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FIELD MANUAL FOR THE DETERMINATION OF CHLOROPHYLL AND PRIMARY PRODUCTION IN LAKE TANGANYIKA RESEARCH

by

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PREFACE

The Research for the Management of the Fisheries on Lake Tanganyika project (LTR) became fully operational in January 1992. It is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Finnish International Development Agency (FINNIDA).

LTR's objective is the determination of the biological basis for fish production on Lake Tanganyika, in order to permit the formulation of a coherent lake-wide fisheries management policy for the four riparian States (Burundi, Tanzania, Zaïre and Zambia)

Particular attention is given to the reinforcement of the skills and physical facilities of the fisheries research units in all four beneficiary countries as well as to the buildup of effective coordination mechanisms to ensure full collaboration between the Governments concerned.

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1. INTRODUCTION

Phytoplankton primary production is generally the major supply of food for the pelagic food chains of large lakes. The present estimates of primary production in Lake Tanganyika are based on extrapolations from limited data (Hecky & Fee, 1981). Therefore, one important goal of the carbon—energy subcomponent of the Lake Tanganyika Research Project is to obtain more reliable information on primary production. However, the rapid changes of chlorophyll and primary production in response to varying light conditions in Tanganyika make the determination of production difficult (Salonen & Sarvala, 1994).

The estimates of primary production can be improved by relating regular *in situ* measurements of the production rate at different depths at three field stations to the corresponding chlorophyll and irradiance levels. These data will be complemented, during the lake—wide cruises of the research vessel *R/V Tanganyika Explorer*, with incubator measurements of primary production at different light intensities.

The purpose is to describe the dependence of primary production on chlorophyll and light with a regression model. This model can then be combined with technically easier measurements of the vertical distribution of chlorophyll and irradiance at the field stations and the vertical chlorophyll profiles derived from fluorometer measurements during the research vessel's cruises to produce more realistic estimates of primary production of Tanganyika ('simulated *in situ* production'; e.g. Lohrenz et al., 1992). The production processes will be further elucidated with small—scale special studies, which cannot be included in the LTR routine, because they require the use of expensive equipment.

Measurements of phytoplankton chlorophyll, primary production and incoming solar radiation (photon flux) should be included in the routine biological monitoring programme of LTR, and the present guide contains a general overview of the relevant methodology.

2. PROCEDURES FOR THE DETERMINATION OF CHLOROPHYLL

2.1. GENERAL

Chlorophyll concentration indicates the trophic level of the lake. It is also a measure of the phytoplankton production potential. Though all autotrophic algae contain chlorophyll, the chlorophyll concentration need not be directly correlated with biomass; their relationship is affected, for instance, by illumination, nutrient concentrations and the species composition of the phytoplankton assemblage.

Algal pigments are extracted into organic solvent, in which the concentrations are measured either spectrophotometrically or fluorometrically. Fluorometry may also be used to measure chlorophyll concentrations *in vivo* in untreated water samples or even *in situ.* Quantitatively, the most important algal pigment is usually chlorophyll *a*, which is determined monochromatically. Other chlorophylls (especially bacteriochlorophylls) and their degradation products may absorb partially on the same wavelengths as chlorophyll *a*. Consequently, the observed concentration may include other pigments in addition to chlorophyll *a*. Therefore, the spectrophotometric methods always give higher chlorophyll values than those obtained with methods that measure only chlorophyll *a* (e.g. high performance liquid chromatography HPLC; Meyns et *al.,* 1994).

Ethanol, methanol and acetone have been commonly used in chlorophyll extraction. In many cases nowadays (e.g. the Integrated Monitoring Programme; Keskitalo & Salonen, 1994), chlorophyll is determined using ethanol as the extractant. It is less of an occupational risk than methanol or acetone and has proved more effective than acetone for many algal groups (e.g. blue–greens and greens). During extraction, the samples are often heated, which will inactivate the chlorophyllase enzymes and enhance extraction. However, cold extraction using an extended extraction period is usually sufficient (Arvola, 1981; Jespersen & Christoffersen, 1987).

2.2. APPARATUS AND REAGENTS

- Spectrophotometer (Spectronic 301 with longpath cellholder and 50-mm rectangular cuvette; WL 320-1100 nm)
- Vacuum filtration device (adjustable vacuum pump, filter holder with clamp, vacuum bottle)
- Glass-fibre filters free of organic binder (Whatman GF/F, diameter 47 mm)
- Glass-fibre filters for filtration of extracts (Whatman GF/F or GF/C, diameter 25 mm)
- Extraction vessels, e.g. test tubes with tight screw caps, capacity of 30-50 ml
- Refrigerator
- Ethanol (C_2H_5OH) 94 % (in terms of volume)

2.3. SAMPLING

Water samples for chlorophyll determination are taken in connection with the routine carbon/energy - zooplankton lift - horizontal limnosampling at two-week intervals from station A. Vertical chlorophyll profiles should always be taken in connection with primary production measurements. The sampler should preferably be covered with light-impermeable material to protect phytoplankton from direct sunlight. Three litres of water from each of the depths of 0, 10, 20, 30, 40, 50, 60, 80 and 100 m are taken to the laboratory in large plastic bottles, shaded from direct sunlight.

The vertical attenuation of irradiance (see chapter 3.5.) should be always measured in connection with the vertical chlorophyll series.

2.4 LABORATORY PROCEDURES

Chlorophyll samples must be filtered on the day of sampling. Put a 47-mm Whatman GF/F glass-fibre filter on the filter holder, and place the filter system on the mouth of the vacuum bottle. Filter three litres of water through the filter using a suction vacuum of <30 kPa (<0.3 bar). Low pressure is necessary to avoid pigment losses.

If a 1-litre vacuum bottle is used, it must be emptied twice during filtering. After the filtration is completed (water has disappeared from the surface of the filter), continue filtering for an additional 15—30 seconds to reduce the residual water content of the filter. Remove the filter from the holder, fold (roll) the filter gently with forceps (if this does not help, the filter can also be cut into pieces), and transfer it into an extraction tube. When folding the filter, avoid pressing the upper surfaces tightly together, as this may impair the effectiveness of extraction.

For the extraction of chlorophyll from the algae, the filters are immersed in ethanol. Add 20 ml (exact volume here depends on the volume needed for the 50-mm cuvette!) of 94% ethanol into the extraction tube. Mark each tube to show the sampling depth.

The extraction tube is closed tightly with its screw cap (instead of cap, Parafilm may also be used for air—tight closure of the tubes). The extraction tubes are left overnight in the dark and cold (refrigerator, about +4°C). Next day the tube contents are mixed well, the filter is removed, and the extract is cleared by filtering it through a Whatman GF/F (or GF/C) 25-mm glass-fiber filter into a clean extraction tube (a filtering syringe may be used).

Fill a 50-mm spectrometer cuvette with the filtered extract. The absorbance of the extract is measured spectrometrically at 665 and 750 nm. The blanks are measured with 94% ethanol. Filtered water and extractant volumes should be selected so as to obtain 665 nm absorbance readings in the range of 0.005 - 0.8.

Do not remove the extract from the cuvette. Add 0.1 ml of 0.3M HCl per 10 ml of extract and mix well. Measure the absorbance again at 665 and 750 nm.

Note 1: If it is impossible to perform chlorophyll extraction immediately after filtration, the filters can be dried in darkness and stored frozen (at least —20°C) maximally for 1 month.
Note 2. If spectrometric determinations cannot be continued immediately after extraction, the cleared extracts can be kept overnight at ≤+4^{0}C protected from light (extracts containing the filter must not be stored).

2.5 CALCULATION AND EXPRESSION OF THE RESULTS

The chlorophyll concentration of the sample is calculated both with and without acidification.

Chlorophyll estimates without the acidification step is obtained from the equation

$$C_c = \frac{12.0}{V_s} \times A \times V_e$$

Chlorophyll estimate using the acidification step is obtained from the equation

$$C_a = \frac{29.1}{V_s \times d} \times (A - A_a) \times V_e$$

where:

A = $A_{665} - A_{750}$
A_a = $A_{a\text{-}665} - A_{a\text{-}750}$, and

A_{665} = sample absorbance at 665 nm (absorption maximum of chlorophyll a) before acidification
$A_{a\text{-}665}$ = sample absorbance at 665 nm after acidification
A_{750} = absorbance at 750 nm (turbidity) before acidification
$Aa_{\text{-}750}$ = absorbance at 750 nm after acidification
C_a = chlorophyll *a* concentration of the sample, mg m^{-3} (with acidification)
C_c = chlorophyll concentration of the sample, mg m^{-3} (without acidification)
d = cuvette path length, cm
V_e = ethanol volume used for extraction, mL
V_s = sample volume filtered, L
12.0 = calculation constant (derived using 83.4 as the absorption coefficient of chlorophyll *a* in 94% ethanol; if 90% ethanol is used for extraction as in the ISO standard, the value of the absorption coefficient will be 82, which results in a calculation constant of 12.2)
29.1 = calculation constant (derived using an absorbance ratio A/A_a = 1.7 for a solution of pure chlorophyll *a* which is transformed to phaeophytin by acidification, and the value 83.4 as the absorption coefficient of chlorophyll *a* in 94% ethanol). For extraction in 90% ethanol this constant = 29.6.

The results are expressed as mg m^{-3} (for each depth zone) and mg m^{-2} (integrated for the entire water column). The depth the water column must be given in connection with the integrated value.

2.6. SOURCES OF ERROR

Chlorophyll is sensitive to both light and temperature, which should be kept in mind during all phases of the work. Slow disintegration will take place even on frozen filters. The turbidity of the extract will bias the results, so the absorbance reading at 750 nm should not exceed 0.05 with a 50-mm cuvette.

The bacteriochlorophylls of autotrophic bacteria (e.g. *Chlorobium)* absorb at 665 nm, causing high chlorophyll values in some anoxic waters (Takahashi & Ichimura, 1970), even though chlorophyll *a* may not be present. The possible presence of bacteriochlorophyll in anoxic waters should be checked by plotting the absorption spectrum of the sample and observing the location of the absorption maximum.

Chlorophyll results may also be affected by phaeopigments. To detect such interference, additional absorbance measurements of acidified subsamples are sometimes made. The absorbance ratio of non—acidified/acidified samples is 1.7 for pure chlorophyll a samples at 665 nm and lower for samples affected by phaeopigments (Lorenzen, 1967; Edler, 1979; Marker *et al*., 1980)

The ISO standard (International Standard ISO 10620:1992) includes acidification as a routine part of the determination. However, from detailed chromatographic studies it is known that the acidification procedure does not give a true picture of the proportion of phaeopigments in the original water sample, especially in the presence of chlorophyll *b* (e.g. Welschmeyer 1994). For this reason, acidification has been removed from the Finnish chlorophyll standard (SFS 5772:1993) and from the routine Integrated Monitoring Programme procedures (Keskitalo & Salonen, 1994).

This change should simplify the procedure and may also reduce the variance of the measurements. However, the acidification step gives some additional, even if loosely defined, information, and therefore it may be included in the routine procedure at Lake Tanganyika. To facilitate comparisons, chlorophyll results should be reported both before and after the acidification.

3. PROCEDURES FOR THE MEASUREMENT OF PRIMARY PRODUCTION

3.1 GENERAL

With the energy from sunlight, phytoplankton can assimilate inorganic carbon from water and this results in the formation of organic matter and release of oxygen. What happens in the photosynthesis can be simplified as the following expression:

$$6\ CO_2 + 6\ H_20\ \text{- >}\ C_6H_{12}O_6 + 6\ 0_2$$

According to this formula, primary production can be estimated

using many techniques. In the simplest case, it can be done by measuring the carbon dioxide uptake or oxygen output due to photosynthesis. However, these approaches are often too insensitive to obtain reliable results, and therefore, in waters with low primary production, it is advisable to use radioactive carbon-14 as a tracer to obtain high sensitivity.

With the radiocarbon method, the amount of radiocarbon fixed by phytoplankton is assessed. At the beginning of the experiment, a known amount of inorganic radiocarbon is introduced into a sample bottle. After the incubation, the sample water is filtered and radioactivity in phytoplankton retained on the filter is assessed, or the radioactivity of the unfiltered sample is directly measured, after acidification and exchange of $^{14}CO_2$ with air (Niemi et *al.*, 1983).
The calculation of results is based on the fact that when phytoplankton assimilates inorganic carbon from water the ratio between radioactivity and DIC in water is reflected in primary production of phytoplankton. This results in the following equation:

$$DPM_{DIC} / DIC = DPM_{Phyto} / OC_{Phyto}$$

from which primary production can be solved.

$$OC_{Phyto} = DIC * DPM_{Phyto} / DPM_{DIC}$$

DPM_{DIC}	-	The radioactivity in added radiocarbon solution
DPM_{Phyto}	-	The radioactivity found in phytoplankton after the incubation
DIC	-	Dissolved inorganic carbon in water
OC_{Phyto}	-	Organic carbon fixed by algae during the incubation

3.2 EQUIPMENT AND CONSUMABLES

For each field station (3) and the research vessel:

- Limnos water sampler (if possible use a sampler covered with light
- impermeable material to avoid harmful effects of too bright light)
- Underwater radiation (PAR) measurement system (Li–Cor)
- Acid washed glass liquid scintillation vials with plastic screw cap, volume 20 ml
- Plastic liquid scintillation vials with plastic screw cap, volume 20 ml
- Plastic rack for holding the vials in order on the boat and in the laboratory
- Buoy
- A string with attachments for the glass vials at appropriate depths, including stoppered tubes for the dark vials
- Adjustable pipette (Finnpipette 0-200 µL, with extra long and

narrow tips) for radiocarbon dosing
— Small pipette for phosphoric acid dosing
- Pipette for dosing formaldehyde
- Blotting paper or equivalent liquid absorbing tissue to cover the table space where one is using radiocarbon
- Plastic sheet to be kept under the mentioned absorbing paper
— Disposable gloves
- Thick rubber gloves
- Aluminum foil
- Orthophosphoric acid (concentrated)
- Formaldehyde (concentrated, 38 %)
— File for opening the radiocarbon ampoules
— A holder for the radiocarbon ampoule
- A wide-mouth, screw-cap scrap vial/bottle (e.g. 250 ml)
— Radiocarbon stock solution, glass ampoules. Radiocarbon stock solution is carrier—free and it is supplied in glass sealed ampoules as sodium carbonate. In this state the solution can be stored infinitely. To avoid problems from rapid contamination of radiocarbon stock solution in the tropical climate, the ampoule size is adjusted so that one ampoule contains enough radiocarbon for one vertical series of primary production measurements.

3.3 SAFETY PRECAUTIONS

Radioactive isotopes are potentially hazardous. Some isotopes disintegrate rapidly, but the half life of radiocarbon is ca. 5000 years, so that in terms of the length of human life it remains forever. Radiocarbon emits weak beta radiation whose penetration energy is low. This means that radiation from the amounts of radioactivity used for the determination of primary production cannot affect human health. Therefore the main concern is to prevent radiocarbon from penetrating the human body. The main mechanisms how radiocarbon might enter the human body are: 1) ingestion, 2) breathing and 3) absorption through skin.
To avoid contamination of the human body, strict rules should be followed.

1) Never eat or drink when handling radioisotopes. Food stuffs must never be in the vicinity of radioisotopes. Direct ingestion is, in practice, the only possibility of badly contaminating the human body. To give some idea about the degree of theoretical health hazard, it may be mentioned that according to the regulations valid in Finland, the maximum radioactivity of radiocarbon permitted in human body is ca. 11 MBq (300 µCi). This is equivalent of one 10 ml ampoule of 1.1 MBq (30 µCi) mL’ stock solution.

2) Remember that inorganic radiocarbon solution with a pH less than 9.5 exchanges carbon dioxide with the atmosphere. Therefore exposure time of any radiocarbon solution to air must be minimised. Good ventilation at the working place prevents the accumulation of gaseous radiocarbon. On the other hand, exchange with atmospheric carbon dioxide is an efficient way of eliminating any unintended contamination by inorganic radiocarbon within a couple of days.

3) Absorption through skin can be prevented by protecting hands with disposable plastic gloves.

To avoid the spreading of radioisotopes, some other general precautions should also be followed. The handling of radioisotopes in the laboratory should be limited to a restricted area. The work table area should be covered by a protective layer, consisting of a plastic sheet at the bottom and absorption paper on the top. All equipment used in the handling of radioisotopes should be dedicated for this work only.

What to do in case of contamination

In spite of all precautions radioactivity may sometimes spread accidentally. In such cases the following instructions should be followed:
- If liquid has spilled on a surface which can be directly flushed (for example the deck of boat), flush it several times with water.
- If radioactive liquid spills in a place which cannot be flushed or rinsed, the spilled liquid should be blotted with absorptive paper (using protective gloves), which should then be burnt.
- If gloves have become contaminated, rinse under running tap water (but do not contaminate the tap with your gloves!).
— If the protective cover on the working table, etc. is contaminated you can let it dry. However, in cases where large volumes of radioactive sample water or any amount of the radiocarbon stock solution have been spilled, roll the absorptive paper together with its underlying plastic sheet, and locate it somewhere outdoors to dry. After drying the waste should be burnt.

Handling off radioactive waste

The level of radioactivity present in the incubation vials is low. Used plastic vials, contaminated gloves and protective absorbing paper can be destroyed by burning. Then radiocarbon in organic matter is converted to CO_2 which escapes into the air. Empty and dry glass ampoules can also be finally cleaned by burning, after which they can be reused or treated as normal waste glass. Liquid waste can be rinsed into a sink with excess (ca. 10 times of the liquid volume) tap water.

3.4 FIELD WORK

The field work, i.e. the preparations for the experimental incubations, should be performed as quickly as possible. The liquid scintillation vials used for incubations should be arranged in a plastic rack according to depth. The depth of incubation should be permanently marked on the top of the caps of the vials (e.g. 10L, 10D, 20L, 20D: number for depth, L for light, D for dark incubations). Never mark vial body, as this will shade the sample.

Primary production is measured twice a month at the routine sampling station A (carbon/energy - zooplankton lift -horizontal limnosampling). The measurement can be combined with limnology sampling or hydrodynamics, or be performed on a separate day. In any case, primary production measurements should always be combined with chlorophyll sampling and measurement of irradiance. Data on temperature, pH and alkalinity are needed for the calculation of production results, and measurements of the main nutrients would also be advisable.

Water for the experiments is taken with a Limnos water sampler, utilizing the same samples for chlorophyll determinations, and, if appropriate, for physical and chemical determinations. To avoid oil contamination, always take samples from that side of the boat which is opposite to the exhaust tube of the engine. Water samples are taken from the depths of 5, 10, 20, 30 and 40 m. Do not expose sample water to direct sunlight. All steps must be made in the shade and during the temporary storage of samples before incubation, vials should be kept in dim light at a constant temperature (e.g. cooling box with no coolant).

1) Begin sampling from the depth of 5 m.

2) Take 2 (3 for 5 and 40 m to obtain controls) vials from the rack, open their caps and fill them by putting the draining tube of the sampler to the bottom of each vial and allowing the overflow of 2-3 vial volumes of water. After filling close the caps, put the vials back into the rack and cover with black plastic or other light-impermeable material (e.g. aluminum foil). Take a 3-litre sample for the determination of chlorophyll from the same Limnos sampler.

3) When all depths have been sampled, radiocarbon is added to the samples. Take the radiocarbon stock solution ampoule and scratch its neck with a file or glass knife. Be sure to knob all radiocarbon down to the main body of the ampoule. Take the ampoule in a piece of paper tissue able to temporarily absorb 5 mL of water in case the ampoule accidentally breaks. Keep tough rubber gloves on to protect your hand from the sharp glass edges, and bend the narrow top end of the ampoule so that the neck opens first on the scratched side. The detached top should be placed in a scrap vial, and the opened ampoule placed in a special holder which keeps it in upright position.

Starting from the 5—m sample, remove ca. 0.5 mL of water and inject 100 µL (0.11 MBq [3 µCi]) of radiocarbon solution ($Na_2{}^{14}CO_3$) with a dosing pipette (this pipette should not be used for any other purpose; see special remarks on safety aspects) into each of the two vials. This is done so that the pipette tip is immersed in sample water.

First, empty the volume of radiocarbon by pressing the piston of the pipette to the bottom of the first step. Then slowly press the piston to the ultimate bottom and simultaneously draw the tip of the pipette out of water. This practice prevents the entrance of lake water into the tip. Some practice is be needed

in advance, with water not containing radiocarbon, to learn this procedure. After the introduction of radiocarbon into each vial close the cap tightly, turn the vial upside-down once, and put it back into the rack. Immediately after closing the cap of the second vial, put it into the dark incubation tube and close the tube.

4) Take the two control vials for the depths of 5 and 40 m, remove ca. 1.5 ml of water and introduce 1 ml of 38% formaldehyde solution into both with a separate pipette. Thereafter introduce the radiocarbon solution. (The controls are needed to ascertain that radiocarbon solution is not contaminated.)

5) The used pipette tip is discarded into the scrap bottle. The stock solution ampoule (which is now almost empty and contaminated by formaldehyde) is acidified with a drop of H_3P0_4 and placed in the plastic scrap bottle, which is closed and transported to the laboratory.

6) When radiocarbon solution has been introduced into all the vials, the uncovered vials are attached to the suspending system by pushing the vial cap into the attachment holes. The closed tubes containing the dark incubation vials are attached below each light vial.

7) The attachment system is connected to the buoy which is left to float freely for the incubation period. Mark the starting time of the incubation in the diary.

8) After the appropriate incubation period (4-6 hours, starting in the morning soon after sunrise; a 4—hour incubation starting at about 9:00 is recommended as the routine) the buoy is taken back to the vessel, the vials detached, put into the rack and protected from light, as described earlier.

9) Add one drop of concentrated orthophosphoric acid to each vial, close the vials carefully and shake to mix the contents. This step acidifies the samples to pH 2. Mark the time at the end of the incubation in the diary. Any observations with possible importance for the determination should also be written down.

During transport, the vials should be kept in insulated boxes to prevent them from heating up.

3.5 MEASUREMENT OF IRRADIANCE

Using the Li-Cor radiation sensor with datalogger, the integrated radiation during the incubation (exact time! - alternative: hourly rate of photon flux during the incubation is sufficient, then the datalogger can be used in the data logging mode with hourly integration period) is measured on the research boat.

To avoid disturbing shadows, the sensor of the radiation meter should be then placed on the roof of the vessel cabin. To

circumvent the interference from reflected light, the sensor should be installed in an upwards opening housing made of, or covered with unreflecting material. The vertical attenuation of radiation is measured immediately after the incubation from the sunny side of the vessel (measuring depths 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45 and 50 m; instantaneous operation mode using continuous running average read from the LCD display). Thereafter the radiation meter is again installed on the roof of the vessel's cabin and integrated radiation recorded until sunset.

Between the primary production expeditions, the radiation meter (with its housing) should be installed in an open safe place, close to the shore, and hourly integrated radiation recorded for each day. These hourly radiation values should then be correlated with total radiation data from the projects' weather stations, or with those obtained from Kigoma airport. The aim of these comparisons is to make possible extrapolations of the primary production estimates for the whole annual cycle using data on daily radiation, proportion of light reaching each depth, chlorophyll levels at the corresponding depths, and a photosynthesis–irradiance relationship obtained from own measurements at the field stations and the research vessel.

If a flat cosine collector is available, it should be used for all irradiance measurements in air. The cosine collector should then also be used as a surface reference while measuring underwater attenuation of irradiance with the spherical sensor.

3.6 LABORATORY WORK

In the laboratory, you should have a series of disposable plastic liquid scintillation vials, marked correspondingly to the vials used in the field. In addition to the identification information in each field vial, you should mark the date and station code on the top of the vial cap. No manipulations should be made in bright light.

1) 6 ml of each sample is transferred with a dosing pipette into the corresponding plastic liquid scintillation vial. If needed, use a suction bulb. Never suck by mouth!

2) The vials are closed tightly and sent to Finland for the radioactivity count. The radioactivity of the incubated samples is so low that it will not penetrate through the bottle walls.

3) The sample water remaining in the glass incubation vials is poured into the sink with running tap water. After emptying, the vials and caps are rinsed with abundant tap water. Then they are acid-washed and rinsed several times with distilled (or deionized) water. After drying, the vials can then be reused for the next incubation.

4) The scrap bottle is taken to a safe place outdoors, protected from rain and human and animal interference, opened, and left for at least two days during which the remaining radioactivity in CO_2 is exchanged with air. Then the contents are rinsed into

the sink with tap water, and the pieces of glass and plastic are collected on paper and discarded.

3.7 COUNTING OF RADIOACTIVITY

The radioactivity is determined with a liquid scintillation counter. This phase of the work will take place at the Lammi Biological Station in Finland. Modern scintillation liquids (often called safe fluors) are not flammable and pose little health risk. The procedure is described here for information:

The acidified samples are allowed to outgas in a vacuum hood for two days (Niemi et *al.,* 1983). After that, 10 ml of fluor are added to each plastic liquid scintillation vial and thoroughly mixed. The vials are placed into a liquid scintillation counter, and counted to at least a 10000 cpm preset count, or for 10 mm. All necessary identification markings are added on the printer sheet, the results are collected, and the vials are removed from the counter rack.

For correct results the liquid scintillation counter is calibrated for quenching following the instructions given in the instrument manual. The external standard channels ratio is used for calibration.

3.8 DETERMINATION OF DISSOLVED INORGANIC CARBON

Dissolved inorganic carbon in lake Tanganyika consists mostly of carbonates and is therefore rather stable. It is adequate to measure its concentration from the pH and alkalinity (and the temperature during their laboratory measurement) of 0, 10 and 20 m samples only. The required information is obtained as part of the routine chemical limnology determinations.

3.9 CALCULATION OF RESULTS

Assimilated carbon at each sample depth is to be calculated according to the following equation:

$$OC_{Phyto} = 1.05 \times DIC \times \frac{DPM_{Phyto/light} - DPM_{Phyto/dark}}{(DPM_{DIC} - DPM_{control}) \times 6/V}$$

Where:

OC_{phyto}	—	concentration of assimilated inorganic carbon during the incubation, mg m^{-3}
1.05	—	correction coefficient to compensate for the slower uptake of radiocarbon than normal carbon
DIC	—	concentration of inorganic carbon in the sample, mg m^{-3}

$DPM_{Phyto/light}$	—	radioactivity of the light sample (average of two determinations, dpm)
$DPM_{Phyto/dark}$	—	radioactivity of the dark sample, dpm
DPM_{DIC}	—	radioactivity added to the sample, dpm (average of two determinations, calculated to the same volume as other dpms)
$DPM_{control}$	-	mean radioactivity of the two dark control samples (5 m, 40 m), dpm.
V	—	mean volume of the glass incubation vials, mL

The results are expressed as assimilated carbon mg C $m^{-2}d^{-1}$ (for each analyzed depth) and as mg C $m^{-2}\,d^{-1}$ (value integrated for the water column). Daily values are calculated from short incubations by multiplying the observed primary production by the ratio between total daily radiation and incubation time radiation. According to later experience with the relationship between chlorophyll and primary production, it may be possible to finely adjust the estimates accordingly. Lake-wide extrapolations will be based on chlorophyll and radiation data.

3.10 SOURCES OF ERROR

Care must be taken not to allow any radiocarbon in the stock solution to be exchanged with atmospheric carbon dioxide. The pH of the solution must remain above 9.5, otherwise radiocarbon starts to exchange with atmospheric carbon dioxide finally resulting in complete loss of radioactivity. To prevent this, avoid prolonged exposure of the isotope solution to the atmosphere.

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