 5RR.

Figure. 4

Map of Swithland Reservoir



Water flows into the southern basin of the reservoir, which therefore tends to act as a large settling pond for the main (northern) basin, from two sources:-

a) Cropston Brook (River Lin) which carries any overflow from Cropston reservoir.

b) Swithland Brook and its tributaries which drain the catchment area.

There are no direct discharges of trade or sewage wastes into these feeder streams. However, there is a minor discharge of sewage into a sludge area, which under adverse conditions could percolate into a feeder steam (Clayfield 1974, personal communication).

Water is abstracted from the reservoir via a valve tower near the dam of the reservoir. There are two draw-off (abstraction) levels at 3.5 m and 6.4 m.

With the exception of a limited amount of private shore line fishing, the reservoir serves no recreational purpose. Like Cropston reservoir, Swithland reservoir is a locally important resting and feeding area for many wildfowl, both migratory and non-migratory, especially in winter.

The shoreline of Swithland reservoir is predominantly woodland, but the horizon develops as woodland only at the northern end and southernmost tip of the reservoir. Elsewhere the horizon develops as pastureland. The catchment area of 1420 Ha is mainly a mixture of woodland and pastureland and includes a small part of Bradgate Park and the villages

of Swithland and parts of Rothley Plain and Cropston, (populations 208, 240 and 141, respectively).

1.2.2. Thornton Reservoir

The formation of Thornton reservoir is illustrated in Figure 5. Some physical parameters of the reservoir may be found in Table 3 and some chemical parameters of the reservoir water are tabulated in Table 4.

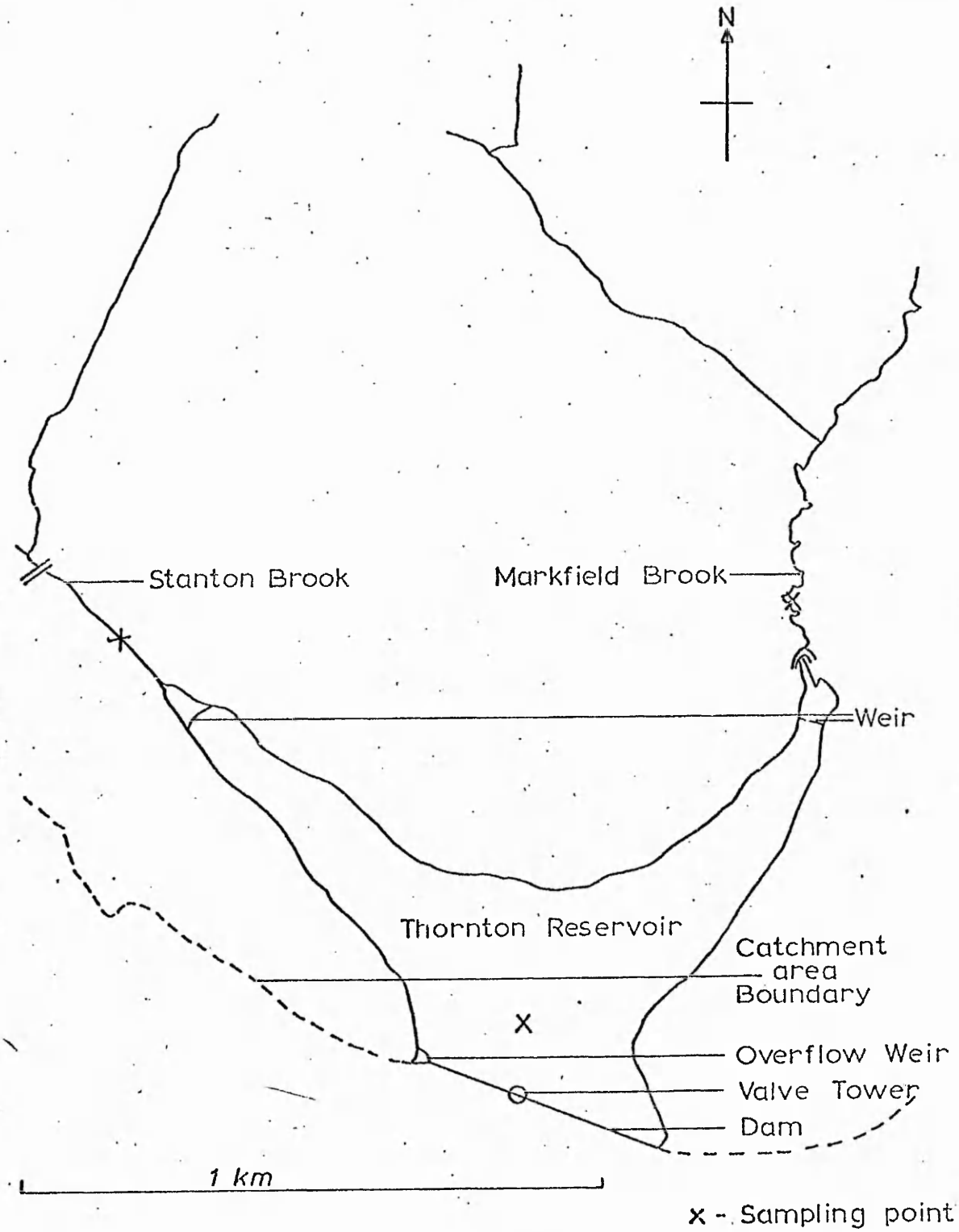
Thornton reservoir is the highest, smallest and deepest of the three reservoirs investigated. Small settling ponds formed by the construction of concrete weirs are located at the two 'finger tips' of the reservoir.

Thornton reservoir has the highest electrical conductivity of the three reservoir waters and this is reflected by the relatively high concentrations of sodium, potassium and chloride ions in the water. The levels of nitrate and carbonate and bicarbonate ions are also relatively high in Thornton reservoir water.

Water flows into the reservoir via two streams, Stanton and Markfield Brooks, which enter the western and eastern tips of the reservoir respectively. There are no direct discharges of trade or sewage wastes into either feeder stream, but there is the possibility of the discharge of inert mineral matter and of land drainage from farm areas into the streams (Clayfield 1974, personal communication).

Figure 5

Map of Thornton Reservoir.



Water is abstracted from the reservoir for treatment at the valve tower which has three draw-off levels at 2.7 m, 5.5 m and 8.2 m.

A limited amount of private fishing is allowed on Thornton reservoir, but this is the only recreational use of the reservoir. Thornton reservoir is not an important local feeding or resting site for wildfowl.

The northern and western shorelines of Thornton reservoir are wooded, but the horizon develops as woodland on the northern shore only. Along the reservoir's western shore the horizon develops as pastureland and recreational land into the village of Thornton. The eastern shoreline and horizon development is pastureland.

The reservoir's catchment area of 1160 Ha is the smallest of the three reservoirs and is predominantly pastureland, but includes four villages:- Markfield and Stanton-under-Bardon (joint population 3164), Thornton (population 816) and a part of Field Head (population estimated as about 80). Thornton reservoir catchment area therefore has the highest population density of the three reservoirs.

SECTION 2

METHODS

2.1 METHODS

The sources of materials referred to in this chapter are given in Appendix 1. The sources of cultures used in this investigation are given in Appendix 2. Appendix 3 is a list of the names and addresses of manufacturers of scientific equipment used in this investigation.

The analysis of micronutrients in natural waters presents acute problems. In particular contamination can very easily occur. In order to guard against contamination, all glassware used in this investigation was Pyrex and all inorganic chemicals used were analytical reagent (A.R.) grade. Glassware and plasticware were thoroughly cleaned after use by the method described in Appendix 4. Glassware and plasticware were handled so that skin contact with inner surfaces was avoided. Water samples were handled in such a way that skin contact with any part of the sample was avoided, both during collection and during all subsequent treatment and analytical stages. Glass-distilled water was used throughout and was stored for nc longer than 24 hrs. Other precautions, such as using internal standards and water blanks, were also taken, and are described in the relevant sections of this chapter.

2.2 SAMPLING METHODS

2.2.1 Reservoir Water

6.5l water samples were taken at weekly intervals, between 11.00 and 13.00 hours, from a depth of 2m in the deepest part of the reservoirs investigated. The approximate positions of the sampling points in the reservoirs are shown in Figures 3.4 and 5.

The choice of sampling apparatus was restricted by the necessity to avoid samplers with metal or rubber components, as a precaution against trace element contamination of samples (Golterman 1971). For this reason, and because of the relative simplicity and ability of the apparatus to take large samples, a tube sampler was constructed, along the lines described in Golterman (1971), from a 2m length of polyvinyl-chloride tubing, 64mm in diameter and fitted with airtight plastic screw caps at either end. The apparatus could therefore be used either as an integrating sampler of the upper 2m of a water column, or for collecting samples from a depth down to 2m. The apparatus was used to collect samples from a depth of 2m for two reasons. Firstly, Golterman (1971) advises against collecting samples for trace element analysis from the surface layer. This is because trace metals may be concentrated at the surface by wind induced foaming of the water for example. Secondly, 2m approximates the middle of the euphotic zone in most fresh waters (Prescott 1969).

The sampling tube was flushed with reservoir water prior to collecting the actual sample. On return to the shore the sample was immediately transferred to a 5l polyethylene bottle, using the spare 1.5l in the tube to rinse the bottle with sample prior to complete filling. This transfer

effectively mixed the sample, and a 250ml portion for the subsequent analysis of dissolved vitamins was immediately removed and filtered under negative pressure through a Whatman GF/C glass-fibre filter paper. The filtered portion was transferred to a 250ml polyethylene bottle, using the first 50 ml to rinse the bottle, and then quickly frozen by placing the bottle in a solid carbon dioxide/methanol mixture. The filter pad, for subsequent particulate vitamin analysis, was transferred to a 100ml wide topped polyethylene bottle and, together with the frozen, filtered portion, transported back to the laboratory with a packing of solid carbon dioxide.

The remainder of the 5l sample was transported back to the laboratory as quickly as possible (with 2 to 3 hours) where the samples were immediately prepared for chlorophyll, phaeophytin and trace element analysis as described in the relevant sections of this section. Frozen samples for later vitamin analysis were transferred to a deep-freeze on return to the laboratory and stored at -20°C.

2.2.2 Feeder Stream Water

5l water samples were taken at monthly intervals between 11.00 and 13.00 hours using a plastic bucket. Both bucket and polyethylene sample bottle were rinsed with stream water prior to collecting the actual sample. This method of collection was considered adequate, since the streams are small and shallow at the sampling points. The approximate positions of the sampling points are shown on Figures 3, 4 and 5, and are the nearest points to the reservoirs with reasonable access.

Portions of the sample for subsequent vitamin analysis were immediately removed from the well-mixed 5l sample and treated as in Section 2.2.1. Frozen samples for subsequent vitamin analysis and the remainder of the 5l sample were transported back to the laboratory as quickly as possible and immediately prepared for chlorophyll, phaeophytin and trace element analysis as described in the relevant sections of this chapter, or stored at $-20^{\circ}C$ for later vitamin analysis.

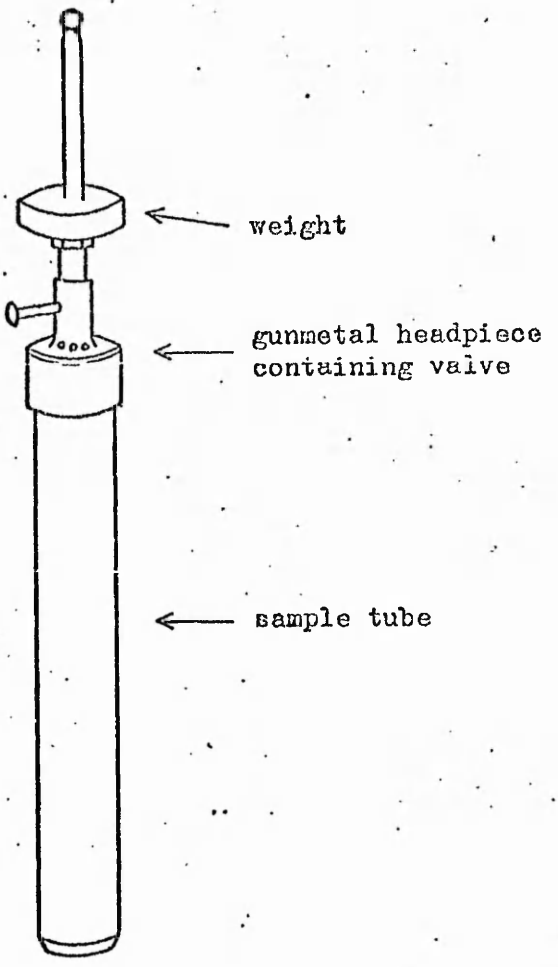
2.2.3 Reservoir Sediments.

Samples of reservoir sediment were taken with a Gilson Mud Sampler, alternatively known as a Freshwater Biological Association, Automatic Mud Sampler, kindly loaned by the Freshwater Biological Association. The apparatus is illustrated in Figure 6; details of its design may be found in Macan (1970) . The sampler was loaded with weights (about 6kg) and used on a line to take relatively undisturbed short cores (10 to 20cm) of sediment together with 500 to 800 ml of the accompanying, overlying water. Samples were taken from the same positions in the reservoirs as the reservoir water samples: see Section 2.2.1 and Figures 3, 4 and 5.

The sediment sample and overlying water were transported back to the laboratory in the sample tube (using rubber bungs in plastic bags to seal both ends of the tube) as quickly as possible (within 2 to 3 hours). The overlying water was siphoned off. A 100ml

Figure 6

Gilson Mud Sampler*



* It should be noted that the headpiece of this apparatus is made of gunmetal and the weights are made of lead. These components must be considered a possible source of contamination of the sample with trace elements. However, the actual sample tube, which comes into direct contact with the sediment, is made of perspex. Distilled water, stored in the complete apparatus for a representative period, was not measurably contaminated with trace elements investigated.

portion was filtered through a Whatman GF/C glass-fibre filter paper and the filtrate frozen and stored at -20°C for later vitamin analysis. The remainder of the overlying water was prepared for trace metal analysis as described in Section 2.4.1.1

The sediment core was carefully pushed out of the sample tube with a perspex piston. The core was sectioned into 2cm lengths as it was extruded from the sampling tube. The 2cm length nearest the sediment/water interface was designated the 'B' layer. (Many of the important exchange reactions between water and sediment probably occur between the water and the first few centimeters of sediment (Collins 1974, personal communication). The next four 2cm sections were pooled and designated the 'C' layer. (The depth of mixing in sediments with a well defined sediment-water interface probably extends to between 5 and 10 cm below the interface, although this mixing may not be complete (Lee 1970b).

The 'B' and 'C' sediment layers were thoroughly mixed and approximately 10g portions of each layer were stored at -20°C in polyethylene bottles for later vitamin analysis. Further 10g portions were removed and accurately weighed before and after drying at 105°C until no further loss of weight was noted (about 24 hrs.). These portions were stored in 100ml conical flasks with plastic caps for later trace element analysis: see Section 2.4.2.

2.3 CHLOROPHYLL AND PHAEOPHYTIN ESTIMATION

In this investigation the biomass of phytoplankton in reservoir and feeder stream water samples was estimated by determination of the concentration of chlorophyll in the samples. The method described below is easy and rapid in use, but the results must be interpreted with care for two reasons. Firstly, quantitative extraction of chlorophyll from algae, especially green algae, is difficult. Secondly, the two most common chlorophylls, a and b, both absorb light at 663 nm and their extinction coefficients are not accurately known (Golterman 1971). However, chlorophyll a is usually quantitatively the most important chlorophyll in green plants (Golterman 1971).

Chlorophyll and phaeophytin were estimated in samples by the method described in Golterman (1971).

A 1l portion of a well mixed 5l water sample from a reservoir or feeder stream was filtered under negative pressure through a Whatman GF/C glass-fibre filter paper coated with a film of magnesium carbonate. The film of magnesium carbonate increases the retention of particles by the filter paper and was produced by drawing 5ml of a 5% suspension of magnesium carbonate in distilled water through the filter paper. The filter paper was then ground in 5ml of 90% acetone to release the pigments from the algal cells and the resulting slurry quantitatively transferred to a centrifuge tube. The centrifuge tube was sealed with Parafilm and stored at $4^{o}C$ in the dark overnight. The slurry was then centrifuged and the supernatant carefully pipetted

off and made up to 10ml with 90% acetone. The optical density of the pigment extract was then measured in a spectrophotometer at 663nm and 750nm before and after acidification with 0.1ml of 4N hydrochloric acid.

The concentration of chlorophyll and phaeophytin in the original sample was then calculated as follows:-

let the optical densities of the pigment extract at 663nm and 750nm, before acidification, be A663 and A750 respectively;

let the optical densities of the pigment extract at 663nm and 750nm, after acidification, be B663 and B750 respectively.

Then the unacidified, corrected optical density (A) and the acidified, corrected optical density (B) are calculated by the expressions:-

$$A = \frac{A663 - A750}{\text{lightpath (cm)}}$$

$$B = \frac{B663 - B750}{\text{lightpath (cm)}}$$

The optical density due to chlorophyll (C) is then calculated by the expression:-

$$C = 2.43\ (A - B)$$

The optical density due to phaeophytin (P) is then calculated by the expression:-

$$P = (A - C)$$

The concentration of chlorophyll or phaeophytin in the original sample is finally calculated from the formula:-

$$\mu g/l \text{ Chlorophyll (or Phaeophytin)} = C \text{ (or P)} \cdot \frac{1000}{KC \text{ (or KP)}} \cdot \frac{\text{volume of extract (ml)}}{\text{volume of filtrate (l)}}$$

where:- KC is the extinction coefficient of chlorophyll (=89)

and KP is the extinction coefficient of phaeophytin (=56)

2.4 INORGANIC MICRONUTRIENTS

2.4.1 Preparation of Samples for Trace Metal Estimation by Atomic Absorption Spectrophotometry (AAS)

2.4.1.1 Reservoir water

Although the sensitivity of conventional AAS is high, the concentration of trace metals in natural waters is usually too low (less than 100µg/l) to allow their direct estimation without sample concentration.

Sample concentration for conventional AAS can be accomplished by several methods including simple evaporation or freeze drying with dissolution in acid, partitioning of metal chelates in smaller volumes of organic solvent or collection of metal ions on ion exchange or chelating resins (Minear & Murray 1973; Willey, Duke, Wojcieszak & Thomas 1972).

In this investigation, simple evaporation and ion exchange techniques were used to concentrate water samples from the reservoirs by a factor up to 40 times.

In order to gain some information about the forms in which the trace metals selected for study exist in the reservoir waters investigated, the concentration step was designed to allow the differentiation of three fractions of each metal:-

a) inorganic free metal ions.

b) soluble organic metal complexes or chelates.

c) particulate inorganic and organic metal.

To do this, samples of reservoir water were concentrated by the following methods:-

a) Inorganic, free metal ions

A 1l portion of the well-mixed 5l sample was filtered under negative pressure through a Whatman GF/C glass fibre filter paper. The filtrate was then allowed to run through a 15cm long and 1.3cm diameter column of Dowex 50W-X8 ion exchange resin in the hydrogen form at the rate of 250ml/hr. The sample was washed through the column with 100ml of distilled water and the column was then regenerated with 240ml of 2N hydrochloric acid run through the column at the rate of 2ml/min. The first 5ml of elutant were allowed to run to waste. The next 25ml were collected and stored in a tightly stoppered glass vial for later analysis by AAS: see Section 2.4.2. The remaining 210ml of 2N hydrochloric acid were allowed to run through the column to waste, thus completing the regeneration of the column. The column was washed with 100ml of distilled water prior to application of the next sample.

b) Inorganic and Organic Soluble Metal

A 1l portion of the well-mixed 5l sample was filtered under negative pressure through a Whatman GF/C glass fibre filter paper. The filtrate was transferred to a 2l conical flask and 2ml of 12N hydrochloric acid added to the filtrate. If necessary the acidified filtrate was stored

at 4°C until the next stage could be begun. The filtrate was then evaporated on a hot plate until the volumn was reduced to about 50ml. The concentrated filtrate was then quantitatively transferred to a 100ml conical flask and evaporated to dryness, without boiling, on a hot plate. The residue was redissolved in 2ml of hot 16N nitric acid. Approximately 20ml of distilled water were then added and precipitated silica removed by centrifugation. The supernatant was made up to a volume of 25ml with distilled water and stored in a tightly stoppered glass vial for later analysis by AAS: see Section 2.4.2.

c) Total Inorganic and Organic Metal

1l of the well-mixed 5l sample was treated and concentrated as described in Section 2.4.1.1b except that the initial filtration stage was omitted.

2.4.1.2 Feeder Streams

1l of the well-mixed 5l sample was treated and concentrated as described in Section 2.4.1.1c.

2.4.1.3 Sediment Samples

Metals were extracted from the 'B' and 'C' layers of sediment cores by a slight modification of the method described by Mathis & Cummings (1973).

15ml of a mixed concentrated acid solution (five volumes of 16N nitric acid and one volume of 14N (70%) perchloric acid) were added to the dried sample (see Section 2.2.3) and the mixture heated at a low heat for approximately 24 hours. The mixture was then allowed to settle and the supernatant carefully pipetted off into a 100ml conical flask with a plastic cap to exclude dust. Three more similar extractions with 10ml of the mixed acid solution were made and the supernatants pooled. 15ml of distilled water were added to the sample to extract the remaining acid from the sediment. The pooled acid and water supernatants were then gently heated until the solution turned faint yellow or colourless and then evaporated to approximately 5ml. The solution was then made up to 50ml with distilled water and stored in a tightly stoppered glass vial for later analysis by AAS: see Section 2.4.2.

2.4.2 Trace Metal Estimation by AAS

2.4.2.1 Preparation of Stock Standard Trace Metal Solutions

Standard trace metal solutions were prepared according to the methods described in Golterman (1971) and stored in tightly stoppered polyethylene bottles.

2.4.2.2 Preparation of Trace Metal Standards for AAS

Standard solutions containing 0, 1, 2.5, 5, 10, 25, 50, 75, 100, 250 and 500µg/l of each of the trace metals to be analysed were prepared

by diluting suitable volumes of intermediate standards, freshly prepared from the stock standard solutions, in distilled water. These standards were concentrated for trace metal analysis in exactly the same way as reservoir water samples (see section 2.4.1.1), that is were concentrated after filtration by ion exchange and evaporation and by evaporation of unfiltered standards.

Additional distilled water blanks were prepared by storing distilled water in the tube sampler (see section 2.2.1), Gilson mud sampler (see section 2.2.3) and polyethylene sample bottles for representative periods of time. These blanks were concentrated by evaporation (see section 2.4.1.1c) for later analysis by AAS.

Additional standard solutions containing 0, 1, 2.5, 5, 10, 25, 50, 75, 100, 250 and 500ug/l of each of the trace metals to be analysed were prepared by diluting suitable volumes of freshly prepared intermediate standard solutions in an artificial reservoir water. The composition of the artificial reservoir water is given in Table 5 and was designed to simulate the average chemical composition of the three reservoir waters investigated with respect to the concentration of major ions (see Table 4). These standards were concentrated for trace metal analysis by AAS by the method described in section 2.4.1.1c and were considered a necessary precaution against possible interference due to matrix effects caused by the high ionic strength of the concentrated samples (Willey et.al. 1972).

TABLE 5

Composition of Artificial Reservoir Water

Chemical	Concentration
$CaCl_2 \cdot 2H_2O$	213 mg/l
$MgSO_4 \cdot 7H_2O$	216 mg/l
$MgCl_2 \cdot 6H_2O$	31 mg/l
$NaHCO_3$	95 mg/l
K_2CO_3	6 mg/l
KCl	1 mg/l

2.4.2.3 Analysis of Samples and Standards by AAS

An EEL 240 Atomic Absorption Spectrophotometer was used for the measurement of trace metal concentrations in the concentrated water samples and standards and sediment extracts. The instrument settings for each element are given in Table 6, together with an approximate sensitivity value (1% absorption). The instrument was used in the integrating mode to increase the accuracy of readings. The burner position was adjusted for maximum deflection of the galvonometer needle when a standard solution of the metal was aspirated into the flame. The instrument was calibrated with distilled water as a blank and a standard 5mg/l solution of the metal, using the range control to give a galvonometer reading of 75% when the standard 5mg/l solution of the metal was aspirated into the flame.

2.4.2.4 Precision of Trace Metal Analysis by AAS

The precision of the method was determined in the following way:-

Five 1l sub-samples of a standard solution containing 5µg/l of each of the trace metals, and five 1l sub-samples of a standard solution containing 100µg/l of each of the trace metals were taken through each of the three concentration methods described in Section 2.4.1.1.

The standard deviation of each set of results was calculated using the formula given in Section 2.9. The results are shown in Table 7.

Table 6

Instrument Settings for Trace Metal Analysis by Atomic Absorption Spectroscopy

Parameter	Iron	Manganese	Copper	Zinc	Cobalt
Wavelength (nm)	248·3	279·5	324·8	213·9	240·7
Lamp current (mA)	5	5	5	5	5
Slit width	2	2	7	2	2
Burner height	6	6	6	6	6
Fuel gas	air/ acetylene	air/ acetylene	air/ acetylene	air/ acetylene	air/ acetylene
Air pressure	5	5	5	5	5
Acetylene pressure	1.5	1.5	1.5	1.5	1.5
Sensitivity (µg/l)	50	50	50	25	50

TABLE 7

Precision of Trace Metal Analysis by AAS

Trace Metal	Concentration (µg/l)	Standard Deviation (µg/l) Concentration Method		
		Ion Exchange	Evaporation after Filtration	Evaporation
Iron	5	2.0	1.5	1.5
	100	4.0	3.5	3.5
Manganese	5	2.0	1.5	1.5
	100	2.5	2.0	2.0
Copper	5	2.0	1.5	1.5
	100	4.0	3.5	3.5
Zinc	5	2.5	2.5	2.5
	100	4.0	3.5	3.5
Cobalt*	5	-	-	2.5
	100	-	-	3.5

* Only total cobalt was measured in the investigation, see Section 3.1.1.7.

2.4.2.5 Calculation of the results of Trace Metal Estimations by AAS

Standard curves, of % absorption against the trace metal concentration in the standards prepared as in Section 2.4.2.2 were drawn. Interference due to matrix effects was not observed. Few chemical interferences in the analysis of the trace metals investigated have been reported (Price 1972). The interference in the analysis of iron and manganese by AAS due to any residual silica is eliminated by the natural calcium present in the samples (Farnsworth 1972). No correction of of results because of interference effects was therefore required and the concentration of trace metal present in the sample was read directly from the relevant standard curve.

The recovery of trace metals by the concentration techniques employed were calculated and are tabulated in Table 8. The relatively low recovery of trace metals other than zinc by the ion exchange concentration method (see Section 2.4.1.1a) is due to the fact that this method was standardized with zinc. The greater than 100% recovery of zinc in standards concentrated by evaporation after filtration is due to slight zinc contamination of these standards during filtration through Whatman GF/C glass fibre filter papers. This contamination is compensated for in the standard curves. Stored, distilled water blanks (see Section 2.4.2.2) did not become contaminated with the trace metals investigated.

The concentration of the trace metals in each of the three fractions defined in Section 2.4.1.1 was calculated from the results as follows:-

Table 8

Recovery of Trace Metals by the Ion Exchange and Evaporation Concentration Methods

Concentration method	% recovery				
	Iron	Manganese	Copper	Zinc	Cobalt
Ion exchange	55	65	68	100	64
Evaporation after filtration	97	96	97	103	100
Evaporation	97	96	99	100	100

let the concentration of trace metal in the water sample concentrated by ion exchange (see Section 2.4.1.1a) be IE;

let the concentration of trace metal in the water sample concentrated by evaporation after filtration (see Section 2.4.1.1b) be EF;

let the concentration of trace metal in the water sample concentrated by evaporation (see Section 2.4.1.1c) be UE;

then – a) the concentration of inorganic free metal ion in the sample = IE;

– b) the concentration of soluble organic metal complexes or chelates in the sample = EF – IE;

– c) the concentration of particulate inorganic and organic metal in the sample = UE – EF.

2.5 ORGANIC MICRONUTRIENTS OR VITAMINS

The low concentration of vitamins in natural waters precludes the use of chemical methods of analysis. Vitamins in natural waters are therefore usually estimated by bioassay methods.

The principle of all bioassay methods is the same. Using a basal culture medium which is nutritionally adequate and indeed if possible optimal in all respects for the selected bioassay organism, except for the absence of the test substance to be assayed, the growth of the organism is then dependent on the added presence of the test substance. The concentration of the test substance in an unknown may therefore be estimated by comparison of the growth response of the organism in the basal culture medium supplemented with the unknown, with the growth response of the organism in the basal culture medium supplemented with standard concentrations of the test substance.

With the exception of the assay method for thiamine using Monochrysis lutheri Droop, the basic method of bioassay is described in Section 2.5.1. Differences in detail are set out in Table 9. The assay method for thiamine using M. lutheri is described in Section 2.5.4.3.

Further details about the assay organisms, methods of sample preparation and the preparation of stock vitamin solutions may be found in subsequent sections. Further details of the assay methods described in Section 2.5.1 may be found in Kavanagh (1963).

Table 9

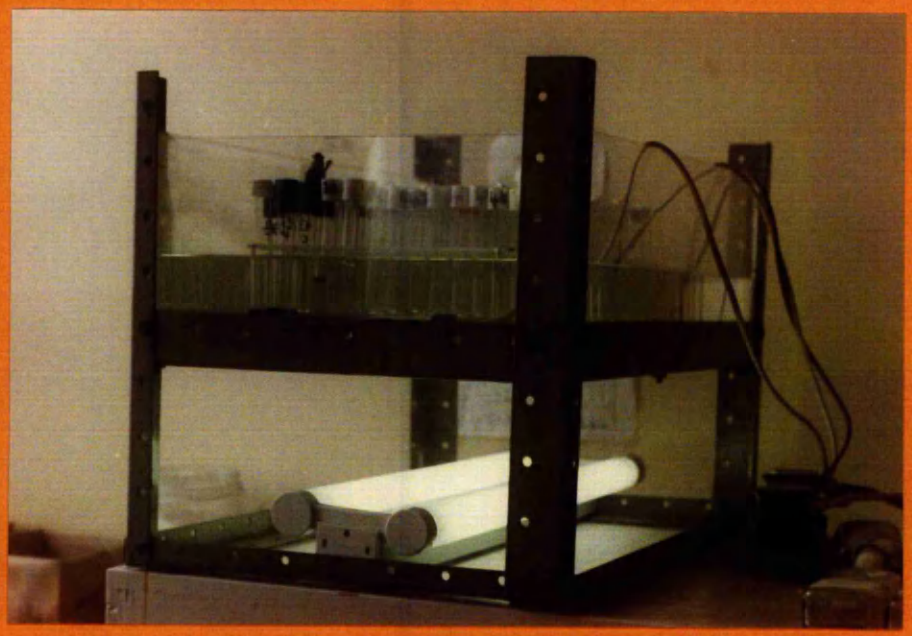
Specifications for the Bioassay of Vitamins B_{12}, Thiamine and Biotin

Vitamin	Assay Organism	Assay Medium	mls./tube double strength assay medium	Standard Vitamin Concentrations	mls./tube of unknown assayed	Vitamin Concentrations added to internal standards	Conditions of Sterilization	Conditions of Incubation	Optical Density Wavelength (nm)
B_{12}	Euglena gracilis Klebs strain z	Bacto Euglena B_{12} Medium	2	0,1,2.5,5,10, 25,50ng/l	a) 2 b) 1	a) 5ng/l b) 20ng/l	Heated in flowing steam for 15 mins.	5 days at 26°C ± 1°C over warm white fluorescent lights* (approx. 3500 Lux).	663
B_{12}	Lactobacillus leichmanii ATCC 7830	Bacto B_{12} Assay Medium USP	5	0,1,2.5,5,10, 25,50ng/l	a) 5 b) 2.5	a) 5ng/l b) 20ng/l	Autoclaved for 5 minutes at 121°C	24 hours at 37°C	540
B_{12}	Ochromonas malhamensis Pringsheim	Bacto B_{12} Ochromonas Medium	5	0,0.5,1,2.5, 5,10,25ng/l	a) 5 b) 2.5	a) 2.5ng/l b) 10ng/l	Autoclaved for 5 minutes at 121°C	5 days at 26°C ± 1°C over warm white fluorescent lights* (approx. 3500 Lux).	540
Thiamine	Lactobacillus viridescens ATCC 12706	Bacto Thiamine Assay Medium LV	5	0,0.1,0.25, 0.5,1.0,2.5, 5.0µg/l	a) 5 b) 2.5	a) 0.25µg/l b) 2.0µg/l	Autoclaved for 5 minutes at 110°C	48 hours at 37°C	540
Biotin	Lactobacillus plantarum ATCC 8014	Bacto Biotin Assay Medium	5	0,1,2.5,5, 10,25,50ng/l	a) 5 b) 2.5	a) 2.5ng/l b) 20ng/l	Autoclaved for 5 minutes at 121°C	48 hours at 37°C	540

* Incubator illustrated in Plate 1

Plate 1

Incubator used for the Bioassay of Vitamin B_{12} with *Euglena gracilis* Klebs strain z and *Ochromonas malhamensis* Pringsheim



2.5.1 Basic Method of Vitamin Bioassay

The dehydrated assay medium (see Table 9) was rehydrated double strength according to the manufacturers' instructions.

Double strength assay medium was pipetted into each of two series of 150 x 16mm test tubes. The volume is specified in Table 9.

To one series of tubes a suitable standard solution of the vitamin and distilled water were added to give the range vitamin concentrations specified in Table 9, in a total volume (per tube) twice that of the assay medium added. Standard curves were constructed each time an assay was run, since conditions of autoclaving, temperature of incubation, size of inoculum etc., which influence the standard curve readings cannot be duplicated exactly from time to time.

To the second series of tubes known volumes of the sample and/or distilled water were added to give a total volume equal to that of the standards. Two dilutions of each sample were assayed. Two further dilutions containing the vitamin concentrations specified in Table 9 were also assayed and used as internal standards. Standards and unknowns were duplicated.

The tubes were capped with plastic test tube caps and sterilized by the method specified in Table 9.

The tubes were cooled to room temperature and inoculated with a vitamin depleted culture of the assay organism. Vitamin depleted cultures were produced in one of two ways:-

1) For Bacteria

Stock cultures were maintained in the assay medium supplemented with the highest (relevant) standard vitamin concentration specified in Table 9. Stock cultures were sub-cultured at weekly intervals, incubated at 37°C for 24 hours and then maintained at room temperature.

Cultures for the inoculation of assay tubes were prepared by inoculating a tube of the stock culture medium with the stock culture. The culture was incubated at 37°C for 24 hours and then depleted of extra-cellular vitamin by washing three times with 0.9% saline. The culture was then diluted 1/100 with 0.9% saline and 1 drop of the well mixed dilution used to inoculate each assay tube.

2) For Algae

Stock cultures were maintained in the assay medium supplemented with the highest (relevant) standard vitamin concentration specified in Table 9. Stock cultures were sub-cultured at weekly intervals and incubated under the conditions of the assay (see Table 9). Cultures for the inoculation of assay tubes were prepared by inoculating a tube of vitamin free assay medium with the stock culture. The culture was incubated under the conditions of the assay for 7 days. 1 drop of the well mixed culture was used to inoculate each assay tube.

The contents of all assay tubes were then thoroughly mixed and incubated under the conditions specified in Table 9.

The amount of growth in each tube was then measured by determining the optical density of the mixed assay tube contents in a spectrophotometer at the wavelength specified in Table 9.

2.5.2 Calculation of Results of Vitamin Bioassays

The concentration of vitamin in the unknown assay tubes was estimated from a standard curve of optical density (of the assay tube contents) plotted against the vitamin concentration of the standards.

The sample dilution and the internal standard which gave an optical density reading nearest to the centre of the standard curve were selected for the calculation of the results using the following formulae:-

$$\text{Concentration of vitamin in original sample} = x \cdot tv/v$$

where - x is the vitamin concentration in the selected dilution, estimated from the standard curve

v is the volume of sample assayed

tv is the total volume per assay tube

Concentration of vitamin in original sample corrected for possible interference = $x/\left[(y - x)/int.\right]$ tv/v

where - y is the vitamin concentration in the selected internal standard estimated from the standard curve

int is the concentration of added vitamin in the internal standard.

Measureable vitamin contamination of distilled water blanks during storage in sampling apparatus and sample bottles, and by the methods of sample preparation (see Sections 2.5.3.1, 2.5.4.1 and 2.5.5.1) was not observed. No corrections to the results had therefore to be made.

2.5.3 Bioassays for Vitamin B_{12}

Several microorganisms have been used to bioassay vitamin B_{12} in natural waters:- Escherichia coli, Lactobacillus leichmanii, Euglena gracilis Klebs, Ochromonas malhamensis Pringsheim, Thraustochytrium globosum Kobayasi & Ookubo, Cyclotella nana Hustedt and Amphidinium Carteri Hulbert.

The most sensitive method for the bioassay of vitamin B_{12} in seawater is that described by Carlucci & Silbernagel (1966a), using C.nana as the bioassay organism. C.nana clone 13-1 responds to a number of vitamin B_{12} congeners (Carlucci 1973) and has a specificity pattern for these congeners of the L.leichmanii type (Provasoli & Carlucci 1974), see Table 2. C.nana clone 3H has a mammalian-type specificity pattern toward vitamin B_{12} congeners (Provasoli & Carlucci 1974,

Carlucci 1973). Golterman (1971) recommended that this method be adapted for freshwater bioassays of vitamin B_{12}. An adaptation of the method for freshwater was described by Carlucci (1973), with a sensitivity about one quarter that of the seawater assay.

For this investigation three organisms were chosen for the bioassay of dissolved vitamin B_{12} in reservoir and feeder stream water samples:- L.leichmanii, E.gracilis and O.malhamensis. These organisms were selected because their specificity patterns toward the congeners of vitamin B_{12} are known (see Table 2) and because commercially prepared basal culture media are available for the bioassays. Bound vitamin B_{12} in reservoir water samples, particulate vitamin B_{12} in reservoir and feeder stream water samples and total vitamin B_{12} in reservoir sediments were determined by the method described in Section 2.5.1 using E.gracilis as the assay organism.

2.5.3.1 Preparation of Samples for Bioassay of Vitamin B_{12}

Distilled water blanks were prepared in ways representative of the preparation methods described in this Section. Distilled water was also stored in the sampling apparatus and polethylene sampling bottles for representative periods. These controls were bioassayed for dissolved vitamin B_{12} by the method described in Section 2.5.1.

a) Dissolved Vitamin B_{12}

Frozen portions of filtered reservoir water and feeder stream water samples, having been stored at -20°C for later vitamin analysis (see

Sections 2.2.1 and 2.2.2), were thawed and mixed. Suitable aliquots were removed for dissolved vitamin B_{12} bioassay by the methods described in Section 2.5.1.

b) Particulate Vitamin B_{12}

The filter pads frozen and stored at -20°C for later vitamin B_{12} analysis (see Sections 2.2.1 and 2.2.2) were allowed to thaw. Vitamin B_{12} in the particulate material retained on the filters was extracted by the method described by Daisley (1969). The filters were ground in 20ml of 0.02M phosphate - 0.01M citrate buffer pH4.6. 0.1ml of a 10mg/l solution of sodium cyanide in distilled water was added to the slurry and the mixture autoclaved at 121°C for 15 minutes. The mixture was then filtered through a Whatman GF/C glass-fibre filter paper under negative pressure and the volume of the filtrate made up to 50ml. Aliquots of the mixed extract were assayed using E. gracilis, by the method described in Section 2.5.1.

c) Bound Vitamin B_{12}

Free vitamin B_{12} and bound vitamin B_{12} in reservoir water samples were separated by the use of ultrafiltration membranes.

It was thought that freezing the reservoir water samples might alter the distribution of vitamin B_{12} between the possible bound and

unbound forms of the vitamin in samples. The ultrafiltration was therefore carried out on an unfrozen portion of the well-mixed 5l reservoir water sample in the laboratory. It should be noted that the storage of unfrozen water samples for later vitamin analysis is not recommended, since the great increase in surface area to volume ratio which results when a sample is enclosed in a relatively small bottle would then be expected to favour the proliferation of bacteria (present in the sample) which may synthesize vitamins, including vitamin B_{12}. However, the period of storage before ultrafiltration was kept as short as possible (2 - 3 hours) and comparative bioassays for vitamin B_{12} on immediately filtered and frozen portions, and portions filtered and stored for two to three hours at room temperature before freezing, were not significantly different.

100ml of the well-mixed reservoir water sample were filtered under negative pressure through a Whatman GF/C glass-fibre filter paper. The filtrate was then forced under a positive pressure of $1kg/cm^2$ nitrogen through a (Millipore) Pellicon PSED ultrafiltration membrane*.

*This membrane has a nominal molecular weight cutoff of 25,000 and does not retain cyanocobalamin (Millpore Preliminary Product Information). Experiments with an Amicon UN-10 ultrafiltration membrane, which has a nominal molecular weight cutoff of about 10,000 showed that approximately 90% of cyanocobalamin was retained by this membrane, emphasizing the importance of molecular shape as well as molecular weight in determining whether a molecule is retained by ultrafiltration membranes.

The ultrafiltrate was then bioassayed for vitamin B_{12} using *E. gracilis*, by the method described in Section 2.5.1. The result is the concentration of free vitamin B_{12} in the sample. This result was subtracted from the result of the bioassay of dissolved vitamin B_{12} in the sample before ultrafiltration (by the same method) to give the concentration of bound forms of vitamin B_{12} in the original sample.

d) Reservoir Sediment Vitamin B_{12}

Vitamin B_{12} was extracted from reservoir sediment samples by a similar method to that used for particulate vitamin B_{12} estimations (see Section 2.5.3.1b) and the extraction of vitamin B_{12} from algae (Natarajan 1973).

Frozen portions of the 'B' and 'C' layers of sediment cores, collected, prepared and defined in Section 2.2.3 were allowed to thaw. 20ml of 0.02M phosphate - 0.01M citrate buffer pH 4.6 were added to the samples. 0.1ml of a 10mg/l solution of sodium cyanide in distilled water was added to the shaken sediment suspension, and the mixture autoclaved for 15 minutes at 121°C. The sediment suspension was centrifuged and the supernatant vitamin B_{12} extract was carefully pipetted off. The extraction was repeated with a further 20ml of 0.02M phosphate - 0.01M citrate buffer supplemented with 0.1ml of a 10mg/l solution of sodium cyanide and the supernatants of the two extractions pooled. The sediment was washed with 20ml of distilled water and the washings added to the vitamin B_{12} extract which was then made up to 100ml with

distilled water and stored at -20°C for later vitamin B_{12} assay using E. gracilis, by the method described in Section 2.5.1.

2.5.3.2 Preparation of Stock and Standard Vitamin B_{12} Solutions

Approximately 11mg of cyanocobalamin crystals were weighed into a glass vial and stored in a dessicator overnight in the dark at 4°C. The dehydrated cyanocobalamin was accurately weighed and dissolved in a sufficient volume of distilled water (about 100ml) to give a final concentration of 100µg/l cyanocobalamin in the solution. This stock, standard cyanocobalamin solution was stored in a tightly stoppered polyetheylene bottle in the dark at 4°C. A fresh stock solution was similarly prepared each month.

Dilute cyanocobalamin standard solutions for use in the bioassay methods described in Section 2.5.1 were freshly prepared from the stock cyanocobalamin standard solution by dilution in distilled water.

2.5.4 Bioassays for Thiamine

Several microorganisms have been used to bioassay thiamine in natural waters:- Lactobacillus viridescens, Lactobacillus fermenti, Cryptococcus albidus (Saito)Skinner, Phycomyces blakesleanus,

Monochrysis lutheri Droop, and a marine phycomycete, isolate S3. Gold (1973) described a method using Crypthecodinium cohnii (Seligo) Schiller (Gyrodinium cohnii) to bioassay thiamine. Baker, Frank, Fennelly & Leevy (1964) described a bioassay method for thiamine using Ochromonas danica Pringsheim.

Golterman (1971) recommended that the method described by Carlucci & Silbernagel (1966b) using M. lutheri as the bioassay organism, be adopted for freshwater bioassays of thiamine.

For this investigation two organisms were used in attempting the bioassay of dissolved thiamine in reservoir water samples:- Lactobacillus viridescens and Monochrysis lutheri Droop.

2.5.4.1 Preparation of Water Samples for Bioassay of Thiamine

Frozen and filtered portions of reservoir water samples stored at -20°C for later vitamin analysis (see Section 2.2.1) were thawed and mixed. Suitable aliqots were removed for thiamine bioassay by the methods described in Sections 2.5.1 and 2.5.4.3.

2.5.4.2 Preparation of Stock and Standard Thiamine Solutions

100mg of thiamine hydrochloride were dissolved in distilled water and diluted to 100ml. This solution contains 1g/l thiamine and was stored in a tightly stoppered polyethylene bottle in the dark at 4°C. A fresh stock solution was prepared each month.

Dilute thiamine standard solutions for use in the bioassay methods described in Sections 2.5.1 and 2.5.4.3 were freshly prepared from the stock thiamine standard by dilution in distilled water.

2.5.4.3 Bioassay of Thiamine with *Monochrysis lutheri* Droop

Maintenance of Stock Culture

Monochrysis lutheri Droop was maintained in the assay medium whose composition is given in Table 10 supplemented with 1µg/l thiamine. The assay medium used was a modified version of the S50 medium described by Droop (1958). The stock culture was subcultured at fortnightly intervals and incubated at 18°C ± 1°C over daylight type flourescent lamps (24 hour day at approximately 4000 Lux), with intermittent reciprocal shaking (120 reciprocations per minute for 2 minutes in every 10 minutes). The incubator is shown in Plate 2.

Assay Method

Monochrysis thiamine assay medium was made up double strength and dispensed in 10ml amounts into two series of 100ml conical flasks. To one series of flasks a 1µg/l thiamine standard solution and/or distilled water were added to give concentrations of 0, 10, 25, 50, 100, 250 and 500ng/l thiamine in a total of 20ml in each flask. Standard curves were constructed each time an assay was run for the reasons given in Section 2.5.1. To the second series of flasks a known volume of sample was added and the volume in each flask

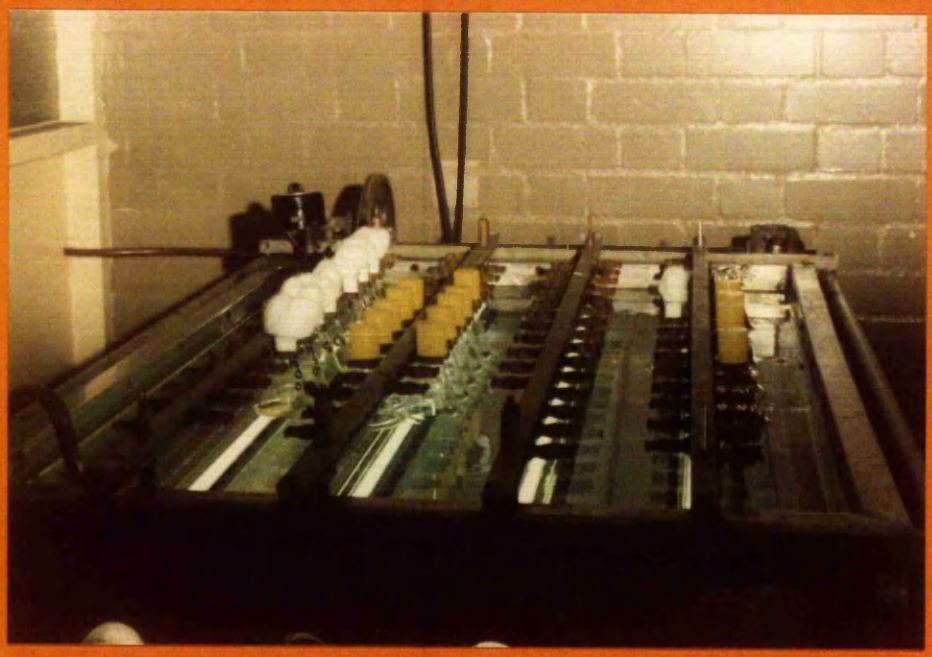
TABLE 10

Composition of Monochrysis Thiamine Assay Medium

	Concentration
NaCl	15g
$MgCl_2 \cdot 6H_2O$	2.5g
KCl	400mg
$CaCl_2 \cdot 2H_2O$	427mg
Na_2SO_4	412mg
Na_2 EDTA	50mg
Fe	500 µg
Mn	50 µg
Zn	5 µg
Cu	5 µg
Co	500ng
Mo	500ng
Glycyl glycine	500mg
Glycine	250mg
KNO_3	100mg
K_2HPO_4	10mg
Vitamin B_{12}	100ng
Distilled water to	1 litre
pH adjusted to	8.0

Plate 2

Illuminated Incubator with Shaking Facility



made up to 20ml with distilled water if necessary. Standards and unknowns were prepared in duplicate.

The flasks were capped with plastic caps and sterilized by autoclaving for 5 minutes at 121°C. The flasks were then inoculated with one drop of a dilute suspension of thiamine-depleted M.lutheri cells. The inoculum was a two week old subculture of the stock culture in thiamine free assay medium, incubated under the same conditions as the stock culture.

The flasks were incubated for two weeks under the conditions described above and then the optical density of the flask contents was measured in a spectrophotometer at 663nm.

Calculation of Results

The concentration of thiamine in the samples was estimated from a standard curve of optical density of assay tube of flask contents plotted against the thiamine concentration of the standards. These estimates were corrected for dilution of the sample in the assay medium to give the concentration of thiamine in the original sample (see Section 2.5.2).

2.5.5 Bioassay for Biotin

Several microorganisms have been used to bioassay biotin in natural waters:- Lactobacillus arabinosus, Lactobacillus Plantarum, Achromobacter species isolate YH-51, Amphidinium carteri Hulbert and Serratia marinorubrum Zobell & Upham.

Golterman (1971) recommended that the method described by Carlucci & Silbernagel (1967) using Amphidinium carteri Hulbert as the bioassay organism, be adapted for the bioassay of biotin in fresh-water. Such an adaptation reduces the sensitivity of the method to about one quarter of the seawater level (see Carlucci 1973) and to a level similar to that of L. plantarum. In this investigation L. plantarum was used as the bioassay organism in attempting to measure dissolved biotin in reservoir water samples.

2.5.5.1 Preparation of Water Samples for Bioassay of Biotin

Filtered and frozen portions of reservoir water samples stored at -20°C for later vitamin analysis (see Section 2.2.1) were thawed and mixed. Suitable aliquots were removed for biotin bioassay by the method described in Section 2.5.1.

2.5.5.2 Preparation of Stock and Standard Biotin Solutions

10mg of D-biotin were dissolved in 100ml of 50% ethyl alcohol in distilled water. This solution contains 100mg/l biotin and is stable for at least a year if kept tightly stoppered at 4°C.

Dilute biotin standard solutions for use in the bioassay method described in Section 2.5.1 were freshly prepared from an intermediate 1µg/l D-biotin standard solution in 95% ethyl alcohol (prepared at monthly intervals from the 100mg/l stock standard solution) by dilution in distilled water.

2.6 MEASUREMENT OF STREAM DISCHARGE

Further details of the method discussed in this section may be found in Němec (1972).

The discharge of a stream may be measured directly or indirectly. Directly, discharge may be measured volumetrically or with the aid of such structures as weirs and flumes. Indirectly, discharge (Q) is estimated by the area-velocity method from the equation

$$Q = A.Va$$

Where A = the cross sectional area of the stream

Va = the average cross sectional velocity.

The discharge of a stream may also be measured by dilution methods.

On smaller streams, discharge is usually measured directly by setting up an artificial control in the form of a weir or flume. The construction of permanent weirs was not feasible for this investigation, and experiments with temporary weirs proved unsatisfactory.

An indirect method of measuring discharge was therefore sought. The average velocity of a stream may be measured directly with a float, current meter, Pitot tube, or other velocity measuring instruments, or indirectly by calculation of the average velocity by a formula such as the Chézy (Manning) equation. In this investigation the latter method was selected, using the Chézy equation.

2.6.1 Method

Within the constraints of accessibility to feeder streams and desirable proximity of the feeder stream sampling points to the reservoirs (see section 2.2.2), a cross section was selected at which to measure discharge which was outside any natural or artificial backwater.

The hydraulic slope of the water surface between points either side of the cross section was measured by normal surveying methods. The breadth of the stream at the cross section was also measured. The average depth of the stream was measured each time a sample was taken.

2.6.2 Calculation of Results

The average velocity of the stream (Va) was calculated from the equation

$$Va = C.\sqrt{R.I}$$

Where C = the Chézy coefficient

R = the hydraulic radius

I = the hydraulic slope

C is calculated from the equation

$$C = 1/n.\ R^{y}$$

Where n = the Manning roughness

y = a function of n

n and y were selected from Tables 26 and 27 in Němec (1972) for each stream. n was selected on the basis of the nature of the

stream bed and banks and general characteristics of the stream as also specified in Němec (1972).

R is calculated from the equation

$$R = A/W$$

Where A = the cross sectional area $(b \cdot d_a)$

W = the wetted perimeter $(b + 2d_a)$

b = the breadth

d_a = the average depth of the stream

The discharge of the stream (Q) was then calculated from the equation

$$Q = A.V_a$$

2.7 ISOLATION OF ALGAE FROM THE RESERVOIRS

The isolation of algae from the reservoirs was made in October/November 1974. It was not possible at this time of year to take advantage of natural blooms of phytoplankton as a starting point for the isolations. Instead, the phytoplankton present in samples of reservoir water were concentrated by aseptically filtering 1l of sample through a Whatman GF/C glass fibre filter paper. The filter pad was transferred to a sterile, 1l conical flask, and approximately 50ml of sterilized filtrate (by membrane filtration) added to the flask, which was then gently shaken to resuspend the phytoplankton retained on the filter pad. This suspension was used to inoculate the culture medium whose composition is given in Table 11. This medium is a modification of the Woods Hole MBL medium described by Nichols (1973), designed to simulate the average chemical composition of the three reservoir waters (see Table 4) with respect to the concentration of major cations and pH.

Woods Hole MBL medium has been successfully used for the culture of Bacillariophyceae, Chlorophyceae, Chrysophyceae and Cyanophyceae (Nichols 1973). Tris buffer is included in the composition of Woods Hole MBL culture medium (where it is of doubtful value as a buffer at the pH of the medium - 7.1 to 7.3), but it has frequently proved toxic to freshwater algae (Smith & Foy 1974). Many algae will grow in this medium even if no buffer is used (Guillard, in Nichols 1973) and Tris was therefore ommitted from the composition of the modified Woods Hole MBL culture medium used.

TABLE 11

Composition of Modified Woods Hole MBL Culture Medium

	Concentration
$CaCl_2 \cdot 2H_2O$	206.00 mg/l
$MgSo_4 \cdot 7H_2O$	207.00 mg/l
$NaHCO_3$	12.60 mg/l
K_2HPO_4	8.71 mg/l
$NaNO_3$	85.01 mg/l
$Na_2SiO_3 \cdot 9H_2O$	28.42 mg/l
$Na_2 \cdot$ EDTA	4.36 mg/l
$FeCl_3 \cdot 6H_2O$	3.15 mg/l
$CuSo_4 \cdot 5H_2O$	10 µg/l
$ZnSo_4 \cdot 7H_2O$	22 µg/l
$CoCl_2 \cdot 6H_2O$	10 µg/l
$MnCl_2 \cdot 4H_2O$	180 µg/l
$Na_2MoO_4 \cdot 2H_2O$	6 µg/l
Thiamine HCl	100 µg/l
Biotin	500 ng/l
Cyanocobalamin	500 ng/l
pH	to 8.0

The inoculum was plated out on the culture medium solidified with 1.5% agar and supplemented with an antibiotic mixture (10mg/l penicillin G and 5mg/l streptomycin) to suppress bacterial growth (Hoshaw & Robowski 1973). The Petri plates were incubated at 20°C under warm white fluorescent lamps (24 hour day at approximately 3500 Lux).

Unialgal colonies were then transferred to 50ml portions of liquid culture medium, supplemented with 1mg/l penicillin G and 0.5mg/l streptomycin, in 100ml conical flasks and incubated at 18°C over daylight type fluorescent lamps (approximately 4000 Lux) with intermittent, reciprocating shaking (120 reciprocations per minute, for 2 minutes in every 10 minutes). The incubator is shown in Plate 2. Stock cultures were subcultured at 14 day intervals.

2.9 LABORATORY CULTURE EXPERIMENTS WITH ALGAL ISOLATES

Laboratory culture experiments were designed to determine the effect of micronutrient concentration on the growth rate of algal isolates. Two defined culture media were used for these experiments: modified Woods Hole MBL with the composition given in Table 10 and a modified Chu No. 10, or EVT medium, used by the Freshwater Biological Association, with the composition given in Table 12. These media were made up to 101% strength without the micronutrient whose effect on the growth rate of the algal isolates was to be examined, and dispensed in 49.5ml amounts into a series of 100ml conical flasks. The micronutrient was then added in a volume of 0.5ml to bring the volume in the flasks to 50ml, the strength of the culture medium to 100% and to give a series of duplicate, standard micronutrient concentrations in the range 0 to 100% of the complete medium. The flasks were capped with plastic caps and sterilized by autoclaving for 10 minutes at 121°C.

The cooled flasks were then inoculated with one drop of a 14 day old stock culture of the algal isolate to be tested and incubated at 18°C ± 1°C over daylight type fluorescent lights (24 hour day, approximately 4000 Lux) with intermittent, reciprocating shaking (120 reciprocations per minute for 2 minutes in every 10 minutes).

The amount of growth in each flask at the end of the incubation period was estimated by determining the concentration of chlorophyll a in the flask using the level 1 method described by Golterman (1971).

TABLE 12

Composition of Modified Chu No. 10 (EVT) Culture Medium

	Concentration
$Ca(NO_3)_2 \cdot 4H_2O$	20.0mg/l
KH_2PO_4	6.2mg/l
$MgSO_4 \cdot 7H_2O$	25.0mg/l
Na_2CO_3	20.0mg/l
Na_2SiO_3	25.0mg/l
N. HCl	0.25ml/l
Added as complex (Na_2.EDTA	2.0mg/l
($FeCl_3$	1.0mg/l
H_3BO_3	2.48mg/l
$MnCl_2 \cdot 4H_2O$	1.39mg/l
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.00mg/l
Vitamin B_{12}	250 µg/l
Thiamine HCl	1mg/l
Biotin	1mg/l
pH to	7.5

The mixed flask contents were filtered under negative pressure, through a Whatman GF/C glass fibre filter paper. The filter paper was then cut into small pieces and transferred to a stoppered test tube. 10ml of boiling 90% methanol was poured onto the pieces of filter pad, the tube was stoppered and kept at $4^{\circ}C$ overnight in the dark. The methanol pigment extract was decanted and the pieces of filter pad washed with 5ml of 90% methanol. The washings were decanted and pooled with the pigment extract. The pigment extract was centrifuged and the supernatent made up to 20ml with 90% methanol. The optical density of the pigment extract was measured in a spectrophotometer at 663nm (OD1) and 750nm (OD2).

The concentration of Chlorophyll a in the flask contents (A) was then calculated from the equation:-

$$\underset{(\mu g/l)}{A} = 13.9 \cdot \frac{(OD1 - OD2)}{\text{lightpath (cm)}} \cdot \frac{\text{volume of extract (ml)}}{\text{volume of filtrate (l)}}$$

2.9 METHODS OF STATISTICAL ANALYSIS SEE ALSO ADDENDUM

The methods of statistical analysis used in this investigation are described in detail by Bailey (1969) and Moroney (1970).

A normal distribution of the data is assumed.

2.9.1 The Mean $(\bar{x})$

$$\bar{x} = \frac{\Sigma x}{n}$$ where - Σx is the sum of individual measurements of the variable x

- n is the number of measurements of x.

2.9.2 The Standard Deviation (s)

The standard deviation is a measure of the variability about the mean

$$s^2 = \frac{\Sigma(x - \bar{x})^2}{n - 1} \quad *$$

$$s = \sqrt{s^2}$$

* 1) s^2 is the variance

2) $\Sigma(x - \bar{x})^2$ is best calculated as:-

$$\Sigma(x - \bar{x})^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

2.9.3 Coefficient of Variation (v)

The coefficient of variation is a standardized measure of the standard deviation. Thus estimates of v may be compared directly with one another.

v is expressed as a percentage.

$$v = \frac{100s}{\bar{x}}$$

2.9.4 Tests of Significance

a) Comparison of the means of two large samples of sizes $n_1 > 30$, $n_2 > 30$

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

The absolute value of d is then referred to Table 13 in order to assess the significance of the difference between the means.

b) Comparison of the means of two small samples (unknown variances assumed equal).

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where s is calculated as follows:-

$$s^2 = \frac{1}{n_1 + n_2 - 2}\left[\Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1} + \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n_2}\right]$$

$$s = \sqrt{s^2}$$

The significance of the difference is determined by referring to a table of the 'Students' t - Distribution (see Appendix 2, Bailey 1969) with $n_1 + n_2 - 2$ degrees of freedom.

TABLE 13 Probability (p) Levels for Different Values of d (see Section 2.9.4a)

d	Probability
1.645	0.10
1.960	0.05
2.326	0.02
2.576	0.01
3.090	0.002
3.291	0.001

c) Comparison of means of two small samples (unknown variances not assumed equal)

The equality of the variances s_1^2 and s_2^2 is tested statistically using the variance ratio or F-test. It is convenient to label the variances so that s_1^2 is larger than s_2^2.

$$F = \frac{s_1^2}{s_2^2}$$

From a table of the F distribution (see for example Appendix 5, Bailey 1969) the appropriate value of F for the chosen level of significance corresponding to $f_1 = n_1 - 1$ degrees of freedom in the numerator and $f_2 = n_2 - 1$ degrees of freedom in the denominator is found. If this tabulated value is exceeded in the data the result is significant. The test then proceeds as follows:-

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

but d is now treated as being distributed approximately like 'Student's' t with f degrees of freedom, the latter being given by

$$f = \frac{1}{\frac{u^2}{n_1 - 1} + \frac{(1-u)^2}{n_2 - 1}}$$

where $u = \frac{s_1^2 / n_1}{s_1^2/n_1 + s_2^2/n_2}$

2.9.5 Regression Analysis

Let $y = a + bx$ be the equation of the true regression line

then b (the slope) $= \dfrac{\Sigma(x - \bar{x})(y - \bar{y})}{\Sigma(x - \bar{x})^2}$

and a (the constant) $= \bar{y} - b\bar{x}$

In order to test for the existence of some degree of association it is necessary to determine whether the observed regression coefficient, b, is significantly different from the hypothetical value zero.
If n is greater than 30 the standard error of b may be calculated as:-

$$\frac{s}{\sqrt{(x - \bar{x})^2}}$$

where $s = \sqrt{s^2}$

and $$s^2 = \frac{1}{n-2}\left[\Sigma(y-\bar{y})^2 - \frac{\left[\Sigma(x-\bar{x})(y-\bar{y})\right]^2}{(x - \bar{x})^2}\right]$$

The ordinary test for normally distributed estimates then applies : for example if b is more than 1.96 standard errors away from zero, then it is significant at the 5 per cent level ($p = 0.05$).

The percentage of the total variation about $\bar{y}$ explained by the regression line (R), may be calculated as follows:-

$$R = \frac{b\left[\Sigma(x - \bar{x})(y - \bar{y})\right]}{\Sigma(y - \bar{y})^2} \cdot 100$$

SECTION 3

RESULTS

3.1 RESULTS OF RESERVOIR SURVEY

3.1.1 Reservoir Water

3.1.1.1 Chlorophyll and Phaeophytin †

The results of chlorophyll and phaeophytin determinations on reservoir water samples over the two year period of the investigation are summarized in Table 14. The results of chlorophyll determinations are illustrated graphically in Figures 7 and 8.

Table 14 shows the rank order in mean chlorophyll and phaeophytin concentrations to be Cropston reservoir < Swithland reservoir < Thornton reservoir. The statistical significance of the differences between reservoirs in the mean concentrations of chlorophyll and phaeophytin was tested by the method described in section 2.9.4a with the following results:-

Difference Between	in Chlorophyll concentration	in Phaeophytin concentration
Cropston and Swithland reservoirs	NS *	NS
Cropston and Thornton reservoirs	SS (P=0.001)	SS (P=0.01)
Swithland and Thornton reservoirs	SS (P=0.001)	NS

*Key:- NS - Not (statistically) Significant

SS - Statistically Significant, probability level (P) given in brackets.

† see also also addendum

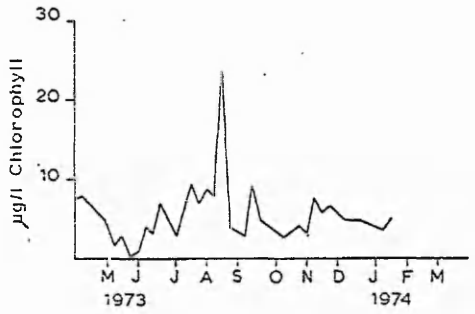
Table 14

Summary of Results of Determinations of Chlorophyll and Phaeophytin in Reservoir Water Samples taken in the Period April 1973 to March 1975

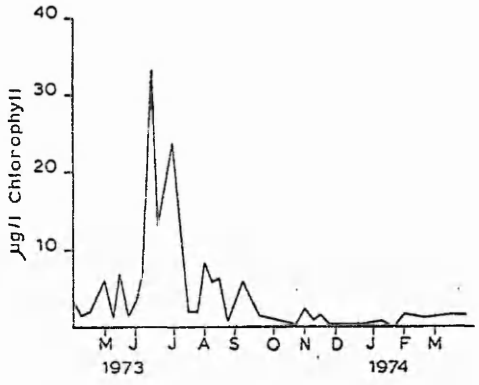
Chlorophyll (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
Mean	4.2	4.5	12.5
Range	0.0-23.5	0.0-48.9	0.3-95.8
Standard Deviation	3.97	7.83	17.0
Coefficient of Variation	94%	173%	136%

Phaeophytin (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
Mean	0.5	0.9	1.7
Range	0.0-6.6	0.0-22.3	0.0-23.0
Standard Deviation	0.93	2.75	3.34
Coefficient of Variation	199%	299%	201%

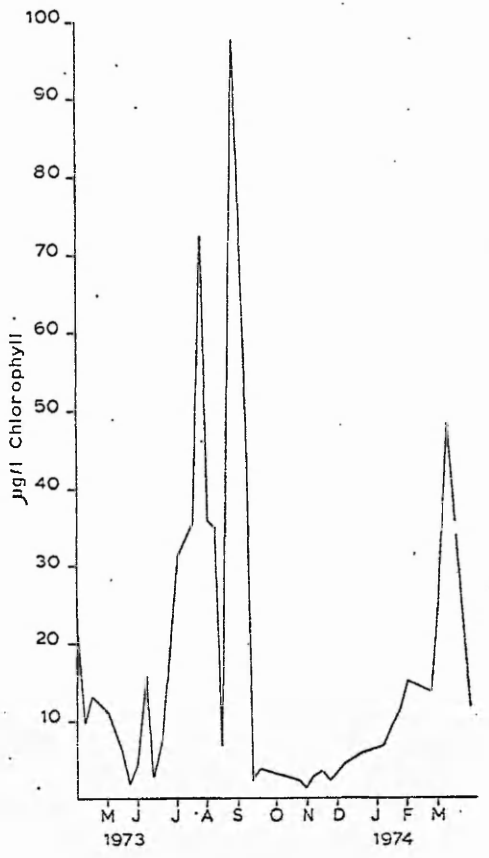
Figure 7 Temporal Variation of Chlorophyll 1973/1974



Cropston Reservoir

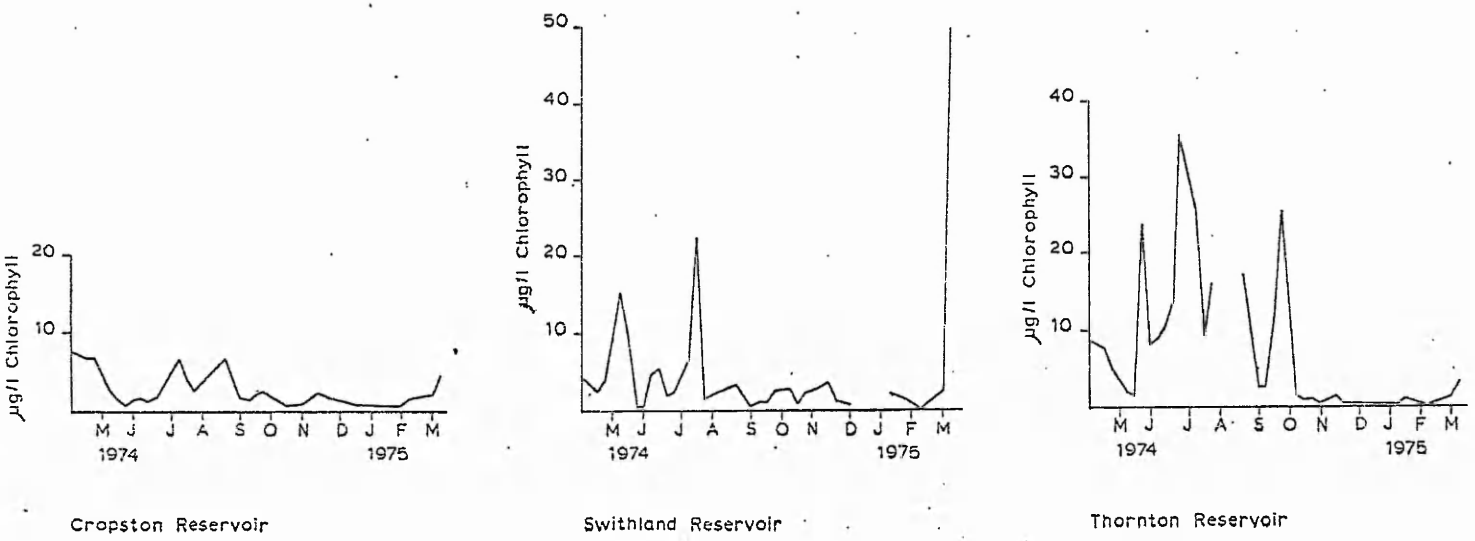


Swithland Reservoir



Thornton Reservoir

Figure 8 Temporal Variation of Chlorophyll 1974 / 1975



3.1.1.2 Vitamin B_{12}

Significant levels of interference (as judged by the use of internal standards) were rarely found in bioassays for vitamin B_{12}. The results presented in this section are, however, corrected for interference, when appropriate, by the method described in section 2.5.2.

3.1.1.2.1 Dissolved Vitamin B_{12} †

The results of bioassays for dissolved vitamin B_{12} on reservoir water samples taken over the two year period of the investigation are summarized in Table 15 and illustrated graphically in Figures 9 to 14. Chlorophyll data are incorporated into these graphs to facilitate visual assessment of the association between the two variables.

Table 15 shows that for all three methods of bioassay, the mean concentration of dissolved vitamin B_{12} follows the same rank order as chlorophyll, that is, Cropston reservoir < Swithland reservoir < Thornton reservoir. The statistical significance of the differences between reservoirs in the mean concentrations of dissolved vitamin B_{12} was tested by the method described in section 2.4.9a with the following results:-

Difference Between	Assay Organism		
	E.gracilis	L.leichmanii	O.malhamensis
Cropston and Swithland reservoirs	SS(P=0.02)	SS(P=0.01)	NS *
Cropston and Thornton reservoirs	SS(P=0.001)	SS(P=0.001)	NS
Swithland and Thornton reservoirs	SS(P=0.001)	SS(P=0.01)	NS

* Key:- NS - Not (statistically) Significant.

SS - Statistically Significant, probability level (P) given in brackets.

† see also addendum

Table 15

Summary of Results of Bioassays for Dissolved Vitamin B_{12} in Reservoir Water Samples taken in the period April 1973 to March 1975

Vitamin B_{12} (ng/l)	RESERVOIR		
	Cropston	Swithland	Thornton
E.gracilis assay			
Mean	24.5	29.3	41.1
Range	4-68	6-62	10-150
Standard Deviation	10.7	12.2	24.2
Coefficient of Variation	44%	42%	59%
L.leichmanii assay			
Mean	14.0	18.9	26.6
Range	2-56	4-45	6-84
Standard Deviation	8.71	9.87	18.4
Coefficient of Variation	62%	52%	69%
O.malhamensis assay			
Mean	2.67	3.11	3.21
Range	0.0-12.0	0.0-24.0	0.0-17.0
Standard Deviation	3.35	3.15	2.24
Coefficient of Variation	126%	101%	70%

Figure 9 Temporal Variations of Vitamin B_{12} (Euglena assay) and Chlorophyll (dotted line) 1973/1974

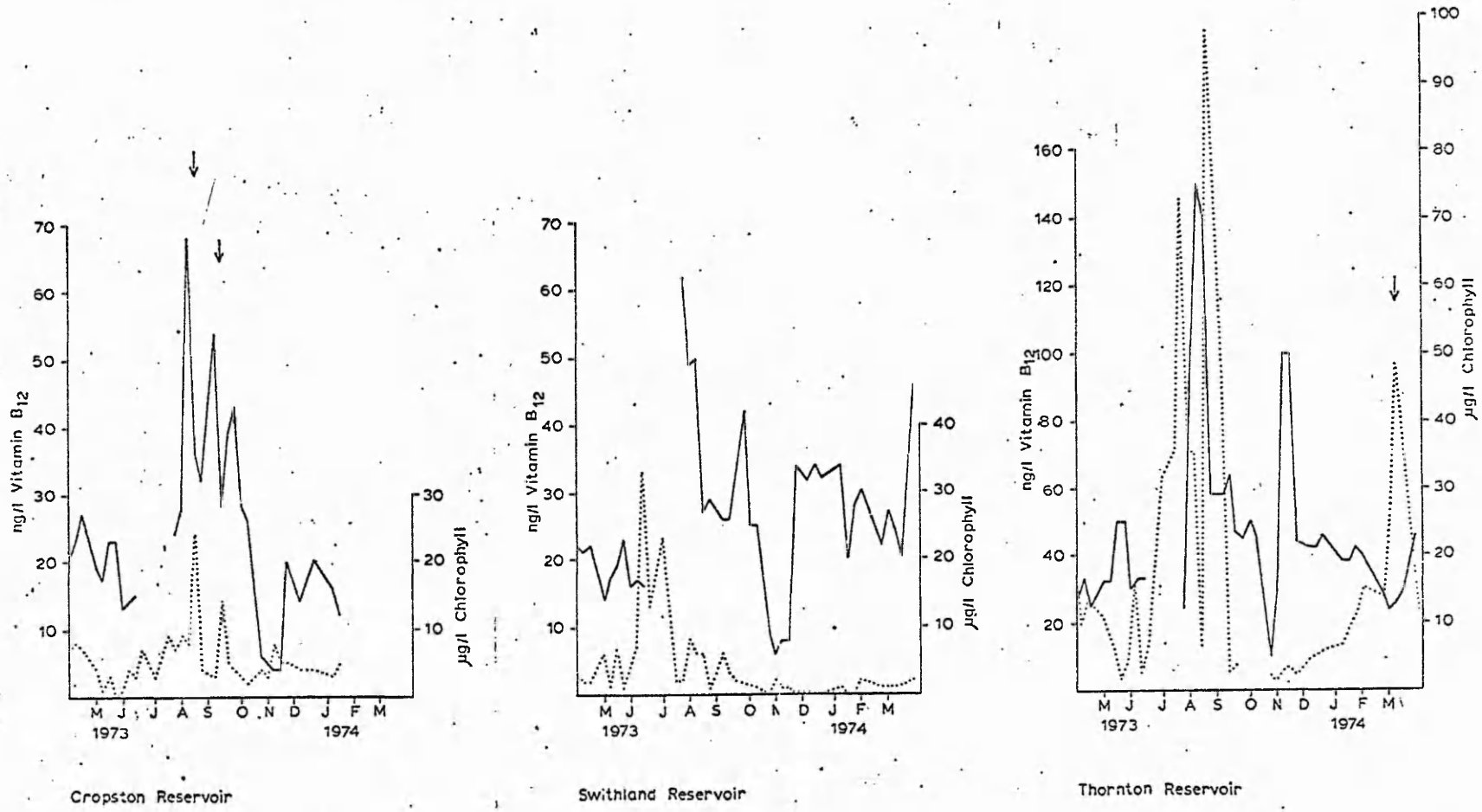


Figure 10 Temporal Variations of Vitamin B_{12} (*Euglena* assay) and Chlorophyll (dotted line) 1974/1975

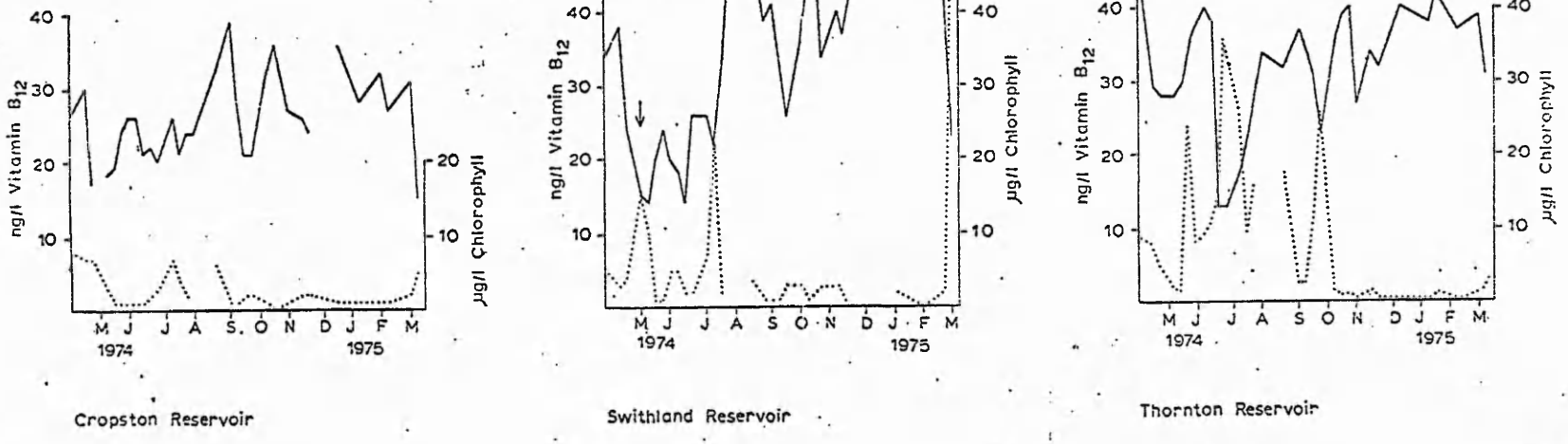


Figure 11 Temporal Variations of Vitamin B_{12} (Lactobacillus assay) and Chlorophyll (dotted line) 1973/1974

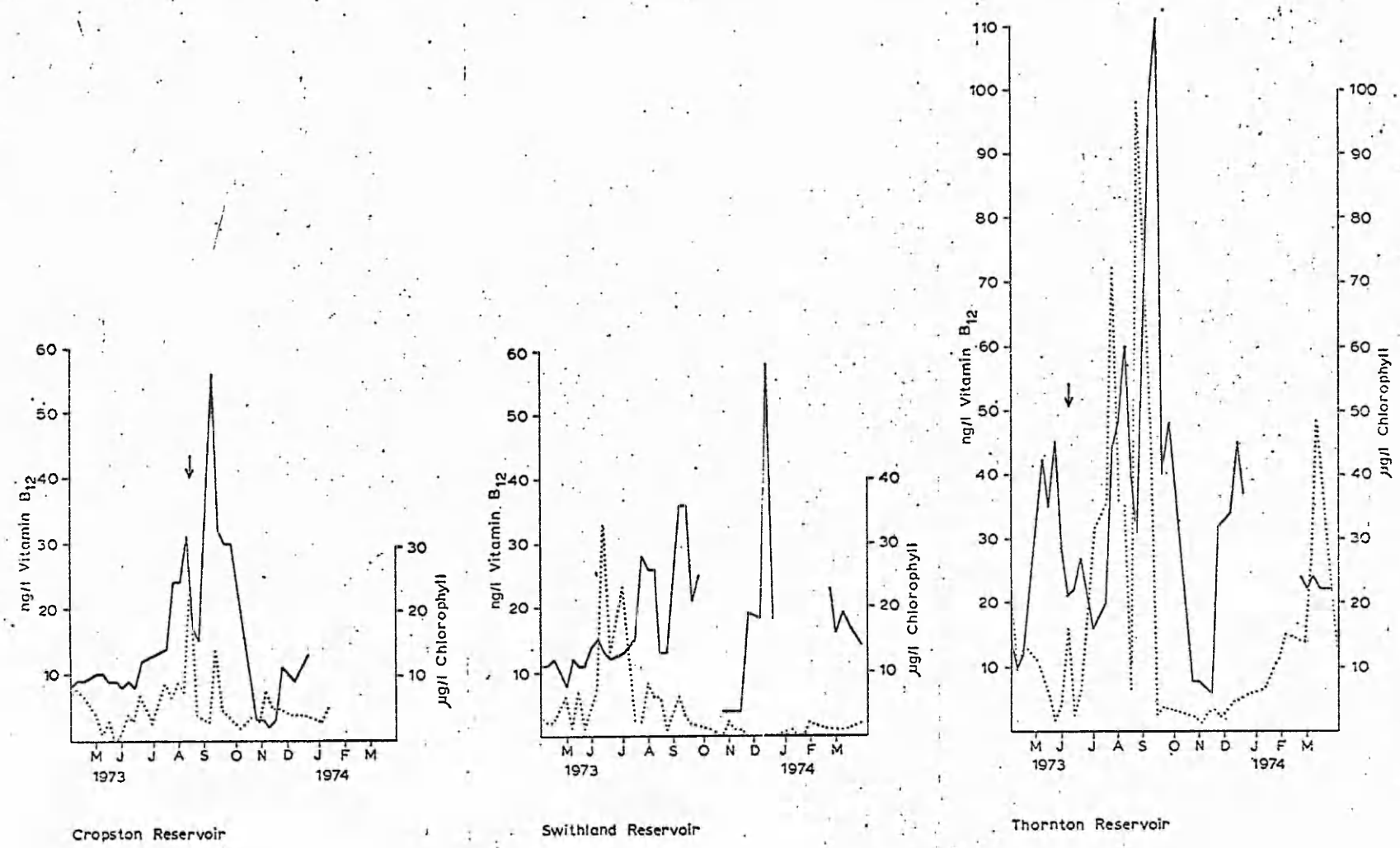
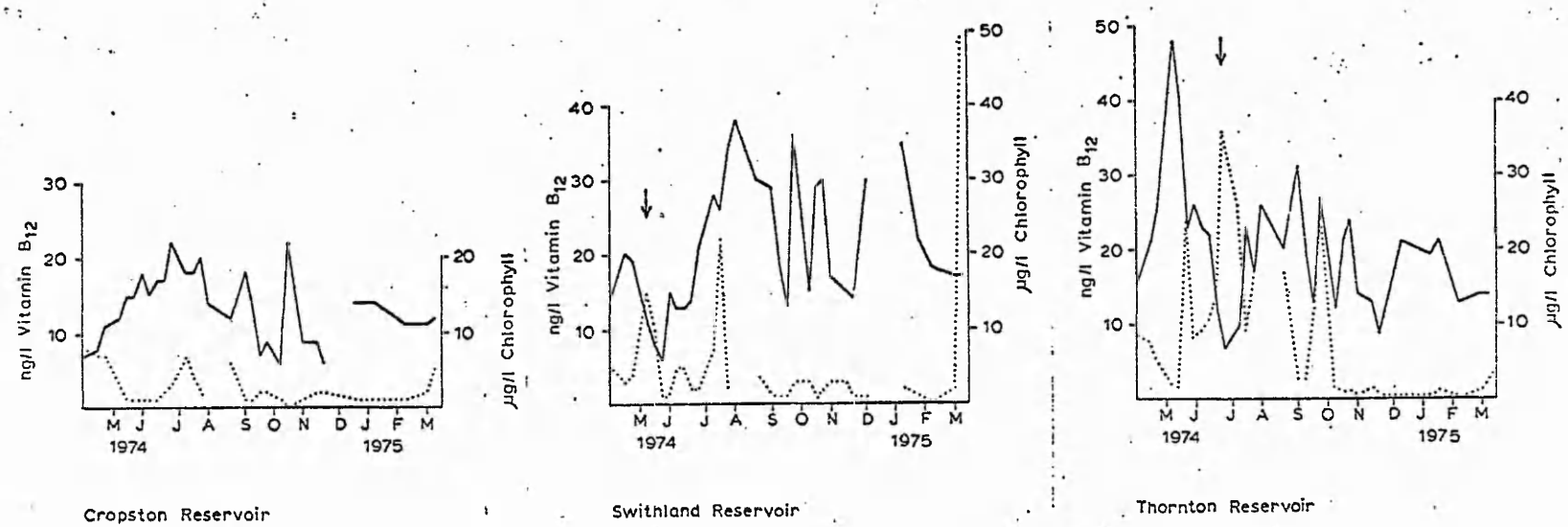


Figure 12 Temporal Variations of Vitamin B_{12} (Lactobacillus assay) and Chlorophyll (dotted line) 1974/1975



Cropston Reservoir

Swithland Reservoir

Thornton Reservoir

Figure 13 Temporal Variations of Vitamin B_{12} (Ochromonas assay) and Chlorophyll (dotted line) 1973/1974

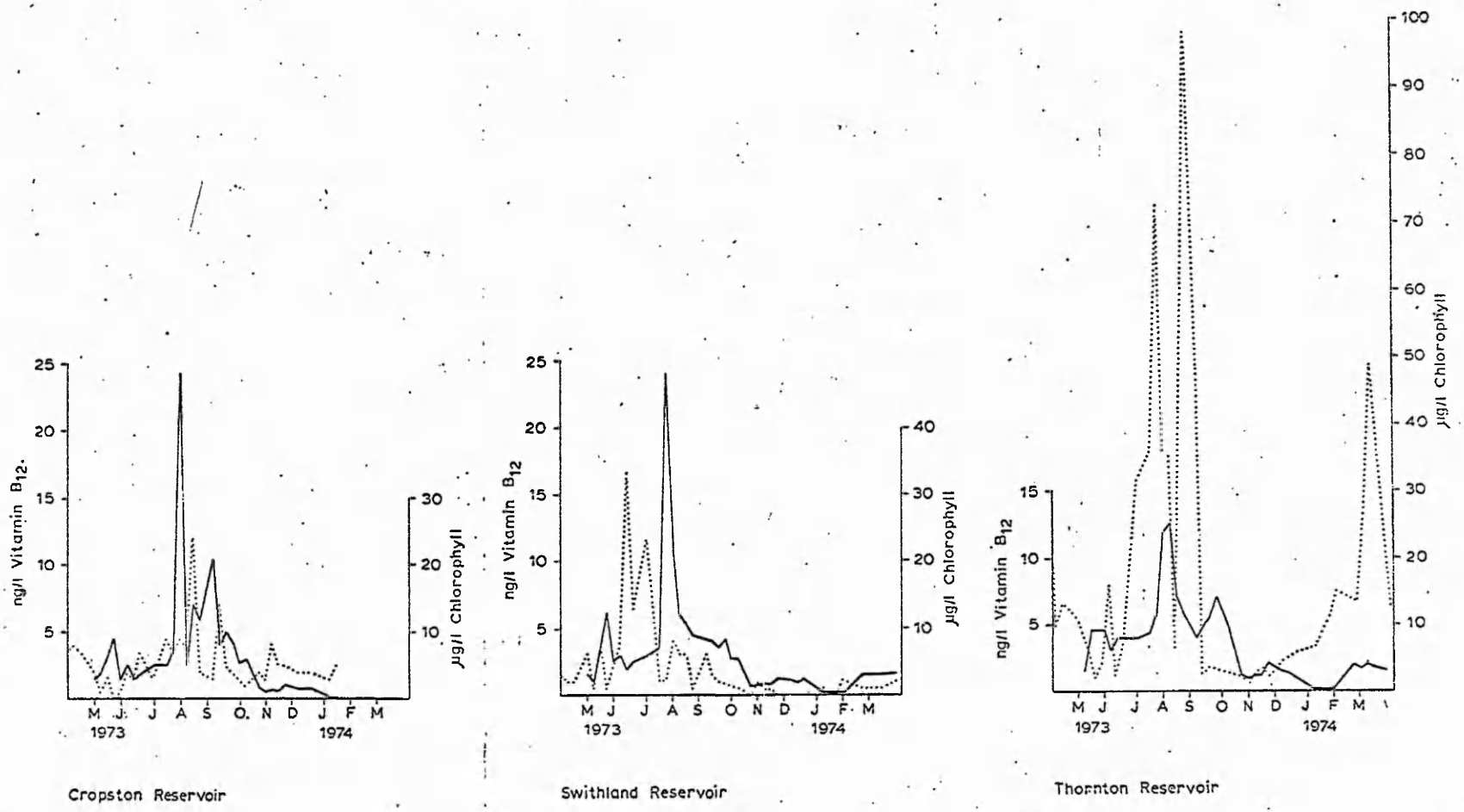
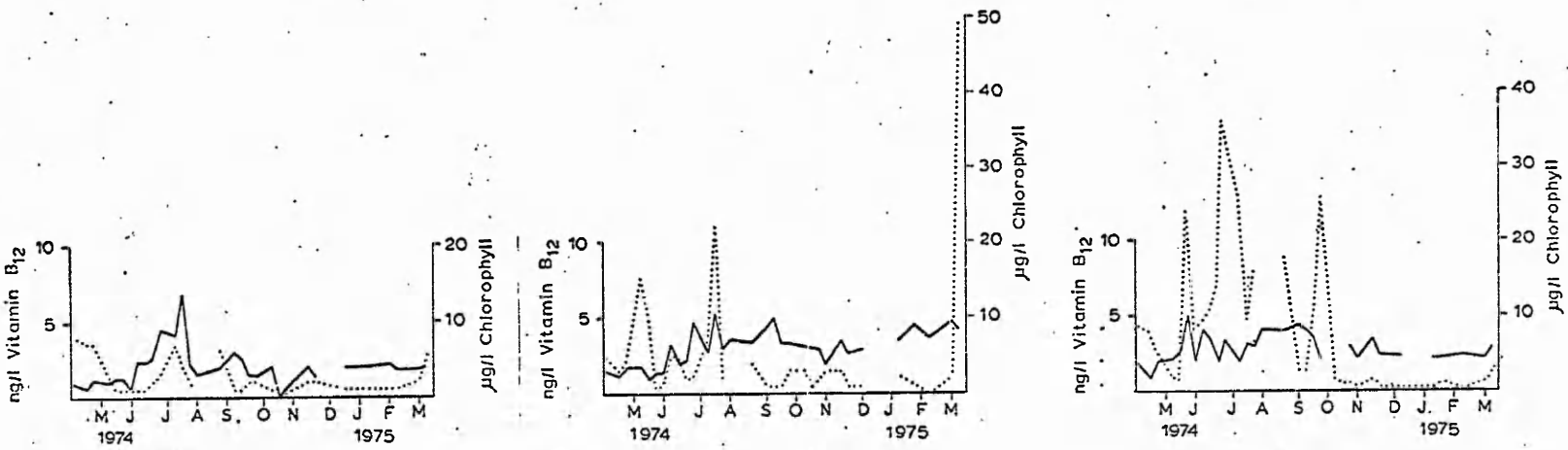


Figure 14 Temporal Variations of Vitamin B_{12} (Ochromonas assay) and Chlorophyll (dotted line) 1974/1975



Linear regression analysis was used to test the degree of association between the individual results of dissolved vitamin B_{12} and chlorophyll determinations. The chlorophyll data was transformed before regression analysis, by taking logarithms to the base 10. Since the raw chlorophyll data included some zero values, it was necessary to add a positive constant (0.1) to the data before making the transformation.

The statistical significance of the calculated regression line was assessed by determining whether the slope of the line was significantly different from zero. The percentage of the variance in the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5. The following results were obtained:-

Regression Between Chloropnyll and Dissolved Vitamin B_{12}	RESERVOIR					
	CROPSTON		SWITHLAND		THORNTON	
	Slope	R^2	Slope	R^2	Slope	R^2
1. E.gracilis assay	NS	0.3%	NS	1.4%	NS *	0.2%
2. L.leichmanii assay	NS	0.5%	NS	1.4%	NS	2.8%
3. O.malhamensis assay	NS	3.1%	Positive SS (P=0.1)	5.2%	Positive SS (P=0.05)	6.8%

* Key:- NS - Not (statistically) Significant

SS - Statistically Significant, probability level (P) given in brackets.

The slopes of both statistically significant regression lines in the above table are positive. Dissolved vitamin B_{12}, as assayed with O.malhamensis, cannot therefore be considered a limiting nutrient in either Swithland or Thornton reservoirs. However, within reservoirs, the concentration of dissolved vitamin B_{12} did show changes which sometimes preceded, coincided with, or followed peaks in the concentration of chlorophyll (arrowed in Figures 9-14).

Figures 9 to 14 show no evidence of any consistent seasonal variation in the concentration of dissolved vitamin B_{12} in the reservoirs.

Within reservoirs, the statistical significance of the differences in mean dissolved vitamin B_{12} concentration assayed with the three different organisms was tested by the method described in section 2.9.4a. The following results were obtained:-

Difference Between	RESERVOIR		
	Cropston	Swithland	Thornton
E.gracilis and L.leichmanii methods	SS(P=0.001)	SS(P=0.001)	SS*(P=0.001)
E.gracilis and O.malhamensis methods	SS(P=0.001)	SS(P=0.001)	SS (P=0.001)
L.leichmanii and O.malhamensis methods	SS(P=0.001)	SS(P=0.001)	SS (P=0.001)

* Key:- SS - Statistically significant, probability level (P) in brackets.

A comparison of the mean concentrations of dissolved vitamin B_{12} assayed with the three organisms shows that in all reservoirs, the E.gracilis assay gave the highest results, the O.malhamensis assay gave the lowest results, and the L.leichmanii assay intermediate results. The mean concentrations of dissolved vitamin B_{12} assayed with O.malhamensis were 8-11% of the comparable E.gracilis results, and 12-19% of the comparable L.leichmanii results, suggesting that approximately 10% of dissolved vitamin B_{12} assayed with E.gracilis and approximately 16% of dissolved vitamin B_{12} assayed with L.leichmanii was 'true' vitamin B_{12} (see Table 2).

The specificity patterns of L.leichmanii and E.gracilis (see Table 2) show that L.leichmanii responds to more vitamin B_{12} analogues than does E.gracilis. It was therefore somewhat surprising to find the mean concentration of dissolved vitamin B_{12}, to be higher in all

three reservoirs, when assayed with E.gracilis, than when assayed with L.leichmanii.

3.1.1.2.2 Soluble (Free) and Complexed (Bound) Vitamin B_{12} †

The results of bioassays with E.gracilis to determine the distribution of dissolved vitamin B_{12} among soluble and complexed fractions of the vitamin in reservoir water samples are summarized in Table 16. Determinations were made at monthly intervals during 1974.

As tested by the methods described in sections 2.9.4b and 2.9.4c, the differences between reservoirs in the mean concentrations of free and bound vitamin B_{12} are not statistically significant.

The results do indicate however that bound forms of the vitamin do exist in the reservoirs studied and may account, on average, for between 10 and 15% of the total dissolved vitamin B_{12}. This observation may partially explain the unexpectedly higher mean concentration of dissolved vitamin B_{12} assayed with E.gracilis compared with assays with L.leichmanii, since complexed forms of the vitamin may be made available to E.gracilis but not to L.leichmanii during the respective methods of bioassay.

† see also addendum

Table 16

Summary of Results of Determinations of Soluble (Free) and Complexed (Bound) Vitamin B_{12} in Reservoir Water Samples

Vitamin B_{12} (ng/l)	RESERVOIR					
	Cropston		Swithland		Thornton	
	Free	Bound	Free	Bound	Free	Bound
Mean	22.1	4.4	26.9	3.42	28.3	5.27
Range	15-29	1-10	18-38	0-10	10-42	0-13
Standard Deviation	4.81	2.83	6.56	3.34	9.71	4.61
Coefficient of Variation	22%	64%	24%	98%	34%	88%

3.1.1.2.3 Particulate Vitamin B_{12} †

The results of bioassays for particulate vitamin B_{12} with E.gracilis are summarized in Table 17 and illustrated graphically in Figures 15 and 16. Chlorophyll data are incorporated into these graphs to facilitate visual assessment of the association between the two variables.

Table 17 shows that the mean concentration of particulate vitamin B_{12}, like the mean concentrations of chlorophyll, phaeophytin and dissolved vitamin B_{12} (all methods of bioassay), was higher in Thornton reservoir than in either Cropston or Swithland reservoirs. The statistical significance of the differences between the mean concentrations of particulate vitamin B_{12} was tested by the method described in section 2.9.4a with the following results:-

Difference Between	in mean particulate vitamin B_{12} concentration
Cropston and Swithland reservoirs	NS *
Cropston and Thornton reservoirs	SS(P=0.01)
Swithland and Thornton reservoirs	SS(P=0.01)

* Key:- NS - Not (statistically) Significant

SS - Statistically Significant, probability level (P) in brackets.

Linear regression analysis was used to test the degree of association between the individual results of particulate vitamin B_{12} and chlorophyll determinations. The chlorophyll data was first transformed as described in section 3.1.1.2.1. The statistical significance of the calculated regression line was assessed by determining whether the slope of the line was significantly

† see addendum

Table 17

Summary of Results of Bioassays for Particulate Vitamin B_{12} in Reservoir Water Samples taken in the period April 1973 to March 1975

Particulate Vitamin B_{12} (ng/l)	RESERVOIR		
	Cropston	Swithland	Thornton
Mean	1.53	1.45	5.35
Range	0.4-5.5	0.2-4.6	0.4-83.2
Standard Deviation	1.05	1.02	11.53
Coefficient of Variation	69%	70%	216%

Figure 15 Temporal Variations of Particulate Vitamin B_{12} and Chlorophyll (dotted line) 1973/1974

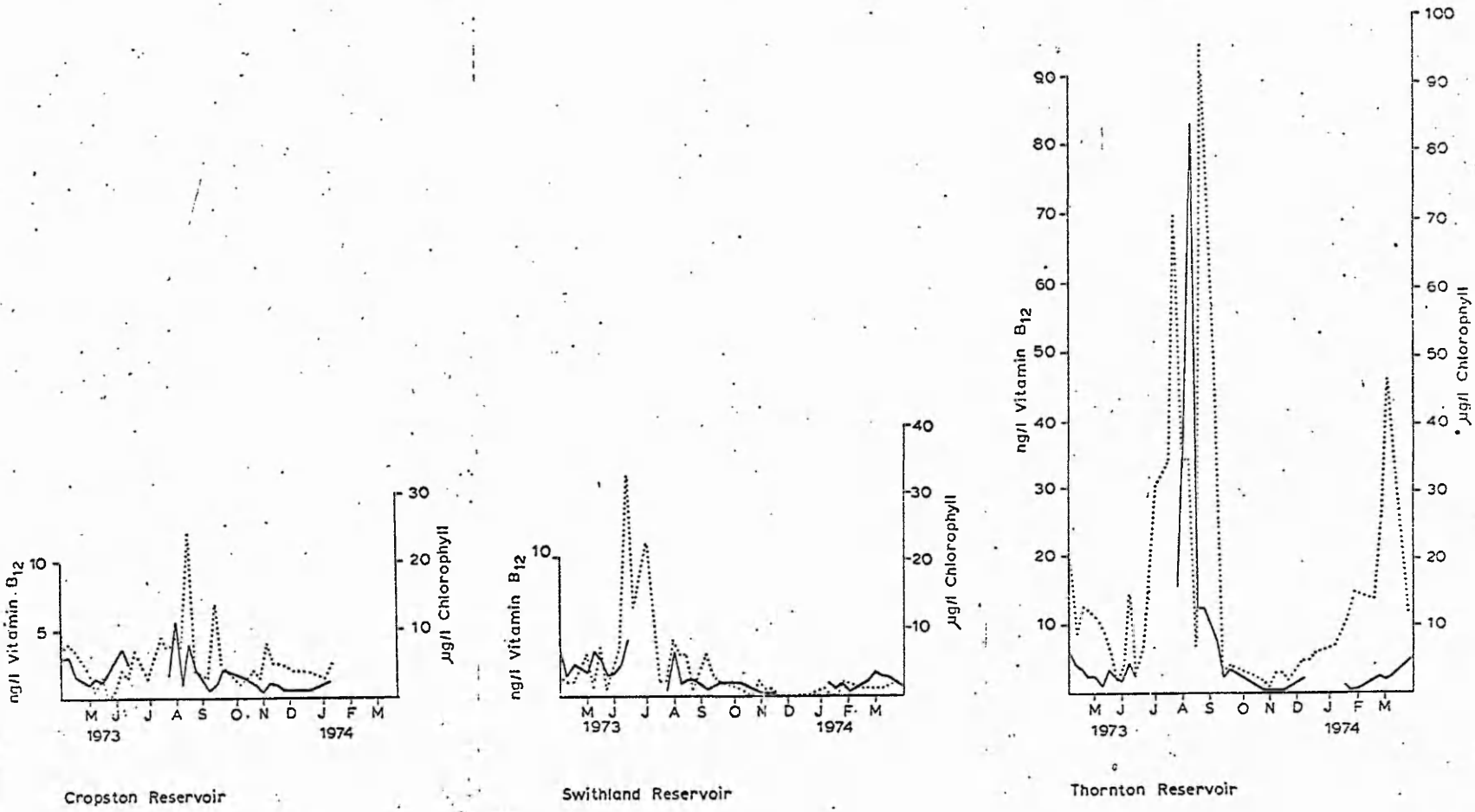
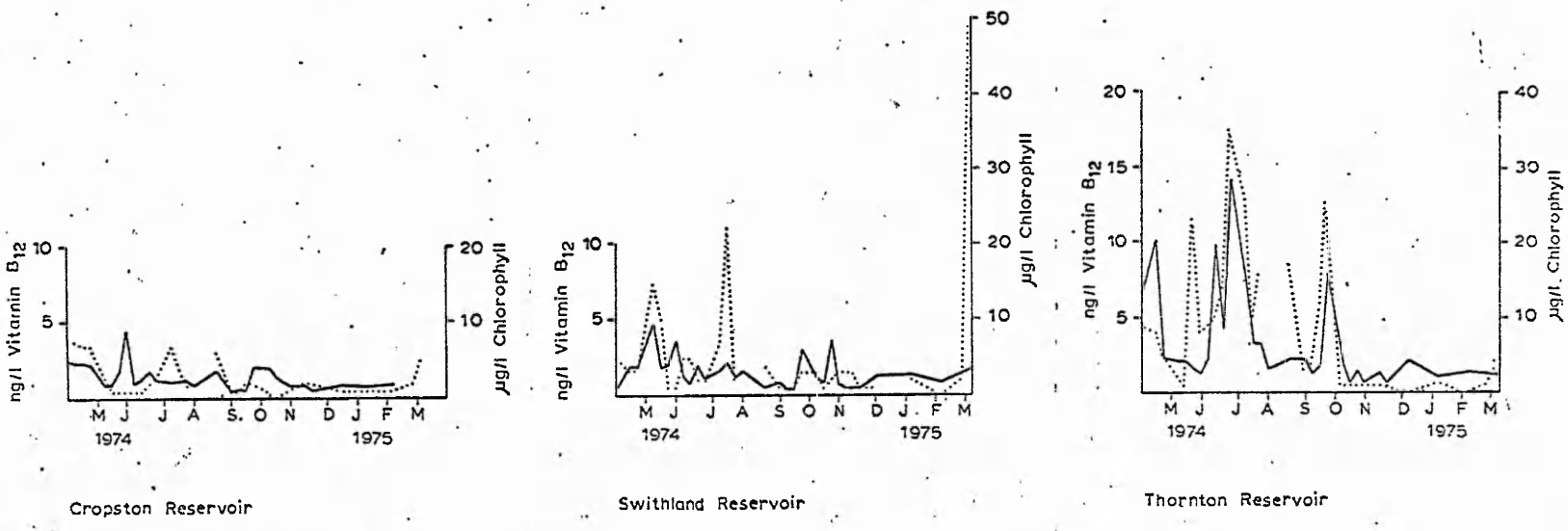


Figure 16 Temporal Variations of Particulate Vitamin B_{12} and Chlorophyll (dotted line) 1974/1975



different from zero. The percentage of the variance in the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5. The following results were obtained:-

Reservoir	Regression between Chlorophyll and Particulate Vitamin B_{12}		
	Slope	Sign of slope	R^2
Cropston	SS(P=0.01)	Positive	11.9%
Swithland	SS(P=0.01)	Positive	13.7%
Thornton	SS(P=0.01)	Positive	13.6%

Although particulate vitamin B_{12} cannot be considered a limiting nutrient the above results do indicate that particulate vitamin B_{12} accounts for a significant proportion of the variance in chlorophyll concentration in each reservoir.

On average, particulate vitamin B_{12} constituted between 5% (Swithland reservoir) and 12% (Thornton reservoir) of the total (dissolved plus particulate) vitamin B_{12} present in the reservoirs. Peaks in the concentration of particulate vitamin B_{12} preceding, coinciding with, and following peaks in chlorophyll concentration were observed. The highest observed concentration of particulate vitamin B_{12}, 83.2ng/l, occurred in Thornton reservoir 2 weeks after a peak in the concentration of chlorophyll on the 28 July 1974 due to a large population of Aphanizomenon.

No evidence of any consistent seasonal variation in the concentration of particulate vitamin B_{12} was observed in the reservoirs (see Figures 15 and 16).

3.1.1.3 Iron†

The results of iron determinations on reservoir water samples over the one year period of investigation are summarized in Table 18 and illustrated graphically in Figures 17 to 19. Chlorophyll data are incorporated into these graphs to facilitate visual assessment of the association between the two variables.

Table 18 shows that the mean concentrations of all iron fractions, like the mean concentrations of chlorophyll, phaeophytin, dissolved and particulate vitamin B_{12}, were higher in Thornton reservoir than in either Cropston or Swithland reservoirs. The statistical significance of the differences between reservoirs in the mean concentrations of the iron fractions was tested by the method described in section 2.9.4a with the following results:-

Difference Between	Iron Fraction			
	Soluble	Complexed	Particulate	Total
Cropston and Swithland reservoirs	SS(P=0.001)	SS(P=0.001)	SS(P=0.001)	SS(P=0.001)
Cropston and Thornton reservoirs	SS(P=0.1)	SS(P=0.01)	SS(P=0.1)	NS
Swithland and Thornton reservoirs	SS(P=0.02)	SS(P=0.002)	SS(P=0.001)	SS(P=0.01)

* Key:- NS - Not (statistically) Significant
SS - Statistically Significant, probability level (P) in brackets.

Linear regression analysis was used to test the degree of association between the individual results of iron and chlorophyll determinations. The chlorophyll data was first transformed as described in section 3.1.1.2.1. The statistical significance of the calculated regression line was assessed by determining whether the slope of the line was significantly different from zero. The percentage of the variance in

† see addendum

Table 18

Summary of Results of Determinations of Iron in Reservoir Water Samples taken in the Period December 1973 to March 1975

Iron (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
a) Soluble			
Mean	12.1	4.7	8.6
Range	0-52	0-35	0-25
Standard Deviation	10.1	6.6	7.2
Coefficient of Variation	84%	140%	84%
b) Complexed			
Mean	73.2	30.8	50.7
Range	23-140	7-127	11-145
Standard Deviation	37.5	24.2	28.9
Coefficient of Variation	311%	79%	57%
c) Particulate			
Mean	79.2	27.3	130.5
Range	7-370	0-124	2-2003
Standard Deviation	66.6	23.3	302.5
Coefficient of Variation	84%	86%	232%
d) Total			
Mean	162.3	61.6	188.9
Range	52-500	15-178	50-2040
Standard Deviation	87.3	34.7	298.3
Coefficient of Variation	54%	56%	158%

Figure 17 Temporal Variations of Soluble Iron and Chlorophyll (dotted line) 1974/1975

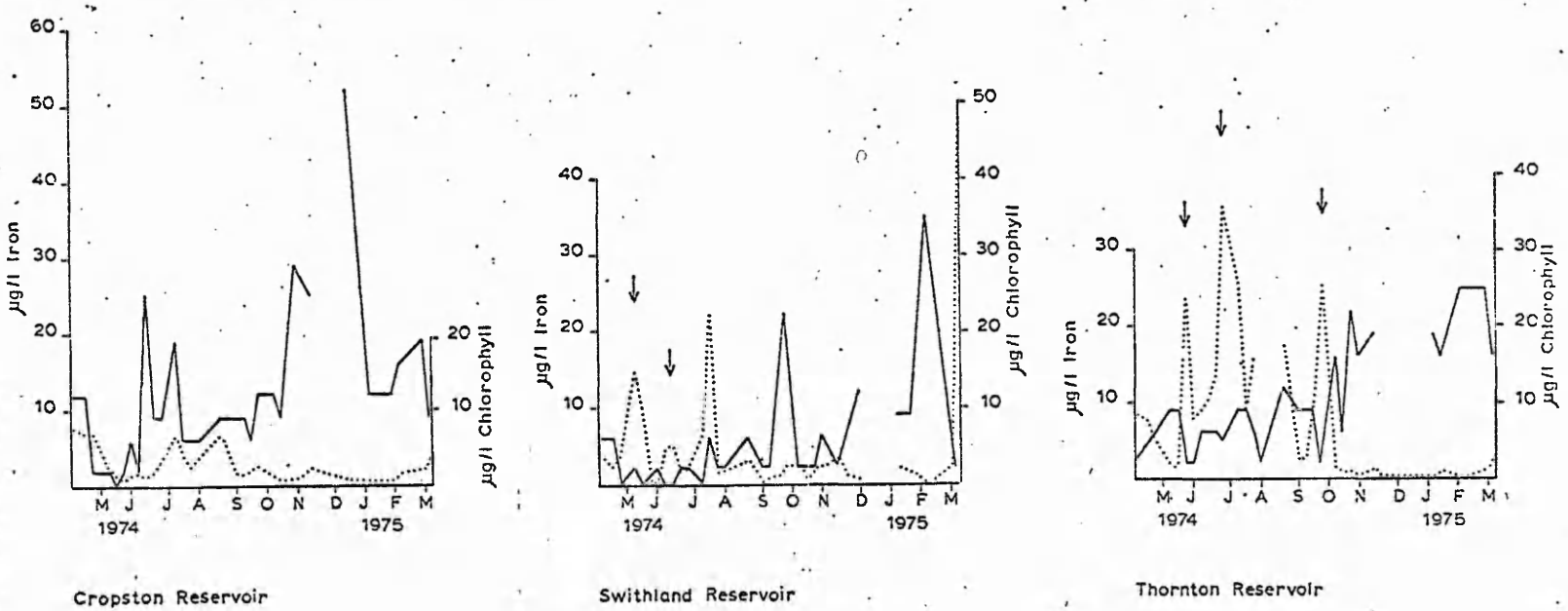


Figure 18 Temporal Variations of Complexed Iron and Chlorophyll (dotted line) 1974/1975

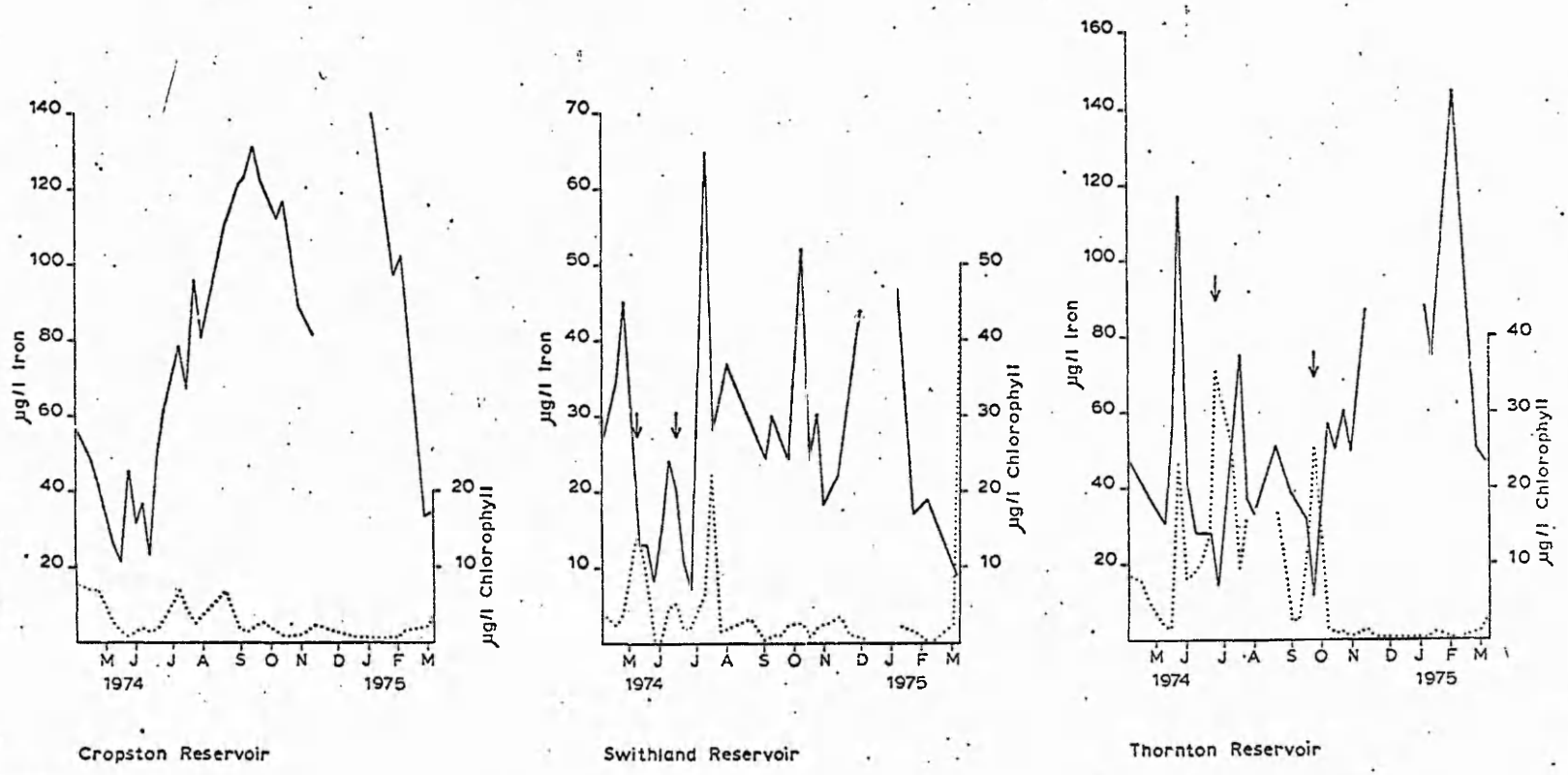
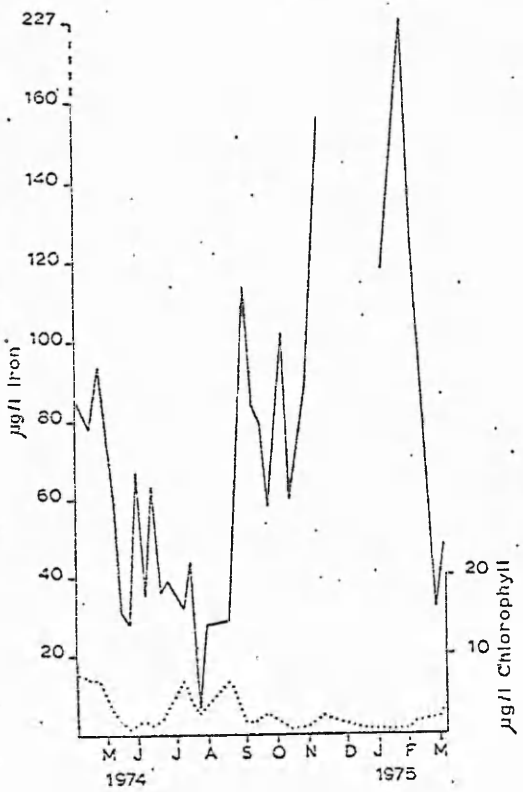
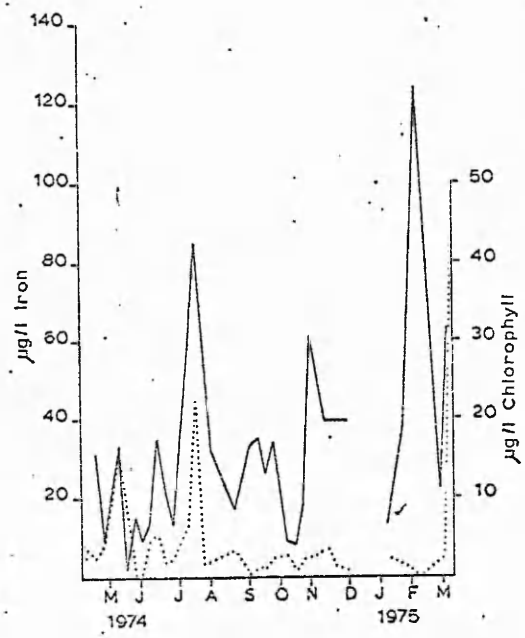


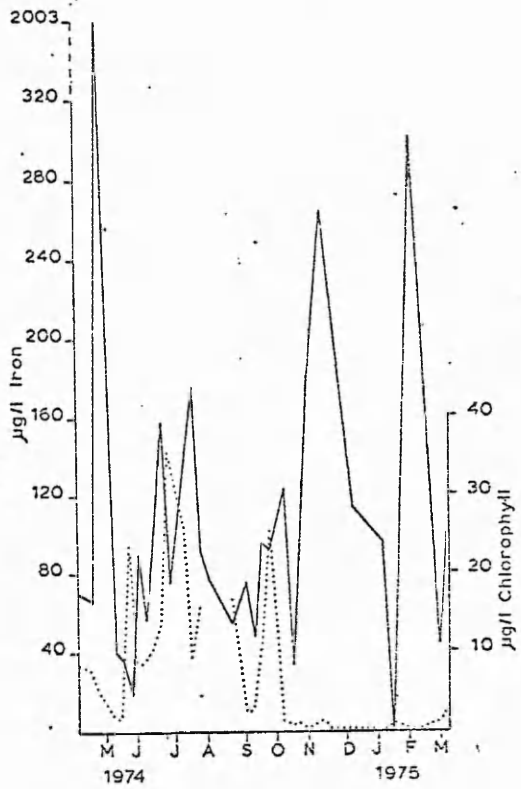
Figure 19 Temporal Variations of Particulate Iron and Chlorophyll (dotted line) 1974/1975



Cropston Reservoir



Swithland Reservoir



Thornton Reservoir

the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5.

The following results were obtained:-

Reservoir and Iron Fraction	Regression Between Chlorophyll and Iron		
	Slope	Sign of Slope	R^2
CROPSTON			
a) Soluble	NS		1.2%
b) Complexed	NS		3.8%
c) Particulate	NS		1.8%
d) Total	NS		0.0%
SWITHLAND			
a) Soluble	NS		5.1%
b) Complexed	NS		0.2%
c) Particulate	NS		2.8%
d) Total	NS		0.9%
THORNTON			
a) Soluble	SS(P=0.001)	Negative	50.9%
b) Complexed	SS(P=0.002)	Negative	23.0%
c) Particulate	NS		0.4%
d) Total	NS		1.2%

The results strongly suggest that soluble and complexed fractions of iron in Thornton reservoir were acting as limiting nutrients during the study period. It is interesting to note that the total concentration of iron in Thornton reservoir showed no statistically significant association with chlorophyll over the same period. On two occasions in Thornton reservoir (in June and September 1974) the concentrations of soluble and complexed iron decreased as the concentration of chlorophyll increased (see Figures 17 and 18).

Similarly, on two occasions in Swithland reservoir (in May and June 1974) the concentration of complexed iron decreased as the

concentration of chlorophyll increased (see Figure 18).

There is good reason to believe that the mean concentration of iron in Cropston reservoir was higher than normal during the study period. Firstly the reservoir was emptied in the Winter of 1973 to 1974 to allow the installation of a scour main. As the water level in the reservoir decreased, the concentration of iron increased. The reservoir was allowed to refill in March 1974 but remained, on average, less than half full during the investigation. Secondly, although the mean concentrations of iron in Swithland and Thornton reservoirs calculated from the records of the Severn Trent Water Authority (for the years 1967 to 1972 inclusive) show good agreement with the means given in Table 18, the mean iron concentration for Cropston reservoir given in Table 18 (162.3 $\mu g/l$) is considerably higher than that calculated from the records of the Severn Trent Water Authority (98 $\mu g/l$). The concentration of complexed iron in Cropston reservoir showed some evidence of a seasonal variation (see Figure 18), but it is not clear what influence the low water level may have had on the results. The concentration of complexed iron decreased rapidly between January 1975 and the end of the study period. This decrease coincided with the rapid natural filling of the reservoir from 60% of total capacity to 100% of total capacity (2.53×10^6 m^3). Clearly the efficiency of mixing of an entire water column will be influenced by its depth.

3.1.1.4 Manganese †

The results of manganese determinations on reservoir water samples over the one year period of investigation are summarized in Table 19 and illustrated graphically in Figures 20 and 21. Chlorophyll data are incorporated into these graphs to facilitate visual assessment of the association between the two variables.

Table 19 shows that the mean concentrations of all manganese fractions, like the mean concentrations of chlorophyll, phaeophytin, dissolved and particulate vitamin B_{12} and all iron fractions, were higher in Thornton reservoir than in either Cropston or Swithland reservoirs. The statistical significance of the differences between reservoirs in the mean concentrations of the manganese fractions was tested by the method described in section 2.9.4a with the following results:-

Difference Between	Manganese Fraction			
	Soluble	Complexed	Particulate	Total
Cropston and Swithland reservoirs	SS(P=0.001)	NS	SS(P=0.1)	SS*(P=0.001)
Cropston and Thornton reservoirs	NS	NS	NS	NS
Swithland and Thornton reservoirs	SS(P=0.1)	NS	SS(P=0.01)	SS(P=0.01)

* Key:- NS - Not (statistically) Significant

SS - Statistically Significant, probability level (P) in brackets.

Linear regression analysis was used to test the degree of association between the individual results of manganese and chlorophyll determinations. The chlorophyll data was first transformed as described in section 3.1.1.2.1. The statistical significance of the calculated regression line was assessed by determining whether the slope of the line was significantly different from zero. The percentage of the variance

† see addendum

Table 19

Summary of Results of Determinations of Manganese in Reservoir Water Samples taken in the Period December 1973 to March 1975

Manganese (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
a) Soluble			
Mean	69.0	34.0	59.7
Range	0-147	3-142	0-316
Standard Deviation	46.5	34.6	77.2
Coefficient of Variation	67%	102%	129%
b) Complexed			
Mean	3.0	3.3	4.0
Range	0-45	0-33	0-56
Standard Deviation	9.1	7.2	10.7
Coefficient of Variation	299%	220%	268%
c) Particulate			
Mean	18.4	10.9	29.9
Range	0-107	0-49	0-193
Standard Deviation	25.3	10.7	42.9
Coefficient of Variation	137%	98%	144%
d) Total			
Mean	90.7	48.0	87.4
Range	30-227	7-204	4-284
Standard Deviation	48.5	44.5	90.4
Coefficient of Variation	53%	93%	104%

Figure 20 Temporal Variations of Soluble Manganese and Chlorophyll (dotted line) 1974/1975

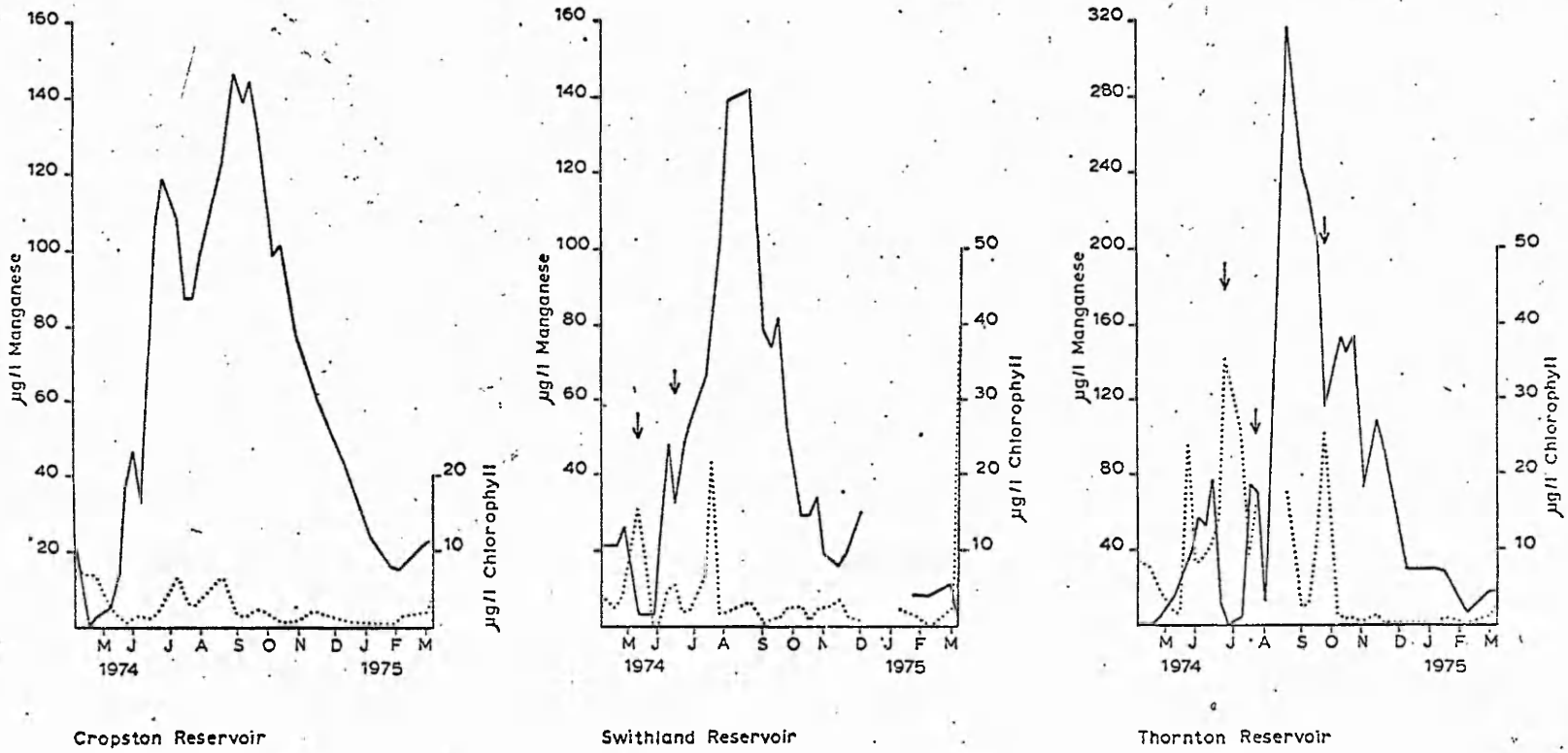
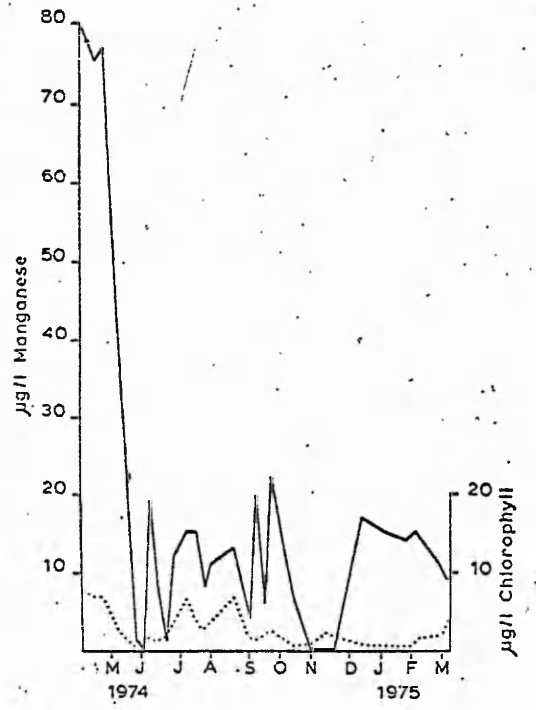
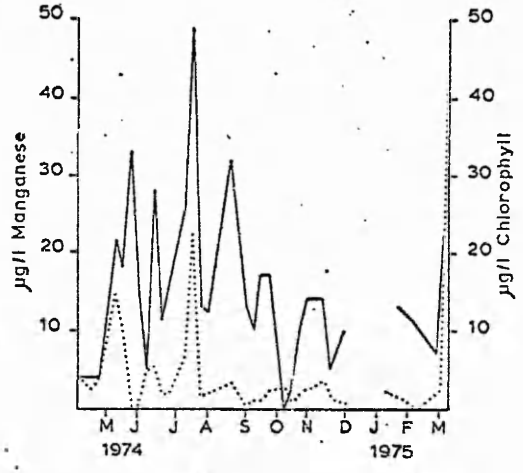


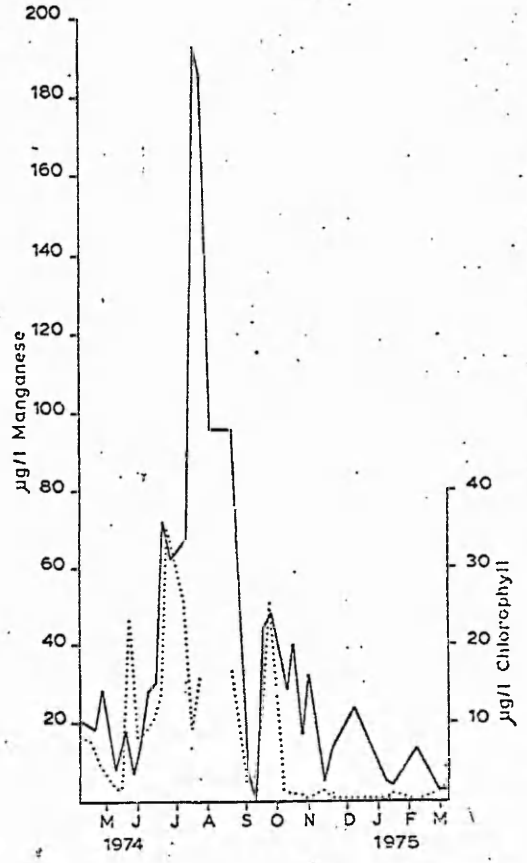
Figure 21 . Temporal Variations of Particulate Manganese and Chlorophyll (dotted line) 1974/1975



Cropston Reservoir



Swithland Reservoir



Thornton Reservoir

in the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5. The following results were obtained:-

Reservoir and Manganese Fraction	Regression between Chlorophyll and Manganese		
	Slope	Sign of Slope	R^2
CROPSTON			
a) Soluble	NS		0.6%
b) Particulate	SS(P=0.001)	Positive	29.6%
c) Total	SS(P=0.05)	Positive	13.7%
SWITHLAND			
a) Soluble	NS		5.9%
b) Particulate	SS(P=0.001)	Positive	31.1%
c) Total	SS(P=0.02)	Positive	12.3%
THORNTON			
a) Soluble	NS		1.8%
b) Particulate	SS(P=0.1)	Positive	7.9%
c) Total	NS		0.0%

The regression lines between chlorophyll and complexed manganese were not calculated because of the high frequency of zero results for this fraction observed in each reservoir.

Although particulate manganese cannot be considered a limiting nutrient, the above results do indicate that this fraction accounts for a significant proportion of the variance in chlorophyll concentration in Cropston and Swithland reservoirs. The variance in chlorophyll concentration accounted for by the concentration of total manganese, although statistically significant in all reservoirs, is probably due almost entirely to the particulate component.

On three occasions in Thornton reservoir (June, August and September 1974) and on two occasions in Swithland reservoir (May and June 1974)

the concentration of soluble manganese decreased as the concentration of chlorophyll increased (see Figure 20), suggesting that soluble manganese may have been acting as a limiting nutrient at these times.

Soluble manganese showed a distinct seasonal variation in all three reservoirs with a peak on 24 August 1974 in Swithland and Thornton reservoirs and a peak two weeks later in Cropston reservoir. Particulate manganese showed a distinct seasonal cycle in Swithland and Thornton reservoirs, but not in Cropston reservoir, with a peak on 20 July 1974 which, therefore preceded the peak in soluble manganese concentration by four weeks.

3.1.1.5 Copper†

The results of copper determinations on reservoir water samples over the two year period of the investigation are summarized in Table 20 and illustrated graphically in Figures 22 to 25. Chlorophyll data are incorporated into the graphs to facilitate visual assessment of the association between the two variables.

Table 20 shows that the mean concentrations of particulate and total copper follow the same rank order as chlorophyll and dissolved vitamin B_{12}, that is Cropston reservoir < Swithland reservoir < Thornton reservoir. With the exception of complexed copper, the mean concentrations of all other copper fractions, like the mean concentrations of chlorophyll, phaeophytin, dissolved and particulate vitamin B_{12}, all iron and manganese fractions, were higher in Thornton reservoir than in either Cropston or Swithland reservoirs. The statistical significance of the differences between reservoirs in the mean concentrations of the copper fractions was tested by the method described in section 2.9.4a with the following results:-

Difference Between	Copper Fraction			
	Soluble	Complexed	Particulate	Total
Cropston and Swithland reservoirs	NS	NS	SS(P=0.01)	NS*
Cropston and Thornton reservoirs	SS(P=0.02)	NS	SS(P=0.01)	SS(P=0.001)
Swithland and Thornton reservoirs	SS(P=0.01)	NS	NS	SS(P=0.01)

* Key:- NS – Not (statistically) Significant
SS – Statistically Significant, probability level (P) in brackets

Linear regression analysis was used to test the degree of association between the individual results of copper and chlorophyll determinations. The chlorophyll data was first transformed as described in section

† see addendum

Table 20

Summary of Results of Determinations of Copper in Reservoir Water Samples taken in the period April 1973 to March 1975

Copper (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
a) Soluble			
Mean	16.7	16.4	25.6
Range	0-130	0-56	4-157
Standard Deviation	18.1	10.8	24.3
Coefficient of Variation	108%	66%	95%
b) Complexed			
Mean	1.4	1.6	1.5
Range	0-12	0-11	0-14
Standard Deviation	2.6	2.6	3.1
Coefficient of Variation	186%	163%	208%
c) Particulate			
Mean	4.5	7.9	9.4
Range	0-27	0-43	0-88
Standard Deviation	5.8	7.7	12.2
Coefficient of Variation	128%	98%	131%
d) Total			
Mean	23.5	26.4	38.1
Range	2-136	6-71	8-158
Standard Deviation	20.4	13.4	28.4
Coefficient of Variation	87%	51%	75%

Figure 22 Temporal Variations of Soluble Copper and Chlorophyll (dotted line) 1973 / 1974.

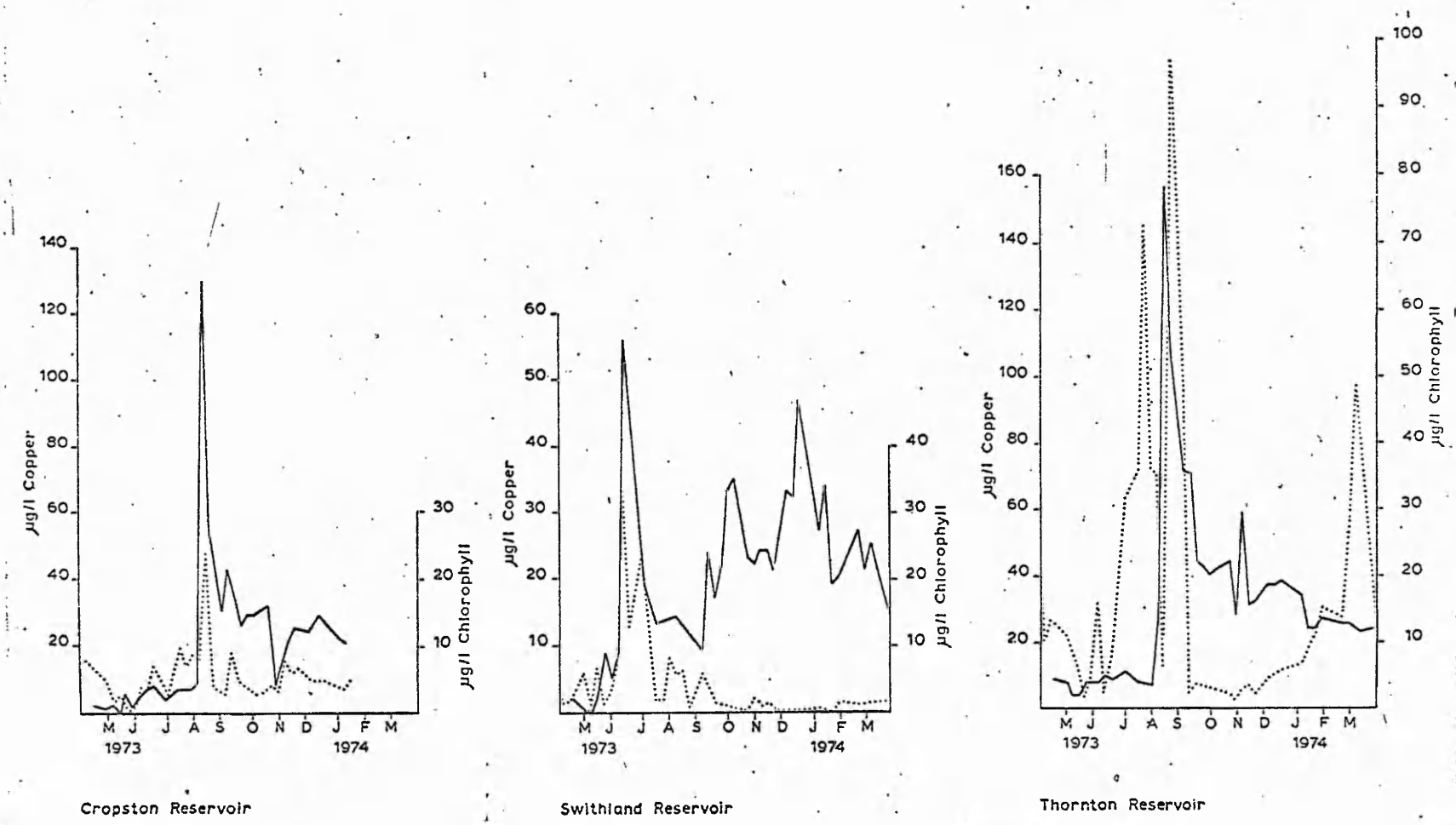
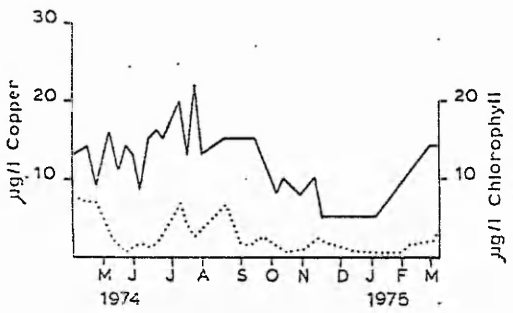
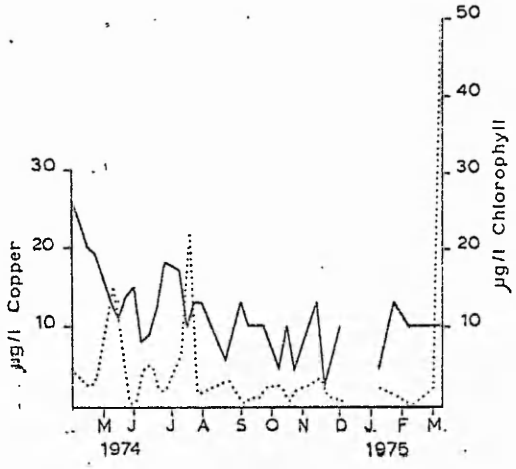


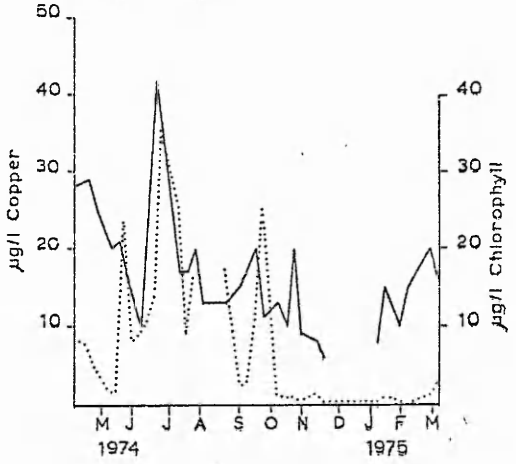
Figure 23 Temporal Variations of Soluble Copper and Chlorophyll (dotted line) 1974/1975



Cropston Reservoir



Swithland Reservoir



Thornton Reservoir

Figure 24 Temporal Variations of Particulate Copper and Chlorophyll (dotted line) 1973/1974

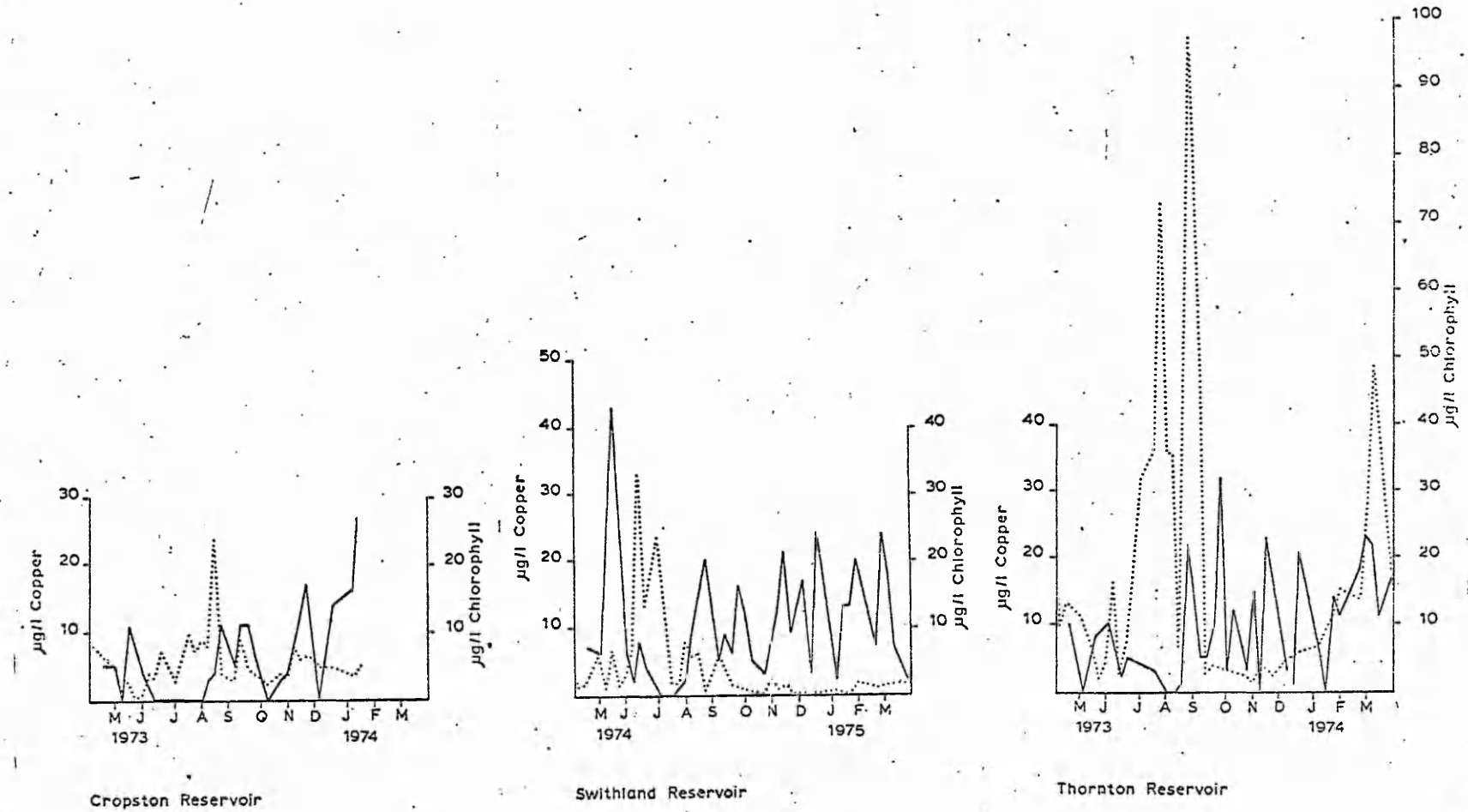
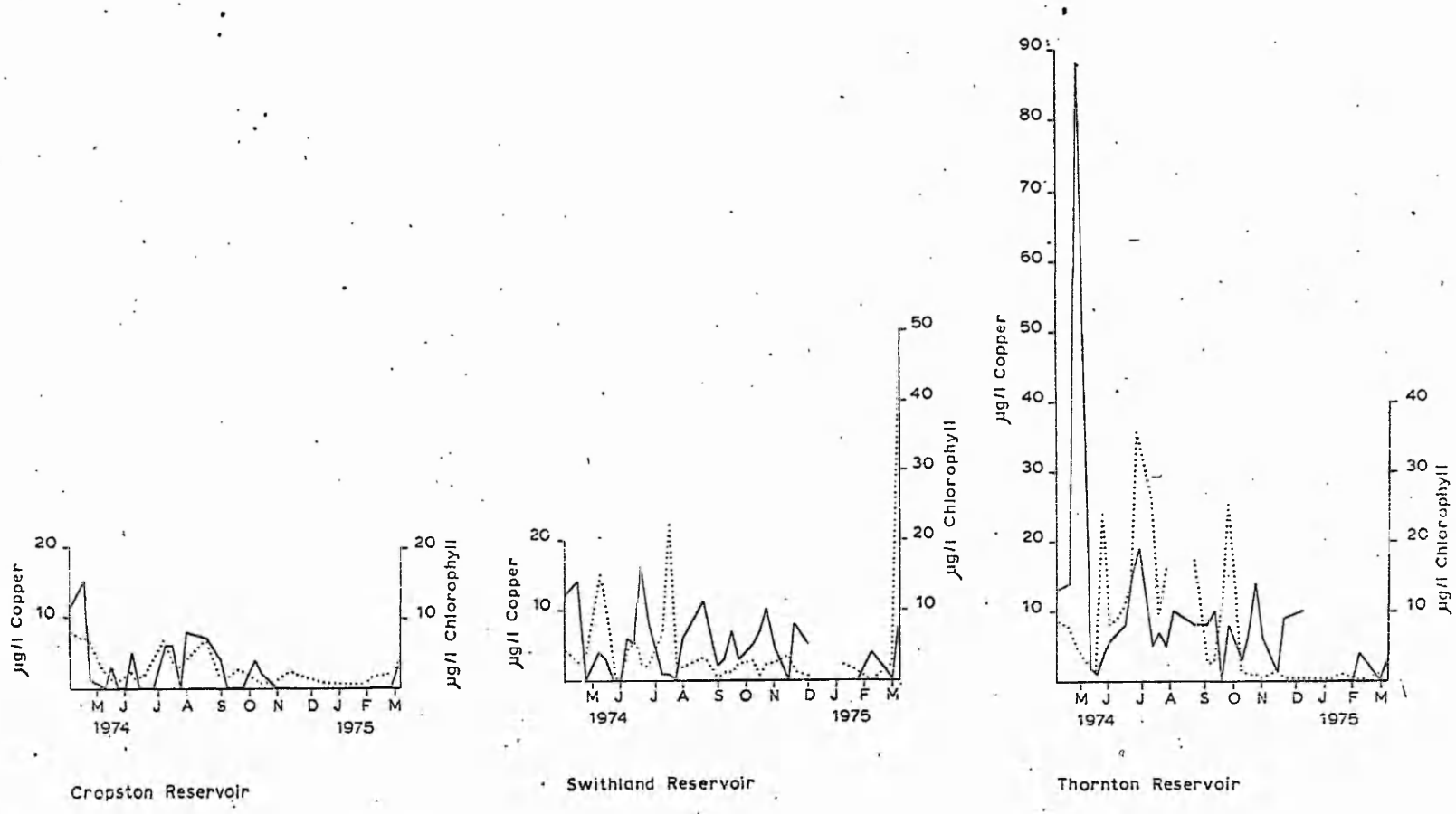


Figure 25 Temporal Variations of Particulate Copper and Chlorophyll (dotted line) 1974/1975



3.1.1.2.1. The statistical significance of the calculated regression line was assessed by determining whether the slope of the line was significantly different from zero. The percentage of the variance in the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5. The regression lines between chlorophyll and complexed copper were not calculated because of the high frequency of zero results for this fraction observed in each reservoir. The following results were obtained:-

Reservoir and Copper fraction	Regression between Chlorophyll and Copper		
	Slope	Sign of Slope	R^2
CROPSTON			
a) Soluble	SS(P=0.002)	Positive	15.5%
b) Particulate	SS(P=0.02)	Positive	11.3%
c) Total	SS(P=0.001)	Positive	22.4%
SWITHLAND			
a) Soluble	SS(P=0.02)	Negative	9.5%
b) Particulate	NS		2.5%
c) Total	SS(P=0.02)	Negative	10.0%
THORNTON			
a) Soluble	NS		2.8%
b) Particulate	NS		1.6%
c) Total	SS(P=0.05)	Positive	6.4%

Although copper cannot be considered a limiting nutrient in Cropston reservoir, the above results do indicate that both fractions of this micronutrient account for a significant proportion of the variance in chlorophyll concentration. In Swithland reservoir, however, the results suggest that soluble copper may have been acting as a limiting nutrient during the study period. It is interesting to note that in Thornton reservoir where the mean concentration of soluble copper was significantly higher than that in Swithland reservoir, the regression of

soluble copper on chlorophyll was not statistically significant.

However, a major cause of temporal variation in the concentration of copper in the reservoirs was caused by applications of copper sulphate to control the growth (or expected growth) of blue green algae. This fact may go some way in explaining the significant positive associations observed between copper and chlorophyll concentrations in Cropston and Thornton reservoirs.

3.1.1.6 Zinc †

The results of zinc determinations on reservoir water samples over the two year period of the investigation are summarized in Table 21 and illustrated graphically in Figures 26 to 29. Chlorophyll data are incorporated into these graphs to facilitate visual assessment of the association between the two variables.

Table 21 shows that the mean concentration of particulate zinc follows the same rank order as chlorophyll, dissolved vitamin B_{12} and total and particulate copper, that is Cropston reservoir < Swithland reservoir < Thornton reservoir. The mean concentrations of particulate and total zinc, like the mean concentrations of chlorophyll; phaeophytin; dissolved and particulate vitamin B_{12}; iron; manganese and soluble, particulate and total copper, were higher in Thornton reservoir than in either Cropston or Swithland reservoirs. The statistical significance of the differences between reservoirs in the mean concentrations of the zinc fractions was tested by the method described in section 2.9.4a with the following results:-

Difference Between	Zinc Fraction			
	Soluble	Complexed	Particulate	Total
Cropston and Swithland reservoirs	NS	NS	NS	NS*
Cropston and Thornton reservoirs	NS	NS	SS(P=0.02)	SS(P=0.1)
Swithland and Thornton reservoirs	NS	NS	SS(P=0.1)	SS(P=0.1)

* Key:- NS - Not (statistically) Significant
SS - Statistically Significant, probability level (P) in brackets.

Linear regression analysis was used to test the degree of association between the individual results of zinc and chlorophyll determinations. The chlorophyll data was first transformed as described in section 3.1.1.2.1. The statistical significance of the calculated regression

† see addendum

Table 21

Summary of Results of Determinations of Zinc in Reservoir Water Samples taken in the Period April 1973 to March 1975

Zinc (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
a) Soluble			
Mean	5.9	5.0	5.5
Range	0-20	0-21	0-15
Standard Deviation	4.0	4.1	3.9
Coefficient of Variation	68%	82%	71%
b) Complexed			
Mean	1.6	2.4	2.5
Range	0-19	0-15	0-24
Standard Deviation	3.0	3.0	3.9
Coefficient of Variation	188%	125%	156%
c) Particulate			
Mean	2.3	4.8	20.1
Range	0-21	0-115	0-344
Standard Deviation	4.8	17.2	55.0
Coefficient of Variation	209%	358%	274%
d) Total			
Mean	11.6	11.8	23.7
Range	3-103	1-125	3-350
Standard Deviation	14.2	17.7	50.0
Coefficient of Variation	122%	150%	211%

Figure 26 Temporal Variations of Soluble Zinc and Chlorophyll (dotted line) 1973/1974

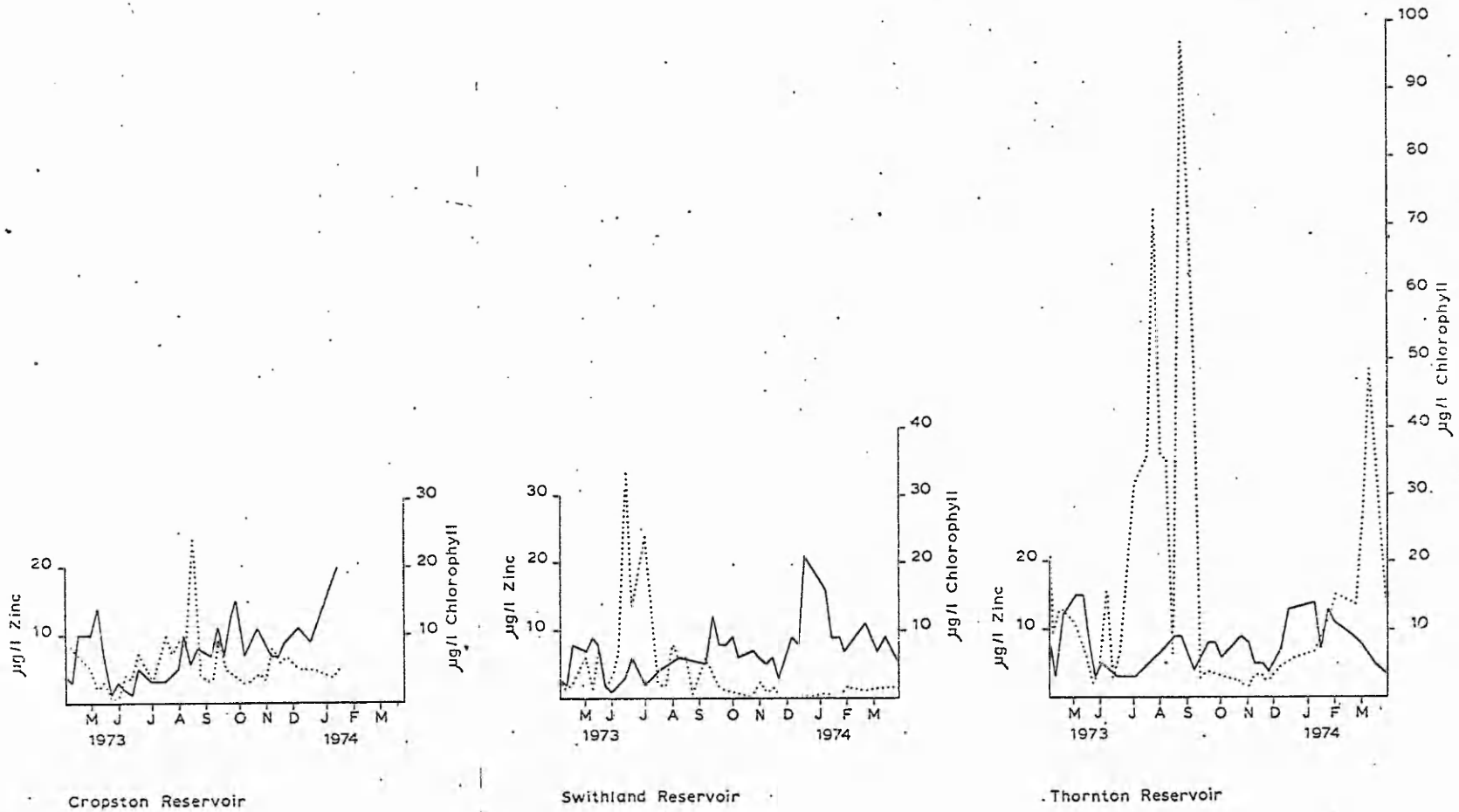


Figure 27 Temporal Variations of Soluble Zinc and Chlorophyll (dotted line) 1974/1975

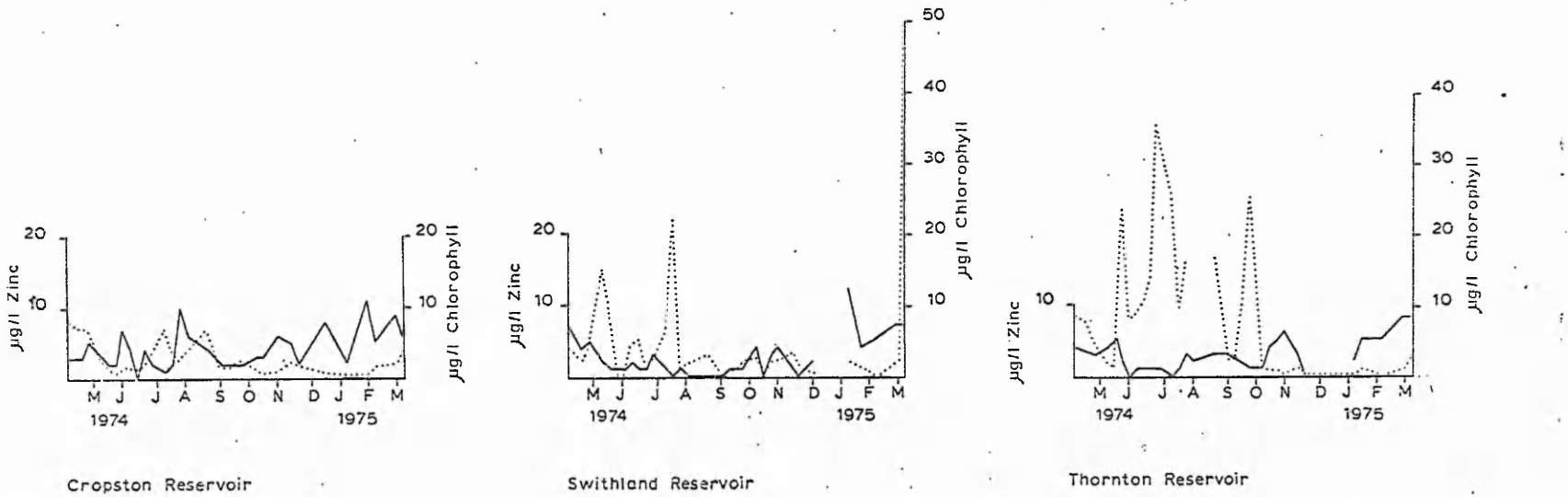


Figure 28 Temporal Variations of Particulate Zinc and Chlorophyll (dotted line) 1973/1974

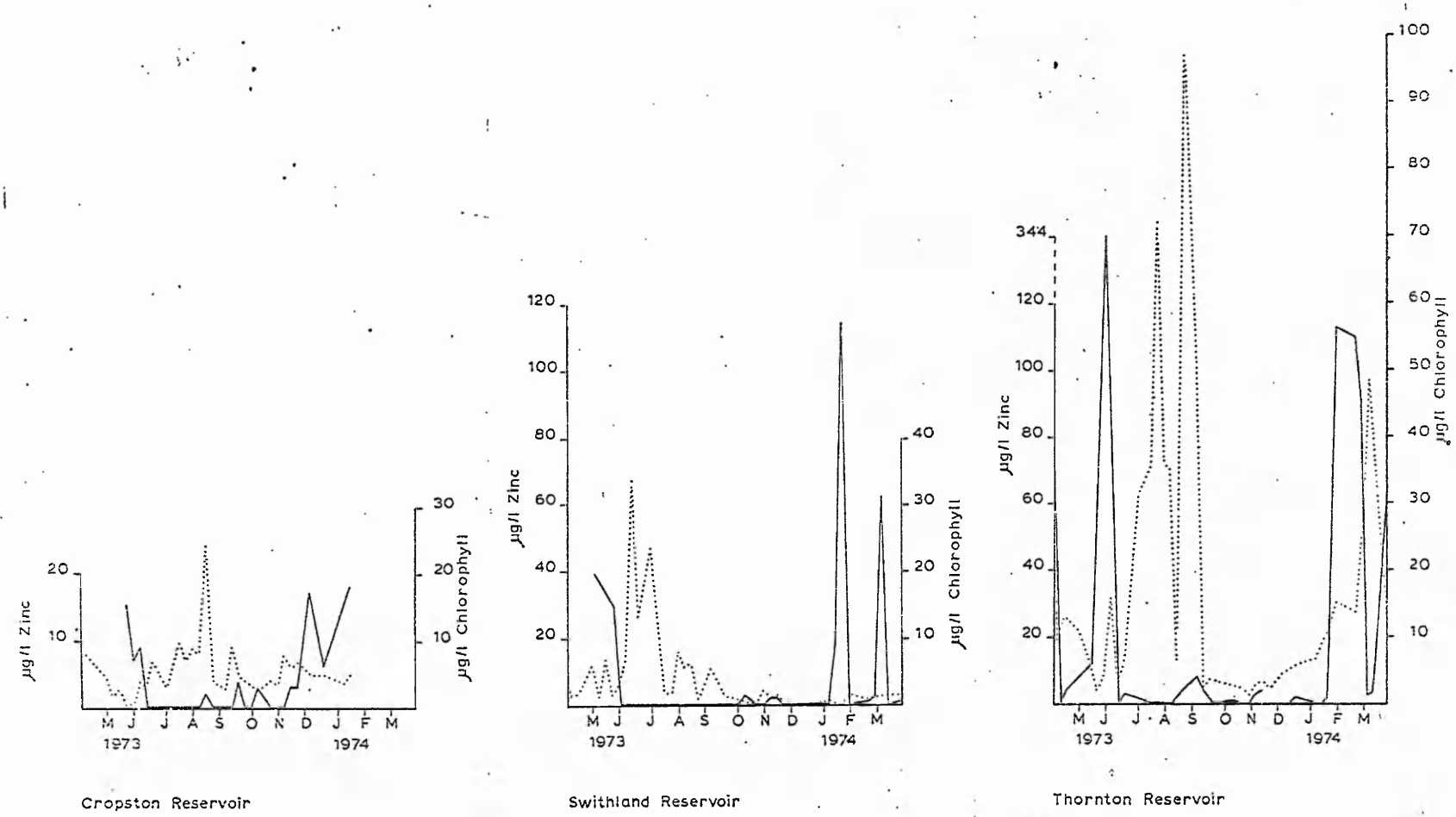
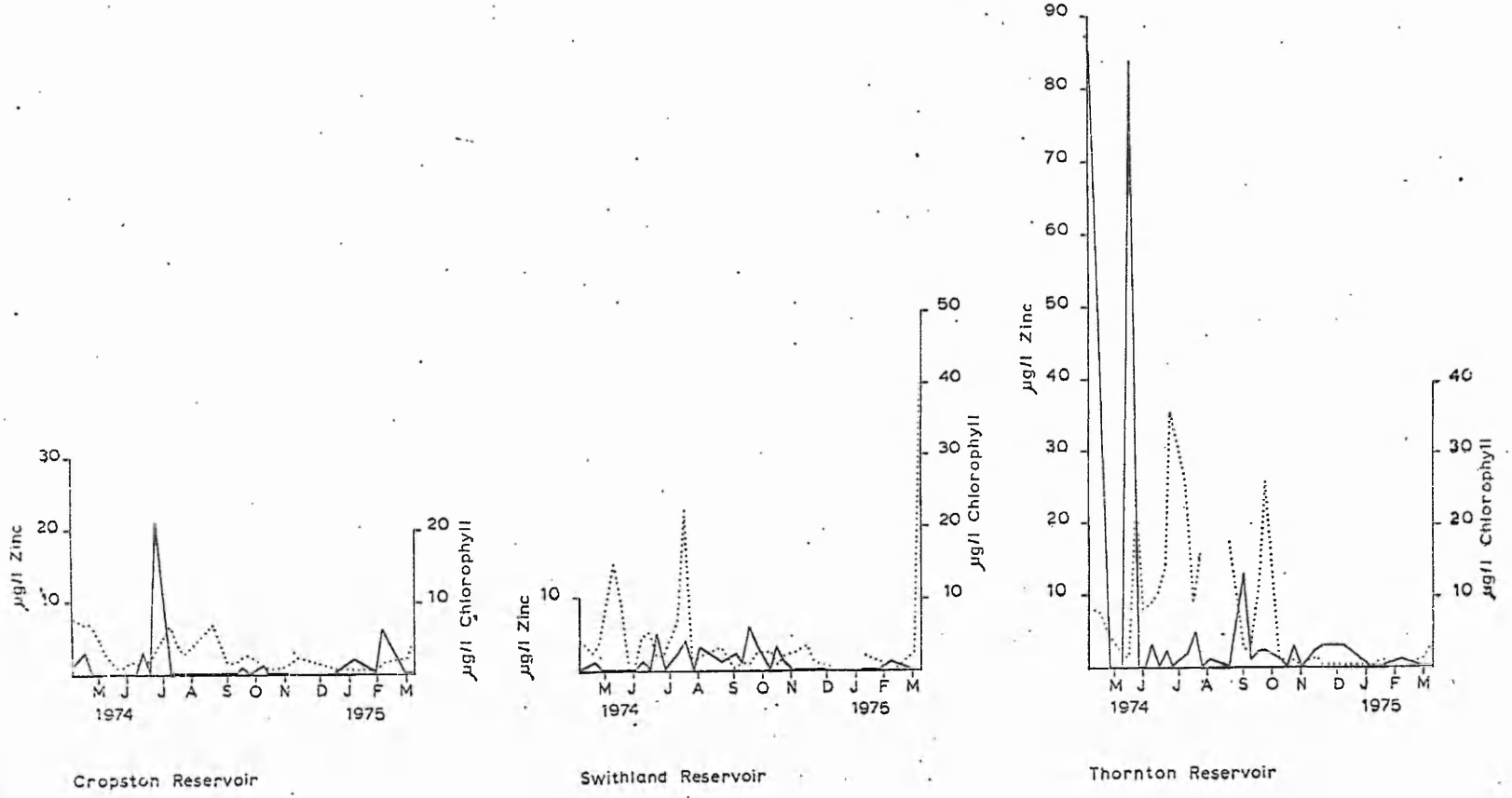


Figure 29 Temporal Variations of Particulate Zinc and Chlorophyll (dotted line)



line was assessed by determining whether the slope of the line was significantly different from zero. The percentage of the variance in the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5. The regression lines between chlorophyll and complexed zinc were not calculated because of the high frequency of zero results for this fraction observed in each reservoir. The following results were obtained:-

Reservoir and Zinc Fraction	Regression between Chlorophyll and Zinc		
	Slope	Sign of Slope	R^2
CROPSTON			
a) Soluble	SS(P=0.1)	Positive	5.3%
b) Particulate	NS		1.3%
c) Total	NS		0.0%
SWITHLAND			
a) Soluble	SS(P=0.01)	Negative	12.2%
b) Particulate	SS(P=0.1)	Negative	5.5%
c) Total	SS(P=0.02)	Negative	9.5%
THORNTON			
a) Soluble	NS		0.9%
b) Particulate	NS		0.4%
c) Total	NS		0.6%

In Swithland reservoir the results suggest that zinc may have been acting as a limiting nutrient during the study period. It is interesting to note that in Thornton reservoir, where the mean concentrations of particulate and total zinc were significantly higher than those in Swithland reservoir, the regression of zinc on chlorophyll was not statistically significant.

No evidence of any consistent seasonal variation in the concentration of zinc was observed in the reservoirs.

3.1.1.7 Cobalt†

The results of cobalt determinations on reservoir water samples over the two year period of the investigation are summarized in Table 22 and illustrated graphically in Figures 30 and 31. Chlorophyll data are incorporated into these graphs to facilitate visual assessment of the association between the two variables.

The analytical sensitivity of the method of determination prevented accurate differentiation of the three cobalt fractions measured, but soluble cobalt probably accounted for at least 90% of the total at all times and in all reservoirs. Table 22 shows that the mean concentration of total cobalt follows the same rank order as chlorophyll, dissolved vitamin B_{12}, total and soluble copper and particulate zinc, that is Cropston reservoir < Swithland reservoir < Thornton reservoir. The mean concentration of total cobalt, like the mean concentrations of chlorophyll; phaeophytin; dissolved and particulate vitamin B_{12}; iron; manganese; soluble, particulate and total copper; particulate and total zinc, was higher in Thornton reservoir than in either Cropston or Swithland reservoirs. The statistical significance of the differences between reservoirs in the mean total cobalt concentration were tested by the method described in section 2.9.4a with the following results:-

Difference Between	Total Cobalt
Cropston and Swithland reservoirs	SS*(P=0.01)
Cropston and Thornton reservoirs	SS(P=0.001)
Swithland and Thornton reservoirs	SS(P=0.05)

* Key:- NS - Not (statistically) Significant
SS - Statistically Significant, probability level (P) in brackets

Linear regression analysis was used to test the degree of association between the individual results of zinc and chlorophyll determinations. The chlorophyll data was first

† see addendum

Figure 30 Temporal Variations of Total Cobalt and Chlorophyll (dotted line) 1973/1974

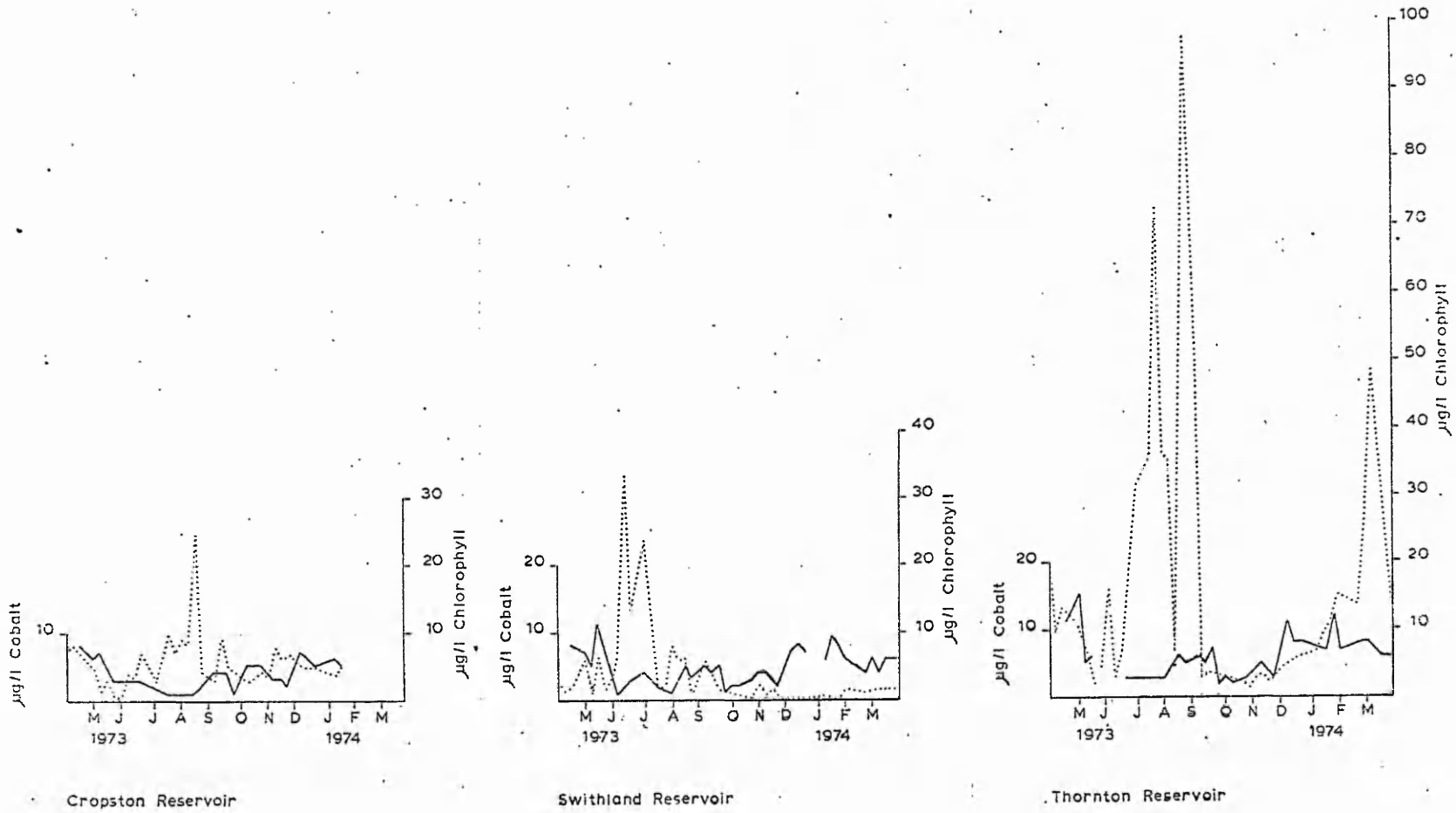
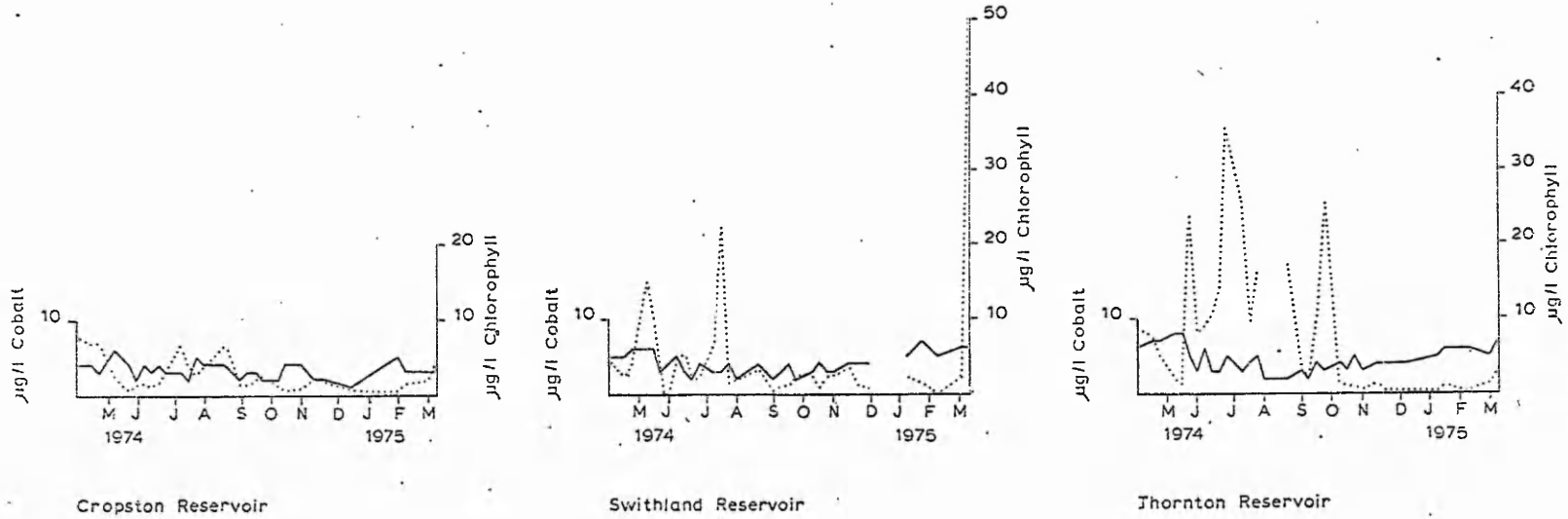


Figure 31 Temporal Variations of Total Cobalt and Chlorophyll (dotted line) 1974/1975



transformed as described in section 3.1.1.2.1. The statistical significance of the calculated regression line was assessed by determining whether the slope of the line was significantly different from zero. The percentage of the variance in the chlorophyll data accounted for by the regression (R^2) was also calculated, see section 2.9.5. The following results were obtained:-

Reservoir	Regression between Chlorophyll and Cobalt		
	Slope	Sign of Slope	R^2
Cropston	NS		0.0%
Swithland	NS		2.9%
Thornton	NS		1.0%

No evidence of any consistent seasonal variation in the concentration of total cobalt was observed in the reservoirs.

Table 22

Summary of Results of Determinations of Cobalt in Reservoir Water Samples taken in the period April 1973 to March 1975

Total Cobalt (µg/l)	RESERVOIR		
	Cropston	Swithland	Thornton
Mean	3.6	4.4	5.3
Range	1-8	1-11	2-15
Standard Deviation	1.6	2.0	2.6
Coefficient of Variation	44%	45%	49%

3.1.1.8 Thiamine and Biotin

Dissolved thiamine and biotin were consistently undetectable by the bioassay organisms used. Only a limited number of bioassays for thiamine with M.lutheri were made, but all failed to detect the vitamin in the reservoir water, despite the greater sensitivity of this method compared with bioassays using L.viridescens. These zero results suggest that the concentration of these vitamins in the reservoirs was less than the sensitivities of the methods - 5ng/l for thiamine with M.lutheri, 50ng/l for thiamine with L.viridescens and 1ng/l for biotin, since the reservoir waters were not found to be inhibitory to the organisms used (as shown by results from internal standards).

3.1.1.9 Summary of the Results of the Reservoir Water Survey

The results presented in sections 3.1.1.1 to 3.1.1.7 show the mean concentration of chlorophyll, and the mean concentrations of one or more fractions of each of the micronutrients studied, to be significantly higher in Thornton reservoir than in either Cropston or Swithland reservoirs.

Of the micronutrients and micronutrient fractions measured, only particulate vitamin B_{12} and particulate manganese showed a consistent and statistically significant (positive) association with chlorophyll in all three reservoirs (as judged by linear regression analysis).

Linear regression analysis of the data also suggests soluble copper and soluble zinc to be limiting nutrients in Swithland reservoir, and soluble and complexed iron to be limiting nutrients in Thornton reservoir.

Dissolved vitamin B_{12} (*E.gracilis* and *L.leichmanii* assays), complexed iron and soluble manganese also showed occasional decreases in concentration in Swithland and Thornton reservoirs, at the same times as increases in chlorophyll concentration, suggesting that these micronutrients may, at times, act as limiting nutrients in these reservoirs.

3.1.2 Reservoir Sediments †

The results of micronutrient determinations on reservoir sediment samples taken in the period December 1974 to March 1975 are summarized in Table 23.

Three layers in the sediment cores could typically be distinguished. The first layer at the sediment/water interface was usually only a few millimetres thick and consisted of easily disturbed, flocculent, coarse particulate matter. This layer overlaid a second layer (B), typically about 2cm thick, of soft brown clayey material. This layer could be distinguished only from the third layer (C), of dark grey clayey mud which it overlaid, in Swithland and Thornton reservoirs. The lack of any visual differentiation between the B and C layers in Cropston reservoir is reflected by the absence of any statistically significant difference between the mean concentrations of the metal micronutrients in the two layers; see Table 24.

All micronutrients, with the exception of copper in Swithland reservoir, show no statistically significant difference between reservoirs in their mean concentration in the C sediment layer. The mean concentrations of vitamin B_{12}, iron, manganese and copper in the B sediment layer of Swithland reservoir are significantly higher than those in Cropston or Thornton reservoirs, see Table 25.

† see addendum

Table 23

Summary of Results of Micronutrient Determinations on Reservoir Sediment Samples

Micro-nutrient	Reservoir	Sediment layer	Micronutrient Statistics *			
			Mean Conc.	Range of Conc.	S	V
Vitamin B_{12} (ng/g)	Cropston	B	49	37-59	11.2	23%
		C	34	33-35	1.0	3%
	Swithland	B	436	397-485	44.8	10%
		C	38	32-50	10.4	27%
	Thornton	B	239	136-375	122.9	51%
		C	58	9-87	42.9	74%
Iron (mg/g)	Cropston	B	45	43-47	2.1	5%
		C	42	39-52	8.8	21%
	Swithland	B	38	37-39	0.8	2%
		C	36	32-39	3.7	10%
	Thornton	B	47	41-52	5.4	12%
		C	43	30-57	19.2	45%
Manganese (mg/g)	Cropston	B	1.2	1.1-1.2	0.04	3%
		C	0.9	0.7-1.2	0.22	24%
	Swithland	B	2.9	2.3-3.5	0.58	20%
		C	0.8	0.7-0.9	0.10	13%
	Thornton	B	1.1	1.0-1.2	0.06	6%
		C	0.7	0.5-0.9	0.27	39%
Copper (µg/g)	Cropston	B	209	203-219	8.5	4%
		C	179	109-243	67.2	38%
	Swithland	B	915	779-1005	119.8	13%
		C	487	413-557	72.1	15%
	Thornton	B	684	622-788	90.6	13%
		C	296	77-515	309.7	105%

Continued

Table 23 (Continued)

Micro-nutrient	Reservoir	Sediment layer	Micronutrient Statistics *			
			Mean Conc.	Range of Conc.	S	V
Zinc ($\mu g/g$)	Cropston	B	294	283-300	9.3	3%
		C	281	253-325	39.0	14%
	Swithland	B	361	347-370	12.3	3%
		C	250	215-275	31.4	13%
	Thornton	B	355	326-389	31.8	9%
		C	186	113-259	103.2	56%
Cobalt ($\mu g/g$)	Cropston	B	23	22-24	1.2	5%
		C	21	19-24	2.9	14%
	Swithland	B	23	20-26	3.1	14%
		C	17	15-19	2.0	12%
	Thornton	B	24	22-26	2.0	8%
		C	21	15-27	8.5	41%

* Key S - Standard deviation

V - Coefficient of variation

Table 24

Statistical Significance of Differences (within reservoirs) Between the Mean Sediment Micronutrient Concentrations in the 'B' and 'C' Layers

Micronutrient	Reservoir		
	Cropston	Swithland	Thornton
Vitamin B_{12}	SS*(P=0.1)	SS(P=0.001)	SS(P=0.1)
Iron	NS	NS	NS
Manganese	NS	SS(P=0.01)	SS(P=0.1)
Copper	NS	SS(P=0.01)	NS
Zinc	NS	SS(P=0.01)	SS(P=0.1)
Cobalt	NS	SS(P=0.1)	NS

* Key NS - Not (statistically) Significant

SS - Statistically Significant, probability level (P) in brackets.

Table 25

Statistical Significance of Differences Between Reservoirs in Mean Sediment Micronutrient Concentrations

Difference Between	Sediment Layer	Micronutrient					
		Vitamin B_{12}	Iron	Manganese	Copper	Zinc	Cobalt
Cropston and Swithland reservoirs	B	SS* (P=0.001)	SS (P=0.01)	SS (P=0.01	SS (P=0.001)	SS (P=0.002	NS
	C	NS	NS	NS	SS (P=0.01)	NS	NS
Cropston and Thornton reservoirs	B	SS (P=0.1)	NS	NS	SS (P=0.001)	SS (P=0.05)	NS
	C	NS	NS	NS	NS	NS	NS
Swithland and Thornton reservoirs	B	SS (P=0.1)	SS (P=0.1)	SS (P=0.02)	SS (P=0.1)	NS	NS
	C	NS	NS	NS	NS	NS	NS

* Key NS - Not (statistically) Significant

SS - Statistically Significant, probability level (P) in brackets.

3.2 FEEDER STREAM SURVEY †

The results of micronutrient determinations on feeder stream water samples taken at monthly intervals between April 1974 and March 1975 are summarized in Table 26.

Calculations of flow rates along the brooks using the area-velocity method described in section 2.6 gave highly improbable results for the streams feeding Cropston and Swithland reservoirs. The reason for this was the difficulty of accurately measuring the small gradients along the streams. Unfortunately, the improbability of the results did not become apparent until figures for the volumes of water abstracted from the reservoirs during the study period became available from the Severn Trent Water Authority.

In order to be able to construct micronutrient budgets for the reservoirs, the minimum volume of water entering Cropston and Swithland reservoirs was calculated from the total of the volume abstracted and the net change in water volume in the reservoirs over the period April 1974 to March 1975. I am indebted to the Severn Trent Water Authority for these figures. Since similar calculations of the minimum volume of water entering Thornton reservoir were approximately 85% of that estimated by the area-velocity method, the actual volume of water entering Cropston and Swithland reservoirs was calculated as 100/85 times the minimum volume. The resulting water budgets for the reservoirs are presented in Table 27. It should be noted that the volume of water abstracted from Cropston reservoir includes the volume abstracted from Swithland reservoir since these reservoirs are operated together for water supply purposes.

Micronutrient budgets for the reservoirs were then calculated from the data presented in Tables 15, 18, 19, 20, 21, 22, 26 and 27. The results are presented in Table 28. The mean micronutrient concentrations of water entering the reservoirs are presented in Table 29. It is realized that Tables 28 and 29 are based on

† see addendum

Table 26

Summary of Results of Micronutrient Determinations on Feeder Stream Water Samples

Dissolved Vitamin B_{12} (ng/l)	Name of Brook			
	Bradgate	Swithland	Stanton	Markfield
a) *E.gracilis*				
Mean	30.0	16.4	30.4	28.0
Range	12-56	6-34	11-78	13-38
S*	14.2	8.5	18.4	8.0
V	47%	52%	61%	28%
b) *L.leichmanii*				
Mean	15.0	12.4	25.6	18.7
Range	2-38	4-38	5-50	9-39
S	11.1	9.7	15.5	9.4
V	74%	78%	61%	50%
c) *O.malhamensis*				
Mean	2.68	2.71	3.04	2.46
Range	1.0-8.0	1.0-7.6	1.4-5.4	1.2-4.6
S	2.01	2.19	1.62	1.03
V	75%	81%	53%	42%

Continued

Table 26 (Continued)

Micronutrient (µg/l)	Name of Brook			
	Bradgate	Swithland	Stanton	Markfield
Iron				
Mean	564	326	467	468
Range	132-3400	82-1260	122-1080	111-1460
S*	945	354	282	395
V	168%	109%	60%	84%
Manganese				
Mean	69.7	34.0	57.3	46.7
Range	33-316	18-79	22-87	28-138
S	87.1	17.4	17.6	33.7
V	125%	51%	31%	72%
Copper				
Mean	10.6	13.4	14.4	13.4
Range	3-17	6-26	2-27	7-19
S	4.7	6.8	7.2	4.1
V	45%	51%	50%	30%
Zinc				
Mean	11.5	7.3	7.9	9.0
Range	3-70	4-19	4-18	4-18
S	19.5	4.4	4.4	5.2
V	170%	61%	55%	58%
Cobalt				
Mean	4.4	4.9	6.7	6.3
Range	0-6	4-7	4-10	4-9
S	1.6	1.3	2.0	2.2
V	37%	26%	30%	34%

* Key S - Standard deviation

V - Coefficient of variation

Table 27

The Water Budgets of Cropston, Swithland and Thornton Reservoirs between April 1974 and March 1975

	RESERVOIR		
	Cropston	Swithland	Thornton
Volume abstracted $x10^6$ m^3	6.09	2.48	2.07
Net change in reservoir volume $x10^6$ m^3	+2.20	+0.08	+0.24
Minimum input volume $x10^6$ m^3	5.54	2.56	2.31
Estimated 'actual' input volume $x10^6$ m^3	6.54	3.02	2.76
Relative contributions to 'actual' input volume from feeder streams $x10^6$ m^3	6.54 (Bradgate Brook)	3.02 (Swithland Brook) 0.00* (Cropston Brook)	1.02 (Stanton Brook) 1.74 (Markfield Brook)

* Contibution from Cropston Brook assumed negligible since Cropston reservoir did not overflow during the study period.

Table 28

Micronutrient Budgets for the Reservoirs from April 1974 to March 1975

	grams		kilograms									
	Dissolved Vitamin B_{12} (Euglena assay)		Total Metal									
			Iron		Manganese		Copper		Zinc		Cobalt	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
Cropston Reservoir	281	158	1601	944	459	530	141	110	54	43	36	18
Net Change	123		657			71	31		11		18	
Swithland Reservoir	50	81	985	156	103	151	41	49	22	14	15	10
Net Change		31	829			48		8	8		5	
Thornton Reservoir	80	66	1292	462	140	239	39	63	24	25	18	9
Net Change	14		830			101		24		1	9	

Table 29

Mean Micronutrient Concentrations in the Water Entering the Reservoirs in April 1974 to March 1975

	ng/l Dissolved Vitamin B_{12} (*Euglena* assay)	µg/l Total Metal				
		Iron	Manganese	Copper	Zinc	Cobalt
Cropston Reservoir	31	221	47	14	6	4
Swithland Reservoir	16	326	34	13	7	5
Thornton Reservoir	29	467	51	14	9	7

Table 30

Statistical Significance of Differences between Feeder Streams in Mean Micronutrient Concentrations

Difference Between	Micronutrient					
	Vitamin B_{12} (*E.gracilis*)	Iron	Manganese	Copper	Zinc	Cobalt
Stanton and Markfield Brooks	NS	NS	NS	NS	NS	NS
Stanton and Swithland Brooks	SS (P=0.05)	NS	SS (P=0.01)	NS	NS	SS (P=0.05)
Stanton and Bradgate Brooks	NS	SS (P=0.1)	SS (P=0.05)	NS	NS	SS (P=0.01)
Markfield and Swithland Brooks	SS (P=0.01)	NS	NS	NS	NS	SS (P=0.1)
Markfield and Bradgate Brooks	NS	NS	NS	NS	NS	SS (P=0.05)
Swithland and Bradgate Brooks	NS	NS	NS	NS	NS	NS

inadequate data, since the number of samples taken was small, since the flow rate and micronutrient concentration probably varied a great deal each month, and because the measurement of flow rates in the feeder streams proved very difficult. It is however believed that some meaningful conclusions can be drawn from the results. Table 29 shows that with the exception of vitamin B_{12} the mean concentrations of all micronutrients entering Swithland reservoir were less than those entering Thornton reservoir. The statistical significance of the differences between feeder streams in mean micronutrient concentrations are shown in Table 30 and were assessed by the methods described in sections 2.9.4b and 2.9.4c.

Table 28 shows that iron and cobalt accumulated in all three reservoirs in 1974 to 1975, whereas manganese showed a net loss.

3.3 LABORATORY EXPERIMENTS WITH ALGAL ISOLATES

Laboratory experiments on algal isolates from the reservoirs were designed to determine the effect of micronutrient concentration on the growth of these isolates. Unialgal cultures of Chlorella sp. and Scenedesmus quadricauda (Turpin) Brébisson were isolated from Thornton reservoir. These green algae were frequently dominant members of the phytoplankton in all three reservoirs during the study period. It was hoped that it would also be possible to isolate a Fragilaria sp. (probably Fragilaria crotonensis (Edwards) Kitton from either Cropston or Swithland reservoirs, in which this alga was frequently dominant, and an Oocystis sp. which was commonly a dominant member of the phytoplankton in all three reservoirs. Unfortunately this proved impossible in the time available. Instead a culture of F.crotonensis L273 was generously given by the Freshwater Biological Association and a culture of Oocystis apiculata W.West was purchased from the National Culture Collection of Algae and Protozoa. Since the chemical compositions of the two media used in these experiments, EVT and Modified Wood's Hole MBL (see Section 2.8), differ considerably, for comparison purposes cultures of Chlorella L221 and S.quadricauda L232 isolated from lakes in the English Lake District were also generously given by the Freshwater Biological Association. These species are hereafter referred to in the text as Chlorella (FBA) and S.quadricauda (FBA).

The results of the reservoir water survey presented in Section 3.1.1. indicated that the concentrations of dissolved vitamin B_{12}, complexed iron and soluble manganese in the reservoirs sometimes decreased with increases in the concentration of chlorophyll, suggesting that at times these micronutrients may have limited the growth of algae in the reservoirs studied. In order to test this hypothesis, experiments were performed to test the separate effects of the concentrations of dissolved vitamin B_{12}, iron and manganese on the growth of the above-mentioned algal species. The method is described in Section 2.8.

3.3.1 Vitamin B_{12}

No dependence of growth on the concentration of vitamin B_{12} was detected for any of the species tested. Four serial transfers of the algae into media containing no added vitamin B_{12}, thiamine or biotin resulted in no depression of growth compared with controls grown in the complete media, suggesting that the algae tested are autotrophic. However, it must be noted that none of the unialgal cultures tested was axenic, although the concentration of bacteria in the cultures was extremely low. It is however possible that these bacteria could synthesise sufficient vitamin B_{12}, thiamine and biotin to enable the growth of auxotrophic algae in vitamin-free media, since Haines & Guillard (1974) showed that the vitamin B_{12} requirement of several marine diatoms could be satisfied by heterotrophic marine bacteria in vitamin B_{12}- limited laboratory cultures. However, the transfer experiment results agree with those summarized by Provasoli & Carlucci (1974) for Chlorella sp., Scenedesmus sp. and Fragilaria sp. It is not known whether O.opiculata is autotrophic or auxotrophic, but the green algae as a group are predominantly autotrophic (Provasoli & Carlucci 1974). However, it should be noted that the genus Fragilaria contains some species auxotrophic for vitamin B_{12}, and the genus Chlorella some species auxotrophic for thiamine (Provasoli & Carlucci 1974).

3.3.2 Iron

The results of experiments to determine the effect of iron concentration on the growth of the algae selected for study are presented graphically in Figures 32 to 37. Each point on each graph represents the average of duplicated cultures. Details of the inoculum and carryover of micronutrient in the inoculum are recorded on each graph. Iron-depleted inocula were prepared by subculturing the alga in an iron-free medium for 14 days under the conditions described in Section 2.8.

Figure 32 Effect of Iron Concentration on the Growth of *Chlorella* sp.

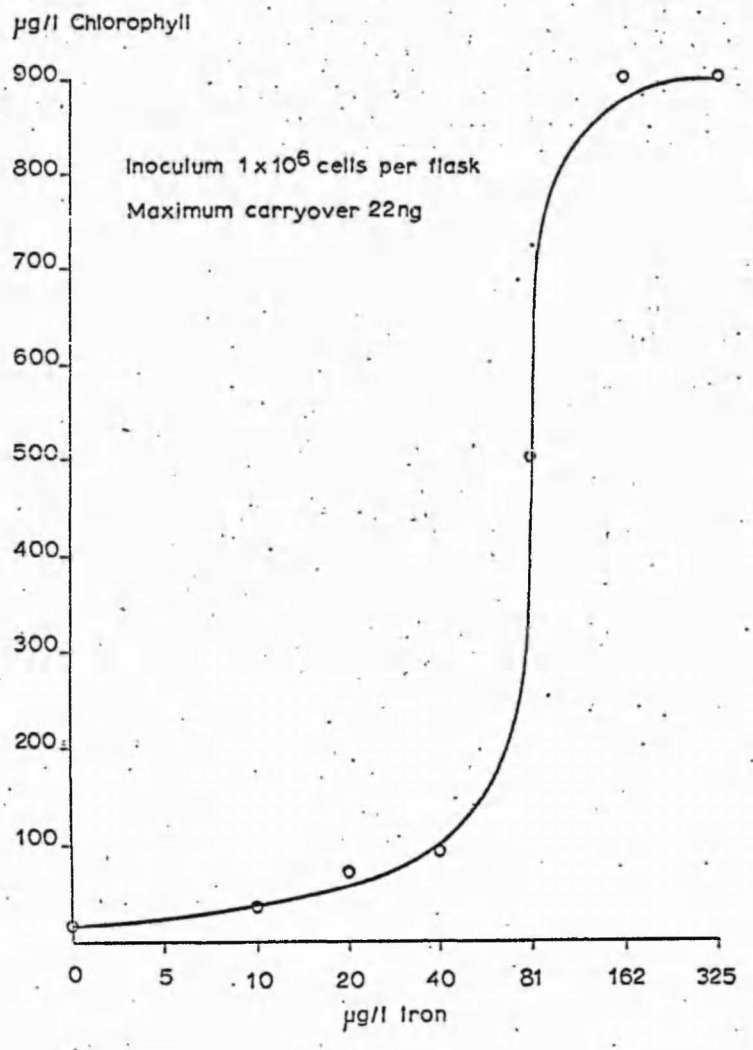


Figure 33 Effect of Iron Concentration on the Growth of *Chlorella* (FBA)

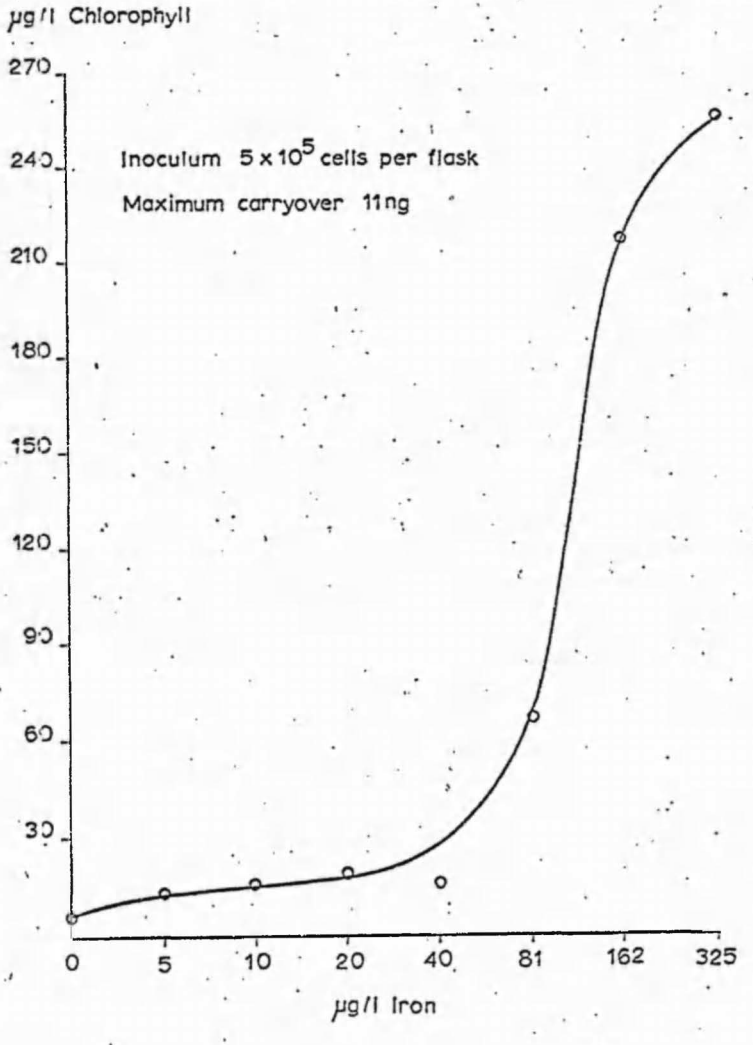


Figure 34 Effect of Iron Concentration on the Growth of *S.quadricauda* (FBA)

Inoculum 7×10^4 cells per flask

Maximum carryover 11ng

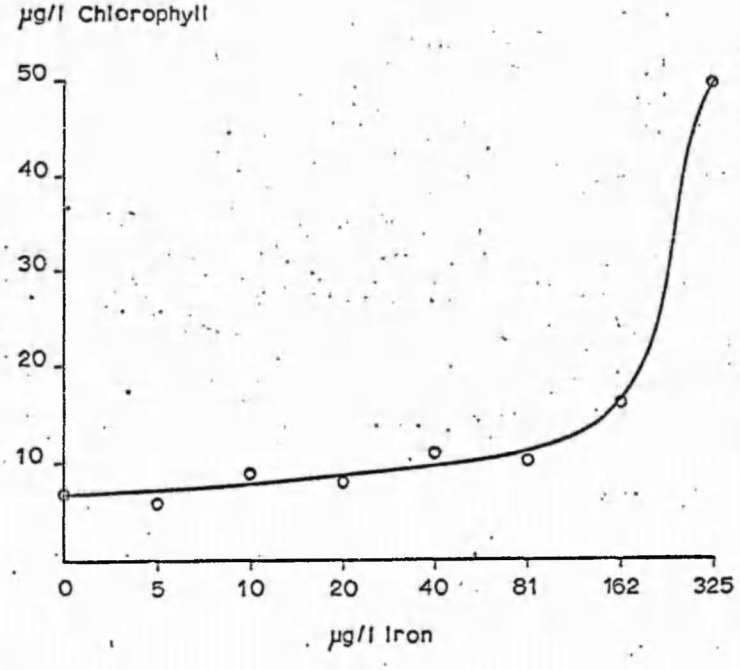


Figure 35 Effect of Iron Concentration on the Growth of *S.quadricauda*

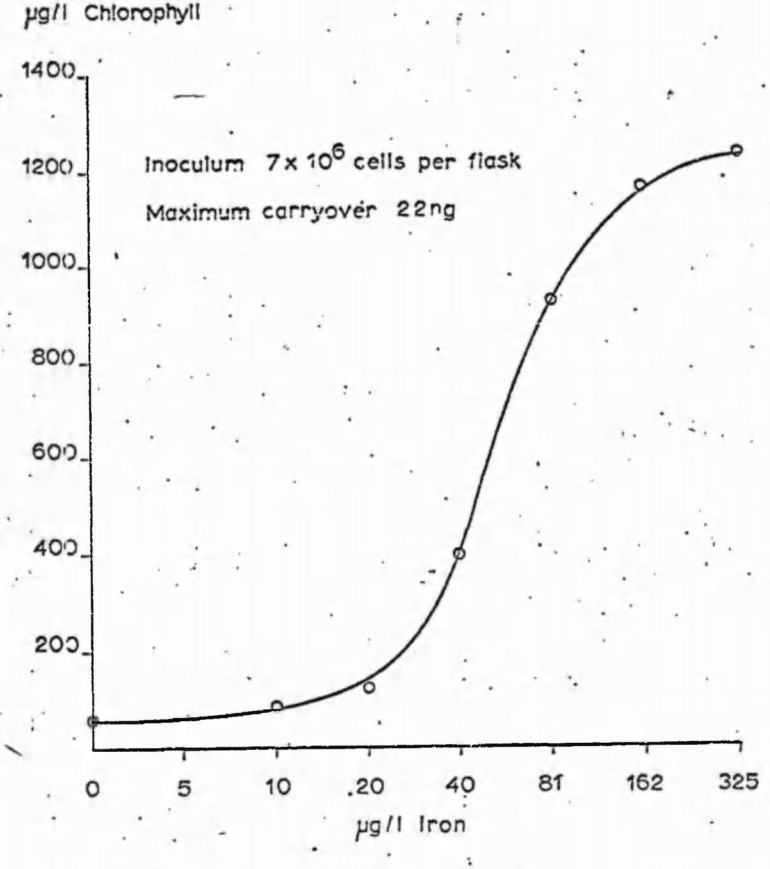


Figure 36 Effect of Iron Concentration on the Growth of *F.crotonensis*

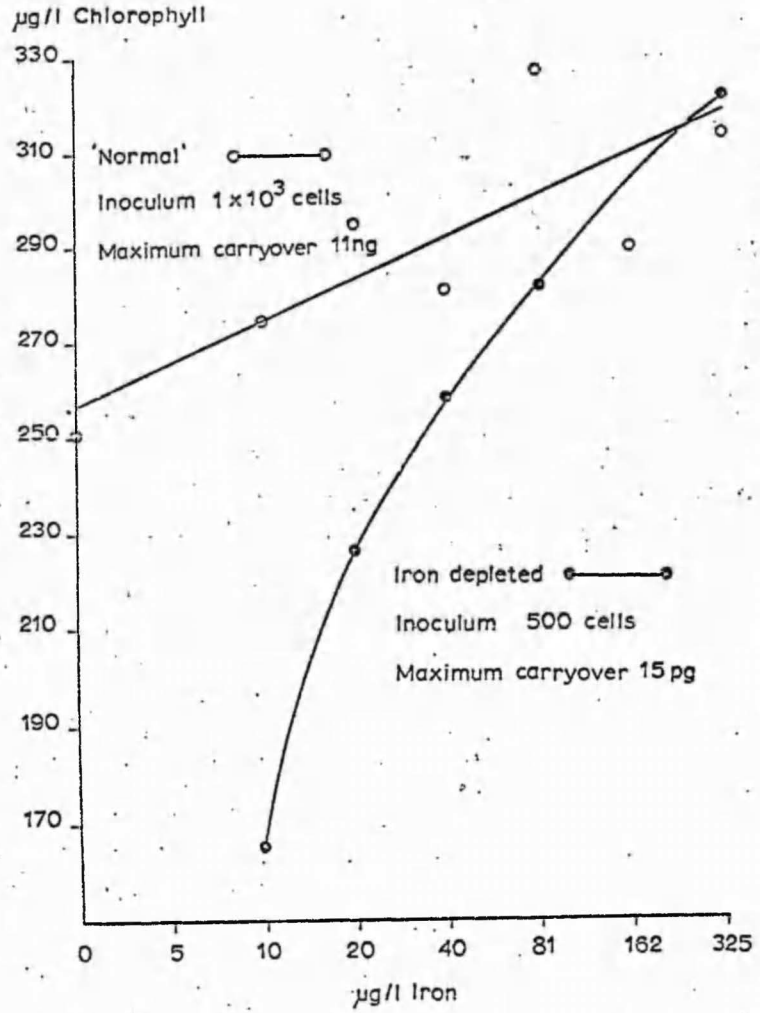
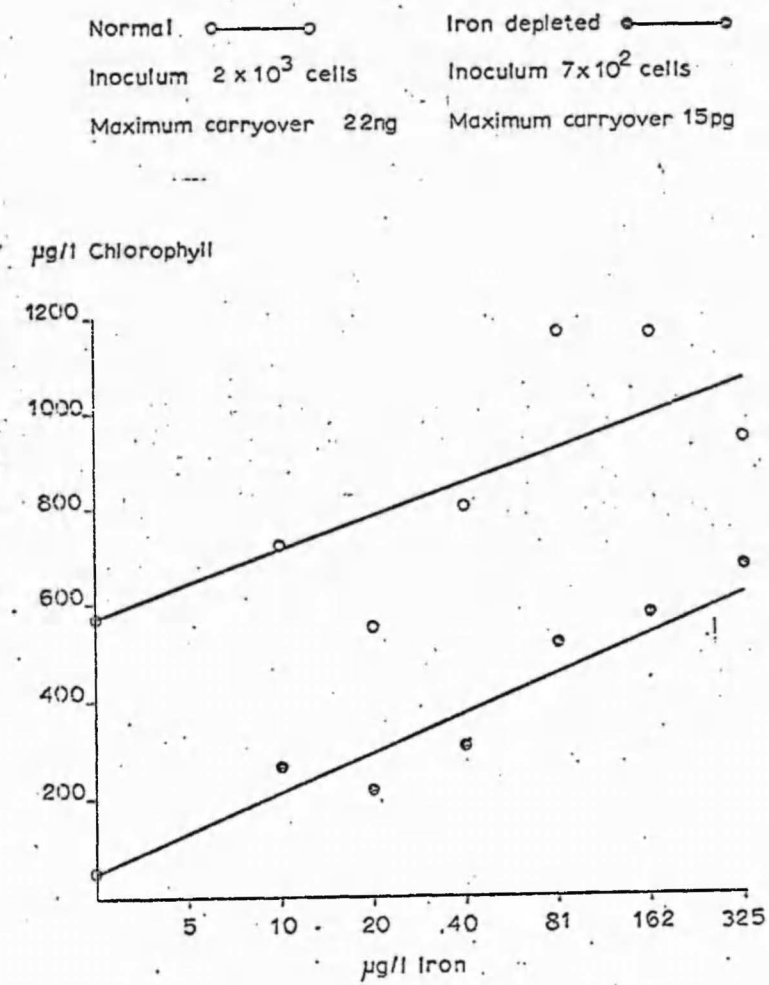


Figure 37 : Effect of Iron Concentration on the Growth of *O.apiculata*



Chlorella (FBA), F.crotonensis and S.quadricauda (FBA) were grown in EVT culture medium and Chlorella sp. O.apiculata and S.quadricauda were cultured in Modified Wood's Hole MBL medium: see Section 2.8.

Chlorella sp. and Chlorella (FBA) showed a marked dependence of growth on the concentration of iron in the medium: see Figures 32 and 33 respectively. Little growth, compared with the zero iron concentration control occurred at iron concentrations of less than 40 µg/l in either culture medium.

S.quadricauda (FBA) and S.quadricauda also showed a marked dependence of growth on the concentration of iron in the medium: see Figures 34 and 35 respectively. S.quadricauda showed little growth, compared with the zero iron concentration control, at iron concentrations less than 20 µg/l. S.quadricauda (FBA) grew relatively poorly at all iron concentrations and showed little difference in growth between the zero iron concentration control and iron concentrations less than 162 µg/l, even after 28 days incubation. However, the concentration of manganese in EVT medium - 386 µg/l, was later shown to be toxic to S.quadricauda (FBA): see Section 3.3.3.

F.crotonensis and O.apiculata showed a much less marked dependence of growth on iron concentration than did the other species tested, even when an iron-depleted culture was used as the inoculum: see Figures 36 and 37 respectively.

3.3.3 Manganese

All algal species tested showed little dependence of growth on manganese concentration, except that concentrations of manganese greater than 100 µg/l were found to be markedly toxic to S.quadricauda (FBA): see Figure 38. This finding explains the relatively poor growth of S.quadricauda (FBA) in experiments to determine the effect of iron concentration on the growth of

Figure 38 Effect of Manganese Concentration on the Growth of *S.quadricauda* (FBA)

Inoculum 3×10^3 cells per flask

Maximum carryover 13ng

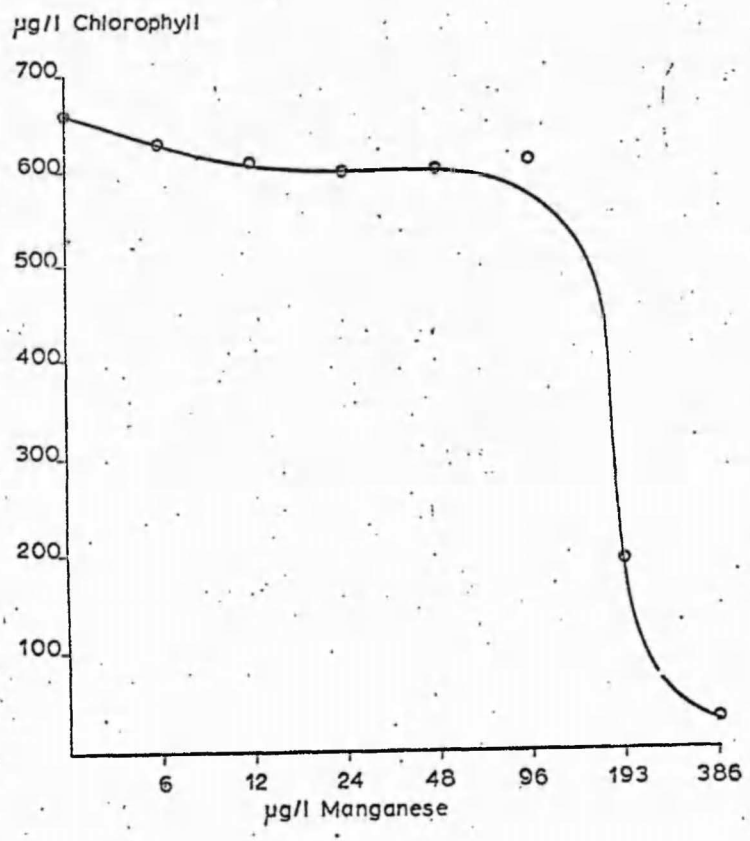


Figure 39 Effect of Manganese Concentration on the Growth of *Chlorella* (FBA)

Inoculum 5×10^5 cells per flask

Maximum carryover 13ng

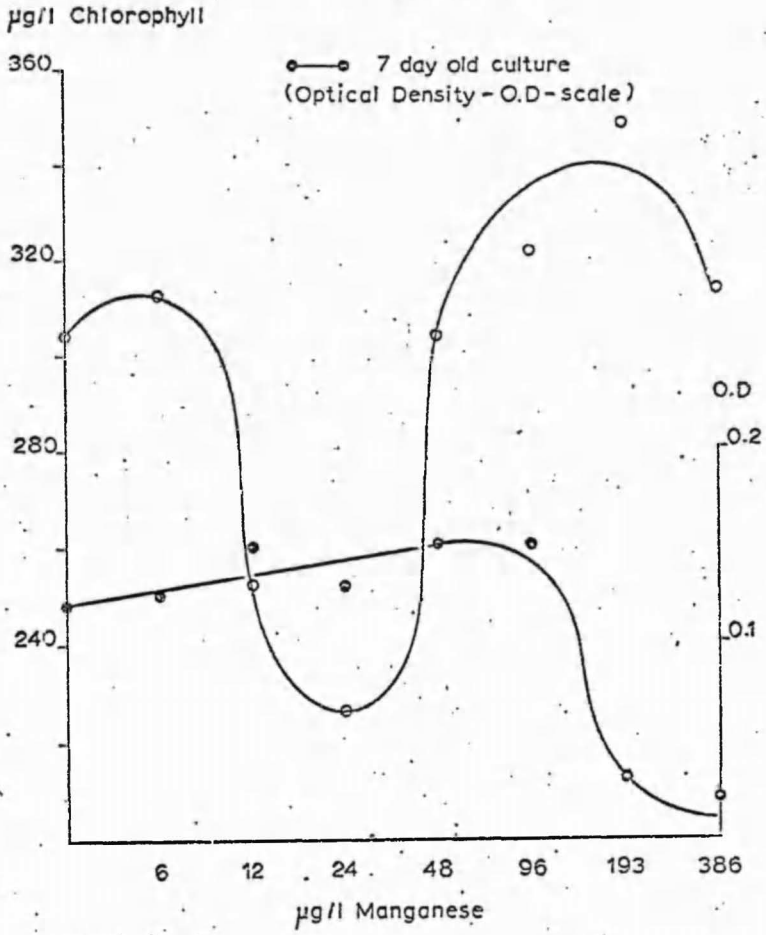
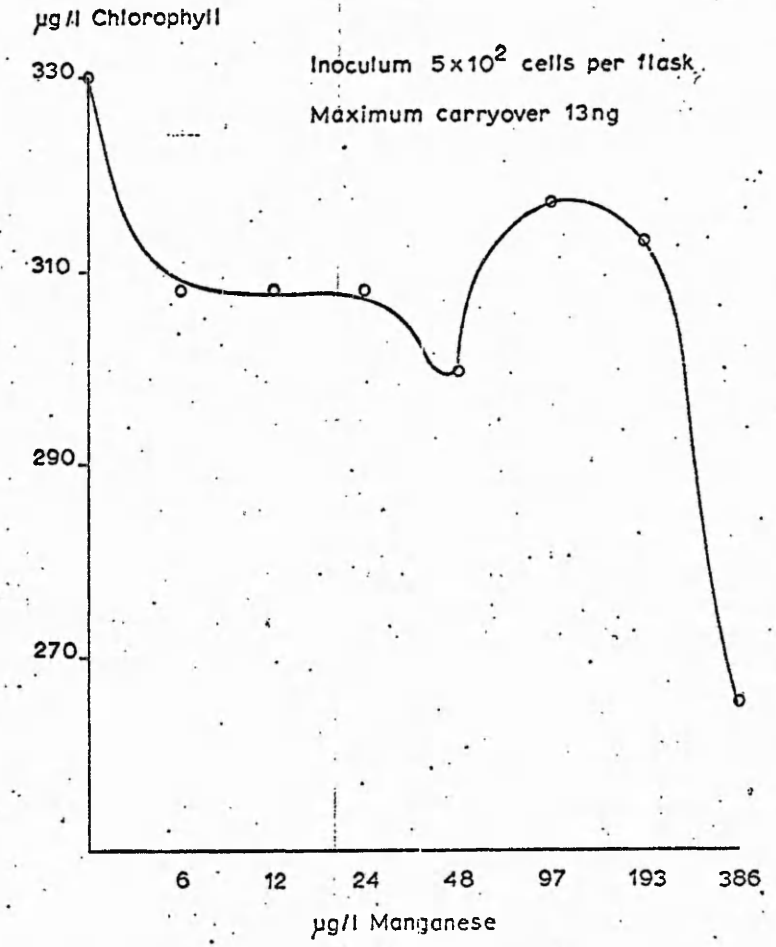


Figure 40 Effect of Manganese Concentration on the Growth of *F. crotonensis*



this alga: see Section 3.3.2. Depression of growth by manganese concentrations greater than 100 µg/l was also observed in cultures of Chlorella (FBA) after 7 days incubation, but these toxic effects apparently disappeared after a further 7 days incubation: see Figure 39. Slight inhibition of the growth of F.crotonensis by manganese concentrations greater than 200 µg/l was observed: see Figure 40. Inhibition of growth by high manganese concentrations was not observed in the experiments on Chlorella sp., O.apiculata and S.quadricauda grown in the Modified Wood's Hole MBL culture medium.

S E C T I O N 4

D I S C U S S I O N

4.1 VITAMIN B_{12}

The ecological importance of vitamin B_{12} to the phytoplankton in the reservoirs studied is suggested by three observations made during the investigation:-

a) minima in the concentration of dissolved vitamin B_{12} frequently coincided with maxima in the concentration of chlorophyll, particularly in Swithland and Thornton reservoirs.

b) the average concentrations of dissolved vitamin B_{12} (*E.gracilis* and *L.leichmanii* assays) and chlorophyll were significantly higher in Thornton reservoir than in either Cropston or Swithland reservoirs.

c) the statistically significant association between particulate vitamin B_{12} and chlorophyll concentrations in all three reservoirs. The positive nature of the association could be interpreted as a reflection of the uptake of dissolved vitamin B_{12} by the phytoplankton.

The questions therefore arise as to whether the growth of auxotrophic algae in the reservoirs may have been limited by vitamin B_{12}, and whether the mean dissolved vitamin B_{12} concentration in the reservoirs is causally related to the mean chlorophyll concentration.

In order to help judge whether vitamin B_{12} may be limiting in nature, Provasoli (1958) tabulated the data on the sensitivity of algae to the vitamin. The sensitivity, that is the lowest vitamin B_{12} concentration giving increased growth compared to a control without vitamin B_{12}, varies from 100pg/l to 5ng/l depending on the alga (Provasoli & Carlucci 1974). Droop (1957) found the sensitivity of *Monochrysis lutheri* Droop to be 100pg/l and concluded that vitamin B_{12} in the sea should at all times be

sufficient to maintain algal growth. Since the concentration of vitamin B_{12} in freshwater is usually greater than that in seawater, it might be concluded that vitamin B_{12} should always be sufficient in freshwater too. However Droop's conclusion has not been widely accepted (Provasoli 1970). The main objections are that Droop's conclusion was based on the needs of only one species of marine alga which may not be typical (but see Guillard & Cassie 1963); and that quantitative data from a chemostat, or indeed from other '*in vitro*' experiments with controlled conditions of nutrient supply, population density, aeration and mixing, may not be an accurate representation of natural conditions.

Droop (1961) showed that the growth rate of *M.lutheri* remained unaltered over a large range of vitamin B_{12} concentrations (0.1 to 100ng/l). However, Ford (1958) found that the division rate of *Ochromonas malhamensis* Pringsheim doubled in the range 13 to 1000ng/l. Clearly algae differ in their ability to capture needed molecules of vitamin B_{12} from dilute concentrations. Other things being equal, *O.malhamensis* would be restricted in growth by a low concentration of vitamin B_{12} (say 1ng/l) which would not be restricting to *M.lutheri*: a possible competitive advantage for the latter organism. Gerome (1971) found that the growth rate of an algal isolate from Lake Leman, France was faster when 20µg/l vitamin B_{12} was added to the culture medium.

Wood (1962, reviewed by Provasoli 1970), showed in chemostat experiments that vitamin B_{12} concentrations less than 8ng/l reduced the growth rate of *Skeletonema costatum* (Grev.) Cleve by 50% or more. Droop (1966) found in chemostat experiments that the growth rate limiting of vitamin B_{12} for *M.lutheri* lay between 2 and 6ng/l, that is at least 20 times the concentration indicated by Droop's (1961) batch culture experiments. Therefore the mean concentration of 'true' dissolved vitamin B_{12} (that is the analogue with a dimethylbenzimidazole nucleotide side chain) in the reservoirs investigated (2.24 to 3.35ng/l - see Table 15) may be limiting to those auxotrophic algae which can only utilise 'true' vitamin B_{12}. In a summary of the 74 species tested, Provasoli & Carlucci (1974) reported that 36 (i.e. 49%) of species can utilise only 'true'

vitamin B_{12}. However, in this present survey, decreases in the concentration of dissolved vitamin B_{12} with increases in the concentration of chlorophyll were usually observed only in the results of bioassays with E.gracilis and L.leichmanii, suggesting that the algae were taking up vitamin B_{12} analogues, the concentrations of these being greater than the concentration of 'true' vitamin B_{12} in the reservoirs (see section 3.1.1.2.1).

However, laboratory sensitivity data are of little value in judging the limiting concentration of a vitamin in natural situations (Provasoli & Carlucci 1974). In nature an organism is seldom operating under conditions of optimal and constant temperature, insolation, salinity and nutrient levels. Daisley (1957) pointed out that in nature the rate of (algal) cell division is more important than the total yield obtainable with a given amount of the vitamin because a certain rate of growth is necessary to build up a bloom that is suffering losses from predation and sinking, for example. Ohwada & Taga (1973) suggested that vitamin B_{12} concentrations of less than 1ng/l (L.leichmanii assay) in the surface waters of Lake Sagami, Japan, might be low enough to affect the photosynthetic and/or growth rates of auxotrophic phytoplankton. Youngman, Johnson & Belling (1973) concluded that on occasions vitamin B_{12} may reach levels low enough to exert a limiting effect on some of the phytoplankton in Farmoor reservoir, Oxfordshire, England. The lowest level observed by these authors was 8ng/l (L.leichmanii assay). In this investigation a similarly low level (7ng/l - L.leichmanii assay) was observed in Thornton reservoir in June 1974 at the same time as peaks in chlorophyll and particulate vitamin B_{12} concentrations. The numerically dominant alga in this bloom was an Oocystis species. The vitamin requirements of Oocystis are not known, but the green algae as a group are predominantly autotrophic (Provasoli & Carlucci 1974). However, large numbers of Rhodomonas were also observed during this bloom. The genus Rhodomonas includes both autotrophic and vitamin B_{12} requiring (auxotrophic) species, but the Cryptophyceae are predominantly auxotrophic (Provasoli & Carlucci 1974). A minimum in the concentration of dissolved vitamin B_{12} in Thornton reservoir in September 1974 also

coincided with a peak in chlorophyll concentration. The numerically dominant species in this bloom (Rhodomonas and Cryptomonas species) were also members of the Cryptophyceae. It is perhaps no coincidence that the abundance of probable vitamin B_{12} auxotrophs, such as Rhodomonas and Cryptomonas, was greater in Thornton reservoir where the mean concentration of dissolved vitamin B_{12} was relatively high.

Little evidence of any consistent seasonal variation in the concentration of dissolved vitamin B_{12} was observed in this investigation. Taken together with the absence of a statistically significant linear and negative association between dissolved vitamin B_{12} and chlorophyll concentrations in the reservoirs, it could be concluded that there is little evidence to support the suggestion that the mean concentration of dissolved vitamin B_{12} determines to some extent the mean chlorophyll concentration i.e. the algal population. The statistically significant association between particulate vitamin B_{12} and chlorophyll concentrations in all three reservoirs, which could be interpreted as reflecting the uptake of dissolved vitamin B_{12} by the phytoplankton, could equally well be due to a third common factor such as the degree of mixing in the reservoir. Any natural environment with conditions favouring bacterial growth is likely to be vitamin rich (Provasoli 1970). The sediments of lakes and reservoirs are such an environment. Gillespie & Morita (1972) found that high concentrations of vitamin B_{12} (up to 1.8 $\mu g/g$ dry sediment) characteristically occurred at the sediment/water interface. In this investigation concentrations of vitamin B_{12} up to 485ng/g (dry sediment) were found in samples of reservoir sediment. Thus factors increasing the degree of mixing in a reservoir might be expected to increase the concentration of particulate matter, including particulate vitamin B_{12}, in the water column, as well as increasing the supply of nutrients to the euphotic zone which could result in increased phytoplankton biomass.

However, it is perhaps naive to expect a simple linear relationship between any one variable and chlorophyll (particularly for a nutrient such as vitamin B_{12} which is not required by all algae), bearing in mind the large number of physical, chemical and biological factors

which affect the ecology of the phytoplankton.

Daisley (1969) also found no evidence of a consistent seasonal variation in the concentration of dissolved vitamin B_{12} in nine lakes of the English Lake District. He also found a positive relationship between the average vitamin concentration and the relative productivity of the lakes. Gerome (1971) in France, similarly found the concentration of vitamin B_{12} in Lake Leman to be less than that in the more eutrophic Lake Nantua. Carlucci & Bowes (1972) found the concentration of vitamin B_{12} in the oligotrophic Lake Tahoe, California USA to be generally undetectable, but found the concentration of the vitamin in the eutrophic Clear Lake, California to be easily measurable. The results of these and other workers suggest that the algae themselves may be responsible for the positive relationship between the mean concentration of dissolved vitamin B_{12} and mean concentration of chlorophyll observed in the reservoirs.

Ohwada & Taga (1973) found that vitamin B_{12} (together with thiamine and biotin) was produced when Anabaena cyanea was actively growing in Lake Sagami, Japan. Gerome (1971) observed elevated levels of vitamin B_{12} in Lake Nantua, France during a bloom of Anabaena and Oscillatoria rubescens de Candolle. Youngman et.al. (1973) and Gillespie & Morita (1972) observed concurrent increases in the concentration of vitamin B_{12} and the standing crop of Aphanizomenon flos-aquae (L.) Ralfs in Farmoor reservoir, Oxfordshire, England and Upper Klamath Lake, Oregon USA respectively. In this investigation a marked increase in the concentrations of dissolved and particulate vitamin B_{12} followed a bloom of Aphanizomenon flos-aquae in Thornton reservoir which reached its peak on 25 July 1974. The fact that the peaks in the concentration of dissolved and particulate vitamin B_{12} followed the peak in chlorophyll concentration by two weeks suggests that bacteria and/or the lysis of A.flos-aquae cells were responsible for the increase in vitamin B_{12} concentration rather than vitamin excretion by the healthy alga. Gillespie & Morita (1972) noted the close, apparently dependant relationship of A.flos-aquae with a number of bacterial species; but since their samples were taken at monthly intervals it is

not possible to discern from their data whether the observed peak in vitamin B_{12} (unfiltered water) followed or coincided with the peak in the standing crop of A.flos-aquae. These workers found that 20 out of 42 bacterial isolates from the water and sediment of Upper Klamath Lake, Oregon were capable of producing 50ng/l or more of vitamin B_{12} per week in culture. Several algae, including ecologically important marine species, also release vitamin B_{12} into their culture medium during growth (Provasoli 1970). It should however be remembered that the production of vitamin B_{12} in culture by a given microorganism does not necessarily mean that it will in nature produce the vitamin in excess of its own requirement (Daisley 1969). Niewolak & Sobierajska (1971) commented that the vast majority of microorganisms in the Ilawa lakes, Poland, do not grow in commonly used laboratory media, which therefore makes it impossible to estimate their share in the production of vitamin B_{12}. Gerome (1971) explained the increase in vitamin B_{12} concentration with depth during the summer stagnation period in terms of the activity of bacteria present in the sediment and water which produce vitamin B_{12}. She also suggested that the greatly increased concentration of vitamin B_{12} at the bottom of Lake Leman in November 1970 was due to increased bacterial activity which accompanied the fall of dead algae from the epilimnion.

The results of the feeder stream survey show no consistent relationship between the mean concentration of dissolved vitamin B_{12} in water entering the reservoirs and that in the surface waters of the reservoirs. The observation that the mean concentration of dissolved vitamin B_{12} in water entering Swithland and Thornton reservoirs was less than that in these reservoirs also suggests the importance of internal sources of vitamin B_{12} in explaining the difference between reservoirs in the mean concentration of dissolved vitamin B_{12}. Ohwada & Taga (1973) similarly suggested that the inflowing water to Lake Sagami, Japan made only a minor contribution to the vitamins in the lake and concluded that the seasonal variation in vitamin B_{12} concentration in the lake water was affected more by biological factors in the lake. Niewolak & Sobierajska (1971) also concluded that vitamin B_{12} in the Ilawa lakes, Poland was of autochonthic origin and

that only an insignificant amount enters with influents to the lakes.

The micronutrient budgets constructed for the reservoirs suggest one other explanation for the lower mean concentration of dissolved vitamin B_{12} in the surface waters of Swithland reservoir compared with Thornton reservoir: that is the net export of dissolved vitamin B_{12} from Swithland reservoir and the net import of vitamin B_{12} into Thornton reservoir (see Table 28). However, a greater net import of dissolved vitamin B_{12} into Cropston reservoir was observed, together with a higher concentration of the vitamin in the inflowing water to the reservoir; compared with that in the surface waters of the reservoir. It would therefore be necessary to postulate a net degradation of vitamin B_{12} in Cropston reservoir in order to explain the relatively low mean concentration of dissolved vitamin B_{12} in this reservoir.

Gillespie & Morita (1972) found that vitamin B_{12} in the sediment of Upper Klamath Lake, Oregon, was degraded by biological and non-biological processes. Photo-destruction at the surface and uptake of vitamin B_{12} by the phytoplankton would tend to reduce the concentration of vitamin B_{12} in a lake or reservoir (Gerome 1971). Carlucci, Silbernagel & McNally (1969) found that solar radiation destroyed about 50% of vitamin B_{12} activity in one week. Since the reservoirs are geographically close to one another, it is assumed that, per unit area, the rate of destruction of vitamin B_{12} by light would be similar in all three reservoirs, but in total would be greatest in Swithland reservoir (largest surface area) and least in Thornton reservoir (smallest surface area). If photo-destruction was the major factor controlling the concentration of vitamin B_{12} in the reservoirs one would not expect the mean overall concentration of dissolved vitamin B_{12} in the reservoirs to be lowest in Cropston reservoir.

It is concluded that the concentration of 'true' vitamin B_{12} O.malhamensis assay) may have been limiting to the growth rate of auxotrophic algae with a mammalian type specificity in all three reservoirs throughout much of the study period. It is further concluded that the growth rate of auxotrophic algae with an E.coli

or L.leichmanii specificity pattern for vitamin B_{12} analogues, may at times have been limited by the concentration of dissolved vitamin B_{12} in the reservoirs. It is possible that the relative abundance of probable vitamin B_{12} auxotrophs such as Rhodomonas and Cryptomonas in Thornton reservoir was due in some part to the relatively high mean concentration of dissolved vitamin B_{12} in the reservoir.

It is also concluded that the observed positive relationship between the mean concentrations of dissolved vitamin B_{12} and chlorophyll in the reservoirs is primarily a result of the algal biomass produced in the reservoir. If, as seems likely from the results of other workers, bacterial synthesis is more important than algal synthesis of the vitamin, then in the absence of organic nutrients input from feeder streams, the amount of bacterial synthesis in the reservoirs will ultimately be dependant on the concentration of organic matter produced in the reservoir by photosynthesis. The positive association between particulate vitamin B_{12} and chlorophyll concentrations in all three reservoirs is interpreted as supporting evidence for this theory.

4.2 IRON

The ecological importance of iron to the phytoplankton in the reservoirs studied is suggested by two observations made during the investigation:-

a) minima in the concentrations of complexed and, less frequently, soluble iron fractions often coincided with maxima in the concentration of chlorophyll in Swithland and Thornton reservoirs.

b) the statistically significant relationships between soluble and complexed iron fractions and chlorophyll in Thornton reservoir. The negative nature of the relationship suggests that iron was acting as a limiting nutrient in Thornton reservoir during the investigation.

The concentration of dissolved iron (that is soluble and complexed iron fractions) decreased to less than 20 µg/l on the two occasions in Thornton reservoir and on the two occasions in Swithland reservoir when a simultaneous increase in chlorophyll concentration occurred in these reservoirs. Experiments designed to determine the effect of iron concentration on algae isolated from the reservoirs (see section 2.8) showed that both Chlorella species tested and both Scenedesmus quadricauda (Turpin) Brébisson isolates tested showed little growth (compared with an iron-free control) at iron concentrations less than 20-40 µg/l. However, in similar experiments with other algal isolates, Fragilaria crotonensis (Edwards) Kitton and Oocystis apiculata W. West showed a much less marked dependance of growth on iron concentration, even when an iron-depleted culture was used as the inoculum. These experiments provide another example of species variability in micronutrient requirements as discussed in section 1.1. Harvey (1939) observed that marine diatoms appear to be covered by a very thin film of ferric iron and produced evidence that such films could provide a source of iron for the diatoms. This could explain the relative independance between growth of F.crotonensis and iron concentration observed in these experiments and the increased dependance observed when an iron-depleted culture of the alga was used as the inoculum. However, for the reasons discussed in section 4.1, there are many problems in attempting to relate the results of

laboratory culture experiments to the natural environment. Even the results of laboratory experiments with the same algal species were shown to vary with the method of culture by Myers (1947). This author found that iron concentrations between 10 and 7400 µg/l gave adequate growth of Chlorella in stagnant culture, but in continuous flow cultures a concentration of 500 µg/l iron limited growth, while concentrations greater than 5000 µg/l were adequate. A greater requirement for vitamin B_{12} in continuous rather than stagnant culture was shown by Wood (1962, reviewed by Provasoli, 1970), see section 4.1.

Eyster (1968) gives the optimal concentration of iron in culture media without a chelator as 50 µg/l and with a chelator (500mg/l EDTA) as 5000 µg/l. If a sufficient excess of EDTA is present in an Fe- EDTA mixture it is known that the iron will be unavailable to algae (Provasoli, McLaughlin & Droop 1957). A similar relationship between iron and the humic acids apparently exists in the natural environment. Sakamoto (1971) observed that the addition of humate-rich stream water to a Canadian experimental lake caused a reduction in the photosynthetic uptake of carbon. Prakash & Rashid (1968) found that the addition of humic acids in concentrations greater than 35mg/l caused a decrease in the growth rate and final yield of Gonyaulux tamarensis Lebour and Gonyaulux catenata (Levander) Cofoid in culture. One may reasonably ask, therefore, whether the concentration of EDTA in the culture media used in the experiments with algal isolates described in section 3.3.2, might significantly affect the interpretation of the results in terms of the natural environment.

Ryhanen (1968) stated that the average concentration of humic substances in lakes and rivers is 13mg/l. On a weight to weight basis this figure is not too dissimilar from the 2mg/l EDTA in the modified Chu No.10 (EVT) medium, and the 4mg/l EDTA in the Modified Wood's Hole MBL medium used in the culture experiments. However, EDTA may not reflect the properties of natural, humic, chelators present in natural waters with which iron is known to be associated (Shapiro 1967a, Ghassemi & Christman 1968). Shapiro (1967b) found a close correlation between the results of bioassays for available iron

using Microcystis aeruginosa Kützing and the concentration of iron which reacted with thiocyanate after the acidity of the membrane filtered samples from Minnesota USA, lakes had been adjusted to pH 1-5. In a later paper Shapiro (1969) showed that iron as a soluble Fe - EDTA complex almost completely reacted with thiocyanate at pH 1.5, from which it may be concluded that such iron is almost completely available to M.aeruginosa. In the same paper, Shapiro showed that a variable percentage of the soluble iron in the lakes did not react with thiocyanate at pH 1.5. Such iron is presumably unavailable, at least to M.aeruginosa, and the variation in the percentage of such iron present may be related to differences in the molecular weight distribution of humic acids in lakes apparent from the work of Shapiro (1967a), Gjessing (1967) and Ghassemi & Christman (1968). Ghassemi & Christman (1968) found that some iron present in water samples from streams and lakes in Washington and Alaska, USA could not be removed by EDTA. This 'resistant' iron may be analogous to Shapiro's 'unavailable' iron. It is clear from such work that the concentration of soluble iron in a lake may not accurately reflect that available to algae, if anything tending to overestimate the available concentration. Indeed Shapiro (1969) concluded that only 30% of the soluble iron present was available to the bioassay organism used (M.aeruginosa) in Lake J, Minnesota, USA.

So far as chelation is concerned it is probable, therefore, that the laboratory culture experiments at least approximated natural conditions, the lower concentration of chelator present in the media possibly compensating, to some extent, for the probability that an unknown percentage of the dissolved iron present in the reservoirs studied is not available to the phytoplankton.

Much of the experimental evidence promoting the importance of iron in algal ecology is based on an experimental approach not used in this investigation for the reasons given in section 1.1, that is enrichment experiments. Unfortunately, much of this important work did not include data on the concentration of iron already present in the lakes that were then experimentally enriched. Such data would have been valuable in assessing whether the iron concentration present

in the reservoirs studied may have occasionally limited the biomass of algae therein. An important exception is the work of Wetzel (1965 and 1966) on marl lakes in Indiana, USA, many of which have a major cation composition similar to the reservoirs studied in this investigation (see Table 4). Wetzel (1965) found that adding iron alone frequently stimulated the photosynthetic uptake of carbon in Crooked Lake where the epilimnetic iron concentration was observed to vary between 10 and 4000 µg/l, a similar range of concentration to that observed in the reservoirs studied in this investigation (see Table 18). Schelske (1962) also observed that iron was the primary limiting nutrient in another marl lake in Indiana, USA. It is tempting to speculate that iron deficiencies may be a common feature of marl lakes.

The highly significant negative relationship between iron (soluble and complexed fractions) and chlorophyll in Thornton reservoir strongly suggests that these fractions may have limited phytoplankton biomass throughout the study period, but does not prove it. Why does not a similar relationship apparently exist in Swithland reservoir where the mean concentration of all iron fractions are significantly less than those in Thornton reservoir (see Table 18)? This question cannot be properly answered without further experimental work. One possible explanation is that the relationship does exist in Swithland reservoir, but is masked by other factors. In the case of soluble iron the explanation may be that the method for the measurement of this fraction was not sensitive enough. The mean concentration of this fraction in Swithland reservoir, 4.7 µg/l, is approximately, only twice the standard deviation of the method (2 µg/l, see Table 7). In the case of complexed iron, the relationship in Swithland reservoir may have been masked by a high proportion of 'unavailable' iron in the complexed fraction. However to repeat the comment made earlier when discussing vitamin B_{12}, it is perhaps naive to expect a simple linear relationship between any one variable and chlorophyll bearing in mind the large number of physical, chemical and biological factors which affect the ecology of the phytoplankton.

The laboratory experiments of Morton & Lee (1974) suggest that a lower mean concentration of iron such as was found in Swithland reservoir compared with that in Thornton reservoir may influence the algal species composition of these reservoirs. These workers found that higher concentrations of iron, even when already present in excess of nutritional needs, favoured the growth of some common blue green algae over some green algae when grown together in batch culture. In this present work it was observed during the study period that blue green algae occurred more frequently in significant numbers in Thornton reservoir, compared with Swithland reservoir where the mean total iron concentration was 37% of that in Thornton reservoir.

It is concluded that dissolved iron may be a major limiting factor in Thornton and possibly also in Swithland reservoirs. Further experimental work is required however before this theory can be proved or disproved.

4.3 MANGANESE

Two observations made during the investigation suggest the importance of manganese in the ecology of the phytoplankton in the reservoirs studied:-

a) the statistically significant association in all three reservoirs between the concentrations of particulate manganese and chlorophyll. The positive nature of the relationship could be interpreted as a reflection of the uptake of soluble manganese by the phytoplankton.

b) the concentration of soluble manganese occasionally decreased (to a concentration of about 5 $\mu g/l$) with increases in chlorophyll concentration in Swithland and Thornton reservoirs.

Eyster (1968) gives the optimal concentration of manganese in algal culture media as being between 5ng/l and 5 $\mu g/l$ in the absence of a chelator and as 5mg/l in the presence of 500mg/l EDTA. These results suggest that in the absence of a chelator, manganese would not limit the growth of algae in the reservoirs even at the lowest concentration observed (about 5 $\mu g/l$). The results of the reservoir survey suggest that, on average, complexed manganese constitutes a relatively small proportion of the mean total concentration in the reservoirs (3% in Cropston, 7% in Swithland and 5% in Thornton reservoir). In any case, the results of the laboratory culture experiments described in section 3.3.3 in which the concentration of the chelator is thought to approximate (quantitatively, but probably not qualitatively) the natural concentration (see section 4.2) also suggest that manganese concentrations of 5 $\mu g/l$ would not severely limit the growth of algal populations, at least the populations of those species tested. The difficulties in interpreting the results of laboratory experiments in terms of the natural environment were briefly discussed in section 4.1. Harvey (1947) suggested that only a fraction of naturally occurring manganese in the sea is in a form available to plant life. As with iron therefore, the concentration of manganese in aquatic habitats may not

accurately reflect the available concentration.

The interpretation of the results of this study are further complicated by the interactions between iron and manganese, and calcium and manganese, observed by Gerloff & Skoog (1957). These workers found that the iron requirement of Microcystis aeruginosa Kützing was positively correlated with the manganese concentration in the medium. This observation, if applicable to other algal species, would lead to the prediction that a higher algal requirement for iron would occur during the summer peak in manganese concentration observed in the reservoirs (see section 3.1.1.4); and whilst not necessarily suggesting a role for manganese as a limiting nutrient does give the element biological significance.

The high concentrations of manganese observed during the summer peaks (maximum 224 µg/l total manganese in Swithland reservoir, and maximum 348 µg/l total manganese in Thornton reservoir) may also have been biologically significant because of their toxicity. The poor growth of Scenedesmus quadricauda (Turpin) Bréhisson (FBA) in EVT culture medium during experiments to determine the effect of iron on the growth of this alga (see section 3.3.2) was later ascribed to the high manganese concentration of this medium. Guseva (reviewed by Lund 1965) and Gerloff & Skoog (1957) suggest that manganese concentrations of 200 µg/l or more in natural waters are toxic to blue green algae and some other phytoplankton species. Patrick, Crum & Coles (1969) confirmed this work and showed manganese to be more toxic to blue-green algae than to green algae and diatoms. However, Gerloff & Skoog (1957) observed that the toxic threshold concentration of manganese of M.aeruginosa increased with the calcium concentration of the medium. With 0.5mg/l calcium in the culture solution, 500 µg/l manganese was slightly toxic and 2000 µg/l manganese stopped growth of the alga completely. However, with 10mg/l calcium present 500 µg/l manganese did not significantly inhibit growth and 4000 µg/l only slightly reduced the growth of the alga. Toxic effects of manganese were not observed in cultures grown in Modified Wood's Hole MBL medium with a calcium concentration of 76mg/l deliberately similar to that of the reservoir water and much higher than that in the EVT medium (3.4mg/l). It would therefore

seem unlikely that manganese would ever be toxic to the algae in the reservoirs studied.

Few field studies of the possible importance of manganese in phytoplankton ecology have been made. The only examples known to me are those by Goldman (1964) and Wetzel (1966). Both of these workers' results were based on the results of nutrient enrichment experiments, an approach not used in this investigation for the reasons discussed in section 1.1. As with iron, these studies did not include detailed data on the concentrations of manganese present in the lakes at the time of the experiments. Goldman (1964) observed a significant manganese deficiency in Lake Coleridge, New Zealand and quotes a manganese concentration in the lake of 4.8 µg/l, matching the 5 µg/l minimal concentration observed in this present study. Wetzel (1966) found some stimulation of photosynthetic carbon uptake by manganese in marl lakes in Indiana, USA and quotes a mean manganese concentration of 21.3 µg/l for the lakes, with a range between 1.0 and 76.0 µg/l. The similarity in major cation composition between these lakes and the reservoirs studied was noted in section 4.2 and the work therefore provides a valuable comparison. Wetzel (1965) concluded however, that at least in two of the Indiana lakes manganese is unlikely to seriously limit algal growth.

To summarise, the data recorded during the survey suggest that manganese may at times have limited algal growth in Swithland and Thornton reservoirs. However, on balance the literature suggests that even the minimum manganese concentrations observed in the reservoirs would not have seriously limited algal growth. The situation is complicated by interactions between iron and manganese; iron may have been more limiting during the summer peak in manganese concentration than is suggested in section 4.2. The potentially toxic effects of high manganese concentrations are probably nullified in the reservoirs by the high calcium concentration of the water. However, the increase in the toxic threshold manganese concentration apparent at high calcium concentrations may also reflect a greater requirement for manganese at high calcium concentrations. These complex interactions led Gerloff & Skoog (1957) to conclude that

the optimal levels of iron and manganese for algal growth must vary over a considerable range and may change abruptly with conditions within a lake or between lakes.

4.4 Copper

The possible ecological importance of copper to the phytoplankton in the reservoirs studied is suggested by two observations made during the investigation:-

a) the statistically significant association between soluble copper and chlorophyll concentrations in Swithland reservoir. The negative nature of the association could be interpreted as showing the uptake of soluble copper by the phytoplankton.

b) the mean concentrations of soluble (and particulate) copper and chlorophyll were significantly higher in Thornton reservoir compared with either Cropston or Swithland reservoirs.

The questions therefore arise as to whether the growth of algae in Swithland reservoir may have been limited by the concentration of soluble copper, and whether the mean soluble (and particulate) copper concentration is causally related to the mean chlorophyll concentration.

The difference between reservoirs in mean total copper concentration is not reflected by the inflowing water to the reservoirs which contained approximately 14 µg/l total copper; significantly less than that observed in the reservoirs. The relative enrichment of the reservoir with regard to copper is no doubt explained by the practice of 'copper sulphating' the reservoirs to control the growth of blue green algae. The concentration of copper in the reservoirs after such treatment reached levels as high as 158 µg/l, see section 3.1.1.5. The higher mean level of copper in Thornton reservoir can be explained by the relatively common appearance of significant populations of blue green algae in the reservoir and the consequent need to treat the reservoir with copper sulphate to check their growth.

Eyster (1968) gives the optimal concentration of copper in algal culture media as being 1 µg/l in the absence of a chelator and 400 µg/l in the presence of 500mg/l EDTA. Mills & Oglesby (1971) quote

the optimal range to be 1 to 20 µg/l. These figures suggest that the copper concentrations observed in the reservoirs would not limit algal growth. Riley (1939) found between 9 and 383 µg/l copper in three Connecticut, USA lakes and concluded that copper could not be regarded as a limiting nutrient (by deficiency) in these lakes. The range of copper concentration observed by Riley (1939) is similar to that observed in this investigation. In the only study I have found where copper was proposed as being, by its deficiency a limiting nutrient, the mean concentration of soluble copper found by Mills & Oglesby (1971) in Cayuga Lake, New York, USA, is at 0.6 µg/l an order of magnitude less than the minimum observed by Riley (1939) and the lowest mean soluble copper concentration observed in the reservoirs. The amount of copper required by algae is very small; Manahan & Smith (1973) calculated that a free Cu^{2+} concentration of only 7.6×10^{-15} g/l and 1.0×10^{-14} g/l produced optimal growth of respectively Chlorella vulgaris Beijerinck and Oocystis masonii Lemmerman in culture. Indeed Steemann-Nielsen and Wium Anderson (1970) showed that in the absence of chelators and significant amounts of colloidal ferric hydroxide, a concentration of only 1 µg/l copper was toxic to Chlorella pyrenoidosa Chick.

If it is assumed that the concentration of copper in the reservoirs was too high for it to act as a limiting nutrient to the phytoplankton by its deficiency, the negative association between soluble copper and chlorophyll observed in Swithland reservoir could be explained by suggesting that soluble copper acted as a limiting nutrient by its toxicity to phytoplankton in the reservoir. It would then be necessary to suggest reasons why a similar relationship was not observed in Cropston reservoir and especially in Thornton reservoir where the mean concentration of soluble copper was significantly higher and therefore potentially more toxic. However, in natural aquatic environments the toxicity of copper may be reduced by several factors such as water hardness (Hutchinson 1957) and the presence of organic matter (Fogg & Westlake 1955, Fitzgerald & Faust 1963). Steemann Nielsen & Wium-Anderson (1970) showed that colloidal ferric hydroxide bound copper so much that even in the absence of EDTA about 50 µg/l copper was necessary to depress the growth of C.pyrenoidosa to the same degree as 1 µg/l copper in

a growth medium containing 6 μg/l iron. It is interesting to note that the mean concentration of soluble iron in Cropston and Thornton reservoirs (which probably consisted mainly of colloidal ferric hydroxide) was significantly greater than that in Swithland reservoir (see Table 18). Further experimental work would be required to test this possible explanation.

4.5 ZINC

The possible ecological importance of zinc to the phytoplankton in the reservoirs studied is suggested by two observations made during the study period:-

a) the statistically significant relationship between soluble zinc and chlorophyll concentrations in Swithland reservoir. The negative nature of the relationship could be interpreted as showing the uptake of soluble zinc by the phytoplankton.

b) the mean concentrations of total zinc and chlorophyll were significantly higher in Thornton reservoir compared with either Cropston or Swithland reservoirs.

Table 21 shows that the mean concentration of soluble zinc was essentially similar in all three reservoirs (about 5 µg/l). The difference in mean total zinc concentration between reservoirs is due mainly to the particulate fraction. Groth (1971) concluded that the phytoplankton was the primary cause of fluctuations in the concentration of zinc in several East Holstein lakes. It is possible that the phytoplankton and the resulting organic debris may provide the explanation for the observed differences between reservoirs in the mean concentration of particulate zinc. Zinc may be accumulated by living organisms by both specific and non-specific mechanisms (Martin et.al. 1971). The positive relationship between the mean concentrations of total zinc and chlorophyll when the reservoirs are compared with one another would not then be seen as causal, but consequential. The statistically significant and positive relationship between particulate zinc and chlorophyll concentrations in Cropston reservoir provides some supporting evidence for this theory. However, Figures 28 and 29 do not support this theory, showing little evidence of a consistent seasonal variation in the concentration of particulate zinc, which would be expected from the theory. These figures suggest that the difference between reservoirs in mean total zinc concentration is due primarily to the frequency and magnitude of sudden increases in particulate zinc concentration unrelated to changes in the concentration of chlorophyll.

Many other factors may be involved in explaining the difference in mean, total zinc concentration between reservoirs. The results of the feeder stream survey, for example, show the mean concentration of total zinc in the inflowing water to the reservoirs to be very similar to that actually in the reservoirs (see Tables 18 and 29), suggesting the possible importance of this source of variation.

Eyster (1968) gives the optimal concentration of zinc in algal culture media as 1 µg/l in the absence of a chelator and as 6mg/l in the presence of 500mg/l EDTA. Mills & Oglesby (1971) quote the optimal range to be 0.5 to 100 µg/l. Assuming a concentration of chelators in natural waters of 13mg/l (see section 4.2) it is probable that the optimum concentration of zinc for algal growth lies nearer the lower limit given by Eyster. Groth (1971) concluded that the concentration of zinc in several East Holstein lakes (2.2 to 19.0 µg/l) did not limit the growth of the phytoplankton. However, Mills & Oglesby (1971) presented presumptive evidence that zinc was at limiting levels in Cayuga Lake, New York, USA. The mean summer concentration of soluble zinc observed by these workers was 2.17 µg/l. Goldman (1964) observed a stimulation of photosynthetic carbon uptake in samples from lakes with a natural zinc concentration of less than 1.2 µg/l (the limit of detection of the method) when enriched with zinc, but no stimulation in a sample from Lake Lyndon, New Zealand which had a natural zinc concentration of 9.3 µg/l. The difference between limiting and non-limiting concentrations of zinc in natural environments may therefore be quite small; the mean concentration of soluble zinc observed in the reservoirs in this investigation falling within this range. This observation may explain the finding of no significant and negative relationship between the concentrations of soluble zinc and chlorophyll in Cropston or Thornton reservoirs, despite the fact that the mean concentration of soluble zinc was not significantly different between reservoirs, because the interaction between zinc and other factors may have made the concentration of soluble zinc relatively more critical to the phytoplankton in Swithland reservoir.

4.6 COBALT

As with vitamin B_{12}, copper and zinc the mean concentration of cobalt appears positively related to the mean concentration of chlorophyll when the reservoirs are compared with one another. No seasonal variation or changes in the concentration of cobalt which could be associated with changes in chlorophyll concentration were observed within reservoirs however. The source of this inter-reservoir variation in mean cobalt concentration may be the inflowing water to the reservoirs in which the mean concentration of cobalt was slightly higher than that in the corresponding reservoir (see Tables 22 and 29).

According to Eyster (1968) the optimum concentration of cobalt in algal culture media is 40ng/l, that is about two orders of magnitude less than that observed in the reservoirs (about 5 µg/l). Taken together with the field data collected during this investigation it would appear that cobalt is not likely by its deficiency to limit the growth of the phytoplankton. Against this conclusion must be set the result of Goldman's (1972) study on Lake Wakatipu, New Zealand where a progressive increase in photosynthetic carbon uptake was observed in water samples enriched with cobalt in increasing concentrations up to 20 µg/l. On the other hand Coleman, Coleman & Rice (1971) suggested that 40ng/l cobalt may be toxic to some algae in culture. Wetzel (1965) found that enrichment of water samples (which had a natural cobalt concentration of less than 2 µg/l) with 5 to 50 µg/l cobalt exerted increasingly inhibitory effects on photosynthetic carbon uptake in one marl lake, but had no effect at all in another. The optimum concentration of cobalt in natural waters is clearly very variable and as concluded by Wetzel (1965) is probably dependent on the quantity (and quality) of complexing capacity present in the water. Clearly the biological significance of cobalt in the reservoirs studied cannot be properly assessed without such information, but it is suggested that in these relatively productive reservoirs sufficient organic material is present to neutralise the possible toxic effects of the natural cobalt concentration.

SECTION 5

CONCLUSIONS

5. CONCLUSION

The results of the investigation showed Swithland reservoir to be biologically and chemically distinct from Thornton reservoir with respect to the mean concentrations of chlorophyll and of the micronutrients studied. Cropston reservoir appears biologically similar to Swithland reservoir and chemically similar with respect to the mean concentrations of chlorophyll and the micronutrients vitamin B_{12}, copper, zinc and cobalt. In this sense the mean micronutrient concentrations of vitamin B_{12}, copper, zinc and cobalt provided a better measure of the productivity of the reservoirs than did either phosphate or nitrate levels (see Table 4) although the latter nutrients are more generally considered to be the major factors limiting primary production in aquatic habitats. For the micronutrients vitamin B_{12} and copper, this broad correlation with phytoplankton biomass was interpreted as being consequential rather than causal, the correlation with copper being almost certainly artificial because of the practice of 'copper-sulphating' reservoirs to control the growth of blue green algae which are generally found in greater abundance in more eutrophic habitats.

However, the correlation with vitamin B_{12} observed in this investigation (and others) suggests that this vitamin could be used as a measure of eutrophication, the concentration of vitamin B_{12} being relatively high in eutrophic waters due to:-

a) increased input into the habitat from external sources such as sewage effluents;

b) a change in the balance between biological producers and consumers of the vitamin within the habitat. Bacteria and some algae, mainly blue green algae, are producers of vitamin B_{12}; other algae, mainly diatoms and dino flagellates, are the main consumers.

The concentration of vitamin B_{12} therefore tends to reflect the heterotrophic activity in, and algal species composition of, a water body.

Soluble and complexed iron, soluble manganese, soluble copper, soluble zinc and dissolved vitamin B_{12} also showed negative associations with chlorophyll, and/or occasional (and sometimes simultaneous) changes in concentration, particularly in Swithland and Thornton reservoirs. This suggests their possible importance as factors influencing phytoplankton biomass in the reservoirs. However, the difficulties in attempting to differentiate between causal and non-causal associations must be taken into account and are amply illustrated in this study. These difficulties are not simply due to inherent problems in the methodology used, but also to the enormous variability between algae in their nutrient requirements. The usually effective scientific approach of dissecting a complex problem into a number of simpler components which can be tackled experimentally (such as the laboratory studies used in this investigation to determine the nutrient requirements of unialgal cultures) cannot provide unequivocal answers in freshwater ecology where unknown antagonistic and synergistic interactions between factors and algae may be important. The interaction between trace metals and chelating or complexing substances is a case in point. Chelation is important in determining the availability or toxicity of micronutrients and in future studies of this kind should be quantified as a factor in its own right (preferably by biological methods such as that suggested by Davey, Morgan & Erickson 1973).

Finally I would suggest that a more extensive but also more integrated programme of field work by a team of researchers might have better satisfied the original aims of the investigation; a programme incorporating both monitoring schedules and the <u>in situ</u> enrichment of the same samples with the nutrients (or factors) being studied. Possible multiple factor limitation of phytoplankton biomass might then be determined by multivariate statistical techniques to analyse the complex problem as a whole rather than as a number of separate components.

Some of the results reported in this thesis were reported to an Advisory Group jointly convened by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations to study and discuss isotopic tracer-aided studies of inland water eutrophication and pollution. The relevant section of the final report is reproduced in Appendix 5.

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A P P E N D I C E S

APPENDIX 1

Sources of Materials

Materials not listed below were obtained from BDH Chemicals Ltd., Poole, Dorset, BH12 4NN, England, or from local laboratory suppliers.

Amicon UM 10 Ultrafiltration Membranes

Amicon N.V.
Mechelaarstraat 11
Oosterhout (N.B.)
Holland.

Bacto B12 Assay Medium USP
Bacto B12 Ochromonas Medium
Bacto Biotin Assay Medium
Bacto Euglena B_{12} Medium
Bacto Thiamine Assay Medium LV

Difco Laboratories
PO Box 14 B
Central Avenue
West Molesey
Surrey
England.

Cyanocobalamin – Glaxo Laboratories Ltd.
891 Greenford Road
Greenford
Middlesex UB6 OHE
England.

^{57}Co - Vitamin B_{12} – Radiochemical Centre
Amersham
England.

Continued/

D - Biotin - Sigma Chemicals Ltd.
Norbiton Station Yard
Kingston on Thames
Surrey KT 2 7BH
England.

Millipore PSED - Ultrafiltration Membranes
Millipore (UK) Ltd.
'Millipore House'
Abbey Road
London NW10 7SP
England.

Norit A - Sigma Chemicals Ltd.
see D - Biotin for address.

Thiamine hydrochloride - Sigma Chemicals Ltd.
see D - Biotin for address.

APPENDIX 2

Sources of Cultures

Chlorella sp. L221 (FBA)

Freshwater Biological Association
The Ferry House
Ambleside
Cumbria LA22 OLP
England

Euglena gracilis Klebs strain Z

National Culture Collection of Algae and Protozoa
36 Storeys Way
Cambridge CB3 ODT
England

Fragilaria crotonensis L273 (FBA)

Freshwater Biological Association
see Chlorella sp for address.

Lactobacillus leichmanii ATCC 7830

Lactobacillus plantarum ATCC 8014

Lactobacillus viridescens ATCC 12706

National Collection of Industrial Bacteria
Torrey Research Station
PO Box 31
135 Abbey Road
Aberdeen
Scotland

Monochrysis lutheri Droop

Scottish Marine Biological Association
Dunstaffnage Marine Research Laboratory
PO Box 3
Oban
Argyll PA34 4AD
Scotland

Continued/

Ochromonas malhamensis Pringsheim

National Culture Collection of Algae and Protozoa
see E.gracilis for address.

Oocystis apiculata W. West

National Culture Collection of Algae and Protozoa
see E.gracilis for address.

Scenedesmus quadricauda L232 (FBA)

Freshwater Biological Association
see Chlorella sp for address.

APPENDIX 3

Manufacturers of Scientific Instruments

EEL 240 Atomic Absorption Spectrophotometer
Evans Electroselenium Ltd.
Halstead
Essex CO9 1BR
England

APPENDIX 4

Method of Cleaning Glass and Plasticware

Glass and plastic equipment was cleaned after use by the following method.

Firstly the apparatus was brushed clean to remove superficial dirt and then immersed in a 2% solution (volume for volume) of a quaternary ammonium detergent and left overnight. The apparatus was then rinsed in hot water and then left overnight immersed in 1N hydrochloric acid. The apparatus was then thoroughly rinsed in five changes of hot tap water and three changes of distilled water before being dried in an oven and finally stored inverted in a cupboard for future use.

APPENDIX 5

AQUATIC PRODUCTIVITY: ISOTOPIC TRACER AIDED STUDIES OF CHEMICAL-BIOLOGICAL INTERACTIONS

REPORT AND RECOMMENDATIONS OF AN ADVISORY GROUP
JOINTLY CONVENED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS,
HELD IN VIENNA, 16–20 JUNE 1975

Vitamins and especially vitamin B12 are micronutrients for about 50% of algae. Comparative studies of the vitamin B12 concentration in lakes and reservoirs indicate that this vitamin could be used as a measure of eutrophication. The concentration of vitamin B12 is relatively high in eutrophic waters. Two factors are involved: (a) increased input into the lake from sewage effluents, (b) a change in the balance between biological consumers and producers of vitamin B12. Bacteria and some algae, mainly blue-green algae are producers of vitamin B12. Other algae, mainly diatoms and dinoflagellates, are the main consumers.

Vitamin B12 therefore reflects the input to heterotrophic activity and algal species composition of a water body. Vitamin B12 may be measured by bioassay and by an isotope dilution method using ^{57}Co-labelled vitamin B12. The method has many practical advantages over more conventional bioassays for this vitamin.

Micronutrients in aquatic ecosystems

Micronutrients are well established as important components of aquatic environments. They may limit primary productivity through deficiency or toxicity. Both of these effects may be greatly modified by the chemistry of a water body, especially the presence of organic matter able to chelate trace metals.

In field and laboratory experiments designed to bioassay the deficiency, toxicity, or availability of micronutrients on the biota of aquatic environments, the uptake of labelled substrates is generally the best method of measuring the response of the organism to chemical changes of the squatic environment.

Trace metal micronutrients may limit autotrophic and auxotrophic primary productivity by deficiency (i.e. as limiting nutrients) or by their toxicity. Toxic effects may effect other organisms in the food chain directly or indirectly (through autotrophic organisms).

Both deficiency and toxicity effects may be greatly modified by the chemistry, especially the presence of organic matter with ability to chelate trace metals. Eutrophic waters may be subject to high inputs of trace metals and organic matter. The organic matter may include man-made chelators such as the polyphosphates used as detergent intermediates. Nitrilotriacetic acid (NTA), a polyphosphate substrate, is a powerful chelator of trace metals and this property must be taken into account when assessing the effects on the environment of replacing polyphosphates with NTA in detergent preparations.

As eutrophication proceeds, the balance between trace metal deficiency and toxicity will change. The net effect on aquatic biota cannot be easily predicted: The increased concentration of trace metals may be potentially toxic, but effectively counteracted by the organic matter also present in increased concentration. Chelation is an important area of limnology which has been relatively neglected, but may be amenable to bioassay techniques.

ADDENDUM

1. Introduction

The statistical methods used in this thesis are in many ways inappropriate and invalid. In this addendum more suitable statistical methods are outlined and where necessary the original data have been re-analysed by these methods. The results of this re-analysis are compared with the original analysis.

Three major criticisms may be made of the statistical methods used.

Firstly, a normal distribution of the data was assumed. This is not justified in many cases by an inspection of the raw data. Therefore the tests of significance described in section 2.9.4 of the thesis are invalid. Also the arithmetic mean is not a representative measure of the average for a highly skewed distribution - the median is much better - and the properties of the standard deviation associated with symmetric distributions no longer apply.

Secondly, the tests of significance described in section 2.9.4 of the thesis are inappropriate. These tests are essentially two sample tests and should not be repeatedly applied to pairs of samples from a multisample experiment.

Finally, correlation analysis rather than regression analysis would have been more appropriate in assessing the degree of association between two variables.

When analysing data which is not normally distributed, non-parametric or 'distribution free' statistical methods can be used.

Thus with highly skewed distributions the median is often reported because it represents the concept of an average better than the mean. The measure of variability associated with the median is the semi-interquartile range. Similarly, the Kruskal-Wallis one way analysis of variance by ranks is an extremely useful non-parametric test for

deciding whether k independant samples are from different populations. Calculation of Kendall's tau, or Spearman's rank correlation coefficient are both useful non-parametric methods of correlation analysis.

Methods for the calculation of the median and semi-interquartile range, together with the Kruskal-Wallis one way analysis of variance by ranks are described below. These methods have been used to re-analyse the raw data and the results are presented and compared with the original analysis.

It is not considered necessary to re-analyse the degree of association between chlorophyll and individual micronutrients by non-parametric methods. However, it is admitted that their use is to be preferred because they are 'distribution free' methods. It could however be argued that regression analysis is more appropriate than correlation analysis since the theory behind and tested in the thesis is that the concentration of chlorophyll in a body of water may be dependant on the concentration of one or more micronutrients in that water. The use of the phrase '.. to test the degree of association between individual results of (for example) dissolved vitamin B_{12} and chlorophyll determinations' in the text of the thesis is somewhat misleading - '.. to test the degree of dependance of chlorophyll concentration on (for example) the concentration of dissolved vitamin B_{12}' is more accurate. Regression analysis also allows an estimate to be made of the predictive value of the independant variable on the dependant variable. Regression does however require that the dependant variable be normally distributed and it will be recalled that the chlorophyll data was transformed by taking logarithms for this purpose. (A logarithmic transformation was found to give a better visual approximation to the normal curve than a square root transformation).

2. Methods

2.1. Statistical Analyses

2.1.1. The median, lower and upper quartiles, the semi-interquartile range

The median is defined as the central value of a set of ranked observations, that is it is the value of the variable which splits the set into two equal parts. The percentiles are the values of the variable which split the observations into 100 equal parts. The median is therefore the 50th percentile and the 25th and 75th percentiles are called the lower and upper quartiles respectively.

The measure of variability associated with the median, the semi-interquartile range (Q), is calculated from the equation:-

$$Q = \frac{Q_3 - Q_1}{2}$$

Where - Q_3 is the upper quartile
- Q_1 is the lower quartile

2.1.2. The Kruskal-Wallis one way analysis of variance by ranks

The Kruskal-Wallis one way analysis of variance by ranks is an extremely useful test for deciding whether k independant samples are from different populations. Further details of the test may be found in Siegel (1956).

For the test each of the N observations are replaced by ranks, that is all of the scores from all of the k samples combined are ranked in a single series. The smallest score is replaced by rank 1 and the largest by rank N, where N is the total number of independant observations in the k samples.

The Kruskal-Wallis statistic H is calculated from the formula:-

$$H = \frac{12}{N(N+1)} \cdot \sum_{j=1}^{k} \frac{R_j}{n_j} - 3(N+1)$$

Where - k is the number of samples

- n_j is the number of observations in the jth sample
- $N = \sum_{j=1}^{k} n_j$
- R_j is the sum of ranks in the jth sample

When ties occur between two or more scores each score is given the mean of the ranks for which it is tied. To correct H for the effect of ties H is divided by:-

$$1 - \frac{\sum T}{N^3 - N}$$

Where - $T = t^3 - t$

- t is the number of tied observations in a tied group of scores

H is distributed approximately as Chi square with k-1 degrees of freedom when n_j is greater than five. When k=3 and the number of observations in each of the three samples is less than or equal to five, the Chi square approximation to the sampling distribution of H is not sufficiently close. Probabilities for these cases may be found in Table O of the appendix in Siegel (1956).

When H is statistically significant, non-parametric multiple comparisons may be effected in a fashion paralleling the Newman-Keuls test (Zar 1974) by using rank sums instead of means in order determine between which of the samples significant differences occur.

2.1.3. The Mann Whitney U test

The Mann Whitney U test may be used to test whether two independant groups have been drawn from the same population.

To apply the test the observations or scores from both groups are combined and ranked in order of increasing size. The value of U is given by the number of times that a score in one group precedes a score in the other group. Further details of the method, together with tables of probabilities associated with values as small as observed values of U may be found in Siegel (1956).

2.2. Analysis of Results

Seasonal data for chlorophyll, phaeophytin, vitamin B_{12}, iron, manganese, copper, zinc and cobalt were summarised by calculation of the medians, lower and upper quartiles, and the semi-interquartile range. Differences between the reservoirs were analysed using the Kruskal-Wallis one way analysis of variance by ranks.

Data for sediment samples were re-analysed. Calculation of the semi-interquartile range was not possible as only three samples were collected in the field. Similarly the median is of little value as an estimate of the average concentration of micronutrients in the sediments. The statistical significance of differences within reservoirs between sediment micronutrient concentrations in the 'B' and 'C' layers was re-assessed using the Mann Whitney U test. The significance of differences in the concentration of micronutrients in the 'B' and 'C' sediment layers between reservoirs was re-assessed using the Kruskal-Wallis one way analysis of variance by ranks.

Results of analyses of micronutrient concentrations in water samples from the feeder streams were summarised by calculation of the

medians, lower and upper quartiles, and the semi-interquartile range. Differences between feeder streams were re-analysed using the Kruskal-Wallis one way analysis of variance by ranks.

To enable re-calculation of micronutrient budgets for the period April 1974 to March 1975, median values of the concentrations of micronutrients in the water entering the reservoirs were calculated.

3. Results

3.1. Reservoir Water

In this section data for the variables measured in the three reservoirs have been summarised by calculation of the median, upper and lower quartiles, and the semi-interquartile range.

Analysis of the statistical significance of the differences in these variables between the reservoirs has been performed using the Kruskal-Wallis one way analysis of variance by ranks.

The results are presented in tabular form followed by a paragraph outlining any differences between the original and re-analyses.

3.1.1. Chlorophyll and phaeophytin

Table 1

Summary of determinations of chlorophyll and phaeophytin from April 1973 to March 1975 in reservoir water samples

Chlorophyll ($\mu g/l$)	Reservoir		
	Cropston	Swithland	Thornton
Median	3.0	2.2	6.6
Lower Quartile	1.4	1.1	2.5
Upper Quartile	6.6	5.2	13.9
Semi-interquartile Range	2.6	2.1	5.7

Table 1 continued

Phaeophytin (µg/l)	Reservoir		
	Cropston	Swithland	Thornton
Median	0.2	0.2	0.5
Lower Quartile	0.0	0.0	0.0
Upper Quartile	0.6	1.0	2.5
Semi-interquartile Range	0.3	0.5	1.3

Table 2

Analysis of the differences in the average concentrations of chlophyll and phaeophytin between reservoirs

Difference between	in Chlorophyll concentration	in Phaeophytin concentration
All three reservoirs	SS* p<0.001	NS
Cropston and Swithland	NS	
Cropston and Thornton	SS p<0.001	
Swithland and Thornton	SS p<0.001	

*Key - SS - Statistically significant at probability level p
NS - Not significant

The results of the original analyses and re-analyses of the data show the concentration of chlorophyll to be significantly higher in Thornton reservoir than in either Cropston or Swithland reservoirs.

3.1.2 Dissolved vitamin B_{12}

Table 3

Summary of determinations of dissolved vitamin B_{12} from April 1973 to March 1975 in reservoir water samples by bioassay

Dissolved Vitamin B_{12} (ng/l)	Reservoir		
	Cropston	Swithland	Thornton
E. gracilis assay			
Median	24	26	36
Lower Quartile	19	20	29
Upper Quartile	28	37	45
Semi-interquartile Range	4.5	8.5	8.0
L. leichmanii assay			
Median	12	15	22
Lower Quartile	9	12	14
Upper Quartile	17	25	34
Semi-interquartile Range	4.0	6.5	10.0
O. malhamensis assay			
Median	2.0	3.0	3.2
Lower Quartile	1.2	1.6	2.0
Upper Quartile	3.0	4.1	4.4
Semi-interquartile Range	0.9	1.3	1.2

medians, lower and upper quartiles, and the semi-interquartile range. Differences between feeder streams were re-analysed using the Kruskal-Wallis one way analysis of variance by ranks.

To enable re-calculation of micronutrient budgets for the period April 1974 to March 1975, median values of the concentrations of micronutrients in the water entering the reservoirs were calculated.

3. Results

3.1. Reservoir Water

In this section data for the variables measured in the three reservoirs have been summarised by calculation of the median, upper and lower quartiles, and the semi-interquartile range.

Analysis of the statistical significance of the differences in these variables between the reservoirs has been performed using the Kruskal-Wallis one way analysis of variance by ranks.

The results are presented in tabular form followed by a paragraph outlining any differences between the original and re-analyses.

3.1.1. Chlorophyll and phaeophytin

Table 1

Summary of determinations of chlorophyll and phaeophytin from April 1973 to March 1975 in reservoir water samples

Chlorophyll ($\mu g/l$)	Reservoir		
	Cropston	Swithland	Thornton
Median	3.0	2.2	6.6
Lower Quartile	1.4	1.1	2.5
Upper Quartile	6.6	5.2	13.9
Semi-interquartile Range	2.6	2.1	5.7

Table 4

Analysis of the differences in the concentration of vitamin B_{12} between reservoirs

Difference between	Assay organism		
	E.gracilis	L.leichmanii	O.malhamensis
All three reservoirs	SS* p<0.001	SS p<0.001	SS p<0.01
Cropston and Swithland	SS p<0.001	SS p<0.001	SS p<0.01
Cropston and Thornton	SS p<0.001	SS p<0.001	SS p<0.01
Swithland and Thornton	SS p<0.001	SS p<0.001	NS

*Key - see page 8

The results of the re-analysis show significant differences between Cropston and Swithland reservoirs, and Cropston and Thornton reservoirs in the concentration of dissolved vitamin B_{12} assayed with O. malhamensis not apparent in the original analysis. However, within reservoirs, re-analysis of the statistical significance of the differences in dissolved vitamin B_{12} concentration assayed with the three different organisms produced exactly the same results as the original analysis (see page 96).

3.1.3. Soluble (free) and complexed (bound) vitamin B_{12}

Table 5

Summary of determinations of soluble (free) and complexed (bound) vitamin B_{12} from February 1974 to March 1975 in reservoir water samples

Vitamin B_{12} (ng/l)	Reservoir					
	Cropston		Swithland		Thornton	
	Free	Bound	Free	Bound	Free	Bound
Median	23	5	27	3	32	3
Lower Quartile	19	2	20	0	20	2
Upper Quartile	25	6	32	6	36	9
Semi-interquartile Range	3	2	6	3	8	3.5

Re-analysis of the differences in concentrations of soluble and complexed vitamin B_{12} showed no significant differences between reservoirs. This result is in agreement with the original analysis (sections 2.9.4a and 2.9.4b in the thesis).

3.1.4. Particulate vitamin B_{12}

Table 6

Summary of determinations of particulate vitamin B_{12} from April 1973 to March 1975 in reservoir water samples by bioassay

Vitamin B_{12} (ng/l)	Reservoir		
	Cropston	Swithland	Thornton
Median	1.32	1.20	2.40
Lower Quartile	0.84	0.67	1.32
Upper Quartile	2.04	2.00	6.25
Semi-interquartile Range	0.60	0.67	2.47

Table 7

Analysis of the differences in the concentration of particulate vitamin B_{12} between reservoirs

Difference between	in particulate vitamin B_{12} concentration :
All three reservoirs	SS* p<0.001
Cropston and Swithland	NS
Cropston and Thornton	SS p<0.001
Swithland and Thornton	SS p<0.001

*Key - see page 8

The results of the original and re-analyses of the data show the concentration of particulate vitamin B_{12} to be significantly higher in Thornton reservoir than in either Cropston or Swithland reservoirs.

3.1.5. Iron

Table 8

Summary of determinations of iron from December 1973 to March 1975 in reservoir water samples

Iron (µg/l)	Reservoir		
	Cropston	Swithland	Thornton
Soluble			
Median	9	2	6
Lower Quartile	6	0	2
Upper Quartile	12	6	12
Semi-interquartile Range	3	3	5
Complexed			
Median	62	23	47
Lower Quartile	37	13	30
Upper Quartile	105	28	57
Semi-interquartile Range	34	7.5	13.5
Particulate			
Median	68	22	74
Lower Quartile	39	13	45
Upper Quartile	94	34	104
Semi-interquartile Range	27.5	10.5	29.5
Total			
Median	137	56	122
Lower Quartile	107	34	93
Upper Quartile	206	69	192
Semi-interquartile Range	50.5	17.5	49.5

Table 9

Analysis of the differences in the concentration of iron between reservoirs

Difference between	Iron fraction			
	Soluble	Complexed	Particulate	Total
All three reservoirs	SS* p<0.001	SS p<0.001	SS p<0.001	SS p<0.001
Cropston and Swithland	SS p<0.001	SS p<0.001	SS p<0.001	SS p<0.001
Cropston and Thornton	NS	SS p<0.05	NS	NS
Swithland and Thornton	SS p<0.001	SS p<0.001	SS p<0.001	SS p<0.001

* Key - see page 8

The results of the original and re-analyses of the data show the concentration of all iron fractions to be significantly higher in Cropston and Thornton reservoirs than in Swithland reservoir. The absence of a significant difference between Cropston and Thornton reservoirs in the concentrations of soluble and particulate iron on re-analysis is explained by the fact that the 0.1 significance level has not been used.

3.1.6. Manganese

Table 10

Summary of determinations of manganese from December 1973 to March 1975 in reservoir water samples

Manganese (µg/l)	Reservoir		
	Cropston	Swithland	Thornton
Soluble			
Median	73	26	29
Lower Quartile	24	11	10
Upper Quartile	107	60	101
Semi-interquartile Range	41.5	24.5	45.5
Complexed			
Median	0	0	0
Lower Quartile	0	0	0
Upper Quartile	1	4	6
Semi-interquartile Range	0.5	2.0	3.0
Particulate			
Median	11	12	18
Lower Quartile	5	4	6
Upper Quartile	20	18	47
Semi-interquartile Range	7.5	7.0	20.5
Total			
Median	81	34	76
Lower Quartile	58	25	22
Upper Quartile	109	70	164
Semi-interquartile Range	25.5	22.5	71.0

Table 11

Analysis of the differences in the concentration of manganese between reservoirs

Difference between	Manganese fraction			
	Soluble	Complexed	Particulate	Total
All three reservoirs	SS* p<0.01	NS	SS p<0.1	SS p<0.01
Cropston and Swithland	NS		NS	SS p<0.01
Cropston and Thornton	SS p<0.05		SS p<0.01	NS
Swithland and Thornton	NS		NS	SS p<0.01

* Key - see page 8

Re-analysis using the Kruskal-Wallis test showed features contrary to the original analysis (see section 3.1.1.4 in the thesis).

Firstly, re-analysis showed a significant difference in soluble manganese concentration between Cropston and Thornton reservoirs, but no significant difference in soluble manganese concentration between Cropston and Swithland reservoirs. The lack of a similarly significant difference between Swithland and Thornton reservoirs is explained by the fact that the 0.1 significance level was not used in the re-analysis.

Secondly, re-analysis showed a significant difference in particulate manganese concentration between Cropston and Thornton reservoirs, but no significant difference in particulate manganese concentration between Swithland and Thornton reservoirs. The lack of a similarly significant difference between Cropston and Swithland reservoirs is explained by the fact that the 0.1 significance level was not used in the re-analysis.

However, with the exception of complexed manganese the conclusion of the original analysis that the concentration of all manganese fractions were higher in Thornton reservoir than in Swithland reservoir, remains valid.

3.1.7. Copper

Table 12

Summary of determinations of copper from April 1973 to March 1975 in reservoir water samples

Copper (µg/l)	Reservoir		
	Cropston	Swithland	Thornton
Soluble			
Median	14	14	20
Lower Quartile	8	9	10
Upper Quartile	20	22	32
Semi-interquartile Range	6.0	6.5	11.0
Complexed			
Median	0	0	0
Lower Quartile	0	0	0
Upper Quartile	2	3	2
Semi-interquartile Range	1.0	1.5	1.0
Particulate			
Median	17	21	26
Lower Quartile	12	15	19
Upper Quartile	30	34	47
Semi-interquartile Range	9.0	9.5	14.0
Total			
Median	17	21	26
Lower Quartile	12	15	19
Upper Quartile	30	34	47
Semi-interquartile Range	9.0	9.5	14.0

Table 13

Analysis of the differences in the concentration of copper between reservoirs

Difference between	Copper fraction			
	Soluble	Complexed	Particulate	Total
All three reservoirs	SS* $p<0.05$	NS	SS $p<0.05$	SS $p<0.01$
Cropston and Swithland	NS		SS $p<0.05$	SS $p<0.05$
Cropston and Thornton	SS $p<0.05$		SS $p<0.001$	SS $p<0.01$
Swithland and Thornton	SS $p<0.01$		NS	SS $p<0.01$

* Key - see page 8

Re-analysis shows a significant difference between the concentration of total copper in Cropston and Swithland reservoirs not apparent in the original analysis. The comments made in section 3.1.1.5 (of the thesis) are not altered by re-analysis of the data.

3.1.8. Zinc

Table 14

Summary of determinations of zinc from April 1973 to March 1975 in reservoir water samples

Zinc (µg/l)	Reservoir		
	Cropston	Swithland	Thornton
Soluble			
Median	5	4	5
Lower Quartile	3	1	3
Upper Quartile	9	5	8
Semi-interquartile Range	3.0	2.0	2.5
Complexed			
Median	0	1	1
Lower Quartile	0	0	0
Upper Quartile	3	4	4
Semi-interquartile Range	1.5	2.0	2.0
Particulate			
Median	0	0	1
Lower Quartile	0	0	0
Upper Quartile	3	2	3
Semi-interquartile Range	1.5	1.0	1.5
Total			
Median	8	7	8
Lower Quartile	5	5	6
Upper Quartile	12	9	15
Semi-interquartile Range	3.5	2.0	4.5

Table 15

Analysis of the differences in the concentration of zinc between reservoirs

Difference between	Zinc fraction			
	Soluble	Complexed	Particulate	Total
All three reservoirs	NS*	NS	NS	NS

* Key - see page 8

Table 14 shows the concentration of all zinc fractions to be very similar in all three reservoirs. This is reflected in Table 15 which shows no significant differences in the concentration of zinc between reservoirs in contrast to the original analysis. This is explained by the fact that the 0.1 and 0.02 significance levels were not used in the re-analysis.

3.1.9. Cobalt

Table 16

Summary of determinations of cobalt from April 1973 to March 1975 in reservoir water samples

Total Cobalt (µg/l)	Reservoir		
	Cropston	Swithland	Thornton
Median	3	4	5
Lower Quartile	2	3	3
Upper Quartile	5	6	6.5
Semi-interquartile Range	1.5	1.5	1.8

Table 17

Analysis of the differences in the concentration of cobalt between reservoirs

Difference between	in total cobalt
All three reservoirs	SS* p<0.01
Cropston and Swithland	SS p<0.01
Cropston and Thornton	SS p<0.01
Swithland and Thornton	SS p<0.05

* Key - see page 8

The comments made in section 3.1.1.7 of the thesis are not affected by re-analysis of the data.

3.2. Reservoir Sediments

Since only three sediment samples were taken from each reservoir, calculation of the semi-interquartile range is not possible and the median is of little value as an estimate of the average concentration of micronutrients in the samples.

Re-analysis of the statistical significance of differences within reservoirs between sediment micronutrient concentrations in the 'B' and 'C' layers was carried out using the Mann Whitney U test. The results are presented in Table 18 as exact probabilities in order to help the reader evaluate where differences might lie had the samples been bigger.

Table 18

Analysis of the differences between the micronutrient concentrations in the 'B' and 'C' layers of sediment within reservoirs

Micronutrient	Reservoir		
	Cropston	Swithland	Thornton
Vitamin B_{12}	α=0.05	α=0.05	α=0.05
Iron	α=0.35	α=0.20	α=0.60
Manganese	α=0.10	α=0.05	α=0.10
Copper	α=0.35	α=0.05	α=0.10
Zinc	α=0.35	α=0.05	α=0.10
Cobalt	α=0.20	α=0.05	α=0.60

Thus re-analysis shows additional differences (at α=0.05) between the 'B' and 'C' sediment layers in the concentration of vitamin B_{12} in Cropston and Thornton reservoirs.

Re-analysis of the statistical significance of differences between reservoirs in the concentration of micronutrients in the 'B' and 'C' sediment layers was carried out using the Kruskal-Wallis one way analysis of variance by ranks. Since only three samples were taken from each reservoir, the Chi-square approximation to the sampling distribution of H is not sufficiently close. For such cases, exact probabilities are given in Table O of the appendix in Siegel (1956). Where H is statistically significant, non-parametric multiple comparisons between pairs of reservoirs have been made by a technique paralleling the Newman-Keuls test (Zar 1974). The results are presented in Table 19.

Compared with the original analysis, re-analysis shows no significant difference between Swithland and Thornton reservoirs in the concentrations of iron and copper in the 'B' sediment layer. This is explained by the fact that the 0.1 significance level was not used in the re-analysis.

Re-analysis also showed no significant difference in zinc concentration in the 'B' sediment layer between Cropston and Swithland reservoirs. This may be explained by the relatively low exact probability for H (0.086) calculated for this micronutrient. However, it is apparent from Table 23 of the thesis that in terms of actual concentration, the size of the differences (in mean zinc concentration in the 'B' sediment layer) between Cropston and Swithland, and Cropston and Thornton reservoirs are almost identical.

Table 19

Analysis of differences in the micronutrient concentrations in the 'B' and 'C' sediment layers between reservoirs

Difference between	Sediment layer	Micronutrient					
		Vitamin B_{12}	Iron	Manganese	Copper	Zinc	Cobalt
All three reservoirs	B	α=0.004	α=0.05	α=0.011	α=0.011	α=0.086	NS*
	C	NS	NS	NS	NS.	NS	NS
Cropston and Swithland	B	SS* p<0.05	SS p<0.01	SS p<0.05	SS p<0.05	NS	
Cropston and Thornton	B	SS p<0.05	NS	NS	SS p<0.05	SS p<0.01	
Swithland and Thornton	B	SS p<0.05	NS	SS p<0.05	NS	NS	

* Key - see page 8.

3.3. Feeder Streams

In this section data for the variables measured in the four feeder streams to the reservoirs have been summarised by calculation of the median, upper and lower quartiles, and the semi-interquartile range (Table 20).

Analysis of the statistical significance of the differences in these variables between feeder streams has been performed using the Kruskal-Wallis one way analysis of variance by ranks (Table 21).

The results are presented in tabular form followed by a paragraph outlining any differences between the original and re-analyses.

Table 20

Summary of results of micronutrient determinations on water samples from the feeder streams

Micronutrient (µg/l)	Name of stream			
	Bradgate	Swithland	Stanton	Markfield
Iron				
Median	264	244	488	402
Lower Quartile	232	120	260	186
Upper Quartile	390	452	680	587
Semi-interquartile range	79	166	210	201
Manganese				
Median	44	30	59	35
Lower Quartile	36	24	41	28
Upper Quartile	51	40	68	49
Semi-interquartile range	7.5	8.0	13.5	10.5

Continued

Table 20 continued

Micronutrient	Name of stream			
	Bradgate	Swithland	Stanton	Markfield
Copper (µg/l)				
Median	10	14	14	14
Lower Quartile	7	8	12	10
Upper Quartile	16	24	19	17
Semi-interquartile range	4.5	8.0	3.5	3.5
Zinc (µg/l)				
Median	6	6	6	8
Lower Quartile	4	5	8	5
Upper Quartile	8	9	9	10
Semi-interquartile range	2.0	2.0	0.5	2.5
Cobalt (µg/l)				
Median	5	4	7	7
Lower Quartile	4	4	5	4
Upper Quartile	5	7	8	8
Semi-interquartile range	0.5	1.5	1.5	2.0
Vitamin B_{12} (ng/l)				
a) *E. gracilis*				
Median	24	16	26	27
Lower Quartile	23	10	14	24
Upper Quartile	41	21	39	36
Semi-interquartile range	9.0	5.5	12.5	6.0

Continued

Table 20 continued

Micronutrient	Name of stream			
	Bradgate	Swithland	Stanton	Markfield
Vitamin B_{12}				
b) L.leichmanii				
Median	13	8	21	14
Lower Quartile	6	7	13	11
Upper Quartile	18	17	41	23
Semi-interquartile range	6.0	5.0	14.0	6.0
Vitamin B_{12}				
c) O.malhamensis				
Median	1.9	2.0	2.4	2.3
Lower Quartile	1.5	1.1	1.6	1.7
Upper Quartile	3.0	3.9	4.8	3.0
Semi-interquartile range	0.8	1.4	1.6	0.7

Re-analysis of differences in the micronutrient concentrations of feeder streams (Table 21) differs from the original analysis in not showing any significant difference between all four streams in the concentration of cobalt and no significant difference between Markfield and Swithland streams in the concentration of vitamin B_{12}.

Table 21

Analysis of differences in the micronutrient concentrations of feeder streams

Difference between	Micronutrient					
	Vitamin B_{12} (*E.gracilis*)	Iron	Manganese	Copper	Zinc	Cobalt
All four streams	SS* p<0.05	NS	SS p<0.05	NS	NS	NS
Stanton and Markfield	NS		SS p<0.01			
Stanton and Swithland	SS p<0.01		SS p<0.05			
Stanton and Bradgate	NS		SS p<0.01			
Markfield and Swithland	NS		NS			
Markfield and Bradgate	NS		NS			
Swithland and Bradgate	SS p<0.001		NS			

* Key – see page 8

Table 22

Micronutrient Budgets for the Reservoirs from April 1974 to March 1975

	grams		kilograms									
	Dissolved Vitamin B_{12}		Total Metal									
			Fe		Mn		Cu		Zn		Co	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Cropston Reservoir	157	146	1727	834	288	493	65	104	39	49	33	18
Net Change	11		893			205		39		10	15	
Swithland Reservoir	48	65	737	139	91	84	42	52	18	17	12	10
Net Change		17	598		7			10	1		2	
Thornton Reservoir	75	75	1198	253	121	157	39	54	19	17	19	10
Net Change	0		945			36		15	2		9	

Table 23

Average Micronutrient Concentrations in the Water Entering the Reservoirs in April 1974 to March 1975

	Dissolved Vitamin B_{12} (ng/l)	Total Metal (µg/l)				
		Fe	Mn	Cu	Zn	Co
Cropston Reservoir	24	264	44	10	6	5
Swithland Reservoir	16	244	30	14	6	4
Thornton Reservoir	27	434	44	14	7	7

3.4. Nutrient Budgets

Using the median rather than the mean, micronutrient budgets for the reservoirs have been re-calculated. The results are presented in Table 22. The median micronutrient concentrations in the water entering the reservoirs are given in Table 23.

4. Summary

Reflecting the positively skewed nature of the underlying frequency distributions, determination of the median rather than calculation of the mean has in general produced lower estimates of the average concentrations of chlorophyll and micronutrients in the reservoirs and feeder streams.

Comparison of the results of the parametric and non-parametric tests of significance show few differences where the number of observations is large, as in the reservoir water survey. Many of the differences that do exist only do so because the 0.1 significance level was not used in the re-analysis. The differences that remain are listed below:-

a) The results of re-analysis show significant differences between Cropston and Swithland and Cropston and Thornton reservoirs in the concentration of dissolved vitamin B_{12} assayed with O.malhamensis not apparent in the original analysis.

b) The results of re-analysis show no significant difference between Cropston and Swithland reservoirs in the concentration of soluble manganese, and no significant difference between Swithland and Thornton reservoirs in the concentration of

particulate manganese.

c) Re-analysis shows a significant difference between Cropston and Swithland reservoirs in the concentration of total copper not apparent in the original analysis.

d) Re-analysis shows a significant difference in vitamin B_{12} concentration between the 'B' and 'C' sediment layers of Cropston and Thornton reservoirs

e) The results of re-analysis show no significant difference between Cropston and Swithland reservoirs in the concentration of zinc in the 'B' sediment layer.

f) Re-analysis shows no significant difference in the cobalt concentration of all four feeder streams.

g) Re-analysis shows no significant difference between Markfield and Swithland streams in the concentration of vitamin B_{12}.

The differences shown by re-analysis in no way affect the interpretation of the results presented in the discussion and conclusion sections of the thesis.

5. References

Siegel S. (1956)
Nonparametric statistics for the behavioural sciences. McGraw-Hill, New York.

Zar J.H. (1974)
Biostatistical analyses. Prentice-Hall, Englewood Cliffs.