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Pollution Special Study (PSS)

Standing instructions for the field, laboratory and data analytical and reporting activities of the Lake Tanganyika special study on pollution (eutrophication)

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2000

Pollution Control and Other Measures to Protect Biodiversity in Lake Tanganyika (RAF/92/G32)
Lutte contre la pollution et autres mesures visant à protéger la biodiversité du Lac Tanganyika (RAF/92/G32)

Le Projet sur la diversité biologique du lac Tanganyika a été formulé pour aider les quatre Etats riverains (Burundi, Congo, Tanzanie et Zambie) à élaborer un système efficace et durable pour gérer et conserver la diversité biologique du lac Tanganyika dans un avenir prévisible. Il est financé par le GEF (Fonds pour l'environnement mondial) par le biais du Programme des Nations Unies pour le développement .	The Lake Tanganyika Biodiversity Project has been formulated to help the four riparian states (Burundi, Congo, Tanzania and Zambia) produce an effective and sustainable system for managing and conserving the biodiversity of Lake Tanganyika into the foreseeable future. It is funded by the Global Environmental Facility through the United Nations Development Programme.

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D R Congo: Ministrie Environnement et Conservation de la Nature
Tanzania: Vice President's Office, Division of Environment
Zambia: Environmental Council of Zambia

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STANDING INSTRUCTIONS FOR THE FIELD, LABORATORY AND DATA ANALYTICAL AND REPORTING ACTIVITIES OF THE LAKE TANGANYIKA SPECIAL STUDY ON POLLUTION (EUTROPHICATION) AND ITS EFFECTS ON BIODIVERSITY

1. INTRODUCTION

This paper describes a selection of the numerous field and laboratory activities involved in the Lake Tanganyika Biodiversity Project (LTBP) Pollution Special Study (PSS). It relates particularly to the following components of the PSS:

- marshalling the equipment and human resources to carry out the tasks
- field sampling and recording of environmental conditions, sample location and type
- where necessary, treating the samples in the field (e.g. filtering, fixing, storing)
- making up reagents
- cleaning and storage of laboratory glassware and plastic containers
- the use and maintenance of balances
- using and maintenance of water stills
- analysing chemical and biological samples in the laboratory

The 'core' determinands and factors concerned with general limnological studies, eutrophication and the ecology of the phytoplankton are as follows:

- weather, especially the wind regime
- water level
- water clarity
- water temperature
- conductivity
- dissolved oxygen
- pH
- total and dissolved fractions of phosphorus
- nitrate-nitrogen
- dissolved silica
- total phytoplankton abundance (chlorophyll$_a$)
- the diversity/richness and population densities of planktonic algal species and of the more common microscopic zooplankton e.g. Protozoa[1]
- incidence of healthy and parasitised or grazed algae

[The nature of the zooplankton, including possibly Rotifera, as well as calanoid and cyclopoid micro-Crustacea and maybe filter-feeding Cladocera were dealt with by the BioSS team.]

In addition, the anglophone laboratories analysed for carbonate and bi-carbonate alkalinity.
In recognition of the main focus on heavy pollution in and around Bujumbura, the Francophone laboratories also paid more attention than the other stations to heavy metals such as Cadmium and Lead. It should be borne in mind that the Bujumbura laboratories also

[1] As a reflection of the environmental indicator value of algal assemblages, we intended to examine the algae attached to substrates such as sand, stones, cobbles, rocks and submerged plants, but in terms of just species numbers rather than population densities; however, little progress was made in this area

used somewhat different i.e. 'HACH'-type methods for their analyses in contrast to the more standard spectrophotometric procedures. This document describes the methods adopted in Tanzania and Zambia – although issues relating to many of the field practices and phytoplankton studies are applicable to all three countries. Before embarking on the detailed procedures, it is stressed that this document is not all-embracing. There is already an enormous body of literature on limnological methods relevant to the PSS programme, and each laboratory was given a copy of the following:

MACKERETH, F. J. H., HERON, J. and TALLING, J. F. (1978). Water Analysis: some revised methods for limnologists, Freshwater Biological Association Scientific Publication, 36.

As such, the present document details the analyses of only three chemical factors i.e. total phosphorus (TP), total soluble phosphorus (TSP) and soluble reactive phosphorus SRP). The main thrust is on physical factors and aspects of the phytoplankton. Throughout, the PURPOSE for carrying out a particular observation or analysis is outlined.

2. WEATHER CONDITIONS AND PHYSICAL MEASUREMENTS OF WATER

Purpose: It is important to record as many aspects of the weather at the time of sampling as possible. This is because the weather determines much of the nature of the physical environment of the lake organisms, and thus to a large extent the very nature (species composition) of the biota at different times of the year.

As examples, wind, temperature and water movement determine whether the water column is isothermal (i.e. mixed and of uniform temperature) or stratified (with cooler, denser water at depth). Under stratified conditions, motile organisms such as flagellate algae or protozoans, and cyanobacteria can adjust their buoyancy and thus move up and down through the water to their preferred light and nutrient zones. Under the same conditions the relatively heavier diatoms may sink out of the lighted zone.

Cloud cover is another important factor in that it affects sunlight energy reaching the lake and thus, the photosynthetic potential of the primary producers, the phytoplankton.

Rainfall, or the lack of it, is the major factor influencing river flows.

Water clarity measurements provide valuable information on the underwater light climate, which can be correlated with data on chlorophyll$_a$, total suspended solids and phytoplankton abundance. In conjunction, the appearance of the surface water could give an indication of its chemical/biological state (by its colour, the presence of visible aggregations of algae, suspended sediment etc.).

Water level records could provide information on seasonal changes in seiches (internal waves) and upwellings which can lead to changes in phytoplankton diversity and abundance - as recorded at Mpulungu in May 1998.

Whilst it may not be necessary to record the weather conditions at all sampling sites, some attempt to do this should be made where the distances between one site and another exceed,

say, 10km. The teams should take full advantage of the existing weather stations, as for example at Mpulungu, since these can provide more-or-less daily, and even continuous records.

Determinands and methods:

(not all of these are attainable for all stations)

Wind force and direction

The strength of the prevailing wind can be fairly readily described using terms such as 'nil', 'light', 'light-to-moderate', 'moderate', 'moderate-to-strong', 'strong' (though presumably the latter two terms are likely to be very little-used, since there probably should not be any open-water sampling attempted during those conditions!).
The direction from which the wind is blowing can be more specifically identified by its 8-point compass bearing,
e.g. SE, N, NW etc.

Daily run of the wind

The daily total 'amount' of wind, recorded using a cup-counter anemometer.

Daily rainfall

Measured using a standard meteorological rain gauge.

Air temperature

Ideally daily maximum and minimum air temperatures are best measured using a screened mercury-in-glass maximum-minimum thermometer. Where this is not possible, and certainly on the boat at sampling sites, the air temperature is measured using an ordinary thermometer shaded from direct sunlight.

Water temperature

The surface water temperature is measured using a mercury-in-glass thermometer in a bucketful of water, to eliminate the possibility of loss of the thermometer over the side of the boat. Where temperatures at depth are required, a probe with a sufficient length of cable is necessary.
(Both the HANNA instruments HI 9023 pH meter and the HI 9143 dissolved oxygen meter measure and display temperature - via a separate probe in the case of the 9023 and an integral sensor in the probe with the 9143)

Water level

Daily readings can be taken from metric staff-gauges fixed at convenient points, usually in close proximity to the LTBP laboratories, e.g. to landing-stages or piers. Ideally, the gauges should be levelled, i.e. fitted in positions relative to points of known altitude, so that the

changes in water level that are recorded at these gauged sites around the lake are directly comparable.

Water clarity

Measured using a Secchi disc. This is a 20cm diameter, quartered black-and-white-painted metal or plastic disc attached to a rope and a weight through a hole in the disc's centre. The weighted disc is lowered into the water on the side of the boat away from direct sunlight and the depth at which the disc just disappears from view is recorded. The rope may be marked with a scale to facilitate measurement, and readings should be taken by two different people for comparison.

As with describing the strength of the prevailing wind, this procedure is highly subjective, so it is recommended that at least one of the persons taking the measurement on any particular week also did so on the previous week.

3. PHOSPHORUS ANALYSES

Soluble reactive phosphorus (SRP)

Purpose:

The determination of SRP in lake studies is important in providing an estimate of the amount of the nutrient phosphorus that exists in a form that can be readily utilised by the algae. Generally speaking, once the supply of SRP ceases, algal growth and production of new cells are likely to stop. Observed low concentrations of SRP, however, do not necessarily indicate a decrease in supply since the nutrient could be being taken in by the algae as fast as it becomes available, thus not being detected in solution. In this case the complete picture of events is given by an increase both in the algal populations and in the phosphorus contained within the cells, the particulate phosphorus (PP).

Many species of algae are able to store phosphorus in their cells, in excess of their immediate needs. This explains why some algal populations can continue to grow (at least for a short time) while SRP levels in the water are virtually undetectable *and* the supply is diminished.

Summary of method: Some forms of phosphate in acidic molybdate solution react to form a yellow phospho-molybdate complex which is reduced with ascorbic acid to a more stable blue complex that has a higher optical absorption than the yellow one. The reaction is catalysed by antimony and the blue colour is formed in amounts proportional to the SRP present.

Apparatus:
All glassware and plastic ware, including pipettor tips, weighing boats and magnetic stirrer bars, coming into direct contact with chemicals or solutions, must have undergone the laboratory washing-up procedure described in Appendix (I) before use. This is especially important because the SRP concentrations that are likely to be encountered in most of the Lake Tanganyika samples will be so low that if we do not maintain clean laboratory ware, we can have no confidence in our results.

Plastic or glass test-tubes (10-15ml capacity), and racks
Glass volumetric flasks
Glass measuring cylinders
Glass volumetric pipettes with pipette fillers
Glass conical flasks
Glass beakers
Repeating pipettes (5ml and 1ml, with appropriate tips)
4-point analytical balance with polystyrene weighing boats and stainless-steel spatula
Magnetic stirrer/hotplate and stirrer bars
Rotormixer
Spectrophotometer (for readings at 882nm) with 4cm glass cuvettes (or, preferably, a 4cm flowcell)

Stock phosphate solution and working standard phosphate solution:

The stock phosphate solution must be prepared very accurately.

Place a clean, dry, polystyrene weighing boat onto the clean, dry, pan of the analytical balance and tare to zero. According to the procedures described in Appendix (II), weigh out exactly 0.2195g of potassium dihydrogen orthophosphate (KH_2PO_4) or 0.2812g of di-potassium hydrogen orthophosphate (K_2HPO_4) and, using a clean glass 500ml beaker and a clean glass 500ml volumetric flask, prepare exactly 500ml of solution. This stock phosphate solution now contains exactly 100mg P l^{-1}, and can be transferred to a glass bottle with screw-cap for storage in a refrigerator where it will keep for up to six months.

To prepare the working standard solution of 100µg P l^{-1} draw up exactly 0.5ml of the stock solution into a clean and dry glass volumetric pipette using a pipette filler and transfer this to a clean and dry 500ml glass volumetric flask. Add distilled water up to the 500ml graduation mark to give the 100µg P l^{-1} working standard solution, which can be poured into a clean and dry 500ml glass conical flask for use.

This working standard solution must be made up fresh for each batch of analyses.

Reagents:

2.5M sulphuric acid solution (H_2SO_4):
Prepare, in advance, some ice (possibly by carefully scraping some that may have built up inside the freezer), break it into smallish pieces and add it to a bucket half-filled with tap-water and position this beside or even in a sink. Place approximately 350ml of distilled water into a clean and dry 500ml glass conical flask. Using a clean ***and dry*** 25ml glass volumetric pipette, fitted with a pipette filler, **very carefully** measure out, in stages, 70ml of concentrated sulphuric acid and run each stage slowly into the conical flask (e.g. 20ml followed by 20ml followed by 20ml followed by 10ml). Whilst the concentrated acid is running into the distilled water keep the conical flask dipped into the bucket of iced water and swirl it gently around. **The addition of concentrated sulphuric acid to water generates a lot of heat so it is essential to cool the process.**

NEVER add water to concentrated sulphuric acid, ALWAYS add the acid to the water

Wear rubber gloves and eye protection and have running water near at hand, hence the sink. When the 70ml of acid have been added and the solution has been allowed to cool to room temperature, dry the outside of the conical flask and pour the solution into a clean and dry 500ml glass measuring cylinder. It is now possible to add distilled water up to the 500ml graduation mark and pour the 500ml of solution back into the conical flask to mix it. Transfer to a screw-topped glass bottle for storage out of direct sunlight, i.e. preferably in a cupboard. Provided the bottle remains tightly capped between occasions of use, this solution should keep indefinitely.

Ammonium molybdate solution:
This solution is also used in the analysis for total phosphorus (TP), so if both SRP and TP analyses are being carried out on the same day just one batch of this reagent need be prepared, sufficient for both sets of analyses.

Because this solution does not keep very well, it is best to make it up fresh on each occasion. In that case, to avoid undue wastage, add up the total number of test-tubes of samples, distilled water blanks and working standards. This will give the volume(s) of mixed reagent(s) that is/are to be prepared, 1ml of mixed reagent being required for each test-tube (see the appropriate SRP and TP/TSP 'Recipes' tables). From the 'Recipes' tables, find out how much ammonium molybdate solution is required to prepare the mixed reagent(s) and prepare a slight excess.

For example for 30 tubes of SRP analysis, 40ml of SRP mixed reagent requires 6ml of ammonium molybdate solution. For 30 tubes of TP analysis again 40ml of TP mixed reagent requires 6ml of the molybdate solution, making a combined total of 12ml of molybdate solution required. In this instance prepare 15ml of the ammonium molybdate solution by weighing out 0.6g of ammonium molybdate according to the procedure in Appendix (II) for the preparation of general reagents - it is not necessary to be as exactly accurate as for the preparation of the stock phosphorus solution.

The small quantity remaining should be poured to waste and washed away with tap-water.

Potassium antimonyl tartrate solution (P.A.T.):
Following the procedure in Appendix (II) for the preparation of general reagents, weigh out 0.274g of PAT - *with care because it is harmful if inhaled or swallowed* - and dissolve it, with stirring, in 100ml of distilled water in a clean glass beaker or conical flask.

Transfer to a stoppered or screw-capped dark glass bottle for storage, out of direct sunlight i.e. preferably in a cupboard. If a dark glass bottle is not available, a clear one can be used provided it is totally wrapped up in aluminium foil.

As with the 2.5M sulphuric acid, this solution keeps indefinitely.

Ascorbic acid:
Make up fresh for each batch of analyses according to the SRP 'Recipes' table.

So, for example, to prepare 30ml of SRP mixed reagent, weigh 0.18g of ascorbic acid into a clean, dry, weighing boat and transfer to a clean and dry 25ml glass conical flask. Add a clean and dry magnetic stirrer bar, **but do not add the 9ml of distilled water until you are ready to add the finished mixed reagent to the sample tubes.**

Method:

Using a marker pen, label clean, dry, test tubes to receive samples, standard solutions and distilled water blanks. Using a pipettor fitted with a clean, dry tip, transfer 5ml of filtered samples, standard solution and distilled water to the appropriately labelled tubes. Once all the samples, standards and distilled water blanks are ready, prepare sufficient mixed reagent according to the SRP 'Recipes' table below. Measure the appropriate volumes of 2.5M sulphuric acid solution, ammonium molybdate solution and P.A.T. solution into a clean glass measuring cylinder and transfer to an appropriate-sized clean glass conical flask. It is pointless using a 250ml flask if the final volume of solution is only going to be 50ml. Add the appropriate amount of distilled water to the ascorbic acid (using the same glass measuring cylinder, if you like) and stir to dissolve, on the stirrer/hotplate. Stir only, do not heat as this would destroy the ascorbic acid. Once all the crystals have dissolved, pour the entire contents of the flask including the stirrer bar into the flask containing the other three ingredients and give the overall mixture a brief stir on the stirrer/hotplate (again, no need to heat).

The resulting mixed reagent should have a pale yellow colour and must be used immediately as it does not keep.

Add 1ml of this mixed reagent **to each test-tube** using a pipettor fitted with a clean, dry tip. When all the tubes have received the mixed reagent, each is mixed well using a rotormixer. Allow to stand for at least 20 minutes (half an hour is probably better).

Compare the absorbances of the samples with that of the standard solution (corrected for the distilled water blank) at 882nm using the spectrophotometer and 4cm cuvettes, or preferably a 4cm flowcell.

Total phosphorus (TP) and total soluble (i.e. dissolved) phosphorus (TSP)

Purpose:

A measure of TP is valuable in assessing the overall amount of phosphorus in all forms, both in solution and in solid form, present in lake- and river-waters, effluents and sediments. Dissolved phosphorus can be present in both organic and inorganic molecules, whilst phosphorus is found in the solid phase both in the cells of living organisms such as phytoplankton, zooplankton and protozoa, as well as in non-living particulate material.

Summary of method:

Total phosphorus (TP), which includes all inorganic and organic forms, both dissolved and in the solid particulate phase, can be measured by using a sulphuric acid-potassium persulphate digestion on an unfiltered sample to convert all forms of P to orthophosphate. This is then measured as orthophosphate by the addition of ammonium molybdate and ascorbic acid as in

the soluble reactive phosphorus (SRP) method. Total soluble phosphorus (TSP) is determined in the same way, using the digestion as above, but on a filtered sample. The phosphorus in the solid phase, particulate phosphorus (PP), is obtained from: PP = TP - TSP

Apparatus:
All glassware and plastic ware, including pipettor tips, weighing boats and magnetic stirrer bars, coming into direct contact with chemicals or solutions, must have undergone the laboratory washing-up procedure described in Appendix (I) before use. This is especially important because the phosphorus concentrations that are likely to be encountered in most of the Lake Tanganyika samples will be so low that if we do not maintain clean laboratory ware, we can have no confidence in our results.

Glass or polypropylene test-tubes (10-15ml capacity), and racks
Glass volumetric flasks
Glass measuring cylinders
Glass volumetric pipettes with pipette fillers
Glass conical flasks
Glass beakers
Repeating pipettes (5ml and 1ml, with appropriate tips)
4-point analytical balance with polystyrene weighing boats and stainless-steel spatula
Pressure-cooker and electric hotplate
Magnetic stirrer/hotplate and stirrer bars
Rotormixer
Spectrophotometer (for readings at 882nm) with 4cm glass cuvettes (or, preferably, a 4cm flowcell)

Stock phosphate solution and working standard phosphate solution:
This is the same solution as prepared in the method for SRP analysis.

Reagents:
2.5M sulphuric acid solution (H_2SO_4):
This is the same solution as prepared in the method for SRP analysis.

30% sulphuric acid solution (H_2SO_4):
This reagent is prepared following the cooling procedure used for the 2.5M sulphuric acid described in the method for SRP analysis. About 50ml of distilled water are placed in a clean and dry 250ml glass conical flask which is then cooled in a bucket of iced water whilst 30ml of concentrated sulphuric acid is **slowly and carefully** added in two batches of 15ml from a clean and dry glass 25ml volumetric pipette fitted with a pipette filler. Wear rubber gloves and eye protection and have running water near at hand. Allow to cool to room temperature then dry the outside of the flask and pour the solution into a clean and dry 100ml glass measuring cylinder and add distilled water up to the 100ml graduation mark. Pour the solution back into the conical flask to mix it and transfer it to a screw-capped glass bottle for storage out of direct sunlight, i.e. preferably in a cupboard.
Provided the bottle remains tightly capped between occasions of use, this solution should keep indefinitely.

Ammonium molybdate solution:
This is the same solution as prepared in the method for SRP analysis. Refer to this method for details of the amount required.

Potassium antimony tartrate solution (P.A.T.):

This is the same solution as prepared in the method for SRP analysis.

Potassium persulphate solution:
From the TP/TSP 'Recipes' table, below, determine how much of this reagent you will require, given that 0.5ml of solution are added to each test-tube containing sample, working standard and distilled water blank and also that a slight excess is prepared to allow for any evaporation during preparation. For example, if there are a total of 42 tubes, say, then prepare 30ml of potassium persulphate solution for a slight excess. In this instance weigh 2.4g of potassium persulphate into a clean, dry, weighing boat and transfer to a clean, dry, 50ml glass conical flask. Add a clean, dry, magnetic stirrer bar. When you are ready to dispense the persulphate solution, add 30ml of distilled water from a clean 50ml glass measuring cylinder. If there are 20 tubes then prepare 15ml of solution and so on. Stir to dissolve on the magnetic stirrer/hotplate with heating, but avoid excessive heating as evaporation could significantly reduce the volume. The solution must be used immediately, whilst still hot.

RECIPES

RECIPES FOR SRP MIXED REAGENT					
add 1ml to 5ml of filtered sample					
mixed reagent required (ml)	2.5M H_2SO_4 solution (ml)	ammonium molybdate solution (ml)	P.A.T. solution (ml)	ascorbic acid (g)	distilled water (ml)
30	15	4.5	1.5	0.18	9
40	20	6.0	2.0	0.24	12
50	25	7.5	2.5	0.30	15
60	30	9.0	3.0	0.36	18
70	35	10.5	3.5	0.42	21
80	40	12.0	4.0	0.48	24
90	45	13.5	4.5	0.54	27
100	50	15.0	5.0	0.60	30
110	55	16.5	5.5	0.66	33
120	60	18.0	6.0	0.72	36
130	65	19.5	6.5	0.78	39
140	70	21.0	7.0	0.84	42
150	75	22.5	7.5	0.90	45
160	80	24.0	8.0	0.96	48
170	85	25.5	8.5	1.02	51
180	90	27.0	9.0	1.08	54
190	95	28.5	9.5	1.14	57
200	100	30.0	10.0	1.20	60
210	105	31.5	10.5	1.26	63
220	110	33.0	11.0	1.32	66
230	115	34.5	11.5	1.38	69
240	120	36.0	12.0	1.44	72
250	125	37.5	12.5	1.50	75

Reference:
*Murphy, J. and Riley, J.P., Analyt. Chim. Acta **27** (1962), 31-36, AA modified single-solution method for the determination of phosphate in natural waters.*

RECIPES FOR TP/TSP ANALYSIS: potassium persulphate solution

Add 0.5ml to 5ml of sample

volume of solution to be prepared (ml)	potassium persulphate (g)
10	0.8
15	1.2
20	1.6
25	2.0
30	2.4
35	2.8
40	3.2
45	3.6
50	4.0
55	4.4
60	4.8
65	5.2
70	5.6
75	6.0
80	6.4
85	6.8
90	7.2
95	7.6
100	8.0
105	8.4
110	8.8
115	9.2
120	9.6
125	10.0
130	10.4
135	10.8
140	11.2

Ascorbic acid:

Make up fresh for each batch of analyses according to the TP/TSP 'Recipes' table, below. Weigh the appropriate amount of ascorbic acid into a clean, dry, weighing boat and transfer to a clean, dry, 25ml, 50ml or 100ml glass conical flask as appropriate. To prepare 30ml of TP/TSP mixed reagent, for example, weigh 0.18g of ascorbic acid into a clean, dry, weighing boat and transfer to a clean, dry 25ml glass conical flask. Add a magnetic stirrer bar **but do not add the 15.9ml of distilled water (from a glass measuring cylinder) until you are ready to add the finished mixed reagent to the sample tubes.**

RECIPES FOR TP/TSP MIXED REAGENT					
add 1ml to 5ml of sample					
mixed reagent required (ml)	2.5M H_2SO_4 solution (ml)	ammonium molybdate solution (ml)	P.A.T. solution (ml)	ascorbic acid (g)	distilled water (ml)
30	8.1	4.5	1.5	0.18	15.9
40	10.8	6.0	2.0	0.24	21.2
50	13.5	7.5	2.5	0.30	26.5
60	16.2	9.0	3.0	0.36	31.8
70	18.9	10.5	3.5	0.42	37.1
80	21.6	12.0	4.0	0.48	42.4
90	24.3	13.5	4.5	0.54	47.7
100	27.0	15.0	5.0	0.60	53.0
110	29.7	16.5	5.5	0.66	58.3
120	32.4	18.0	6.0	0.72	63.6
130	35.1	19.5	6.5	0.78	68.9
140	37.8	21.0	7.0	0.84	74.2
150	40.5	22.5	7.5	0.90	79.5
160	43.2	24.0	8.0	0.96	84.8
170	45.9	25.5	8.5	1.02	90.1
180	48.6	27.0	9.0	1.08	95.4
190	51.3	28.5	9.5	1.14	100.7
200	54.0	30.0	10.0	1.20	106.0
210	56.7	31.5	10.5	1.26	111.3
220	59.4	33.0	11.0	1.32	116.6
230	62.1	34.5	11.5	1.38	121.9
240	64.8	36.0	12.0	1.44	127.2
250	67.5	37.5	12.5	1.50	132.5

Method:

Using a marker pen, label the clean, dry, test tubes to receive samples, standard solutions and distilled water blanks. Using a pipettor, fitted with a clean, dry tip, add 0.1ml of 30% H_2SO_4 to each tube. Now transfer 5ml of unfiltered samples, filtered samples, standard solution and distilled water to the appropriate tubes. It is important to add the 0.1ml of the sulphuric acid solution first to the empty test-tubes as this is the only way of being able to see which tubes have received their 0.1ml. Unless you are very careful it is very difficult to do this once the test-tubes contain the 5ml samples - a tube containing 5ml of liquid looks identical to one containing 5.1ml, but a tube containing 0.1ml is totally unlike an empty one.

Now prepare sufficient potassium persulphate solution according to the TP/TSP 'Recipes' table and add 0.5ml whilst still hot to each tube using a pipettor. Cover the tubes with aluminium foil and place in suitable containers (e.g. glass or polypropylene beakers, or even empty and well-washed tin cans) into the pressure-cooker. Place enough distilled water into

the pressure-cooker (a depth of 4 or 5cm should be sufficient), fit the lid securely and place onto the electric hotplate, switched on and set to 'high'. When steam issues from the safety-valve allow 30 minutes more heating before switching off, removing the pressure-cooker from the hotplate and allowing it to cool to room temperature. After cooling, remove the tubes and place them in order in a rack.

Once all the samples, standards and distilled water blanks are ready, prepare sufficient 'mixed reagent' according to the TP/TSP 'Recipes' table. Measure the appropriate volumes of 2.5M sulphuric acid solution, ammonium molybdate solution and P.A.T. solution using a clean, dry, glass measuring cylinder and transfer to an appropriate-sized clean glass conical flask. It is pointless using a 250ml flask if the final volume of solution is only going to be 50ml! Add the appropriate amount of distilled water to the ascorbic acid, using the same glass measuring cylinder if you like, and stir to dissolve, on the stirrer/hotplate. Stir only, do not heat as this would destroy the ascorbic acid. Once all the crystals have dissolved pour the entire contents of the flask into the flask containing the other three ingredients and give the overall mixture a brief stir on the stirrer/hotplate (again, no need to heat).

The resulting mixed reagent should have a pale yellow colour and must be used immediately as it does not keep.

Add 1ml of this mixed reagent **to each test tube** using a pipettor fitted with a clean, dry tip. When all the tubes have received the mixed reagent, each is mixed well using a rotormixer and allow to stand for at least 20minutes (as for the SRP method half an hour is probably better). Compare the absorbances of the samples with that of the standard solution (corrected for the distilled water blank) at 882nm using the spectrophotometer and 4cm cuvettes, or preferably a 4cm flowcell.

Reference:

Standard Methods for the Examination of Water and Wastewater, 14th edn., American Public Health Association, Washington, 1975 pp 476, 481-2.

4. PHYTOPLANKTON STUDIES

It is important to assess the phytoplankton in any lake studied. Distributed throughout much of this lake down to 200m, the phytoplankton is a main assemblage of photosynthetic plants forming the basis of many food chains leading to herbivorous fish and top predators. Only in fringing zones and shallow waters do other plants (rooted macrophytes and micro-algae attached to various substrates) assume this role. Knowledge about the factors controlling temporal and spatial changes in phytoplankton abundance and species composition is extensive. The influences of physical and many weather-related factors, nutrient status (eutrophication), grazing effects and the 'trophic cascade' are perhaps the best understood. In this respect the phytoplankton is a valuable environmental indicator. However, the extent to which these organisms reflect the impacts of other pollutants of concern i.e. heavy metals, pesticides and hydrocarbons, is far from clear.

Assessment of the phytoplankton is not easy. Albeit depending on the *raisons d'être* of a phytoplankton project, sampling has to take account of the wide diversity of these organisms ranging in size from nanometres to millimetres, and densities from the positively buoyant to the relatively heavy. Ultimately at least, sampling programmes have also to recognise the often rapid temporal and spatial shifts in species composition due to changes (over timescales ranging from minutes to decades) in e.g. water movements, clarity, nutrient and other ion content, and grazing pressure. The following guidelines for sampling relate specifically to the requirements of the Special Study that seeks to use the phytoplankton (and other micro-flora) to gauge the effects of pollution on biodiversity. The present recommendations also focus largely on establishing a simple but effective and sustainable routine, which can be expanded as skills are mastered.

Sampling locations

1. At least until sampling procedures become 'routine', locate sampling sites as near as possible to the laboratory in which the samples are to be analysed, i.e. Kigoma and Mpulungu bays.
2. Choose sampling locations that are representative of 'open' water rather than in-shore zones where re-suspension and disturbance of biota more characteristic of substrata such as muds and rocks are likely to dominate.
3. Use bathymetric maps to identify a site that is 'typical/representative' of e.g. the bays.
4. Establish two sampling sites contrasting in pollution status, but otherwise (as far as possible) similar in general character; thus, choose two sites in open water comparable in total depth, but with one positioned significantly nearer than the other to a pollution source e.g. an industrial outfall, or area of intensive human activity.

Sampling frequency

1. Particularly as the various work activities involved in phytoplankton sampling (Table 1) are achieved more efficiently, aim to sample the phytoplankton weekly.

Sampling depth

1. Until the field and laboratory practices have been perfected, collect 'whole-column' (integrated tube) rather than a number of discrete depth (closing water bottle) samples - even if the water column is thermally stratified.
2. The tube, should be clear, 2.5-cm internal diameter (thus holding approximately 0.5 litre of water per metre), and no more than 20m long; it should be weighted at one end (the bottom) to which is attached a thin, strong rope a metre or two longer than the tube itself.

3. Mark the tube at half-metre intervals in order to assess the depth over which samples are collected.

4. Bear in mind that (i) cleanliness is essential especially when working in the comparatively pristine Lake Tanganyika, and (ii) nutrient analyses will be done on the same tube samples. When not in use, the tube should be completely drained, and neatly coiled along with the attached rope, and stored out of direct sunlight.

5. Rinse the tube at the sampling site before taking each sample.

6.Collect tube samples in duplicate at each site and store each of these in separate bottles.

7. Keeping a secure hold on the tube and the rope, lower the tube slowly into the water until the bottom (weighted) end is at the required depth as indicated on the tube. Pull up the weighted end of the tube slowly with the rope, until the tube assumes a 'U' shape, and empty the sample into *a previously rinsed and cleaned* bucket or other wide-mouthed container; this will ensure complete mixing of the sample; transfer into previously labelled, 2-litre container/s. This should be sufficient for a microscopic analysis of the phytoplankton (0.5 l), a chlorophyll$_a$ extraction and a series of nutrient analyses (0.5 l). Collect the duplicate sample in the same way and transfer it to a separate container.

8. Keep the samples in the dark and as cool as possible during transport back to the laboratory.

Sample treatment

1. Immediately on return to the laboratory, store the samples in a cool place - preferably a refrigerator. Shake the samples thoroughly before any water is withdrawn for analysis.

2. Use of a high power microscope, achieving magnifications of up to at least 500-600x, and preferably more, is essential for assessing *population densities and species diversity* of organisms that are generally very small. As a consequence, it is also necessary to bring the organisms 'closer together' - otherwise valuable time is wasted in searching for them. Concentration can be achieved in four ways - by sedimentation, centrifugation, a combination of these two, or by using fine-mesh nets.

3. *Sedimentation:* a 1-litre aliquot of sample should be transferred into a glass cylinder, and 'fixed' with enough drops of Lugol's Iodine (a saturated solution of Iodine in a saturated aqueous solution of potassium iodide) to give a deep red wine colour. This solution preserves the

organisms, stains starch bodies (a feature of green algae, but not all other groups), and increases the relative density of the organisms and thus, accelerates their sedimentation. The cylinder should be covered to prevent dust deposition and minimise iodine evaporation, and left to stand for at least 24 hours. The uppermost majority of the liquid (*ca* 900 ml) is then very carefully withdrawn using a gentle sip or siphon system; the remaining liquid (now with the algae more concentrated than before) is transferred to a smaller e.g. 100-ml cylinder and left for a further 24 hours for the cells to concentrate further. All but the lowermost 10 ml of sample is withdrawn with special care - using a siphon, sip or pipette system. The remaining 10 ml - in which the organisms are now concentrated 100-fold - is then poured into a labelled 15-ml screw-top centrifuge tube until examination. Concentration by sedimentation is commonly favoured over centrifugation, in that it requires little operator time, and the method is unlikely to damage the organisms. However, as indicated above it requires glassware and glassware cleaning facilities - clean water for example.

4. *Centrifugation:* on its own, centrifugation is unlikely to be feasible or effective for concentrating the organisms to the degree required. The organisms from virtually all samples from Lake Tanganyika will be very sparse i.e. a with a few tens of organisms per millilitre rather than the hundreds, thousands, even millions recorded in eutrophic waters. Thus, unless a centrifuge equipped with large e.g. 250-ml containers is available, centrifugation would take an immense amount of time.

5. *Sedimentation combined with centrifugation:* these two methods could be combined. As an example, having concentrated the material some 10-fold by sedimentation, a further 10-fold concentration could be achieved with the centrifuge.

6. *Nets:* the plankton can be concentrated very efficiently using fine (e.g. 25 or 30-µm mesh) nets. What is more, the volume of water from which the organisms are harvested can be estimated e.g. the product of length of tow and the diameter of the mouth of the net. However, pico and nano plankton can pass through all but the finest of nets, and long, spindle-shaped species can pass through very narrow meshes. In any event, net capture efficiency will be lowest at the start of a tow. As a result, the percentage composition of the organisms in a net haul will differ somewhat from the 'real' situation. Still, tow-net samples may well be useful if carried out in a systematic fashion e.g. by slowly driving the boat around in a circular course whilst towing the net, attached to a few metres of thin rope, just beneath the surface for a timed period, say five minutes.

Population density estimation - algal counts

The numbers of individuals of the different species present are estimated by counting. Two main methods have been used for many years. The first of these involves counting the algae sedimented onto the base of a chamber of known volume (or 'effective volume' if the material has been pre-concentrated as outlined above), using an inverted microscope. The second method employs counting chambers of various types but usable on conventional microscopes. Sedgwick-Rafter and Palmer-Maloney chambers have standard capacities e.g. 1 ml. These are of a shallow 'tray'-like construction with a glass cover, and a grid etched on the base to facilitate counting of the organisms in a known number of squares. As the depth of all these counting chambers is known, so is the volume (or 'effective volume') in which the organisms are counted, thereby enabling the assessment of population densities. The present project wishes to

introduce the Lund nano-plankton counting chamber for this work - because it is the only chamber in which the organisms are likely to settle and distribute themselves more or less randomly. Patchy and largely unknown distributions of organisms result with all other chambers - whether of cylindrical or tray-like design. The very special feature of the Lund Chamber is achieved because the sample can be introduced via a glass Pasteur pipette in a single smooth 2- to 3-second operation from one open end, with air being expelled at the other. The chamber consists of a glass slide with glass strips 55mm long, 3mm wide and approximately 0.45 mm deep cemented along each of the long sides and positioned such that they can support a 50mm x 22mm coverslip. The coverslip is placed on the two strips, and the sample is introduced as outlined above. Capillary forces are sufficient to hold the two units together and even light oil immersion objectives can be used.

5. CHLOROPHYLL$_A$

Purpose:
The determination of chlorophyll$_a$ (one of a number of pigments present in phytoplankton cells) provides a broad index of the total amounts ('biomass') of those cells in the water. The emphasis on the term 'broad' is important because the chlorophyll$_a$ content of the algal cells can vary depending on the following:

- the type of algae present: green algae, for example, are generally richer in chlorophyll$_a$ than diatoms or blue-green algae (cyanobacteria)
- their nutrient content: cells deficient in a nutrient such as nitrogen often appear yellow rather than a vivid green owing to a lack of chlorophyll$_a$
- whether the algal cells are bleached/discoloured under conditions of high light intensity

In spite of these irregularities and variations in the chlorophyll$_a$-to-biomass ratios, chlorophyll$_a$ levels will generally increase or decrease with increases or decreases in total algal biomass. The relationships between chlorophyll$_a$ levels and algal biomass expressed as total cell numbers, cell volumes or dry weights per unit volume of water tends to be close where the algal assemblage is dominated by a single species. An example of such a situation in Lake Tanganyika is the 'blooming' of species such as the blue-green alga *Anabaena flos-aquae* which occurred in the Nsumbu and Mpulungu regions of the lake in May 1998. Conversely, the relationship between chlorophyll$_a$ levels, total cell numbers, cell volumes or total dry weights is less distinct where (as is usually the case) many species are present.

Summary of Method:
A measured volume of lakewater is filtered through a glass-fibre filter disc which is then immersed in a 90% methanol solution and allowed to stand overnight in a spark-free cold-store or refrigerator. The chlorophyll$_a$ pigment contained in the phytoplankton cells trapped on the filter is thus extracted by the methanol to yield a green solution whose intensity of colour is proportional to the amount of pigment present.

In all but the most exceptional circumstances, the concentration of the phytoplankton onto the glass-fibre filters and the steeping of the filters in methanol overnight must be done immediately on return to the laboratory with samples.

Important note:
Generally speaking, chlorophyll$_a$ is just one of a series of determinands analysed for in a water sample. The remaining filtrate can be collected and used for the determination of dissolved materials such as phosphorus, silica and nitrate. The method outlined below gives details of its collection.

Apparatus:
Screw-capped polypropylene centrifuge tubes and rack (15ml capacity or thereabouts)
Suction pump (electric or manual)
Filtration apparatus (stainless-steel funnel assembly and, preferably, modified desiccator to facilitate collection of the filtrate)
Whatman GF/C filter pads (4.7cm diameter) and forceps (preferably stainless steel, curved and pointed)
Clean plastic measuring cylinder, at least 1000ml
Clean glass measuring cylinder, 1000ml
Clean plastic beaker, 250ml
Repeating adjustable-volume liquid dispenser and reservoir
Light-proof container
Bench-top centrifuge (2500 revolutions per minute)
Spectrophotometer (for reading at 480, 665 and 750nm) with 4cm glass cuvettes.

Reagent:
90% aqueous methanol.
This reagent is prepared according to the actual volume used for the extraction, to take into account the water that is retained by the GF/C filter (taken to be 0.5ml for the 4.7cm diameter discs). So, for a final extract volume of 11.5ml of 90% methanol, 11ml of a 94.1% solution are dispensed - 94.1 x (11/11.5) = 90.

The solution to be prepared in this case, therefore, is 941ml of analytical quality methanol with distilled water added to make 1000ml of solution. This must be prepared in a clean, preferably glass, measuring cylinder.

This solution is stored in the reservoir of the repeating dispenser in a flame-proof locker.

Method:
Set up the filtration apparatus and label the 15ml screw-capped centrifuge tubes with the sample site, date of sampling and the volume of lakewater to be filtered.

After having shaken the bottle containing the lakewater sample (to ensure thorough mixing), measure a known volume using the clean plastic measuring cylinder.

Pour most of this sample (all but say 200ml) into the filtration funnel and allow it to pass through the system, thereby rinsing the filter disc.

Now open the air vent to allow the vacuum pump to be switched off and discard this first part of the filtrate.

Place the clean plastic beaker inside the filtration assembly, close the air vent and filter the remainder of the sample, collecting the filtrate in the beaker.

Without switching off the vacuum pump, dismantle the filtration funnel and slowly and carefully remove the filter disc. If this is done too quickly, air blasts into the filtration assembly and can blow the filtrate out of the collecting beaker. Trying to avoid touching the filter disc with fingers. Fold it over or roll it up and place into the bottom of an appropriately labelled screw-capped centrifuge tube, which is immediately placed into a light-proof container. The pump can now be switched off and the filtrate removed for subsampling or storing in a refrigerator or freezer, as appropriate, for any other analyses.

Fit a new filter disc, and proceed with the rest of the samples, collecting the filtrate as before. If the chlorophyll$_a$ samples are not to be analysed the following day, remove the tubes from the light-proof container and put them in a polythene bag labelled with the date, and place this bag into the freezer.

If the samples are to be analysed the following day, add 11ml of the 94.1% methanol solution from the liquid dispenser to one of the tubes and, after ensuring that the cap is firmly in place, shake the tube a couple of times, ensuring that the filter disc remains totally immersed in the methanol solution after shaking.

Place the tube in the rack in the light-proof container and proceed with the remainder of the samples, placing each in turn into the light-proof container after the addition of the methanol solution. It is important to keep the samples in the dark at all times to avoid degradation, by light, of the chlorophyll$_a$ in the extracts.

Place the light-proof container into a cold-store or refrigerator (not the freezer!) and leave overnight.

The following morning, switch on the spectrophotometer to allow sufficient warm-up time (consult the instruction manual).

Remove the light-proof container from the refrigerator and, whilst wearing clean disposable latex/vinyl gloves, take out a tube and give it a shake. Carefully remove the filter disc from the solution using a clean spatula and carefully roll and squeeze the disc between fingers and thumb (this technique requires a little practice but is soon mastered) to return any absorbed solution back into the tube, which is then re-capped. The gloves must be worn whilst doing this because methanol is poisonous and can be absorbed through unprotected skin.

The squeezed filter disc can now be discarded and the tube returned to the light-proof container.

When all the filter discs have been removed in this way, the capped tubes are placed into the centrifuge (taking care to have equal numbers of tubes on opposite sides of the rotor for balance) and spun for 10 minutes at 2500rpm.

Carefully remove the tubes from the centrifuge so as not to disturb the now cleared solution, and place them in the rack in the light-proof container.

Add 11ml of the methanol solution from the repeating dispenser to a clean 4cm glass cuvette and, after ensuring that the optical surfaces are clean and dry, place it into the spectrophotometer.

This is the methanol blank.

It is very important not to touch the optical surfaces of the cuvette with fingers as this would leave marks which would affect the absorbance readings. Handle only the opaque ground-glass sides. The optical surfaces may be gently wiped using a soft tissue if necessary.

Set the wavelength to either 750 or 480nm and set the absorbance to zero. Consult the instruction manual since spectrophotometers may vary in their methods of operation.

Carefully unscrew the cap on the first sample tube and in one smooth movement pour most of the clear, hopefully green, extract into a second clean 4cm glass cuvette. This operation must be performed carefully so as not to disturb the plug of material at the bottom of the sample tube - any material that is re-suspended will alter the absorbance of the solution and will necessitate centrifugation again.

After ensuring that the optical surfaces are clean and dry place the cuvette in the spectrophotometer and read and record the absorbance.

Now alter the wavelength to 665nm and, with the methanol blank in the lightpath, set the absorbance to zero.

Place the extract in the lightpath and read and record the absorbance.

Set the wavelength to the third remaining value (depending on the first chosen wavelength) and zero the absorbance with the methanol blank before taking and recording the final reading for the sample extract.

Dispose of the extract (being mostly methanol, this could be collected in an empty screw-topped glass bottle and used for starting fires, taking care with storage) and proceed with the remaining samples.

The used sample tubes may be re-cycled by the standard washing-up procedure.

The concentration of chlorophyll$_a$ in the original lakewater sample is given by the equation:

$[chlorophyll_a]\mu g.l^{-1} = (O.D._{665} - O.D._{750}) \times \{(13.9 \times v)/(V \times L)\}$

where:
$O.D._{665}$ = optical density (absorbance) at 665nm wavelength, a distinctive peak for $chlorophyll_a$
$O.D._{750}$ = optical density at 750nm, a correction for any background turbidity
v = the volume of the extract in millilitres (e.g. 11.5ml)
V = the volume of lakewater filtered in litres
L = the path-length of the cuvettes used in cm (e.g. 4cm)
13.9 = an absorption coefficient (a constant) for $chlorophyll_a$

The ratio: $(O.D._{480} - O.D._{750})/(O.D._{665} - O.D._{750})$ gives a figure known as the Carotenoid Index,
which is an indication of the 'health' of the phytoplankton population.

Reference:
Some problems in the estimation of $chlorophyll_a$ in phytoplankton

J.F. Talling and D. Driver, Proceedings of the Conference on Primary Productivity Measurement, Marine and Freshwater, held at the University of Hawaii, August 21-September 6, 1961. U.S. Atomic Energy Commission, Division of Technical Information, 1963, pp 142-146.

6. PHYTOPLANKTON ASSEMBLAGE SIZE SPECTRA ('P.A.S.S.') AND SPECIES RICHNESS AND DIVERSITY

Introduction:

This note concerns a simple and reasonably rapid method for assessing quantitative (size) and qualitative (species richness and diversity) aspects of phytoplankton assemblages (and other microscopic organisms e.g. algae and protozoa) on the surfaces of stones and cobbles etc. The method is also repeatable such that results obtained from different places can be compared directly, as can results from the same place at different times. This is supremely important in the context of the LTBP in that by monitoring the lake using this method, change (OR LACK OF CHANGE) in e.g. species composition can be assessed. When combined with the results from chlorophyll and algal population density information, change or lack of change in the amounts of phytoplankton and the different species can also be assessed.

The focus on size (length) of the organisms reflects the fact that size determines many major features of the phytoplankton - and these features in turn can tell us something about the physical, chemical and other biological features of the lake environment at the instant of sampling. Thus, the types and amounts of species we observe are acting as environmental indicators. A few examples are as follows - BUT NOTICE THAT THERE ARE FEW HARD AND FAST RULES - BECAUSE THE INTERACTIONS BETWEEN THE PHYSICAL AND CHEMICAL FACTORS THAT DETERMINE THE SPECIES PRESENT ARE NUMEROUS AND VERY COMPLEX:

- when large species predominate it suggests that grazing by zooplankton on small species is heavy.
- on the other hand, when large species such as the nitrogen-fixing blue-green alga *Anabaena flos-aquae* dominates the scene (as in mid-June 1998 in Mpulungu Bay) AND the specimens contain the cells (heterocysts) that are fixing nitrogen from the gaseous N dissolved in the water, it is likely that nitrogen concentrations in the forms of nitrate and ammonia are very low.
- where small species dominate, it is likely that grazing is less evident; however, owing to their larger surface area to volume ratio, small species are also generally more efficient at taking up nutrients - this might explain the predominance of very small organisms (<2 micrometres in greatest dimension - picoplankton) in some samples taken from the remote Nsumbu area.
- in general, large 'heavy' diatoms are less likely to survive during prolonged calm periods because they will sink out of the upper, better-illuminated euphotic zone - i.e. the zone extending from the surface down to the depth at which gains in energy due to photosynthetic fixation of carbon by the phytoplankton just balance losses due to respiration. However, this zone can be very deep in Lake Tanganyika except in areas of high sediment or other particulate concentrations. In the Kigoma area for example, dense growths of attached photosynthetic algae can be collected from underwater cliff faces extending down to 30m or more.

Of course, phytoplankton size cannot explain or account for all features of the freshwater environment, but it is worth bearing in mind that the species we see, and the way in which the

species change in time and space, can be viewed as the outcome of the interactions between a host of physical and chemical factors, i.e.

physics + chemistry = biology

In the case of phytoplankton, the important physical factors include water temperature, movement and depth of mixing; water colour/particle content and thus, clarity and spectral composition; day length is important but (in contrast to the situation in Scotland where it varies from 5.5 h in mid-December to 18.5 h in mid-June!), the values over Lake Tanganyika differ by less than 1 h around a mean value of approximately 12 h.

Chemical factors are potentially as numerous as the many hundreds of chemical substances entering the lake. However, whatever the situation, phytoplankton and other microscopic algae require nitrogen (N) in the form of nitrate or ammonia (with some blue-green algae also able to use gaseous N as indicated above); phosphorus (P) in what is termed soluble (molybdate) reactive P; and in the case of diatoms especially, but also some chrysophyte flagellates and possibly dinoflagellates, dissolved silica (SiO_2). These nutrients are viewed as especially important in that utilisation of them by e.g. phytoplankton leads to occasional depletion - and this is also reflected in the succession of phytoplankton species over the year. A prime example in Temperate zones concerns the depletion of silica by diatoms; their demise leaves more opportunities for other non-silica using species e.g. blue-green algae to flourish. It is possible that silica is less important in Lake Tanganyika because of the ample inputs from the catchment.
Major ions such as Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), Chloride (Cl), Carbonate (CO_3) and Bicarbonate (HCO_3) in addition to carbon dioxide (CO_2) are all needed to some extent and in the correct proportions according to species; however, many of these are generally present in excess although the seasonal and spatial fluctuations in their availability contribute (like the changing physical conditions) to determining the phytoplankton.
Other organisms can also affect the nature of the phytoplankton, and examples referring to the zooplankton are given above. A major contrast between many African and European waters is in the importance of direct links between fish and phytoplankton; there appear to be many more instances of Tropical fish species feeding directly on planktonic algae - and also on algae attached to rocks, stones etc. The importance of algae as food for fish in European waters is less well-documented, but it is likely that few (at least adult) fish feed on phytoplankton.

Stage 1: Equipment and procedures for sampling

- 25 or 30-micrometre mesh net - to be towed SLOWLY for 5 minutes (or 10 minutes if the 'catch' is very sparse) in a wide circle within the upper 2 metres of the water column; at the end of the 5 or 10 minutes carefully agitate the net up and down to concentrate the material in the cup at the base of the net; AS LONG AS THE MATERIAL IS WELL MIXED, it is not necessary to retain all the sample - 15 ml should be sufficient.
- sample tubes (15-ml) including a spare at each sampling site (ZM2 for example) for a subsample that can be examined live back in the laboratory.
- labels and pens for marking the sample tubes.
- cool box

Stage 2: Equipment and procedures for analysis in the laboratory

- compound binocular microscope fitted with 10x magnification eyepieces, a 0 to 100 linear scale eyepiece measuring graticule, and a 40x objective lens; the actual length of each division on the scale will have been calculated using a stage graticule: for example, each division of the scale on the eyepiece measuring graticule in the Olympus CH30 microscope in the Mpulungu LTBP station is 2.5 micrometres.
- Lund nanoplankton counting chamber and a 22mm by 50mm coverslip; NOTE THAT THE CHAMBER NEED NOT BE CALIBRATED FOR THE 'PASS' ANALYSIS; chambers only need to be calibrated if they are to be used for algal counting and population density estimations.
- a glass Pasteur pipette for filling the chamber with the WELL SHAKEN sample.
- plastic/glass beakers for cleaning the pipettes between samples.
- stationery: proformas for recording the species and their lengths at the microscope: REMEMBER TO RECORD SAMPLE SITE, SAMPLING DATE, OPERATOR AND DATE ON WHICH SAMPLE WAS ANALYSED.

Stage 3: The microscopic analysis

- set up the chamber with the glass coverslip
- SHAKE THE SAMPLE THAT IS TO BE ANALYSED, unscrew the sample container and, with the Pasteur pipette, carefully withdraw a small volume (0.5-1.0ml) and fill the chamber steadily and smoothly. You will learn by experience as to the volume needed - practice with water; the chambers hold approximately 0.5ml.
- place the filled chamber in a Petri dish containing a piece of tissue-paper soaked in water (to slow down evaporation) for 5 minutes to allow the organisms to settle onto the base of the chamber.
- transfer the chamber to the microscope, and set up the focusing and illumination to suit your requirements.
- start the analysis by placing the objective anywhere over the chamber and name, sketch and measure the length of the organism whose CENTRE POINT IS NEAREST THE '50' IN THE MEASURING GRATICULE; move the objective over the chamber to the left or right or up or down and repeat the process until you have recorded the details of 50 organisms. MEASURE ONLY THOSE PHYTOPLANKTON ORGANISMS THAT ARE 10 OR MORE MICRONS IN LENGTH i.e. 4 divisions of the measuring graticule IF USING THE MICROSCOPE DESCRIBED ABOVE.
- IGNORE DEAD (EMPTY) SPECIMENS.
- In the early stages at least, it is vital that you sketch the things that you record. It is unlikely that you will be able to identify (i.e. name) more than a few, if any, organisms when you start - and there will be many occasions on which you will be unable to judge whether a specimen is even an alga - let alone the major group (Blue-green, Diatom, Desmid, Green etc.) or Genus (e.g. *Cosmarium, Aulacoseira, Anabaena)* to which it belongs, or the actual species (e.g. *Anabaena flos-aquae*). THIS IS WHY, IN ADDITION TO SKETCHING THE ORGANISM, IT IS ESSENTIAL TO NOTE, WHEREVER POSSIBLE, THE REFERENCE (BOOK, PAGE NUMBER AND FIGURE) WHICH YOU USED FOR NAMING YOUR SPECIMEN.

Names might therefore be as broad as e.g. 1. Pennate Diatom with pointed end, 2. colony of many very tiny cells distributed at random, 3. a crescent-shaped organism with blunt ends, 4. a ribbon of cells, 5. a group of 4 cells each with a spine at each end and 6. a group, or groups of 8 cells joined by fine strands of mucilage. Sooner or later, and especially if you come across a number of specimens of the same organism you will be able to be much more specific - and possibly recognise the above descriptions as 1. *Navicula*, 2. *Aphanocapsa*, 3. *Cosmarium*, 4. *Fragilaria*, 5. *Scenedesmus* and 6. *Dimorphococcus*.

Stage 4: The data analysis

From the sets of 50 length measurements and 'names', there are at least 3 types of information that can be obtained:

- the size distribution of the phytoplankton assemblage (or collection of material from the surfaces of stones for example).
- the number of different species in the arrays of 50 specimens.
- at least 2 types of species diversity indices and species richness indices.

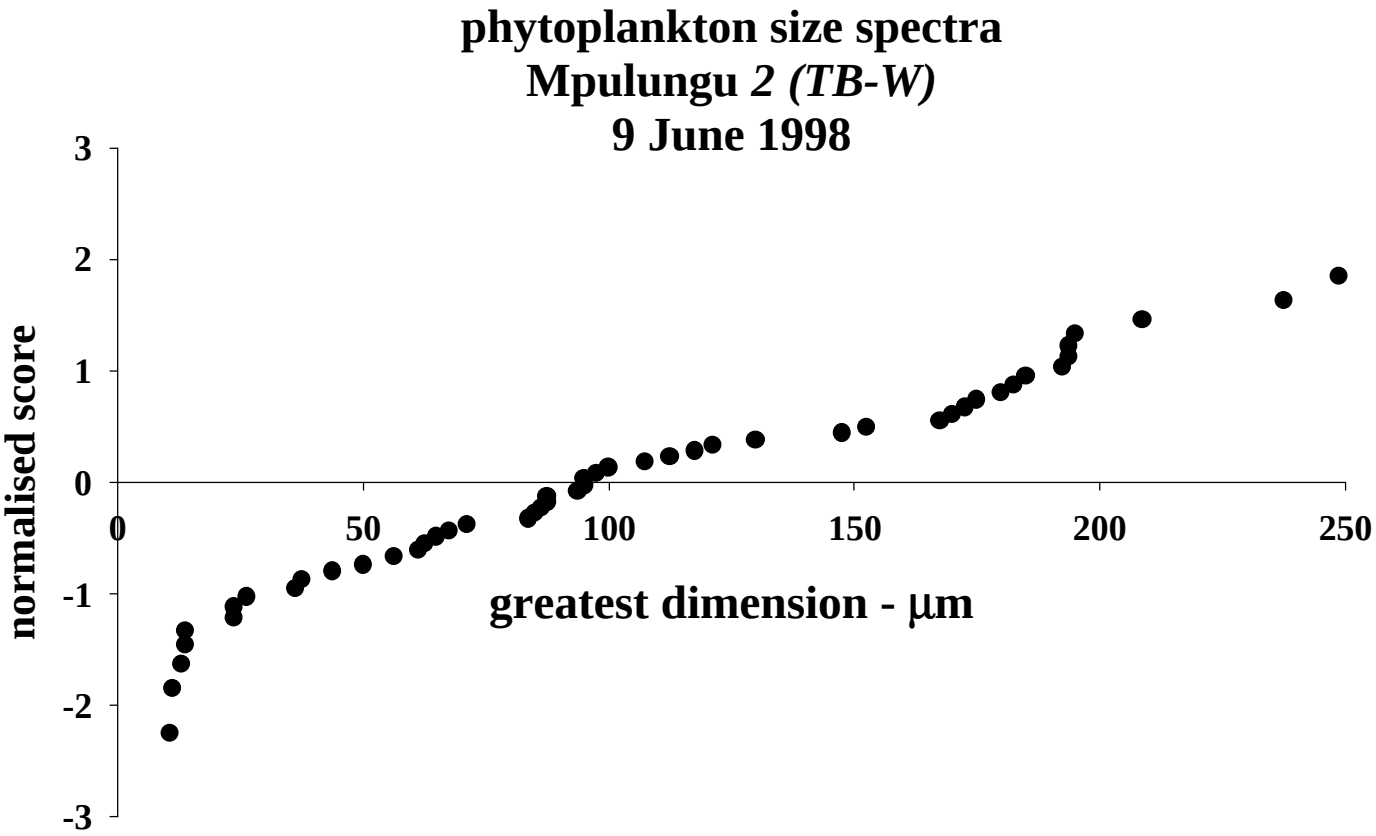
This document is concentrating on the first of the above, and this is known as the 'rankit' method of displaying the size frequency distributions of the organism lengths. Fuller details of the underlying the statistical method involved will be sent by mail shortly.

Meanwhile, refer to Table 1 which shows a data array, and Figure 1 which is the plot of those data for sample ZM2 (Zambia, Mpulungu site 2) collected 9 June 1998.

Table 1. Column 1: rankit numbers for 50 measurements; Column 2: the 50 measurements (converted to micrometres by multiplying by 2.5) ranked in ascending order; Column 3: the 50 4-letter codes 'names' corresponding to the length measurements. Data for Site ZM2, 9 June 1998

-2.25	10.625	ug	0.67	172.5	nz
-1.85	11.25	pdxx	0.74	175	nz
-1.63	13.125	ygf	0.80	180	nz
-1.46	13.75	ygf	0.87	182.5	nz
-1.33	13.75	ygf	0.95	185	nz
-1.22	23.75	faxx	1.03	192.5	nz
-1.12	23.75	chro	1.12	193.75	nz
-1.03	26.25	pdxx	1.22	193.75	nz
-0.95	36.25	saxx	1.33	195	nz
-0.87	37.5	chro	1.46	208.75	nz
-0.80	43.75	faxx	1.63	237.5	nz
-0.74	50	aafa	1.85	248.75	pdxx
-0.67	56.25	saxx	2.25	257.5	nz
-0.61	61.25	aafa			
-0.55	62.5	aafa			
-0.49	65	aafa			
-0.44	67.5	aafa			
-0.38	71.25	saxx			
-0.33	83.75	saxx			
-0.28	85	saxx			
-0.23	86.25	nz			
-0.18	87.5	nz			
-0.13	87.5	aafa			
-0.08	93.75	aafa			
-0.03	95	nz			
0.03	95	nz			
0.08	97.5	nz			
0.13	100	aafa			
0.18	107.5	saxx			
0.23	112.5	nz			
0.28	117.5	nz			
0.33	121.25	aafa			
0.38	130	aafa			
0.44	147.5	nz			
0.49	152.5	nz			
0.55	167.5	nz			
0.61	170	Nz			

Figure 1: The graph of the data from Table 1.



APPENDIX I - CLEANING AND STORAGE OF LABORATORY GLASS- AND PLASTIC-WARE

For any laboratory analysis to be successful, the analyst must be totally confident that the results obtained are genuine values for the determinand being measured and are not false representations brought about by undesirable external factors, the most common of which is contamination of the glassware being used. Contamination takes a variety of forms, from traces of reagents or previous samples to the more common 'enemy' of air-borne dust. Since the constituents of this dust can comprise, amongst other things, dead skin cells (human skin is constantly being renewed, with the surface layer of old cells being shed), and particles of soil. Both of these components are potential sources of the nutrient phosphorus and will interfere with the analysis for phosphorus of water samples with existing very low levels of P, such as is generally the case with Lake Tanganyika.

By following relatively simple procedures, however, it is possible to completely eliminate the uncertainty brought about by contamination, and to maintain a store of ready-to-use glass- and plastic-ware.

'Decon 90' is a specialist laboratory detergent that is entirely phosphorus-free and is fully bio-degradable. It is ideally suited for the rigorous cleaning of all glass- and plastic-ware, requiring that the articles to be cleaned be merely soaked in a 2% solution of the detergent in water (scrubbing with a test-tube brush may however be necessary with heavily-soiled items).

Extreme care should be exercised when handling the concentrated detergent, any splashes onto the skin must be immediately washed off with plenty of running water.

Due to its strongly alkaline nature, 'Decon 90' is not suitable for cleaning certain metal articles, so to avoid any possible problems its use should be limited to glass- and plastic-ware.

It should be readily obvious that in order for the 'Decon 90' to be able to perform its function it must be in contact with the surfaces to be cleaned. The easiest way of achieving this is to prepare a large (25litres or more) container of the solution (2% strength should be adequate, i.e. 500ml of the concentrated 'Decon 90' added to 25 litres of water) into which the articles to be cleaned are sunk. A quick rinse with tapwater before placing the articles into the detergent solution is advised, to avoid excessive build-up of contaminants in the solution.

The manner in which the articles to be cleaned are placed into the detergent solution is of critical importance, since the detergent must be in contact with all of the surfaces to be cleaned - i.e. there must be no trapped air bubbles at all in any of the items being soaked.

In order to achieve this, the items must be carefully and systematically placed into the detergent solution, not merely dropped in. Plastic ware needs particular care in this respect as it has a natural tendency to float and thus must be weighted down with heavier glassware.

Frequent rinsing of the hands under running water should be sufficient to prevent any potential skin problems caused by contact with the detergent solution - alternatively domestic rubber gloves may be worn.

For routine use the items are left soaking at least overnight before being individually removed and rinsed under running water (preferably warm) until all traces of the detergent are removed. Especial care must be taken with small items such as pipettor tips.

A final rinse with distilled water completes the washing process - it not being necessary to completely fill each item with distilled water, a brief swirl around should suffice.

Having gone to such lengths to clean the items it is now important to allow them to dry in such a way that they do not pick up any contamination. Small items such as pipettor tips and magnetic stirrer bars may be placed inside clean plastic sandwich boxes with the lids on loosely to allow some ventilation. Volumetric pipettes must be placed inside clean containers (plastic measuring cylinders are ideal, as are cleaned plastic water bottles that have had their tops cut off) and into a drawer or a cupboard as protection from dust. Test-tubes are best inverted in racks to dry after the distilled water rinse, before being stored in cleaned plastic sandwich boxes. Larger items such as beakers, flasks and measuring cylinders must also be allowed to dry inverted. This is best achieved using a glassware drying rack, but not by placing the clean items onto the prongs of the rack. The rack is very probably dusty and the last thing you need is for this to be introduced into your newly-cleaned flask or cylinder! Glass and plastic items can be placed onto the drying rack in such a way that no part of the rack comes into contact with the inside of any item, the cleaned articles being suspended between the prongs of the rack not on them.

Once dry, these items may be stored in cupboards or even on the bench provided that they are stoppered or sealed in some way - 'clingfilm' being a very effective method to cover the mouths of beakers and conical flasks.

The effective 'life' of the 2% detergent solution is largely dictated by frequency of use and the degree of soiling of the articles being cleaned, but generally speaking the solution should continue cleaning for at least 3 to 4 months before needing to be replaced - generally indicated by extreme cloudiness of the solution and development of a degree of 'stringiness'.

Being totally bio-degradable, the old solution may be tipped to waste down the sink.

APPENDIX II USE OF THE FOUR-POINT ANALYTICAL BALANCE (and preparation of solutions)

The analytical balance capable of reading to four decimal places (which is equivalent to one-tenth of a milligram) is a highly sensitive piece of laboratory equipment that must be used with great care and observance of correct procedures. These procedures relate not only to the operation of the balance itself but also to the handling of the materials being weighed.

In the first instance, the balance must be correctly positioned on the bench with sufficient room beside it for working, and away from any draughts. The balance must be correctly set up (levelled using the adjustable feet and the built-in spirit-level), **and the pan must always be clean and dry before use**. It must be switched on in good time to allow any necessary warm-up period, and calibrated according to the instruction manual.

The procedure for weighing out a certain amount of a particular chemical depends on whether or not that weight has to be accurate.

When preparing a stock solution from which working standard solutions are made up, the weight must be **exactly** what the 'recipe' calls for, since the working standard is the solution against which all the unknown samples are to be compared. If this is not prepared accurately then all results obtained will be meaningless.

More often than not, the weight of the chemical to be used for the stock solution is given to four decimal places, signifying the need for that level of accuracy.

In this case the correct procedure is as follows:
with the balance correctly set up, warmed-up and the pan clean and dry, place a clean and dry weighing boat onto the pan and tare to zero (see the instruction manual as necessary).

We do not need to know the weight of the weighing boat, only the amount of material it is going to contain.

Now, using a clean and dry stainless-steel spatula, very carefully add the chemical to the weighing boat until the required weight is registered. (You should **always** wipe the spatula before use, with a clean paper tissue, to eliminate any possibility of introducing contamination into the container from which you are removing the chemical being weighed - if it is 'Analytical Quality' it should remain 'Analytical Quality'!)

Care must be taken not to spill any material onto the pan beside the weighing boat as this will register on the weight displayed but not be contained in the weighing boat, with the result that the stock solution will have less than the intended concentration. The required weight must be that which is registered with the sliding doors of the balance closed, to totally eliminate draughts and thus give a steady reading. This may differ from what appears to be the correct weight immediately after you have finished adding the chemical with the doors open, so careful adjustment must be made. Either more chemical has to be added or some removed, which, as the spatula is known to be absolutely clean (see above), may be returned to its container.
Once the required weight is achieved, carefully remove the weighing boat and tip the chemical into a suitable-sized beaker or conical flask (depending on what volume of solution is to be

prepared) in which has been placed a clean and dry magnetic stirrer bar. Now, using a wash-bottle containing distilled water, rinse the inside surface of the weighing boat into the beaker or flask to remove all traces of the chemical. This way you can be certain that all of the chemical that you have weighed will have been transferred to the beaker or flask.

The chemical can now be dissolved by adding more distilled water, to a quantity that is less than the final desired volume, and stirring on the magnetic stirrer/hotplate. The solution can now be made up accurately by transferring **the liquid only** to a suitable volumetric flask (the magnetic stirrer bar can be 'trapped' inside the beaker or conical flask by holding a magnet, or another stirrer bar, against the outside of the beaker or conical flask). Rinse the beaker or conical flask (together with the magnetic stirrer bar) with a little distilled water and add this to the volumetric flask, again keeping the stirrer bar inside the beaker or conical flask. Now add distilled water to the volumetric flask up to the graduation mark.

Pouring the contents of the volumetric flask back into the beaker or conical flask effectively mixes the solution which can now be transferred to a suitable reagent bottle for storage, again withholding the stirrer bar.

When weighing out chemicals for the preparation of reagent solutions, four-point accuracy is generally not required, so long as the working standard solution is included in every batch of analyses and the same reagent mixture is added to the distilled water blanks, the working standards and the samples.

A little thought here should clarify the issue:

the working standard solution is **known** to be of a certain concentration because of the method of its preparation, i.e. with utmost care at all of the stages involved. If the reagents used in the analysis were prepared with the same degree of care then, assuming that the distilled water used in the solutions and blanks is always of exactly the same quality, and the pipettors used to dispense the distilled water blanks and working standards deliver exactly the same volumes each time, the absorbance readings of the working standards would always be identical. If, however, the reagent solutions were prepared to a slightly lesser degree of accuracy, the composition of those solutions would vary slightly as would the absorbance of the working standards. For example, a 100$\mu g.l^{-1}$ phosphorus standard solution might give an absorbance reading of 0.250 on one occasion and 0.240 or 0.260 on other occasions. But since the concentration of this solution is known exactly, the actual values of the absorbance readings make no difference whatsoever to the ultimate measurement of the concentrations in the lakewater samples since these will also have received exactly the same reagent mixtures as the working standards and would thus exhibit a parallel degree of variation.

For general weighing of reagents, therefore, the procedure is as follows: place a clean and dry weighing boat onto the clean and dry pan of the balance and tare to zero.

Using a clean and dry spatula carefully add the chemical to the weighing boat until the required weight is registered. The weighing boat can now be removed, there being no need to close the sliding doors as above, and the chemical can be tipped into a beaker or conical flask as appropriate. A gentle tap on the bottom of the up-turned weighing boat should ensure that virtually all of the chemical will have been transferred. Add a magnetic stirrer bar and measure in

the required volume of distilled water using an appropriate measuring cylinder. Stir to dissolve and the solution is ready for use or can now be transferred to a reagent bottle for storage.

It is good laboratory practice to always clean the pan of the balance after use, and also to lift the pan off to check if any material has found its way underneath. Some chemicals are harmful and may cause damage if left for long periods of time.

APPENDIX III USE AND MAINTENANCE OF THE BIBBY 'DISTINCTION' STILL

Distilled water is an essential ingredient in most analytical chemistry laboratories, being used for a variety of purposes including the final rinsing of detergent-cleaned glass- and plastic-ware and in the preparation of solutions of chemical reagents, stock and standard solutions.

The Bibby 'Distinction' is a relatively simple glass still that can deliver up to 4 litres per hour of high quality distilled water, provided it is properly maintained and is operating efficiently.
There are 4 potential sources of problems that must be overcome to guarantee success:

#1 - electricity supply
This must be constant and stable throughout the distillation process otherwise the still will not function or will automatically switch off.

Continuity of electricity supply is something that cannot be guaranteed without a permanent independent supply, but stability can be virtually assured with a voltage stabilizer/surge protector.

#2 - water supply
The still requires a minimum tap-water supply rate of 1 litre per minute, otherwise it will automatically shut down, by the activation of a thermal cut-off switch, owing to the boiler overheating. Increases in the rate of supply, however, can be tolerated, leading to a decrease in distilled water output through loss of efficiency, or, at worst, minor flooding in the lab!

N.B. It would be best to keep checking at intervals of say 10-15 minutes that the still is functioning correctly so that, in the event of an automatic shut-down, it can be re-started and the water supply adjusted as required.

#3 - build-up of lime-scale inside the boiler and on the heating element
The chemical composition of Lake Tanganyika water (which constitutes the water supplied to the taps) is such that prolonged evaporation (such as occurs in the still) results in the deposition of lime-scale which greatly reduces the efficiency of the still's operation by reducing heat transfer from the heating element. Periodic removal of these deposits with a solution of hydrochloric acid, followed by thorough rinsing with water, is essential.

#4 - collection and storage of the distilled water
There is no point in making distilled water if it is to be exposed to contamination by contact with a dirty collection vessel or from dirt or dust entering that vessel during and/or after production. The collecting vessel must be scrupulously clean and be kept covered at all times (indeed, it is best to have a vessel whose sole use is the collection and storage of distilled water). Likewise, the dispensing tap of this vessel must be kept dirt-free to avoid contamination of the distilled water as it is being dispensed.

Procedure for the removal of lime-scale:

With the still switched off, close the drain stopcock and allow the boiler to completely fill with water. Now open the drain stopcock and allow about 250ml of water to escape. Fully close the stopcock and pour about 100ml of concentrated hydrochloric acid (the 'specified laboratory

reagent' **NOT** the 'analytical reagent') into the boiler *via* the integral funnel. Now let the boiler completely fill with water, turn off the inflow and allow the acid solution to bubble away, digesting the built-up deposits. When all effervescence stops (or at least slows right down) after maybe 15-20 minutes, and most if not all the lime-scale deposits have dissolved, open the drain stopcock and empty the boiler. Now repeatedly fill and empty the boiler with water several times to rinse out all traces of the acid. When you are confident that the still is clean, allow it to fill with water and it is ready for use.

When this de-scaling process has to be carried out depends very much on the frequency and duration of use of the still - it should be self-evident!