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ANGLIAN REGION

STANDARD METHODOLOGIES

Freshwater Phytoplankton

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C. TEST METHODS AND PROCEDURES **Freshwater Phytoplankton**	**ISSUE DATE:Sept 1995**

F I N A L D R A F T

PHYTOPLANKTON
STANDARD METHODOLOGIES

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1. INTRODUCTION

The identification and enumeration of phytoplankton is becoming an increasingly important component of biological work programmes. Long term monitoring of algae, in respect of eutrophication, has occurred in the Anglian Region for many years. More recently the monitoring of blue-green algae, toxic and nuisance marine species has become important.

In addition there are two EC Directives, the Urban Waste Water Treatment Directive [91/271/EEC] and Nitrates Directive [91/676/EEC] which have a requirement for monitoring phytoplankton in estuarine waters, coastal waters, still freshwaters and running freshwaters.

The use of phytoplankton densities, in conjunction with chemical and physical data, is necessary for the effective control of eutrophication. Toxic algae, such as blue-greens and *Prymnesium*, are of great economic importance. *Prymnesium* can have a devastating effect on fisheries. Blue-green algae can cause death to livestock, and the potential health risk often forces closure of recreational facilities. Some marine dinoflagellates are the cause of shellfish poisoning (paralytic, diarrhetic, neurotoxic and amnesic shellfish poisoning). Filter-feeding shellfish consume toxic dinoflagellates and in doing so, accumulate the algal toxins in their own tissue. These toxins can then be transferred to humans on consumption. Other marine species, such as *Phaeocystis*, can cause foaming on bathing waters, while some marine diatoms are responsible for fish deaths.

1. 1 Sources Of Error

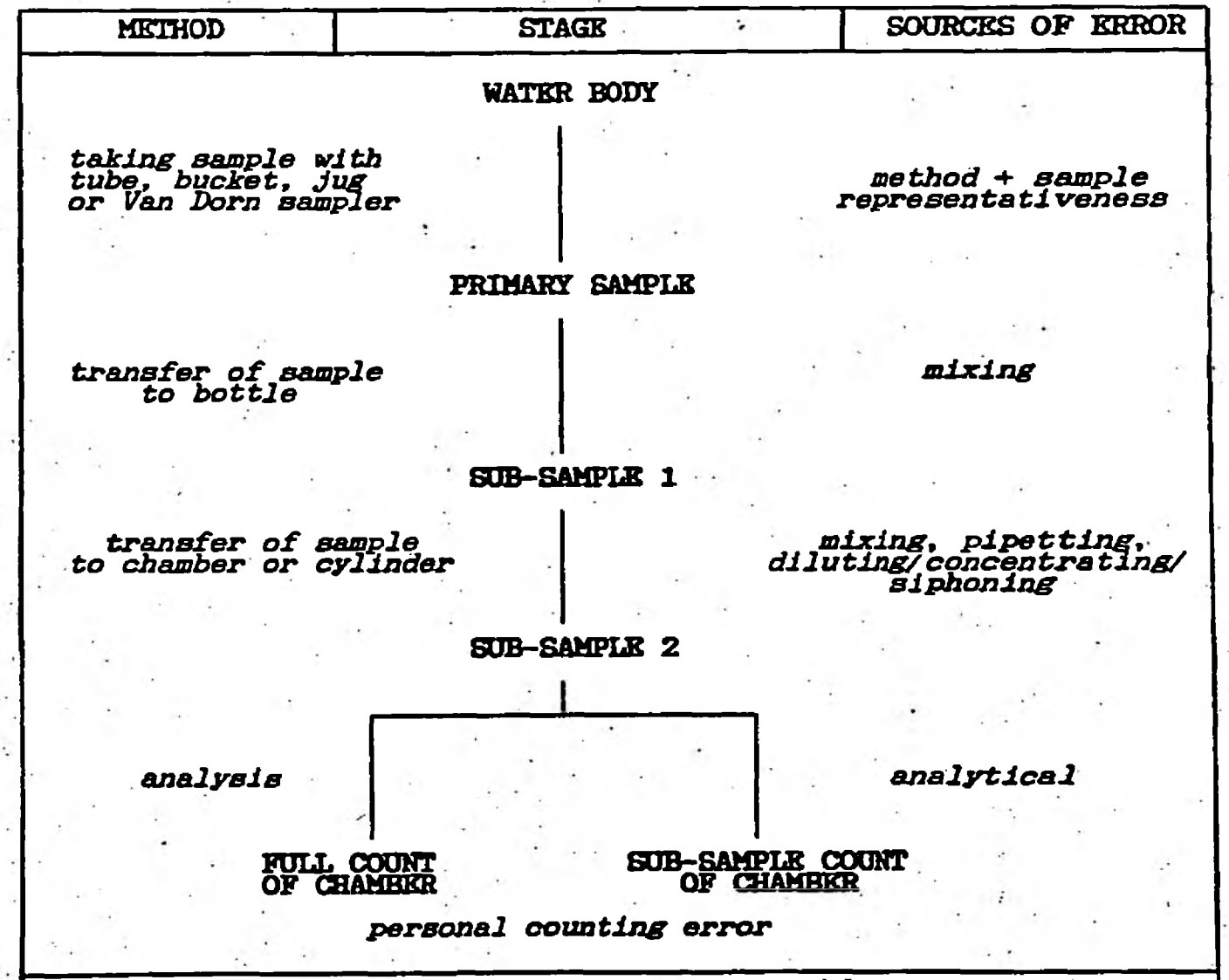


Figure 1. 1 Schematic representation of possible sources of error in phytoplankton analysis and sampling.

Figure 1. 1. shows a schematic representation of some of the principal sources of error in phytoplankton sampling and analysis. The left hand column shows the methods, the central column the stages and the right hand column the sources of error.

2. DEFINITION

Plankton are those organisms which are adapted to suspension, in the sea or fresh water, and which are liable to passive movement by wind or current. Phytoplankton are the 'plant' component of the plankton, although the classification is often arbitrary with some taxa, such as *Peridinium*, being classified both as phytoplankton and protozoa.

Phytoplankton encompass a wide range of cell sizes and cell volumes. Ranging from the largest, which can be visible to the naked eye (500 - 1500 μm eg. *Volvox*), to the smallest, being less than 1 μm in diameter. The larger forms are often referred to as net-plankton, they are normally larger than 60μm, and are so named because they are retained by the mesh of a commonly used phytoplankton net (mesh apertures ranging from 35 to 60 μm). Nano-plankton range from 5 to 60 μm and normally pass through the mesh of a phytoplankton net, however because the pores of nets are prone to clogging nano-plankton can be collected in great numbers. Phytoplankton smaller than 5 μm are often classed as ultra or pico-plankton.

These classifications are arbitrary and there is no one recognised system in use, however the distinction between net and nano-plankton can be useful and is adopted throughout.

3. PRINCIPLE

Phytoplankton are monitored for many different reasons. For long term surveys the results are mainly used to examine comparative trends, whereas, with the analysis of toxic or nuisance species, quantity is the prime consideration.

In brief, the first stage of the basic method is to collect a representative sample from the water body being studied. In routine lake surveys the vertical distribution of phytoplankton, within the upper water column, is homogenized using an integrated tube sampler. In other cases spatial distribution is unimportant (eg. blue-green algae scum samples), or discretely sampled (eg. depth profile samples). On collection the sample is sub-sampled and stored in a bottle containing a suitable preservative. Finally, in the laboratory, the sample is again sub-sampled (which can involve concentration or dilution) and the final aliquot analysed. Analysis normally involves a series of examinations, within a counting chamber, at different magnifications, using a different technique for each. The success of the method, thus, depends on, the representativeness of the primary sample, the efficiency of mixing at the sub-sampling stages, the accuracy of the final enumeration and, finally, the skill of the operator.

As many levels of sub-sampling are involved in the process of sampling and analysis, the quality of the final result depends on the combined quality of each stage. To satisfactorily monitor the final quality of phytoplankton data, and apply the most cost effective methods, every stage of the process, from sampling to final result, requires quality evaluation.

A suitable quality control system will include regular evaluation of all possible sources of error, the documentation of such evaluations and clear action procedures to correct any aberration.

4. CHEMICAL HAZARDS ASSOCIATED WITH COLLECTION AND ANALYSIS

4. 1. Inventory of chemicals

4. 1: 1 **Water**

Although dilution and dispersion are considerable within the aquatic/marine environments, where sewage or other discharges occur, localised pollution may pose a significant health hazard. The risks associated with Leptospirosis and other water borne diseases must be appreciated (Appendix 5). Physical contact should be avoided by use of appropriate clothing including long PVC/rubber gauntlets. All wounds should be covered with a waterproof dressing.

4. 1: 2 **Other Chemicals**

	COSHH No.	Nature of risk
Acetone	0009	Flammable, Avoid Contact.
Formaldehyde	0106	Toxic, Irritant, Flammable
Glycerol	0167	Irritant
Methanol	0152	Toxic, Irritant, Flammable
Decon 90	0440	Mild Irritant
Lugol's Iodine	0513	Toxic, Corrosive See Appendix 1
10% HCl	0018	Corrosive
Ethanol	0005	Flammable
Isopropanol	0157	May be Harmful, Flammable Irritant

4. 2. COSHH Assessments

For detailed information on each of the chemicals listed in section 4. 1: 2 and their handling refer to the relevant COSHH assessments, held in the laboratory. For Lugol's Iodine and formaldehyde also see appendix 2 - Fixatives and Safety.

5. PHYSICAL HAZARDS

5. 1. Clothing

Clothing should be suitable for the worst potential weather. A dry-suit and suitable 'bear' are ideal for wet and/or cold boat work. In colder weather gloves and a suitable hat or hood should be worn to prevent excessive heat loss. A pair of industrial 'Marigold' or diving gloves worn over thin thermal gloves are ideal protection from cold water and heat loss, and still allow enough movement and sensitivity of the fingers for most jobs to be carried out normally.

Some form of waterproof footwear should be worn. Waders are not recommended for boat use and chestwaders are not allowed as their buoyancy properties may hold an operator in an inverted position if they were to fall into deep water. Footwear should have adequate tread, especially for boat work.

A life-jacket, eg. NRA approved twin chamber automatic inflation - 'crewsaver', and not a buoyancy aids must be worn at all times by all persons on board a vessel or when working near water.

The operation of the jacket must be fully understood before departure. Life-jackets must be tested/serviced at least once a year.

5. 2. Procedures

A procedure exists for recording the time that the biologist(s) leaves for, and is expected to return from, field-work. Details of the route, locations and estimated times of departure and arrival should be recorded and left with a designated person, or on a movements board. Any significant changes from the plan should be reported, for example: delays of 1 hr or more, changes in the sites/area to be visited. When the work is completed the designated person should be told. If staff have not reported in by the expected time (+ 1 hr) then the designated person should set the emergency search procedure in motion. The procedure will involve search parties who will check the areas intended to be visited.

For each site and situation, assessment of the hazards should be made and suitable precautions taken. Such assessment should include prevailing weather and provision for abandoning work should conditions change.

N.B. Refer to safety information held in the laboratory or see your Safety Officer for further details.

5. 3. Boat Handling

Boat handling information is contained in the NRA Code of Practice - Marine Activities (in draft at present time):

Part I: Use of sea-going vessels
Part II: Use of small vessels

At least one member of each crew should be a trained boat handler.

5. 3: 1 **Checklist of Safety And Emergency Equipment**

1. A suitable anchor and rope, should be made fast to the vessel and be ready for use at all times.
2. A rope (painter) attached to the bow.
3. Alternative means of propulsion, for tidal activities a back-up engine is appropriate, for other use oars/paddles are necessary.
4. A knife,. spike or pliers, in an appropriate case for use in an emergency to cut moorings or to clear a fouled propeller.
5. Flares as per Department of Transport schedule, emergency foghorn (marine sampling).
6. A throw line.
7. A waterproof torch.
8. A bailer.
9. A compass (in case of poor visibility).
10. A VHF radio/portable telephone.
11. A charts of the operational area, adjacent waters and coastline, preferably in a waterproof wallet (marine sampling).

12. First aid kit (offshore RYA first aid kit is appropriate).

13. Waterproof watch.

14. Boat-hook.

6. SAMPLING METHODS AND EQUIPMENT

6. 1 Sampling Programme Design

Although a detailed account of sampling design is beyond the scope of this manual, a few key points are appropriate. Before commencing a sampling programme make a rigorous statement of objectives. This statement should include the ultimate goal of the survey, precision desired, analysis required, the hypothesis to be tested, etc. This is one of the easiest questions to ask but perhaps one of the most difficult to answer. However, this question must be answered if an efficient and effective sampling programme is to be implemented. One way of approaching sample programme design, if time and resources permit, is to carry out a pilot study. The three most popular sampling strategies are as follows:

1. Simple Random Sampling: Each sampling unit in the population has an equal and independent probability of being selected.

2. Stratified Random Sampling: The population is divided into strata and one or more samples are collected at random from each stratum.

3. Systematic Sampling: Samples are collected from the population at regular intervals, and there is no element of randomization involved.

For a fuller treatment of sampling strategies, design and analysis see the following: Venrick E L, in Sournia (1978) - Cassie R M, in Edmondson & Winberg (1971) - Irish A E & Clarke R T (1984).

6. 2 Sampling

6. 2:1 **Qualitative Sampling**

Qualitative and semi-quantitative samples can be taken using a phytoplankton net (recommended mesh size 53 μm). In some quantitative studies nets have been used for sampling larger taxa, this practice is not advisable as the filtering properties vary in an unpredictable way. A vertical or horizontal net haul is a quick and effective method for collecting a concentrated sample of the large and rare taxa. Although in some cases (eg. most Norfolk Broads and bathing water samples) the algae can be so concentrated or there is so much debris present that the use of a net is impractical.

6. 2.1:1 ***Semi-quantitative Sampling Using Plankton Net***

If the sample is to be used for semi-quantitative analysis then attention should be paid to consistency of sampling method. For vertical hauls the net should be weighted at the base of the cone so that filtering only takes place during the haul, and haul depth should be kept consistent (eg. 5 metres). The net should be hauled very slowly otherwise most of the water will go around the net rather than through it. For horizontal hauls the towing speed should not exceed 1 m.s-1 and the net should be towed for a consistent time period.

6. 2.1:2 ***Net Maintenance***

Nets should be washed as soon as possible after use, and should not be allowed to dry before washing. This is especially important when used in salt water. When algae are abundant the net can be pre-washed in the field. This is done by removing the net filter or cone and hauling the net quickly through the water. This method may remove the obvious soiling but it is not a substitute for correct cleaning. In the laboratory the net should be back-washed using tap water and allowed to air dry before storing. Occasionally the net can be soaked over-night in dilute decon 90 (about 5% by volume - See Chemical Hazards), after it has been thoroughly cleaned. When dry the net should be examined for holes and replaced/repaired as necessary.

6. 2:2 Quantitative Samples

Quantitative samples are essential if accurate quantitative results are required (eg. algal units/ml, etc). It is important to consider what the results are going to be used for. They may be part of a long term trend analysis or a one off sample. Before sampling we need to know how many samples are required to estimate the abundance of common taxa, to the required level of precision. We may also wish to assign confidence limits to the population estimate. These questions can only be answered through experimentation and regular assessment of sampling efficiency.

The principle of quantitative sampling is to collect a representative sample from a preselected sample site. A volume of 250 - 500ml is normally sufficient, and the samples should be fixed straight away in the field. It is best to have the sample bottle ready, labelled and with Lugol's Iodine inside, before the sample is taken. The sample bottle should be completely filled, mixed by shaking or inversion, and stored out of direct sunlight. Dark glass is the most suitable material for sample bottles although clear plastic (PEP) bottles are more often used today.

6. 2.2:1 *Surface Samples*

Surface samples should be taken with a bucket or jug which is dipped into the water body. The sample should then be thoroughly mixed before sub-sampling. The sample bottle should not be dipped directly into the water.

6 2.2:2 *Tube Samples - Deep Water Liable to Stratify*

Obtaining representative samples of lake phytoplankton is complicated by their spatial distribution, some taxa may be scattered over a profile of only a few metres. If the water is completely mixed then a single sample from any depth is sufficient, but a completely homogenous dispersion is rare. To overcome some of the problems of vertical dispersion an integrated tube sampler can be used. The integrated tube is a length (standard length 5 metres - but can be as long as 60 metres) of flexible hose with a weight at one end and ropes connected to both, see Lund et al (1958).

The tube sampler is operated by lowering the weighted end slowly into the water column so that a uniform sample is collected equal to the length of the tube. When the upper end of the tube is at the surface of the water the tube is plugged. The rope connected to the lower end of the tube is then pulled until the tube forms a 'U' shape.

The tube can then be lifted free of the water, unplugged and the sample poured into a bucket (see figure 6. 1). It may be necessary (perhaps even desirable) to take several tube samples to collect enough water for a set of chemical and biological samples. The sample should be thoroughly mixed before any sub-samples are taken. A flexible tube can easily be constructed from a length of PVC hose, two hose clips, two lengths of rope, a bung and weight.

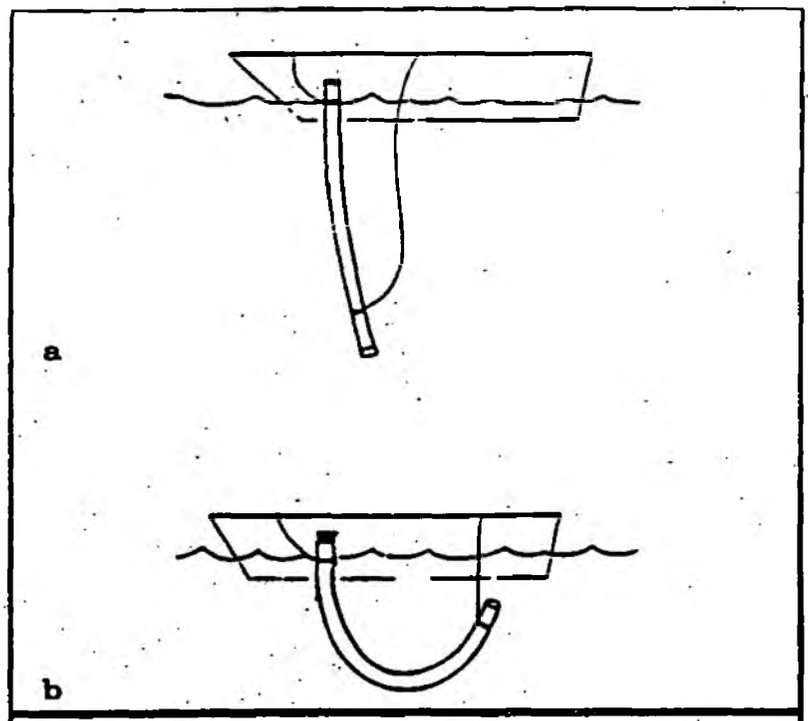


FIGURE: 6. 1 Tube sample taken from a boat. (a) tube being lowered. (b) tube plugged and being formed into a 'U' shape.

An alternative integrated tube sampler can be constructed from several lengths of domestic waste pipe (1¼ - 1½" internal diameter) and suitable screw connectors. The type of pipe that has bonded connections needs to be used as the 'push-fit' type connectors will not be strong enough to take the weight. The rigid tube sampler is operated by lowering the tube vertically and slowly into the water column until the top is level with the surface. The upper end of the tube is then plugged and the tube withdrawn from the water. As the lower end of the tube comes clear of the water a bucket is held in position and the sample retained. The bucket should be marked at a volume equal to a full sampler so that the effectiveness of each sample can be evaluated. If the collected sample is significantly less than expected then the sample should be re-taken after checking for air leaks in the sampler. The sample should be thoroughly mixed before sub-samples are taken. A segmented pipe sampler (SIPS) is described by Sutherland, Leonard and Taylor (1992).

When a hose or pipe sampler is 'home made' it should be thoroughly washed out before use. If samples taken with a 'home-made' sampler are also intended for chemical analysis then it may be wise to test the sampler for chemical leeching prior to use.

6. 2.2:3 ***Profile and Fixed Depth Sampling***

Although it is best to use an integrated tube sampler for routine lake sampling it may be desirable to investigate the vertical distribution of phytoplankton from time to time, using a universal water sampler, Van Dorn water sampler or similar (Fig: 6. 2)

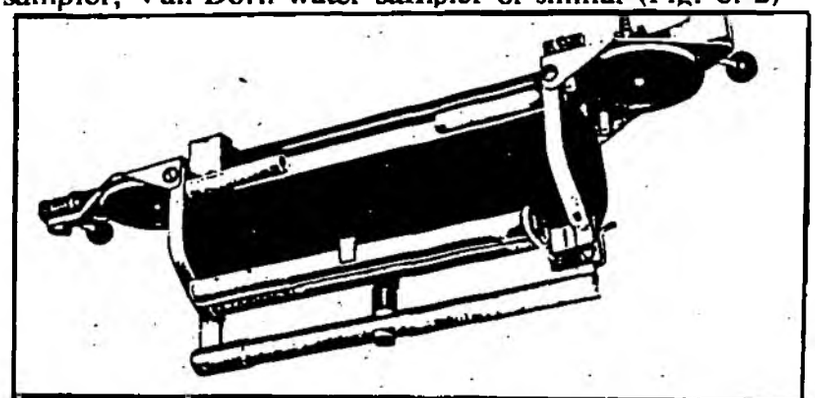


FIGURE: 6. 2 Universal water sampler

The universal water sampler is a tube with a spring loaded lid at each end, a lid release mechanisms and is suspended on a cable. To operate,the open sampler is lowered slowly,through the water column, to the desired depth at which point a messenger is sent down the cable. On contact with the sampler the messenger activates a mechanism which releases the spring loaded lids. The sample can then be retrieved, poured into a bucket, and thoroughly mixed ready for sub-sampling.

6. 3 Blue-green Algae Samples

See NRA Instruction of Blue-green algae monitoring and management of incidents.

Samples taken specifically for blue-green algae counts should be collected as follows. A sampling point on a down-wind shore should be selected. The presence of scums, blooms, water colouration etc. should be noted and the 'Inspection of Standing Waters' form completed (see Appendix 3 - Documentation). A surface dip should be taken from the scum or bloom, if present, and it is best to take both a live and fixed sample (fixed with Lugol's Iodine, see appendix 2 - Fixatives and Safety Precautions). Only a few ml's of each is necessary if a scum is present, but if unsure collect between 250 - 1000 ml of each. If the samples are sent for analysis then the laboratory should be advised that samples are on the way. If a scum is present then a sample should be taken for toxicity testing (see below).

6. 4 Blue-green Algae Samples for Toxicity Testing

If a blue-green algal scum is encountered then a live sample of scum should be collected as described in Table 1. Samples for toxin analysis (NRA Policy Instruction. Blue-green algal monitoring and management of incidents) Appendix 3. The sample should be sent to Dundee University as per the description in the NRA Technical Services Brochure.

6. 5 Bathing Water Samples

Bathing water algal sampling is currently required for the NRA Bathing Waters Monitoring Programme (EC Bathing Water Directive). Samples should be taken if there is any sign of an algal bloom, scums or the water is discoloured. A surface dip should be taken in the bloom or scum area, and every effort should be made to cause the minimum of disturbance to the sediment prior to taking the sample. Two samples should be taken, of about 250 ml each - one live and one fixed with Lugol's Iodine (see appendix 2 - Fixatives and Safety Precautions), packed in a light-tight bag and sent/taken for analysis. If the samples are sent for analysis then the laboratory should be advised that they are on the way. A Toxic Algal Bloom Report should be completed and sent off as per the Marine Algae Monitoring Sampling Methods (see Appendix 3 - Documentation). Completed forms should be sent to the Toxic Algae Scientist, TAPS Centre, Peterborough.

6. 6 Estuarine Sampling

Estuarine sampling is complicated by the possibility of salinity gradients. Phytoplankton and other suspended material tend to accumulate around the halocline, and thus complicate efforts to obtain a representative sample. Therefore, before taking estuarine samples the presence of a salinity gradient should be investigated, by taking a salinity profile. If a salinity gradient is found then a suitable sampling strategy should be adopted, which takes this into consideration, such as profile sampling.

6. 7 *Prymnesium* Samples

Samples taken in waters where the presence of *Prymnesium* is suspected should also have salinity and pH measured, preferably in the field. Samples should be taken from the surface, with a jug or bucket, from the most discoloured area (depending on accessibility).

Two water samples should be taken, of about 250 ml each - one live and one fixed with Lugol's Iodine (see appendix 2 - Fixatives and Safety Precautions), packed in a light-tight bag and sent/taken for analysis. If the samples are sent for analysis then the laboratory should be advised that they are on the way.

6.8 Sample Treatment

6. 8:1 **Fixatives**

The recommended fixative for routine sampling is Lugol's Iodine (see appendix 2 - Fixatives and Safety Precautions). Use the minimum volume necessary to achieve a 'straw' colouration. 1 - 5 drops for a sample $\leq$ 100 ml, dispensed from a dropper bottle, is usually sufficient.

The iodine in Lugol's solution sublimes with time from most types of container, so it can only be used for long term storage with special care. Containers should be air-tight and light-tight, or stored in complete darkness. As little air space as possible should be left in the container and samples should be stored in a cool place.

One of the properties of Lugol's Iodine is that it destroys the gas vacuoles of blue-green algae thus allowing sedimentation. However, gas vacuoles are required intact for the correct identification of many taxa, thus an alternative fixative is required for special taxonomic studies. A concentration of 2.5 to 4% formaldehyde is recommended as a secondary preservative in taxonomic studies (see appendix 2 - Fixatives and Safety Precautions).

6. 8:2 **Live Samples**

It is often useful to collect some live material along with the fixed samples. Identification is often easier with live material and in some cases taxa will be overlooked or misidentified if not seen in the live state (not to mention the aesthetics of observing live phytoplankton). Live samples should be viewed the same day as collected, but if this is not possible then they should be examined within 24 hours. Samples should be stored in water-tight pots which should not be more than one-third full. During hot weather live samples should be stored within a cool box or vacuum flask and transferred to a refrigerator on arrival at the laboratory.

If necessary live samples can be concentrated using a plankton net. If zooplankton are abundant in a live sample then they can be removed using a 140 μm filter. Although some nauplii and most rotifers are small enough to pass through a 140 μm filter the removal of the larger forms will reduce disturbance during observation and prevent consumption of the larger algae. The filtrate should also be stored, as above, for separate examination as some forms (eg. blue-green filaments) may be retained. It is often useful to combine live examination with qualitative/semi-quantitative analysis - see below.

6. 9 General

Samplers, buckets and funnels etc. should be washed out at each sample site prior to use. Occasionally the sampling equipment should be thoroughly cleaned in the laboratory, and an over-night soak in dilute decon 90 (about 5% by volume - See Chemical Hazards) will remove most staining.

Every effort should be made not to transfer algae from one location to another, even if this means taking a drum of 'flushing water' on a sample run. As nets are probably the most likely vehicle for transferring algae from one water body to another, it is recommended that, where possible, a separate net is used for each routinely sampled water body.

'Check Lists' of sampling equipment and materials should be drawn up for all routine sampling programmes and referred to prior to all sample runs. It is wise to take a few extra bottles to account for loss or unexpected events (eg. finding an algal scum, etc.).

6. 10 Sampling Quality Audit

The precision of primary samples and their respective sub-samples should be evaluated regularly. A knowledge of sampling precision is necessary so that resources can be used effectively and as an aid in data interpretation. For example, if a large error is present in the primary sample then rather than counting several sub-samples to a 95% confidence limit of $\leq \pm 20\%$ it may be more accurate, and no more time consuming, to take several primary samples and enumerate them to a 95% confidence limit of $\leq \pm 50\%$. In some cases primary sample precision is relatively unimportant (eg. blue-green scum analysis), but it can be a major source of error.

At regular intervals (at least once annually per sample type/method) a routine sample site should be chosen at random and a set of primary samples and their respective sub-samples taken to evaluate sampling precision, as follows.

1. Take 5 - 10 primary samples and treat as described in (3), below.

2. Take 5 - 10 sub-samples from one primary sample, and treat as described in (3), below.

3. One of the most dominant taxa should be selected and enumerated to a 95% confidence limit $< \pm 25\%$ (or using the 'Basic Method' - section 8). At least two counts should be made per sample/sub-sample, and each should also have its chlorophyll a content measured.

Sample and sub-sample precision should be evaluated and sampling strategies adjusted accordingly. The simplest analysis would be a chi^2 'goodness of fit' (Lund, Kipling & Le Cren - 1958) or an index of dispersion (Elliott - 1977). A more thorough analysis would be to carry out a nested analysis of variance for each sampling and analysis level (Davis - 1956. Water Data Unit Tech. Mem. 20. Venrick, in Sournia - 1981), and the result can be presented graphically, as Irish and Clarke (1984).

In practice the laboratory carrying out the quality audit should conduct the 'quick' tests and a more thorough analysis can be carried out later by the Quality Manager. It is only possible to carry out a thorough analysis of the results if each enumeration is fitted with a confidence limit (see section 8). Results should be kept in a log-book for future reference.

6. 11 Summary

1: Before embarking on a sampling programme a rigorous statement of objectives should be made.

2: Qualitative and semi-quantitative samples can give a quick indication of taxa present and their relative abundance.

3: Quantitative samples are essential if accurate quantitative results are required. Use a jug or bucket (not a bottle) for surface samples. Use a 5m integrated tube sampler in waters liable to stratify, and a Van Dorn (or similar) for discrete depth samples.

4: Samples taken for blue-green algae analysis, blue-green algae toxicity tests, bathing water samples, estuarine samples and *Prymnesium* samples all have special requirements - see text.

5: Use Lugol's Iodine to fix routine samples. Use 1 - 5 drops for a sample $\leq$ 100ml (see appendix 2 - Fixatives and Safety Precautions).

6: Take some live samples as it is often easier to identify living material.

7: All sampling equipment should be kept clean. Nets should be washed before they are allowed to dry.

8: Sampling precision and accuracy should be evaluated regularly.

7. ANALYSIS METHODS AND EQUIPMENT

7. 1 Microscope

The recommended microscope is the Zeiss Axiovert 10, inverted microscope, (or equivalent) fitted with a modified stage plate, to take the Hydro-Bios counting chambers, and a phase contrast condenser. The microscope should be equipped approximately as follows:

1. 3 x10 eyepieces. One with a Whipple graticule, the second with a cross-hair graticule (Figure 7. 1) and a third without a graticule.

2. Low power objective, 5 - 10 x magnification

3. x20 phase contrast objective

4. x40 phase contrast objective - (Differential interference contrast (DIC) would be useful but not essential)

5. x100 oil emersion, phase contrast objective

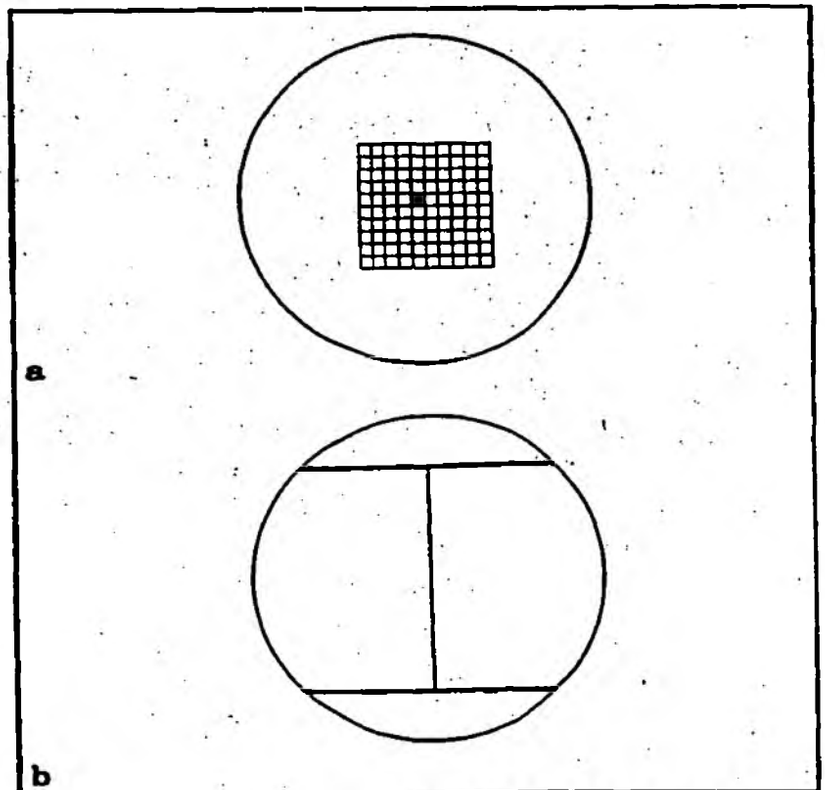


FIGURE: 7. 1 (a) Whipple graticule (b) cross-hair graticule

The microscope should be kept covered when not in use and serviced annually. Lenses should be kept clean by wiping with a lens tissue or medical wipe. Isopropanol can be used to remove emersion oil (see Chemical Hazards), which should be done immediately after use, and applied with a cotton bud. It's a good idea to keep, at least, one spare bulb for the microscope lamp-house. For general microscope operating instructions see microscope handbook or Appendix 4 - Equipment Operating Instructions.

Both graticules should be calibrated, the cross-hair at x200 and the Whipple at all available magnifications.

The cross-hair graticule is calibrated by measuring the distance between the two parallel lines (@ x200) using a stage micrometer. The area covered by the cross-hair graticule is then equal to the distance between the two parallel lines times the length of scan, which is normally the diameter of the chamber (see - Chamber Calibration).

The whipple graticule is calibrated by measuring the length of one whole side, the length of one unit and the length of one sub-unit, at all available magnifications, using a stage micrometer. The area covered by the Whipple graticule is calculated by squaring the length of one whole side (l^2). The length of the whole graticule and its respective units and sub-units, at all available magnifications, should be clearly tabulated and kept near to the microscope, as being able to ascertain measurements is important during taxonomic work (see section 12).

7. 2 Sedimentation Chambers

Twenty to thirty chambers are required for efficient working (one to six per sample - see section 8), and each should be individually marked - by etching or permanent marker. The recommended sedimentation chambers are the 5 and 10 ml Hydro-Bios plankton chambers, or similar (Figure 7.2). These chambers consist of a threaded metal base plate with a screw-in 'plexiglass' tube which holds a glass cover-slip in place. When in use the chamber is covered with a glass slide.

To sediment quantities greater than 10 ml a clean glass cylinder should be used (see Glass Cylinders- below), or alternatively a combination plate. Combination plates are expensive (almost six times the price of a 5 or 10 ml chamber), unreliable and difficult to use. Combination plates can save time, if used successfully, but overall they are not recommended.

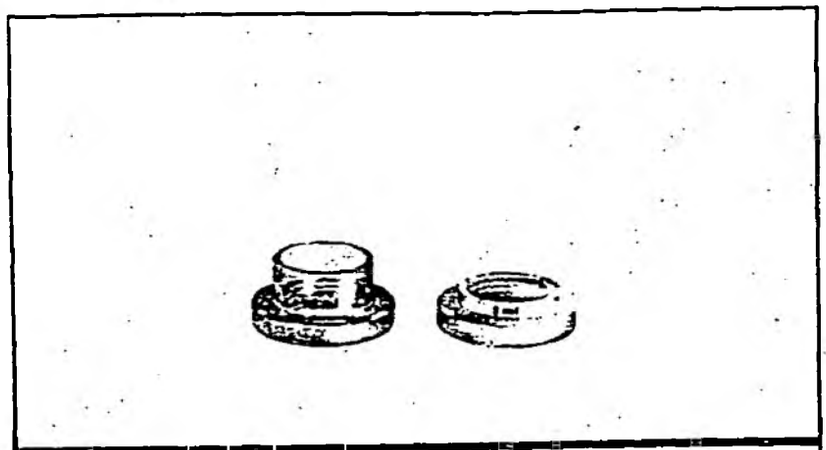


FIGURE: 7. 2 Tubular plankton chambers

Figure 7. 3 shows a cross-section of a combination plate. To sediment volumes greater than 10 ml the cylinder shown would be replaced with a 50 or 100 ml cylinder, although the 100 ml cylinder is best avoided because convection currents can hinder sedimentation, Tangen (1976) - in Sournia (1978).

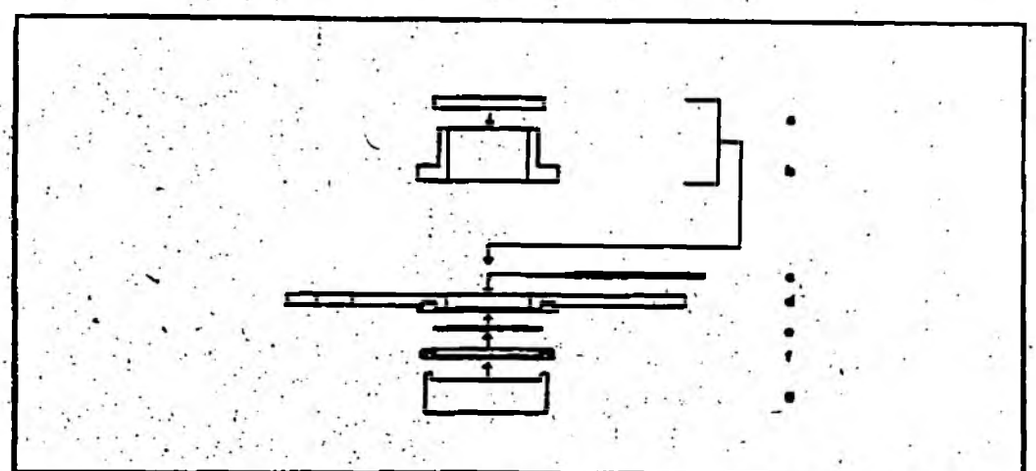


FIGURE: 7. 3 Cross-section of combination plate (a) Top plate. (b) 10 ml sedimentation cylinder. (c) Top plate of bottom chamber. (d) Plate. (e) Cover-slip. (f) Threaded retaining ring. (g) Key.

When filling combination plates the cylinder should be held firmly and squarely in position. The cylinder should be completely filled (to exclude all air) and sealed by a glass slide. The chamber is then left to sediment. Leakage can occur between the cylinder and plate (see - Sedimentation Table, below) and the application of a smear of grease (such as Apiezon AP101) may help. An alternative method of preventing leakage is to hold the cylinder in place with elastic bands during sedimentation. After sedimentation the cylinder is carefully replaced, with one continuous movement, by a glass plate (c - Figure 7. 3) and the supernatant allowed to run to waste. The chamber is then used in the normal way. Methods for using sedimentation chambers and combination plates are fully described by Utermöhl (1958) and Sournia (1978).

7. 3 Chamber Maintenance

The glass cover-slips of sedimentation chambers, or combination plates, should be replaced regularly. This is done by unscrewing the chamber, with the key supplied (5 ml chambers and combination plates only), and carefully depositing the used cover-slip in a 'Cin-Bin' or similar. GREAT CARE should be taken as a thin splinter of glass is often left in the metal chamber base. If a glass splinter remains in the chamber base it should be CAREFULLY removed with a soft object, like a pencil tip, (NOT a finger) and disposed of as above. When all the glass is safely removed the cover-slip seats should be cleaned thoroughly with a medical wipe. A new cover-slip can then be carefully placed in its seat and the cylinder/retaining ring replaced. The retainer should be screwed in firmly but NOT over tightened.

If problems are encountered with leakage through the cover-slip then it can be sealed by very carefully applying a small smear of grease (such as Apiezon AP101) to the contact surfaces prior to assembly.
Sealing with grease is best achieved by applying a smear to the cylinder/retaining ring contact surface and firstly assembling the chamber without a cover slip. Doing this allows excess grease to be removed easily. The chamber is then dismantled, taking care not to accidentally wipe the grease from the contact surfaces, and then reassembled with a cover-slip in place.

After use chambers should be washed out thoroughly with a jet of filtered water (see, Filtered Water - below) dispensed from a wash bottle. The chamber should then be wiped dry with a medical wipe, or similar. If necessary the glass cover-slip can be cleaned with a drop of methanol (see, Chemical Hazards) and a medical wipe. The chambers should be stored inverted or covered when not in use.
Occasionally the dismantled chamber components should be soaked overnight in dilute decon 90 (about 5% by volume - see Chemical Hazards) after which they should be thoroughly rinsed in filtered water and air dried before use.

7. 4 Chamber Calibration

For the purposes of quantitative sub-sampling the analysis area of each chamber (cover-slip aperture) needs to be calculated. Chamber area is calculated by first measuring the diameter of the cover-slip aperture, not the cylinder itself, using either a hand held vernier gauge or the microscope stage vernier. The mean of at least five diameter measurements should be taken and recorded in a log book, along with the chamber number, for future reference. Once the diameter of the cover-slip aperture is established then the analysis area is calculated using the formula Ör².

7. 5 Sedimentation Table

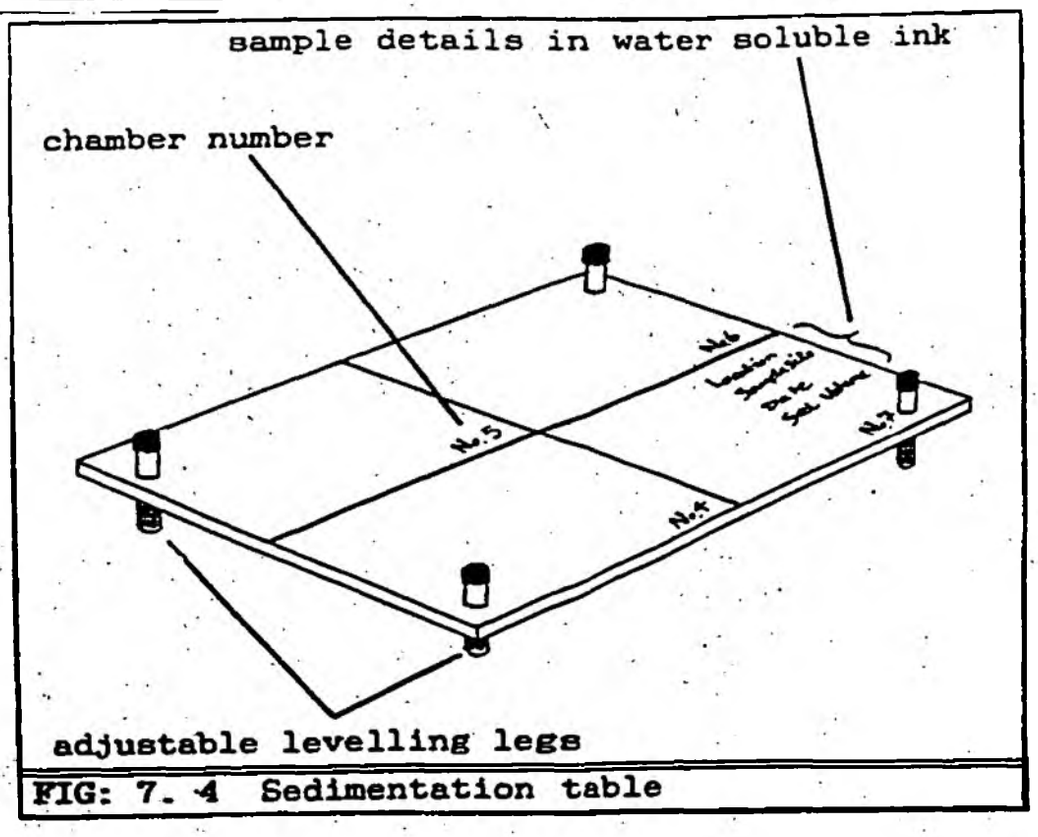


FIG: 7. 4 Sedimentation table

The use of a sedimentation table or board is highly recommended. Figure 7. 4 shows a sedimentation table, note the adjustable legs for levelling.

A sedimentation table need not be this elaborate, a piece of smooth water-proof board (with or without adjustable legs) divided up into labelled sections will do. A white board which will take water-soluble marker is ideal as sample details can be written next to the chamber.

Positioning of the sedimentation table is very important. The table must be level and positioned away from sources of energy - strong lighting, heaters, windows etc. Tangen (in Sournia - 1978), found a more homogenous distribution of sediment when his sedimentation table (Figure 7.4) was completely level. He also found that leakage rarely occurs from sedimentation plate chambers when exactly horizontal. It has been suggested that sedimentation should take place within a darkened cupboard (Bellinger E. G. - pers. comm.), although there is no quantitative data to support this method.

7. 6 Filtered Water

During sedimentation, dilution and general chamber maintenance, a supply of ultra-pure or membrane-filtered water is required. Direct tap water can contain detritus that may hinder analysis, and should be avoided. If a supply of ultra-pure/membrane-filtered water is not available then the cheapest way of filtering water is to use an in-line membrane filter, see Jones (1979). It is a good idea to keep both a wash bottle and dropper bottle of filtered water to hand for routine use. The bottles should be emptied, rinsed and refilled with freshly filtered water at regular intervals, weekly during the spring/summer and monthly at other times. A little Lugol's Iodine (see Appendix 2 - Fixatives and Safely Precautions) added to the dropper bottle should inhibit bacterial and algal growth and reduce the necessity for such frequent changes. Water from wash and dropper bottles should be sedimented occasionally (about 500 ml) and examined for algae, bacteria, fungi, detritus etc. If excessive material is found in the sedimented water then the filter unit should be renewed/serviced or the water bottles require flushing and refilling more frequently. An occasional (about every 4 weeks) overnight soaking in dilute decon 90 (about 5% by volume - see Chemical Hazards) followed by a thorough rinsing is also advisable for wash and dropper bottles. Glass bottles can also be cleaned by washing in 10% HCl acid (see Chemical Hazards).

7. 7 Glass Cylinders

Glass cylinders, used for initial sedimentation, must not be filled above a height five times that of the cylinders diameter, as convection currents can prevent some forms from sedimenting. Cylinders should be thoroughly washed immediately after use, and rinsed in filtered water. An occasional (about every 4 weeks) overnight soaking in dilute decon 90 (about 5% by volume - see Chemical Hazards) followed by a thorough rinsing is also advisable. Glass cylinders can also be cleaned by washing in 10% HCl acid (see Chemical Hazards).

When using glass cylinders for initial sedimentation some form of siphoning apparatus (see below) is required to draw off the supernatant when sedimentation is complete. It is recommended that about 10% is left in the cylinder, after siphoning.

When siphoning is complete the remaining contents should be thoroughly mixed (by rolling between hands) and transferred to a 10 ml counting chamber. The inner sides of the cylinder should be carefully washed down with a very small quantity of filtered water, mixed, and then used to top up the chamber. When transferring sediment from a cylinder to a chamber the cylinder should be washed out several times, therefore the very minimum of filtered water should be used at each stage, otherwise the chamber could be inadvertently overfilled. If a chamber is overfilled then the sediment must be discarded and a new sub-sample set up in a clean cylinder. If necessary (when algal numbers are very low) a series of sedimentations can be carried out. For example a 750ml sample is sedimented in a 1000ml cylinder. After sedimentation the sedimented is then transferred to a 100 ml cylinder re-sedimented and the sediment finally transferred to a counting chamber.

7. 8 Siphoning Cylinders

Some form of siphoning device is required for removing the supernatant with the minimum of disturbance, Figure 7. 5. If a vacuum pump is available then the supernatant can be slowly drawn off via a length of flexible hose and a glass Pasteur pipette, etc. An alternative siphoning method is to use a 'U' shaped glass tube (figure 7. 5). The tube is filled with filtered water which is held in place by sealing one end with a finger or thumb. The open end of the siphon tube is then placed into the supernatant as the sealed end is vented. As the water flows from the tube it creates a siphon pressure and the supernatant is drawn off. This technique requires some practice but is well worth learning as it is much more convenient, and quicker, than using vacuum pumps etc.

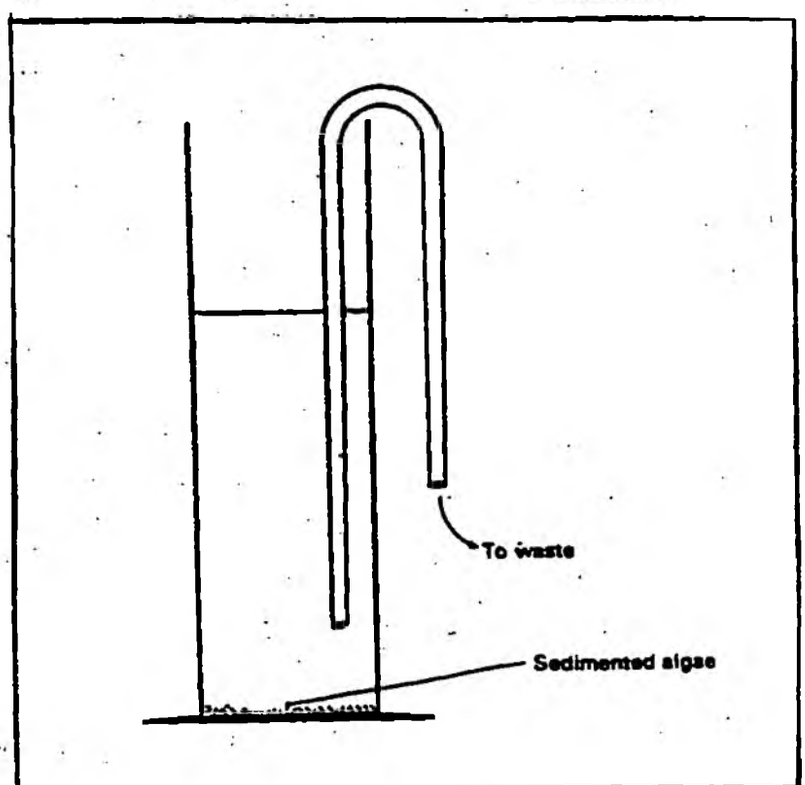


FIG: 7. 5 Siphoning off supernatant.

During siphoning the siphon tube should be lowered very slowly and carefully, keeping the siphoning end just below the surface of the water. If the sediment is accidentally disturbed then the sample must be allowed to re-sediment before the siphoning continues. If any of the sediment is inadvertently siphoned off then the sample must be re-sedimented in a clean cylinder.

Occasionally the efficiency of cylinder sedimentation and siphoning should be evaluated by re-sedimenting collected supernatant and examining for the presence of algae. If algae are present in the re-sedimented supernatant, then sedimentation/siphoning methods and equipment cleaning should re-evaluated. The results of siphoning efficiency tests should be kept in a log book for future reference.

7. 9 Pipettes

Auto-pipettes and a supply of clean tips are required. Variable volume macro-pipettes that deliver 1-5 and 5-10 ml are suitable for larger volumes. Whereas a micro-pipette that delivers between 200 - 1000 μl, or fixed volumes of 250-500-1000 μl, is ideal for the smaller volumes. Pipettes should have an accuracy and precision of less than 10%, and this should be checked regularly. Evaluating pipette accuracy and precision can be done by using the pipette to deliver filtered water, at 20°C, into a vessel placed on a good quality balance (1g = 1ml). A series of ten deliveries is enough to assess performance. If the pipette fails to achieve the manufacturer's values for accuracy and precision then it should be serviced/renewed. Records of pipette accuracy and precision tests should be kept in a log book for future reference.

7. 10 Lund Chamber

An alternative counting chamber, which has the advantage of rapid sedimentation, is the Lund chamber (Lund 1959). The Lund chamber is not recommended and should not be used for routine samples (long-term monitoring sites, trend analysis etc.). Lund chambers can be used for ad hoc samples at the discretion of the operator or if time does not allow the use of a sedimentation chamber. The Lund chamber is recommended by some for all nano-plankton work (Bailly-Watts, pers. comm.) as the sediment forms a random, or even, distribution. Lund however, found a random distribution only if certain precautions were followed. A report on counting methods (AWA, Plankton Methodologies Working Group - 1979) found that cell counts within Lund Chambers often have a highly contagious distribution (variance > mean).

The Lund chamber consists of a standard microscope slide (76x26mm and 1.0/1.2mm thick) which has two raised strips (0.5mm deep) cemented along its long axis, see figure

7.6. A cover slip (22x50mm No. 1½) is placed squarely on the raised strips and the sample allowed to flow into the chamber from a pipette, by capillarity (this stage of filling is critical).

A whipple graticule (Figure 7. 1:a) is recommended for use with a Lund chamber and the chamber can either be used on an inverted or conventional microscope. If it is intended to use a Lund chamber on a compound microscope, then a long working-distance lens is usually required for x40 magnification.

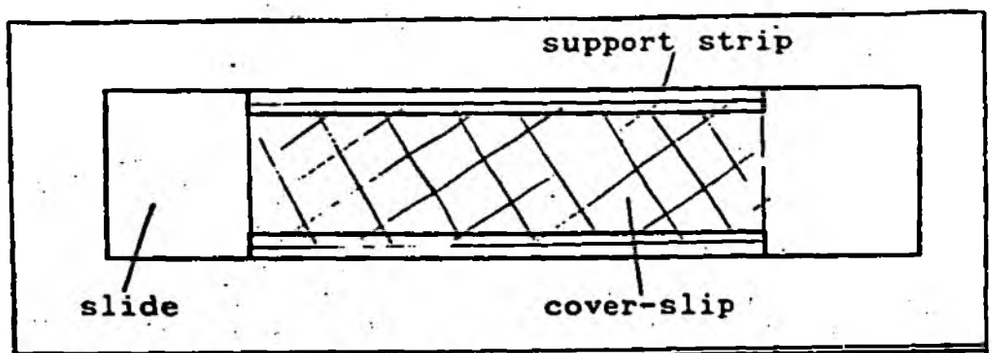


FIG: 7. 6 Lund chamber, from above.

For quantitative analysis the chamber will require calibration of analysis area (length x width) and volume. The length of the chamber will be equal to the cover-slip length, whereas the width will be equal to the average distance between the support strips. Both length and width can be calculated using a vernier gauge. The volume of the chamber can be calculated either by measuring the average support strip height or by filling the chamber several times (filtered water @ 20°C) and calculating the average volume. The latter method can be calculated by weighing the chamber both while empty and full. When measuring the chamber volume care should be taken not to overfill the chamber. Once calibrated the chamber should be individually marked. It is recommended that at least ten measurements of each criteria are made and the results should be recorded in a log book, along with the chamber number, for future reference.

The Lund chamber should be rinsed immediately after use with filtered water, and if necessary, cleaned with a drop of methanol (see - Chemical Hazards), and a medical wipe. For further details of Lund chamber construction and operation, see Lund J (1959). The methods of concentrating and diluting samples, used in conjunction with sedimentation chambers, can also be used with the Lund chamber.

7. 11 Sedimentation Technique

7. 11:1 **Introduction**

The distribution of sedimented phytoplankton within counting chambers is the key to sub-sampling accuracy. If the algae are evenly or randomly distributed within the chamber then a 95% confidence limit of approximately ± 20% can easily be achieved by counting about 100 units, Lund et al 1958). However, an even or random distribution is frequently not achieved in the Hydro-Bios type chambers, and the unpredictable distribution of sediment is probably due to a combination of chemical, physical and biological factors. Sedimented algae often tend towards the edges of chambers, possibly due to convection currents within the chamber during settlement.

Utermöhl (1958) recommended the use of a special filling chamber which has a sieve bottom, in order to produce a homogenous distribution of sediments on the chamber floor. Nauwerck (in Sournia - 1978) found that this procedure made little difference and the homogenous distribution of the phytoplankton could never be guaranteed.

7. 11:2 **Mixing Samples**

Samples should be at room temperature and well mixed before sub-sampling. The importance of sample mixing to obtain a representative sub-sample cannot be over emphasised. A thorough, but gentle, mixing is considered necessary by some. Others recommend 100 to 200 shakes of the sample bottle in order to loosen attached organisms. Utermöhl (1931, in Sournia, 1978) emphasised that shaking should be done in such a manner that circular water movements are avoided. No doubt the amount of mixing required will ultimately depend on both physical and biological factors (eg. size of bottle and taxa present). The only advice that can be given is it is better to mix, probably by repeated inversions, the sample too much than too little. A thorough mixing of the sample could save a great deal of time at the enumeration stage, for example if replicate counts were found to be statistically different.

7. 11:3 **Setting-up Chambers**

There is no way of being able to predetermine the ideal sedimentation volume. It is not sufficient to routinely sediment a set volume on all occasions, as this can lead to unnecessary error. Sedimentation volumes must be varied to suit the enumeration method and phytoplankton density. Suitable sedimentation volumes can vary from less than 0.01ml (achieved through dilution) to greater than 100 ml, and the full range should be used as necessary. Although it is very difficult to predetermine the correct sedimentation volume a

good estimate can be made from previous experience, using chlorophyll a data, Secchi disk depth, colour of sample and previous records. If none of these data are available then set up a range of chambers - 1, 2, 5, 10 and 50 ml, for example. It takes very little time to set up chambers, but achieving a suitable sediment quantity could save time at the enumeration stage. When setting-up chambers a clear record must be kept in a note book/record sheet (as well as on the sedimentation table - see above) of the following details.

SAMPLE LOCATION	:
SAMPLE SITE	:
SAMPLE DATE	:
CHAMBER NUMBER	:
SEDIMENTATION VOLUME:	
SEDIMENTATION DATE	:
SEDIMENTATION TIME	:

When the chosen sedimentation volume equals that of the chamber then the mixed sample can be poured straight into the chamber, to overflowing, and cover-slip fitted. Spilt sample should be mopped up and the cover slip dried if necessary.

If a small volume (less than the chamber volume) is chosen for sedimentation then the chamber will require topping up with filtered water. The sample should be initially mixed in the chamber with a jet of filtered water from a wash bottle, taking care not to overfill the chamber. When the chamber is almost full the glass slide can be fitted leaving a small gap at one side. The chamber should then be gently rocked so that the sample comes in contact with the glass cover, and a meniscus formed. The remaining air space in the chamber can then be filled by carefully adding filtered water from a dropper bottle, drawn into the chamber through capillarity. Alternatively the small air gap can be left and topped up after sedimentation is complete. All air bubbles should be removed from the chamber before analysis as they can interfere with the optical properties of the microscope.

Utermöhl (1958) suggests that great care should be exercised when fitting the glass cover (when used in conjunction with his filling chamber - above) so that currents within the chamber are brought to rest, and thus an even distribution of sediment achieved. He concluded that pushing the glass cover on from the side creates currents in the chamber.

7. 11:4 **Sedimentation Time**

Lugol's Iodine is absorbed by algal cells thus increasing their density and aiding sedimentation. With the 5 and 10 ml counting chambers sedimentation overnight is quite adequate. Some suggest that 3 hours is ample for a 10 ml chamber with 1 hour probably being sufficient (Lund et al, 1958). For larger volumes in glass cylinders or combination plates, a longer settlement time is required. Allow 6 hours per cm height of liquid sedimented, when small diatoms are present, and 3 hours per cm height at other times. For further details of sedimentation times see Furet and Benson-Evans (1982).

7. 12 Dilution of Samples

Volumes smaller than those attainable with a pipette can be achieved by diluting a small amount of sample with filtered water (both at room temperature) thoroughly mixing and then sedimenting a portion of the mixture. Table 7. 1 lists a range of concentrations at three final sedimentation volumes.

Columns one, two and three show the volume of sample, volume of filtered water and final sedimentation volume if 1ml of the mixture is sedimented. Columns four and five show the final sedimentation volume if a 5 or 10ml chamber is filled from the mixture, thus dispensing with the necessity for further pipetting. It may be wise to add a drop of Lugol's Iodine (see Appendix 2 - Fixatives and Safety Precautions) to the mixture, during the spring/summer, to inhibit bacterial and algal growth.

SAMPLE VOLUME ML	FILTERED WATER VOL. ML	1ML SEDIMENTED	5ML SEDIMENTED	10ML SEDIMENTED
1	9	0.1000	0.500	1.00
1	19	0.0500	0.250	0.50
1	29	0.0333	0.170	0.33
1	39	0.0250	0.125	0.25
1	49	0.0200	0.100	0.20
1	59	0.0167	0.083	0.17
1	69	0.0143	0.071	0.14
1	79	0.0125	0.063	0.13
1	89	0.1111	0.056	0.11
1	99	0.0100	0.050	0.10
0.5	99.5	0.0050	0.025	0.05
0.5	199.5	0.0025	0.013	0.03
0.25	99.75	0.0025	0.013	0.03
0.25	199.75	0.0013	0.006	0.01

TABLE 7. 1 Table of dilutions, see text.

7. 12 Summary

1: Use an inverted microscope equipped with x20 x40 & x100 (oil) objectives, 3 x10 eyepieces (one with cross-hair and one with Whipple graticule) and a stage adaptor to take Hydro-Bios counting chambers.

2: Use 5 and 10ml Hydro-Bios type counting chambers.

3: Use glass cylinders to sediment volumes greater than 10ml (or combination chambers if preferred).

4: Chambers and graticules require calibration.

5: Use a level sedimentation table or board and position away from sources of heat and vibration.

6: Use filtered or ultra-pure water for topping up sedimentation cylinders, counting chambers, for dilutions and washing glass ware.

7: After sedimentation in cylinders remove supernatant with a recommended siphoning device.

8: The Lund Chamber is not recommended but can be used for ad hoc samples.

9: Samples should be thoroughly mixed before sub-sampling.

10: When setting up chambers/cylinders a clear record of details must be kept.

11: Sediment for 6 hours per cm height of chamber/cylinder, when small diatom are present, and 3 hours per cm at other times.

12: Dilute samples if necessary.

8. ENUMERATION

8. 1 Introduction

Direct counting has three main advantages over other methods of estimating algal numbers (eg. coulter counter or chlorophyll a analysis). Firstly the algae are actually observed thus allowing changes in appearance to be observed, such as dead or dying cells, fungal attack, presence of spores or heterocysts, etc. Secondly counting allows population estimates of low density taxa, more readily than other methods. Finally counting allows small numbers of specific algae to be distinguished from others or detritus (see, Lund and Talling - 1957).

A population estimate, as its name suggests, has a certain amount of error associated with it. It is the convention to present estimates along with a level of accuracy (eg. 100 ± 10%, a range of 90-110). The required accuracy of results for estimating phytoplankton populations is not normally very high. Most ecological surveys are concerned with generations, or changes in abundance of 100%, therefore a method which can estimate abundance to an overall accuracy of ≤ ±50% is quite adequate. As phytoplankton sampling and enumeration has numerous sources of error it is probably wise to maintain the enumeration error as low as possible within the resources available. An enumeration accuracy of approximately ±20% is attainable without excessive effort and should be the target level, for the dominant algae, in routine samples. Where enumeration is concerned with gross numbers, such as blue-green algae scum analysis, an enumeration target accuracy of ≤ ±50% is quite sufficient. The normal convention for statistical significance is the 95% level, one chance in twenty of being wrong, and is considered quite sufficient by most algologists and statisticians for population estimates. See - Lund, Kipling and Le Cren (1958), and Venrick - in Sournia (1978).

The enumeration of the larger taxa, (eg. *Ceratium* and some *Cosmarium* spp.) in Hydro-Bios chambers, is quite straightforward as they can easily be identified at low power (80 - 100x magnification). Working at low power the whole chamber can be scanned, within a few minutes, in a series of horizontal transects (Figure 8. 1:a). As this method covers the whole chamber the only enumeration error is the personal counting error, which should be negligible. Confidence limits, however, cannot be assigned to a full chamber count, unless it can be shown to have come from a Poisson distribution (eg. the algae within the sample bottle are distributed at random), or if several chambers are counted. Therefore a series of replicate counts (at least three) are required, to assess the distribution, otherwise the results from a full chamber count will have a mean value only.

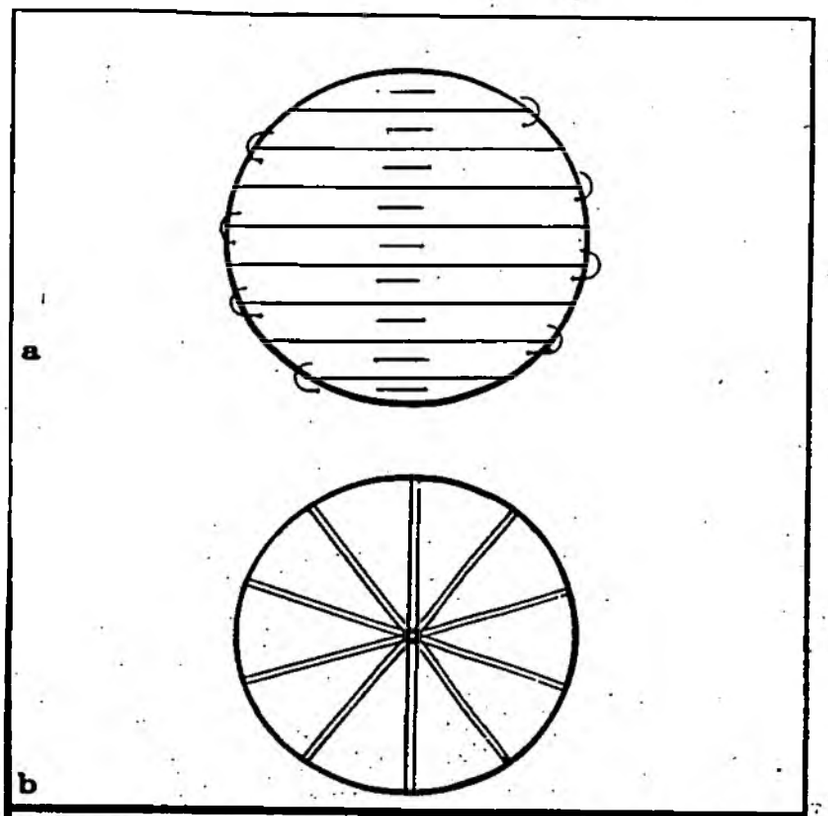


FIG: 8. 1 (a) Strip counting of entire chamber. (b) Five diameter transects.

At higher magnifications (x20° & x40°) scanning the whole chamber becomes impractical (because of the time involved), and sub-sampling strategies are employed. It is at the sub-sampling stage of enumeration that significant errors can occur. If the sediment within the chamber has a random distribution then the error is related to the number of individuals counted.

A count of 100 individuals, from a Poisson distribution, will yield an accuracy of approximately ± 20% (Lund, Kipling & Le Cren, 1958). As the distribution of sediment, within Hydro-Bios type chambers, is often non-random then this type of approach is inappropriate, and the statistical distribution of collected data requires continuous monitoring.

When counting transects or fields, phytoplankton should be counted in 'algal units'. The algal unit is an individual entity, a colony, filament, cell, etc. For example, if a filament of *Aphanizomenon flos-aquae* (consisting of 50 cells) is encountered while counting a transect it should be counted as 'one unit'. The number of cells per unit also requires enumeration and can either be done while sub-sampling or at some later point, whichever is most convenient. The two results, units and cells per unit, should be recorded separately, and the latter used to calculate the average cells per unit (see section 8. 7).

8. 2 Random Transects @ x200

Net-plankton should be counted in diameter transects at x200 magnification. A series of between five and nine, randomly placed diameter, transects should be counted using a cross-hair graticule (Figures 7.1:a and 8.1:b). The graticule is lined up against the left hand outermost circumference of the chamber, or if available, the chamber centring pin is located. To line up against the left hand outermost edge of the chambers, first roughly locate the chamber's left hand circumference and then focus onto it, until a 'sharp' image is visible. The central position of the edge can then be located by moving the stage 'up and down' until the chamber is at its widest point. The microscope can then be re-focused, on the chamber bottom, and the transect count commenced.

During transect counts, the algae that fall within the two horizontal lines, of the cross-hair graticule, are counted as they pass the vertical line (figure 7.1 (b)). Algae that cross one of the horizontal lines have to be treated according to a predetermined counting rule. Normally the algae crossing one of the lines are counted as within the transect whereas those crossing the other are excluded. It does not matter which line is chosen for each criteria, providing the rule is strictly adhered to throughout the count. Algae rarely cross both lines, but if they do then 50% should be counted as within the transect. Some filamentous algae, such as *Fragilaria* sp., form very long ribbons, and care should be taken not to count the same filament several times. The next transect position is located by revolving the chamber, or its base plate, to a randomly chosen position, either by using random numbers or in an ad hoc fashion. Care must be

taken when rotating chambers as this movement is very disruptive and can cause the sediment to become re-suspended. If the microscope stage does not have a centring pin then the chamber should be lined up prior to each transect (as above). If a chamber centring pin is used then its efficiency should be examined regularly.

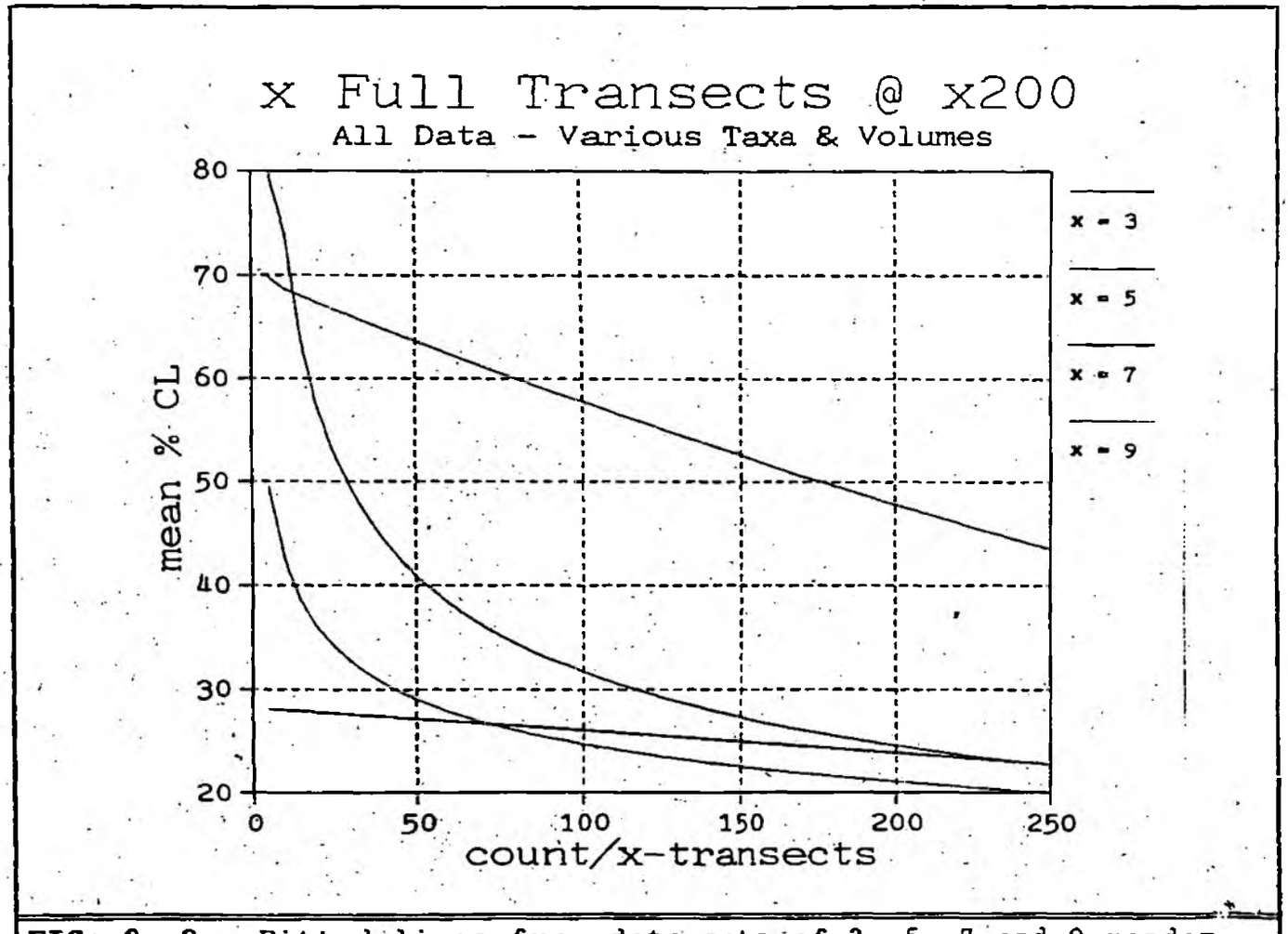


FIG: 8. 2 Fitted lines from data sets of 3, 5, 7 and 9 random transects. The upper and lower straight lines are 3 & 7 transects, respectively, whereas the upper and lower curves are 5 & 9.

A count of about 250 units is necessary within 9 transects, to achieve an error of approximately ±20%. If an error of ≤ ±50% is acceptable, then this should be achievable at a count of 100 units in 5 transects. Fitted lines from data sets of 3, 5, 7 and 9 transects (mean confidence limit vs. count per x transects) are shown in figure 8. 2. The upper and lower straight lines represent 3 and 7 transects, respectively, whereas the upper and lower curved lines are from 9 and 5.

Although all the fitted lines are statistically significant the data spread is still quite wide. In one case an error of < 20% was achieved from a count of less than 100 units in 3 transects, whereas in another case a count greater than 600, in 9 transects, had an error > ±20%. This wide disparity in results is due to the unpredictable distribution of sediment (and small sample number), and illustrates the importance of monitoring the distribution of each count.

The advantage of diameter transects counts is that they eliminate any transverse contagiousness within the chamber. For example, if the distribution of sediment is tending towards the edges of the chamber, as is often the case, then this is unimportant as the whole transect is treated as one sample unit. Diameter transects, however, have two main disadvantages. Firstly, a sample unit number of nine (9 transects) is small, in statistical terms, and thus requires the uniformity of counts, between transects, to be very consistent if a narrow confidence limit is to be achieve. Secondly, diameter transects tend to under sample the edges of the chamber and over sample the middle. This becomes more important as the distribution of algae become more contagious. As algae often sediment towards the edges of chambers then this means that transects tend to underestimate the population.

Analysis has shown that intra-chamber replicate counts are always statistically similar, and therefore, it is only necessary to do a single count from a chamber. This intra-chamber counting stability is probably due to the large area covered by transect counts, 9 transects are approximately equal to a third of the chamber area. Both intra-chamber and inter-chamber replications should be carried out regularly to evaluate analytical and sub-sampling error, respectively. The results of all such evaluations should be recorded in a log-book for future reference. Confidence limits from intra-chamber replicate counts should overlap (statistical test = NS), otherwise the analytical error is unacceptable.

To achieve a suitable count, between 20 and 30 algal units of the dominant taxon, should be achieved per transect. If the count falls short of 40 after two transects then it is best to abort the analysis, and try another chamber, with a greater volume.

8. 3 Random Fields @ x400

Nano-plankton should be counted in randomly placed Whipple fields at x400 magnification. Unlike transect counts, random fields cover a relatively small proportion of the chamber. 100 fields cover an area approximately equal to 1/180 th of the total chamber area. This dictates that selection of sample unit positions, the field, is far more crucial than in transect counts. The importance of random sampling becomes increasingly important as the distribution of sediment becomes more contagious. True random sampling means that any locus within the chamber has an equal probability of being selected, and the selected positions are totally independent. In most cases algologists select random fields in an ad hoc (pseudo-random) fashion, and this is perfectly acceptable if the sediment is at random, but rarely satisfies the criteria for true randomness. As the sediment in Hydro-Bios type chambers is often non-random then particular attention has to be placed on the positioning of random fields. Analysis has shown that intra-chamber replicate counts are sometimes statistically dissimilar. This intra-chamber variation is almost certainly due to analytical error and thus, indicates the importance of ensuring that randomness is maintained.

Both intra-chamber and inter-chamber replications should be carried out regularly to evaluate analytical and sub-sampling error, respectively. The results of all such evaluations should be recorded in a log-book for future reference. Confidence limits from intra-chamber replicate counts should overlap (statistical test = NS), otherwise the analytical error is unacceptable.

8. 3: 1 **Placing Random Fields**

A simple, but not entirely effective, way of assessing randomness is to conduct 'dummy' counts using specially modified cover-slips, figure 8. 3. The cover-slips are made by blanking out sections (of known area) with an opaque translucent material (eg. liquid 'Tip-Ex'). Randomness tests are conducted by placing one of the modified cover-slips into a chamber and conducting a series of pseudo-random movements while a second person records the result of each movement - either 'blank' or 'clear'.

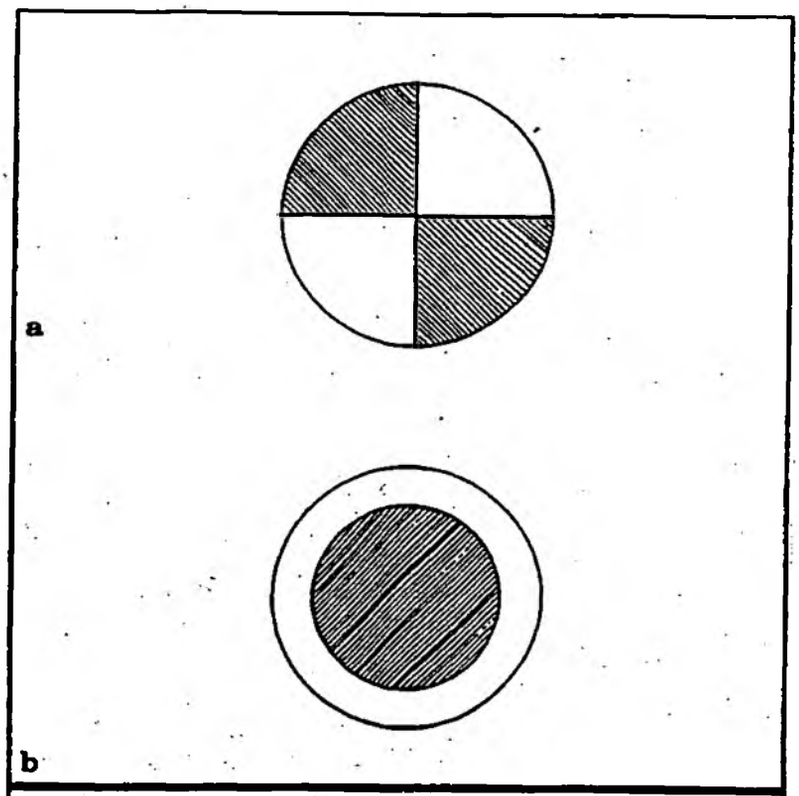


FIG: 8. 3 Modified cover-slips for randomness tests. (a) sectors. (b) Edge/centre. Both modified cover--slips have an equal area of 'blank' and 'clear'.

Several hundred pseudo-random fields need to be selected, so there is no chance of the operator keeping a mental note of the proceedings. After the test the data should be analysed using a simple Chi² test, (observed - expected)²/expected, the expected result for each of the areas will be equal to the proportion of that area. For example, if the blank and clear portions of the modified cover-slip occupy equal areas, and the total count equals 500, then the expected values will be 250.

It is best to start the tests with the sector cover-slip (Figure 8.3:a) and if randomness is not disproved, then move onto the edge/centre test. Another cover-slip could be made up similar to 8. 3:b. but with a 25-75% ratio, as random sampling of the edge area can be of particular importance. This test will show non-randomness and does give a good feel of how randomness should be, but a non-significant result does not prove that sampling is at random. If the pseudo-random method is chosen for positioning fields then regular randomness tests should be carried out, and the results kept in a log-book for future reference.

Using 'x' 'y' coordinates to position random fields is a very time consuming process, being over ten times longer that pseudo-random field selection. The positioning of 'x' 'y' coordinates is complicated by the availability of 'y' coordinate movement being dependent on 'x' coordinate position. A variation on 'x' 'y' coordinates is to have the 'x' axis across the centre of the chamber and the 'y' coordinate as a circular movement of the chamber. This method is considerably faster than standard 'x' 'y' coordinate placing, and has the additional advantage of being fully operational from the seated position (for true 'x' 'y' coordinates the operator has to stand to position each 'y' coordinate). The mayor disadvantage with this method is that repeated rotational movements of the chamber are very disruptive to the sediment. An alternative would be to have a computer program that produced a series of 100 (or any other chosen number) random pairs of coordinates and then sort them into an order that minimises the rotational movement of the chamber. This method has the disadvantage that a full set of fields has to be counted, otherwise the positioning would not be at random. The practicalities of this method have yet to be explored.

A third possible method of attaining randomly placed fields is to use an electronic microscope stage. Electronic stages, which have built in random position controllers, are available for the Axiovert 10, but they are very expensive (cheapest about £6 000). A major advantage of electronic stages is that an individual field can be relocated at the end of the count for high power oil emersion examination. The practicalities of using electronic stages have yet to be explored.

8. 3: 2 Counting Random Fields

When counting in Whipple fields all the taxa that fall within the graticule are identified and enumerated. Those taxa that cross the edge of the graticule have to be treated according to a predetermined counting rule. Normally algae that cross two of the graticule's four edges are included in the count, whereas those that cross the other two are excluded. The edges for each criterion have to be predetermined and adhered to throughout the count. The top and left hand edges are often used as 'inclusive' edges.

A count of about 120 units is necessary, within 100 fields, to achieve an accuracy of approximately $\pm 20\%$, in most cases. Figure 8. 4 shows the fitted lines for 25, 50 and 100 random fields, mean confidence limit vs. count per x fields. The upper line is from a data sets of 25 fields, the middle 50 fields and lower 100 fields.

Although the fitted lines for random fields are statistically significant the data spread is still sufficiently wide to necessitate constant monitoring.

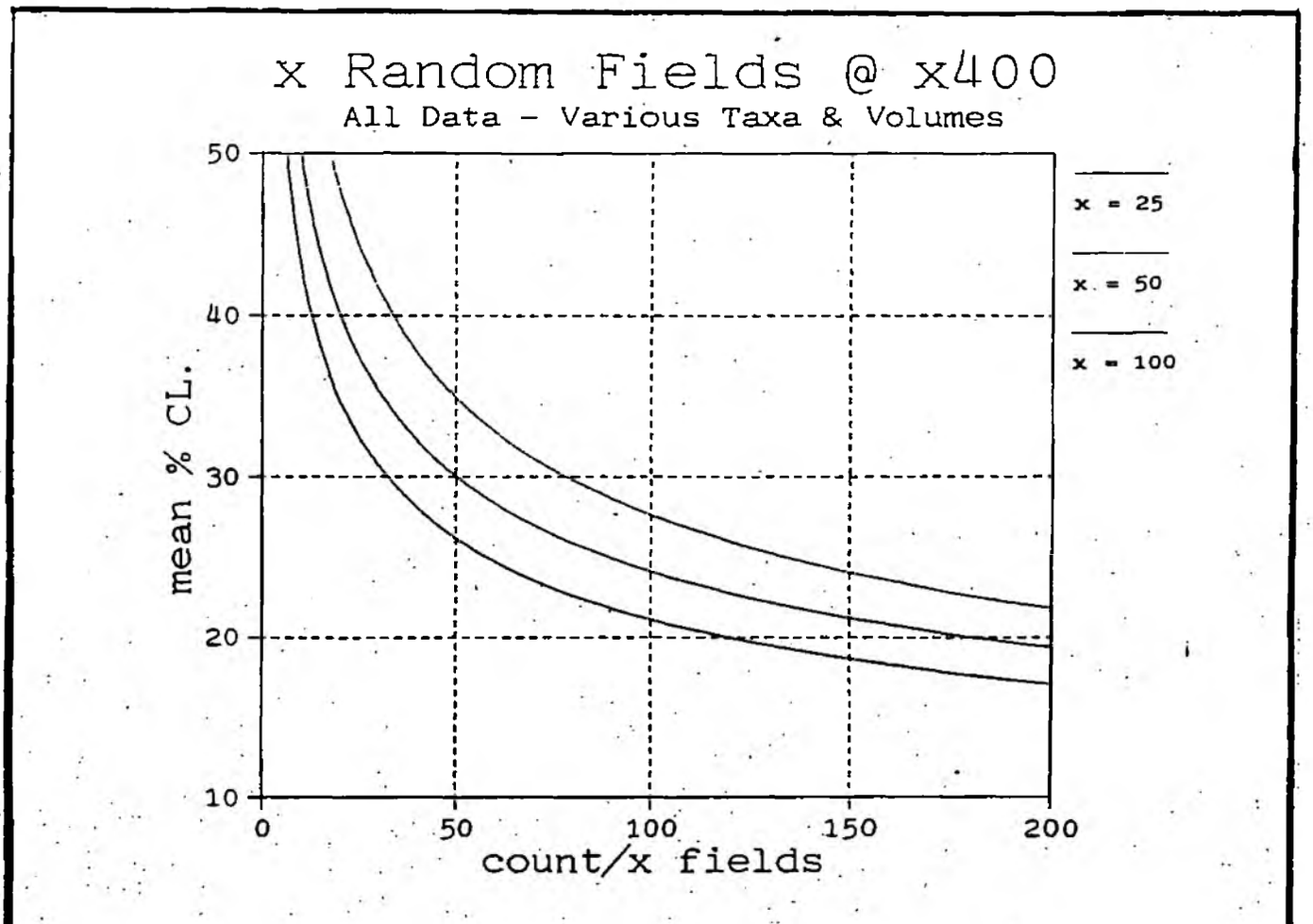


FIG: 8. 4 . Fitted lines from data sets of 25, 50 and 100 random fields. The upper line in for 25 fields the middle 50, fields and lower 100 fields.

8. 4 Lund Chamber Enumeration

Pseudo-random Whipple fields, at x200 and x400, are usually used for phytoplankton enumeration within a Lund Chamber. It is suggested that the ends of the chamber are avoided and should be checked for concentrations of larger taxa, Lund (1959).

8. 5 Calculating Units Per Ml

Quantitative date is usually expressed as units/ml, although some do use units/l. To calculate units/ml, in either sedimentation chambers or the Lund Chamber the following computation is required.

$$\text{units/ml} = \frac{\text{number of units counted}}{\text{number of fields/transects}} \times \left\{ \frac{\text{area of chamber}}{\text{area of field/transect}} \right\} \left\{ \text{sample volume in ml} \right\}$$

or

$$\text{units/ml} = \frac{(\text{number of units counted} \times \text{factor})}{\text{volume of sample in ml}}$$

$$\text{where factor} = \frac{\text{area of chamber}}{(\text{area of field/transect} \times \text{number of fields/transects})}$$

EXAMPLE:

chamber area = 530.9 mm^2
Whipple field area = 0.0292 mm^2
Sample volume = 8ml
Units counted in 100 fields = 105

Factor = 530.9 ÷ (100 x 0.0292) = 181.5

units/ml = 105 x 181.5 ÷ 8 = 2382.2

8. 6 Calculating Confidence Limits For Unit Counts

The arithmetic mean of the population under study is estimated from the sample mean. As the estimated mean is used to determine the total number in the water body it is important that we know the accuracy of this estimate. Table 8. 1 shows data from two sets of three transects, for *Aphanizomenon* and *Cryptomonas* respectively, and the calculations for assigning confidence limits.

		A	B
		Aphanizomenon	*Cryptomonas*
1	Transect 1	69	305
2	Transect 2	51	296
3	Transect 3	126	286
4	n	3	3
5	Σx	246	887
6	Mean (m)	82	295.7
7	Variance (S^2) $= \Sigma(x - m)^2/n$	1533 / ----	90.3 / ---
8	I = n-1(S^2)/m	37	0.61
9	P (95%)	***	NS
10	$\log_{10} x$	Transformed	Data
11	Transect 1	1.84	2.48
12	Transect 2	1.71	2.47
13	Transect 3	2.10	2.45
14	t (95% - n-1)	4.303	4.303
15	Mean	1.888	2.472
16	S^2_Y	0.039	0.0002
17	CL */ factor $10^{(t * \sqrt{S^2_Y/n})}$	3.095 / ----	1.083 / -----
18	UCL = m * CL factor	253.79	320.24
19	LCL = m / CL factor	26.49	273.03
20	mean % CL	147.3	8.00
21	Poisson CLs $m \pm t * \sqrt{m/n}$		
22	UCL	------	338.42
23	LCL	------	252.98
24	% CL	------	14.45

Table 8. 1 Calculating confidence limits, see text.

The difference in data distribution between the two sets of transect counts in table 8 .1 (lines 1-3), is obvious. The *Cryptomonas* count is very consistent, whereas the *Aphanizomenon* count varies widely. However without any confidence limits the resultant means would both be considered to have the same accuracy. A comparison of variance and mean is a quick way of assessing the data distribution. The *Aphanizomenon* count has a variance far greater than the mean and the resulting index of dispersion (row 8) is highly significant. This result indicates that the *Aphanizomenon* data is from a highly contagious distribution. The *Cryptomonas* date has an index of dispersion which is non-significant, and the population could be considered to be at random. Although this may not be an entirely safe assumption with such a small sample.

The transect counts were then transformed using logs, (Table 8. 1 lines 10-13). The main object of the transformation is to stabilise the mean, and allow the use of statistical tests associated with a normal distribution. The transformation is assumed successful, as the transformed variance, for Aphanizomenon (A16), now being lower then the mean (A15). One of the properties of transformation is that the resultant (geometric) mean is usually lower than the arithmetic mean (10^(A15) = 77.3 as opposed to 82 (A6)). One way of overcoming this quirk of transformation is to calculate a confidence limit factor (line 17) and apply it to the arithmetic mean (lines 18 and 19), see Elliott (1977). As logs were used in the transformation the arithmetic mean is multiplied and divided by the factor, rather then the more familiar addition and subtraction, and this results in asymmetrical confidence limits.

The asymmetry of the confidence limits is less biased to the upper confidence limit, when calculated using the geometric mean, as opposed to the arithmetic mean (Elliott - pers. comm). Therefore, where comparison is more important than absolute numbers (eg, trend analysis) the use of geometric means to assign confidence limits may be more appropriate, whereas where absolute numbers are important (toxic algae analysis), the use of arithmetic mean is more suitable.

The confidence limits and mean for the two counts, in table 8.1., are shown on lines 18 and 19, with line 20 showing the mean percentage difference. It is now obvious that the degree of confidence in the two data sets is very different, and this difference is mainly a result of difference in distribution within the two data sets. These two data sets are extreme cases, but intermediate cases are not rare. It can be seen from this example that the data distribution requires continuous monitoring, if it is intended to assign confidence limits, to the final results.
As the *Cryptomonas* data was not shown to deviate significantly from random then confidence limits could be assigned using an alternative formula (lines 21, 22 and 23), which does not necessitate transformation. However the resultant confidence limits are not as narrow as those achieved through transformation.

Although the calculations shown in table 8. 1 are straightforward it is impractical to have to apply them manually to every taxa counted, especially when 100 fields are involved. Although manual computation of confidence limits, for a dominant taxon only, may be practicable in some laboratories (especially when the dominant taxon are usually enumerated in transects - using programmable calculator or spreadsheet), it is best avoided if possible.

One solution is to build data transformation and computation into a direct entry facility, where data is entered directly from the microscope into a computer, then confidence limits could be applied to all data with little or no increase in counting time. It is also important to note that the confidence limits for the *Cryptomonas* count (table 8 .1) is for cells, whereas the *Aphanizomenon* result is for filaments only. Therefore the 147% mean error, for *Aphanizomenon*, still requires the addition of a cells/filament error (which is at least another 26%).

8. 7 Calculating Cells Per Colonial Algal Unit

When enumerating colonial algal units, some measure of the number of cells per unit should be made. The cell numbers of some colonial algae have a consistent distribution, and can have one or several modes. For example *Asterionella formosa* was found by Lund et al (1958) to be at least bimodal, with modes usually at 4 and 8 cells per colony. The cell numbers of other colonial algae have an inconsistent distribution. The cell numbers in *Microcystis* colonies can vary from a few to tens of millions.

For low density colonial units it is most efficient to enumerate the cell per unit at the same time as the unit count, but the dominant taxa can be left until later, if preferred. For the dominant taxa, the number of cells from at least 30 units (selected at random) should be counted, and the average calculated. The cell numbers of most colonial units can be counted directly, but for others it is very difficult, and only an estimate can be made.

The cell numbers of some filamentous forms, such as *Aphanizomenon*, can either be counted directly or by a combined method. The combined method firstly involves averaging the number of cells per unit length of filament (n = 10). Then the lengths of at least thirty filaments are measured and the average found. The average number of cells per filament is then computed as follows.

$$\text{average cells per filament} = \left\{ \frac{\text{average filament length}}{\{\text{unit length used in cell count}\}} \right\} \times \text{number of cells Per unit length}$$

The direct count method is probably more accurate than the combined method, as the latter method involves two sets of averages. However there is so much natural variation between filament lengths that the difference is probably negligible. The second method does supply a little extra information, in that it is possible to calculate the cell length from the data collected (cell length can be used in calculating bio-volumes). Either of these methods is adequate.

With spiral forming filaments, such as *Anabaena circinalis*, another alternative method can be applied. Firstly the average number of cells per gyre is calculated. Then the number of gyres per filament is counted, and then multiplied by the average number of cells per gyre. In some cases the filaments are so long and entangled that only a rough estimate of the number of gyres is possible.

Calculating the numbers of cells in colonies of *Microcystis* presents further problems.. A gross estimate can be made by counting the number of cells per unit area (use Whipple graticule), and then estimating how many of the area units approximate to the area of the colony. The cell count is then calculated as follows: number of area units x number of cells per unit area. This method takes no account of the three dimensional nature of the colony, but the result could be multiplied up to compensate. Another method which has been applied to *Microcystis aeruginosa* is to find the average colony diameter and apply the following regression equation, see Reynolds and Jaworski (1978) and figure 8. 5.

$$\text{Log10 (number of cells)} = 2.99\ \text{Log10 (colony diameter)} - 2.80$$

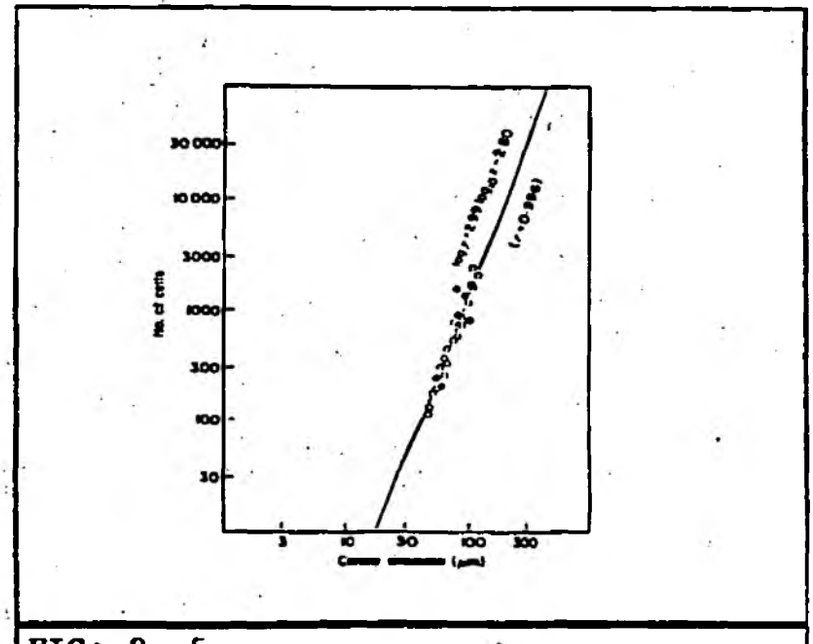


FIG: 8. 5
From: Reynolds and Jaworski (1978)

The regression solution has many limitations, but it is the best 'quick' method available.

Other methods for estimating the cell concentrations of colonial blue-green algae involve the use of ultrasonic disruption and alkaline hydrolysis (Reynolds and Jaworski, 1978), but both are beyond the scope of routine algal work, and this manual.

Calculating confidence limits for cells per colony, can be a time consuming process. As the cell numbers from many forms have an inconsistent distribution it would be necessary to transform data and the resulting confidence limits could be very wide. Without some form of computerised data handling system the best that can be done is to estimate the means, only. It must be remembered that calculating the cell densities of units, adds another source of error to the final result, which in some cases will be considerable.

8. 8 Cell Volumes

Expressing results simply in terms of algal units ml-1 can give a misleading impression of biomass, however transforming cell counts into volumes can compensate for this shortfall. The cell volumes of many taxa can be found in the literature, and in many cases the average cell volumes are very consistent within comparable taxa from different locations. However this consistency cannot be relied upon as the volumes of some taxa vary widely (Bellinger, 1974). A compromise is to use volumes from the literature for infrequent and volumetrically consistent taxa, and calculate actual volumes for the dominant and volumetrically inconsistent forms.

In many cases cell volumes can be adequately approximated by relating the linear measurements of the cell, or parts of, to simple geometric shapes. A simple example is relating a *Microcystis* cell to a sphere ($4/3\grave{O}r^2$), whereas a complex example is where a *Ceratium* cell is related to three different sized cones ($1/3\ \grave{O}r^2h$). Using geometric shapes to calculate cell volumes only gives an approximation of the actual volume and, therefore, introduces error which should be evaluated if the method is used regularly. Another complication is the cell vacuole. For example, some diatoms contain numerous vacuoles, and this should be taken into consideration. Some consider surface area to volume ratio as a more practical measure than just volume, the higher the ratio the greater the cells photosynthetic and nutrient uptake potential and, thus, productive capacity (Bellinger, 1974).

It is unnecessary and impractical to convert all data to volumes, however the use of volumes can be of great value (especially in long term monitoring projects). For example when conveying information to those that are not familiar with the wide range of cell volumes that exist.

In some cases the transformation of cell counts to volumes could be automated within a data-base, using data from the literature for the infrequent/consistent taxa, but a facility should exist to enter/alter volumes for the numerically dominant/inconsistent forms.

8. 9 Count Replication and Chamber Replication/Sub-sampling Error

It is recommended that, occasionally, replicate and second chamber counts are carried out to assess analytical and sub-sampling error. Replicate counts are a second or third count within the same chamber and mainly assesses analytical error (intra-chamber replication). Whereas chamber replication is a count on a second or third different chamber (of same sample), and mainly assess a combination of sub-sampling (pipetting) and analytical error (inter-chamber replication). If enumeration error is not quantified then replication and sub-sampling errors cannot adequately be identified.

Where time does not allow a complete count or chamber replication, it is probably sufficient to enumerate the numerically dominant taxon only, a confirmation count. The confidence limits from replicate counts should overlap (t-Test or Mann-Whitney test, non-significant), otherwise the counts are, in statistical terms, significantly different. When the second count does not differ significantly from the first then the results from the first chamber can be accepted, alternatively the two counts can be pooled. If the confidence limits do not overlap then it may be necessary to fully count a third chamber. Count and chamber replications should be carried out on all stages of enumeration and the results recorded in a log book for future reference.

The mathematics of chamber replications are too time consuming to conduct manually, in most cases, and could only be used efficiently if automated. For example, replicate comparison calculations could be integrated into a direct data entry system.

Only when a good database of count and chamber replication is built up will it be possible to modify enumeration strategies to be most efficient.

8. 10 Chlorophyll a

Although a detailed description of chlorophyll *a* analysis, safety precautions and data interpretation is beyond the scope of this document, a brief account is of value. Comparisons and correlations between chlorophyll *a* levels and cell counts/volumes are useful, both as a means of data interpretation and an enumeration quality check. It must be remembered, however, that chlorophyll *a* concentration differs considerably between algal groups. For example, green algae have a higher chlorophyll *a* content than blue-green algae, for equivalent cell volumes. chlorophyll *a* content also varies according to many other factors, within the same group, such as physiological state and light intensity.

As a quality check cell volumes can be correlated with chlorophyll *a* results. As a rule of thumb 4μg of chlorophyll *a* is approximately equivalent to 1cm^3 of cell volume (Bailly-Watts, pers. comm.). If the chlorophyll *a* result is significantly greater than the combined cell volumes then an error exist.

The dominant taxa responsible for chlorophyll *a* peaks can easily be extrapolated with a simple comparison of chlorophyll *a* and cell count results, or cell volumes. For further information on chlorophyll *a* and other quantitative measures of algal biomass see Lund and Talling (1957).

8. 11 Enumeration Quality Control

As enumeration error (using transects or fields) is dependent on sediment distribution, within the counting chamber, then this distribution must be known for a quantitative assessment of counting efficiency (assigning confidence limits). If sediment distribution is not monitored then each result will have a mean value only, with unknown error, and only a qualitative assessment of counting efficiency can be made (eg. using 'Basic Method' Section 8.12:1(1)). It may be deemed sufficient by some operators/managers to quality assess the dominant taxa only, and in some cases this is possible longhand. In most cases, however, manual computation of confidence limits is totally impractical and the only satisfactory solution is to development of a automated quality control system, which would probably involve direct data entry, from the microscope into a computer. A direct data entry system is described by Cunningham and Purewal (1983).

Enumeration spot-checks and inter-laboratory calibrations should be carried out on a regular basis. The results of any quality checks should be used to identify weak areas, and action their correction. All quality check results should also be recorded for future reference. Enumeration quality audits would only be of limited value if the sediment distribution were not monitored, and precision levels controlled, as precise confidence limits (or data distributions) would not be available for comparison. Enumeration quality checks could be extremely valuable, both to the operators and the quality manager, as they would highlight problem areas and instil operator confidence.

In addition to inter-laboratory calibrations, regular laboratory visits, by the quality manager or her/his deputy, would be necessary to assess each operator, equipment and ensure adherence to standardized methodologies. Many aspects of enumeration quality control are intricately linked to taxonomy quality control (see Taxonomy), and would be carried out simultaneously.

8. 12 Enumeration Overview

A three stage enumeration strategy is recommended as follows:

1. Whole chamber count at low power (80-100 x magnification) for larger taxa only, Ceratium sp., Cosmarium sp., etc.

2. 5-9 randomly placed diameter transects, @ x200 magnification, using a cross hair graticule, for net-plankton.

3. 50-100 randomly placed Whipple graticule fields, @ x400 magnification, for nano-plankton.

No 'hard and fast' rules can be set as to which algae are enumerated at each stage, as this depends on several factors, such as size range, abundance, amount of detritus - etc. For example if a lot of detritus is present then this restricts the number of different taxa that can be enumerated in transects, as the smaller forms will be more easily overlooked. Likewise if the sample is very 'clean' then smaller algae can be identified and enumerated readily in transects. Care should always be taken if the algae have a wide size range as some could be overlooked in transect counts. If unsure as to which taxa to count using each strategy level, always chose the cautious option, and enumerate at high magnification. That is, if unsure of a taxon's suitability at the full chamber count level then try enumerating in transects, and if unsure at the transect level then enumerate in fields.

Although this three tier method is not suitable for all applications some part of it will be. For example stage one may not be possible for samples containing a lot of detritus, such as in-shore marine samples.

Enumeration can either be carried out on a single chamber, counting the dominant taxon with the most appropriate method, or if more information is required, then a series of concentrations can be set up in separate chambers, and a dominant taxon chosen from each, with chamber replication at each stage. As the larger taxa are normally rare then it is usually appropriate to sediment a large volume, where practicable, for full chamber, low power, counts. Precision can only be assigned to full chamber scans if at least three replicates are counted, and even then the percentage error may be large.

8. 12:1 Enumeration Options

The following are the 'basic method' (1) and four suggested enumeration options, each yielding a greater degree of precision and/or information. It is not recommended that the 'basic method' is modified, whereas the other methods are suggestions only. Options 2 - 5 should be modified to achieve the desired level of acceptable precision and information, in view of all sources of error (section 1.2 & 6.10). Equipment and methodology quality checks should not be modified and should be used in conjunction with the chosen enumeration method. All the methods, with the exception of 1 and possibly 2 (in some cases) are only practicable if used in conjunction with an automated system that assesses the data distribution and assigns confidence limits. Confidence limits can either be accepted and recorded, regardless of percentage error, or more preferably an acceptable level of error predetermined, and all samples falling outside that level rejected/recounted.

The following methods are the best options available at the current time. With the development of a direct data entry system and the accumulation of enumeration data it may become necessary to modify the following methods to a greater or lesser extent. All enumeration methods have some inherent problems associated with them. For example, transects counts tend to underestimate the population, but are analytically very stable, whereas pseudo-random field counts can give very accurate results but can be analytically unstable. Only with further developments and experimentation can the methods be 'fine tuned' to be more efficient.

1. Basic Method

One chamber set up per sample and a dominant taxon chosen from either transect or field counts.

a: Full chamber count, in transects @ x 80-100, for larger taxa.

b: 5 (minimum) random diameter transects @ x200, for net plankton, counting about 20 of the dominant taxon per transect (if dominant taxon counted in transects).

c: 50 (minimum) random Whipple fields @ x400, for nano-plankton, counting between 50 to 100 of the dominant taxon (if dominant taxon counted in fields).

d: Find average number of cells per algal unit for all taxa counted. If the dominant taxon is colonial then the number of cells from at least 30 units should be enumerated and the average found.

Although confidence limits cannot be assigned to this method, in most cases the unit error will be $< \pm 50\%$ for the dominant taxon if counted in transects, or $< \pm 40\%$ for the dominant taxon if counted if fields. It is unsafe to assume confidence limits for any of the sub-dominant taxa, and those with low counts should be treated as qualitative only.

2. Confidence Limits Assigned To Dominant Taxon

One chamber set up per sample and a dominant taxon chosen from either transect or field counts.

a: Full chamber count, in transects @ x 80-100, for larger taxa.

b: 5-9 random transects @ x200, for net plankton, counting between 20 to 30 of the dominant taxon per transect (if dominant taxon counted in transects).

c: 50-100 random Whipple fields @ x400, for nano-plankton, counting between 100 to 150 of the dominant taxon (if dominant taxon counted in fields).

d: Sample units (transects/fields) should be recorded separately for dominant taxon and confidence limits calculated manually (programmable calculator or spreadsheet), or using an automated system.

e: Find average number of cells per algal unit for all taxa counted. If the dominant taxon is colonial then the number of cells from at least 30 units should be enumerated and the average found.

The greater the number of sampling units counted the narrower the achievable confidence limits. This is especially important in transects where the sample is relatively small. Confidence limits for the dominant taxon should be monitored during the count, using a programmable calculator or spreadsheet, and the count continued until the desirable level is achieved, counting a minimum of 5 transects and 50 fields. If the desired confidence limits are not achievable then the count should be abandoned and the chamber reset.

3. Confidence Limits Assigned To Dominant Taxon With Replicate Chamber Count For Dominant Taxon

Two chambers set up per sample and a dominant taxon chosen from either transect or field counts.

a: As 2 (above) but a second chamber is enumerated and confidence limits established for the dominant taxon. The confidence limits of the dominant taxon, calculated from each chamber, should overlap or the counts are statistically dissimilar. If the confidence limits do not overlap then a third chamber should be counted and assessed against both counts (section 8. 9). When the dominant taxon from two counts are found to be statistically similar then the results can be pooled, and confidence limits calculated for the pooled data.

This method is probably only practicable if used in conjunction with an automated data system (see section 8. 11).

4. A Series Of Concentrations Set-up In Separate Chambers, With Replicates. Confidence Limits Assigned To Dominant Taxon From Each Chamber, And Replicate Chambers Counted For Dominant Taxon At Each Concentration

Seven chambers set-up at three concentrations, with one replicate each for transect and field counts and three for full chamber counts (at least three chambers require enumeration for full chamber counts). This method is only practicable if used in conjunction with an automated data system (see section 8. 11).

a: Full chamber count, in transects @ x 80-100, for larger taxa. All taxa enumerated in second and third chambers.

b: 5-9 random transects @ x200, for net plankton, counting between 20 to 30 of the dominant taxon per transect.Dominant taxon enumerated in second chamber.

c: 50-100 random Whipple fields @ x400, for nano-plankton, counting between 100 to 150 of the dominant taxon. Dominant taxon enumerated in second chamber.

d: Sample units (transects/fields) should be recorded separately for dominant taxon and confidence limits calculated using an automated system.

e: Find average number of cells per algal unit for all taxa counted. If the dominant taxon is colonial then the number of cells from at least 30 units should be enumerated and the average found.

The replicate counts should be treated as in section 8. 9. When the dominant taxon from replicate counts are found to be statistically similar then the results can be pooled, and confidence limits calculated for the pooled data. If the confidence limits do not overlap then another chamber should be counted and assessed against both previous counts (see section 8. 9). Sub-dominant taxa treated as in example 2.

5. A Series Of Concentrations Set-up In Separate Chambers, With Replicates. Confidence Limits Assigned To All Taxa From Each Chamber And Replicate Chambers Counted For Dominant Taxon, Or All Taxa, At Each Concentration

Seven chambers set-up at three concentrations, with one replicate each for transect and field counts and three for full chamber counts (at least three chambers require enumeration for full chamber counts). This method is only practicable if used in conjunction with an automated data system (see section 8. 11).

a: Full chamber count, in transects @ x 80-100, for larger taxa. All taxa enumerated in second and third chambers.

b: 5-9 random transects @ x200, for net plankton, counting between 20 to 30 of the dominant taxon per transect. Dominant taxon/all taxa enumerated in second chamber.

c: 100 random Whipple fields @ x400, for nano-plankton, counting 100 to 150 of the dominant taxon. Dominant taxon/all taxa enumerated in second chamber.

d: Sample units (transects/fields) recorded separately for all taxa and confidence limits calculated using an automated data system. All taxa with a 95% confidence limit > ±50% treated as qualitative only.

e: Find average number of cells per algal unit for all taxa counted. If the dominant taxon is colonial then the number of cells from at least 30 units should be enumerated, and the 95% confidence limit calculated.

The replicate counts should be treated as in section 8. 9. When taxa from replicate counts are found to be statistically similar then the results can be pooled, and confidence limits calculated for the pooled data. If the confidence limits do not overlap then another chamber should be counted and assessed against both previous counts (see section 8. 9).

8. 13 Specific Enumeration Techniques

8. 13: 1 **Routine Enumeration**

Maintaining enumeration quality is essential for long term monitoring programmes. If methodologies are altered, to improve quality, then this should be well documented and made clear in reports, etc. If resources permit, then a changeover period should be adopted where the 'old' and 'new' methods are run in parallel to assess the differences. Although sampling error will vary with time, it is wise to maintain enumeration error as constant as possible, within predetermined levels.

8. 13: 2 **Blue-Green Algae Enumeration**

The 1990 Blue-green algae enumeration method, 6 units in a two minute scan (NRA Water Quality Series No. 2), is not recommended. This method was never intended as a serious quantitative method, and should only be used in an emergency, or for qualitative results. See Blue-green algae page 1.

If a scum is present, then the 'warning threshold' will always be exceeded, however a count should still be made. With scum analysis sample dilution will be necessary and counting to an error of ±50% is quite acceptable. If the result of a count is close to the threshold level then enumerating to a greater level of accuracy, and a second chamber count, may be advisable. The results of all counts should be entered on the Inspection of Standing Waters Form (see appendix 3 - Documentation), and copies sent to the relevant Catchment and Principal Quality Officers, the Regional Biologist and one retained on file. If a sample exceeds the threshold then the Principle Quality Officer should be informed as soon as possible by fax.

The warning threshold levels for various taxa, both cell and unit counts, can be found in appendix 3 - Equivalence Among Cyanobacteria. If a taxa is encountered which is not in the table then either contact the Toxic Algae Scientist in the TAPS Centre, Peterborough, for advice. As a last resort the warning threshold for a taxon, of equivalent volume, can be used.

8. 13: 3 Enumeration of Bathing Water Samples

Enumeration of bathing water samples should be carried out in accordance to the guidelines laid down in the Marine Algal Monitoring Programme, see appendix 3 - Documentation. Appendix 3 also lists, Abundance Values Relating to Bloom Levels For Common Bloom Forming Algae. If a bloom is identified then the relevant Principle Quality Officer should be notified, as soon as possible by fax. A Significant Blooms Report should be completed for all samples analysed (see appendix 3 - Documentation), and copies sent to the relevant Catchment and Principal Quality Officers, the Regional Biologist and one retained on file.

Phaeocystis colonies are often too large to enumerate using sedimentation chambers, and an alternative method can be employed. 10ml of mixed *Phaeocystis* sample is delivered into a petri dish, over suitable graph paper, and sub-samples counted using a binocular microscope. The calculation for colonies per ml is as follows.

$$\text{colonies/ml} = \text{colonies counted} \times \left\{ \frac{\text{area of petri dish}}{\frac{\text{area analysed}}{10}} \right\}$$

9. QUALITATIVE AND SEMI-QUANTITATIVE ANALYSIS

9. 1 Qualitative Analysis

Qualitative analysis is useful as a rapid assessment method for the more abundant taxa. Either a live or fixed sample is sedimented and scanned at the appropriate magnification for the presence of algae. All taxa present are recorded with a 'P', tick or by name. This method is useful when looking for a particular toxic or nuisance species, or just as a quick indicator of species composition. When used in conjunction with a concentrated net sample (where possible), qualitative analysis is ideal for monitoring the scarce net-plankton, that rarely show up in sedimentation chambers.

9. 2 Semi-Quantitative Analysis

This method is similar to qualitative analysis (above) but the taxa are crudely ranked according to abundance. The chamber is scanned (can be done for a fixed time period) and a mental note kept of abundance. Observing net plankton at x80-x100 can also be a useful indicator of abundance. Five categories are usually used, to indicate abundance, either 1 to 5 or P, S, C, A, & V, and represent the following levels of abundance.

Score	Score	Category	Abundance
1	P	Present	1
2	S	Scare	2 - 10
3	C	Common	11 - 100
4	A	Abundant	101 - 1000
5	V	Very Abundant	1001 +

10. DATA HANDLING

10. 1 Introduction

Where possible all data, especially long term monitoring data, should be kept in a suitable database. Data entry should be simple, logical and include an error correction facility. The database should allow easy sorting, searching and retrieval of data and should be able to produce simple time course plots of individual, or groups of taxa, or interface with a suitable spreadsheet. The database can either contain the bare minimum of information to allow searches, and be supported by a secondary system of fuller information (digital and/or hard copy), or hold all relevant information in one. If a combined system is used then it should interface with an entry program which permits simultaneous data entry to both systems. Whichever approach is adopted it should be possible to work backwards from the final result to the raw data. It is not sufficient to just maintain a database of final results which cannot be referenced or cross-checked against the original notebook or data sheet. Each data base file should hold enough records to facilitate the maximum number of taxa encountered per sample.

10. 2 Taxa Codes

For the sake of simplicity taxa are normally stored within a database as an individual code. Many coding systems are available, all with their own limitations, but the one which is recommended was developed by Whitton, Diaz and Holmes (1979). Although this coding system requires updating, which may involve converting the present six digit code to an eight digit one (to facilitate extra taxa), in most cases it can be used straightaway.

The system uses a six digit number, in which the first two are the Phylum Pair, the second two the Genus Pair, and the last two the species pair. Thus allowing 100 species per genus (00-99). For example the code 013208 represents 01???? for *Cyanophyta*, 0132?? for *Oscillatoria* spp. and 013208 for *Oscillatoria limnetica*. From this example it can be seen that a search can be instigated at the phylum, genus or species level, quite easily.As many of the species pairs are unused there is scope for the use of size classes for unidentified taxa. For example 'small centric diatoms' $< 5\mu m$ in diameter. Since the development of this coded system many more species have now been described, within some genera, and the present species pairs will probably require expanding to a quartet to accommodate them, Whitton - pers. comm.

10. 3 Data Base Structure

The following example shows a data base which has two components, however there is no reason why all the data cannot be kept in one system. The basic data base holds the minimum of information in a form which allows rapid manipulation. The support data is held in a second unit, digital and/or hard copy, and holds the full information in a text form.

10. 3: 1 **Basic Data Base**

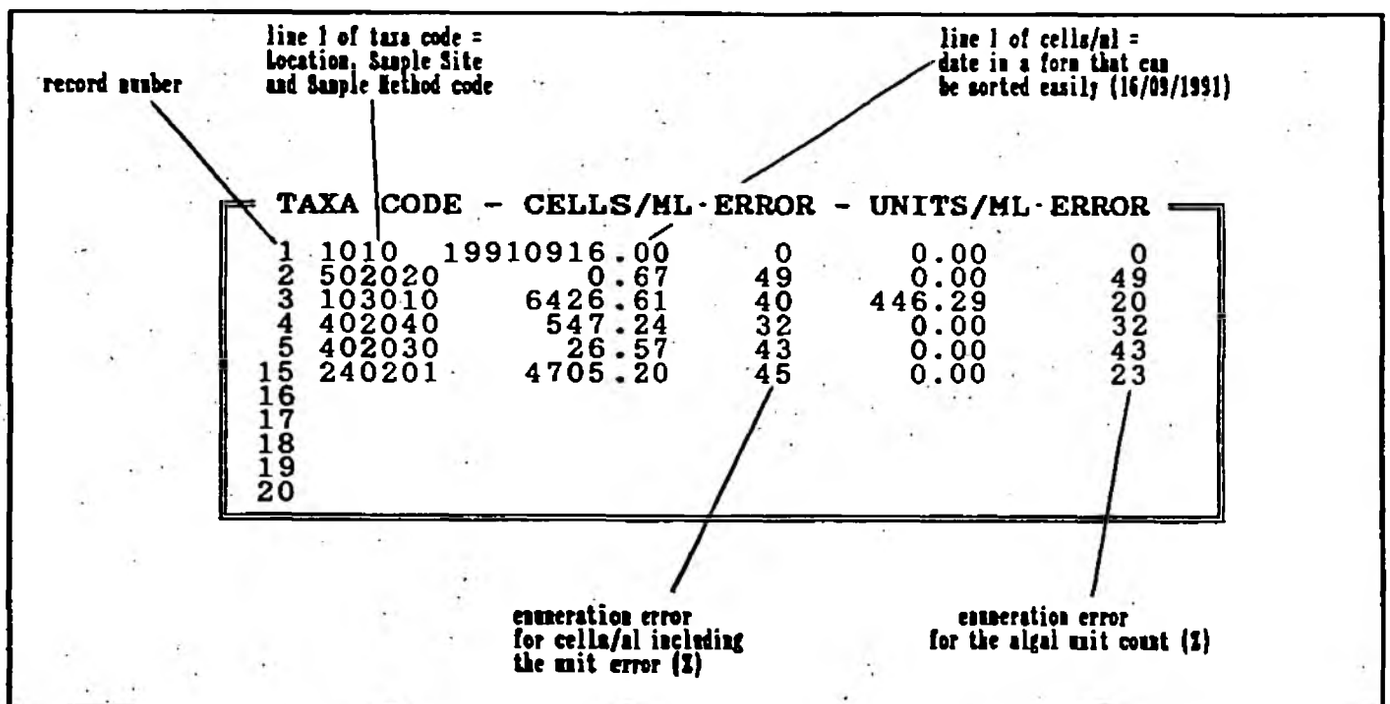


FIGURE 10. 1 Layout of basic data base, showing 20 records. Line 1 holds the location, sample site and sample method code, followed by the data in a form which allows rapid numerical sorting. Unit error is the enumeration error for the units/ml count, whereas the cells/ml error is the cells/unit error combined with the unit enumeration error (where applicable). Lines 16 - 20 are empty.

Figure 10. 1 shows a basic data base structure which holds the minimum of information to allow rapid data manipulation. Line one holds the sample identifiers. Firstly the location, site and sampling method code, followed by the date, in a form which allows rapid numerical sorting. The taxa records are in code form only, this minimizes space and facilitate taxonomic sorting (section 10. 2).

Enumeration error is shown for both unit and cell counts, although these are the same when the unit is a single cell. For example, taxa 103010 and 240201 are both colonial and therefore have an error associated with the units/ml and cells/unit counts. The cell count error is a combination of the unit/ml count and the number of cells/unit. All other taxa are singular and the error is the same in both cases. It may be considered unnecessary to store the error in the basic database. This depends on whether it is intended that the data should be plotted according to its associated precision. If this is not so then error can be stored in the support system only.

10. 3: 2 Support Data Base

Phytoplankton Count

Location:	GRAFHAM WATER	Sample Site:	NEAR DAM WALL
Sample Method:	SURFACE DIP	Sample Date:	12:11:2011
Date Analysed:	13:11:2011	Sample Point Code:	RO2BA22M22
Method:	SEDIMENTATION CHAMBER	Microscope:	ZEISS AXIOVERT 10
Chamber Number:	10	Sample Volume:	10.00ml
Chamber Area:	480.87mm^2	Area 1 T @ x200:	16.40mm^2
Magnification:	T@x200 F@x400	Area 1 F @ x400:	0.20mm^2
Analyst:	A N ALYST		

Comment: STRONG NORTH WESTERLY WIND, WATER TEMP 3°C

Count Type	Taxa	Cells/ml	CL	Units/ml	CL
FULL	*Ceratium hirundinella*	0.5	0	-----	--
T7	*Aphanizomenon flos-aquae*	906.6	40	46.0	19
T7	*Cryptomonas* - B	13.3	18	-----	--
T7	*Microcystis aeruginosa*	136.1	60	3.8	28
T7	*Nitzschia acicularis*	0.5	0	-----	--
F100	*Rhodomonas* sp.	55.0	18	-----	--
F100	Small flagellate <= 5µm	532.0	16	-----	--

(T = Transects F = Fields)
(CL = < enumeration % error @ (95% level) 0 = Unknown or > 99)

FIGURE 10. 2 Record sheet from support data base, see text.

Figure 10. 2 shows a record sheet from a full or support data base. Enough information is present to allow the data to be worked backwards to a raw count. A verification field could be included to indicate that the data has been quality checked, although this information can be kept in a separate file.The importance of clear, concise and unambiguous data is paramount. It is essential that data sheets, used in conjunction with the methods and support manuals, will tell someone everything they need to know about the data. It is not sufficient to simply hold certain key information in mind, or on a scrap of paper somewhere, it should be all written down and kept in a logical order for future reference.

10. 4 Data Base Quality Control

Data held in digital or paper files should be quality checked on a regular basis. Working from the original note book or data sheet (where applicable) entry and computation of results should be checked for the presence of errors. If entry error is excessive then data entry procedures should be reexamined. The data-base should also be quality checked, at regular intervals, to ensure that computations are accurate. A record of all such quality checks should be kept in a log-book for future reference.

11. REPORTING

11. 1 Introduction

Standardisation of reporting, where possible, would simplify report writing, comparison and interpretation. Before writing a report it is important to identify the 'target', and pitch the report at an appropriate level. There is no point in producing a large scientific document if it is not going to be appreciated, or understood. Likewise a summary page is insufficient as an internal laboratory record. Word-processors allow easy modification of the same document to meet a variety of needs.

Feedback from report targets is also important. If no feedback is received, then it is appropriate to enquire if the report was suitable for the purpose intended. Without feedback report style cannot be modified to be most effective.

Although it is inappropriate to make definite recommendations about report layout, as flexibility is very important, a minimum standard to work from is useful. The basic report layout should follow that of a scientific paper.

Abstract / Summary
Introduction
Method
Results and Discussion
Conclusion
Summary
Acknowledgements (if appropriate)
References

Where possible results should be presented graphically, as opposed to tables. If there is not enough algal data to plot then the dominant taxa should be shown, against their appropriate peaks on a chlorophyll *a* plot.

11. 2 Lakes and Reservoirs Liable to Stratify

Where possible reports on lake and reservoirs phytoplankton should include the following physical and chemical data, along with the phytoplankton results.

Chlorophyll *a*
Light penetration (Secchi and/or Meter)
Temperature profile
Dissolved oxygen profile
Total phosphate
Ortho-phosphate
Total oxidised nitrogen
Silica
Ammonia
pH }
Conductivity } profiles if possible

All the above (except profiles) should be shown graphically in the results section, with the 'x' axes as a time scale. Phytoplankton results should be plotted as natural logs against time. Profiles should be shown as isopleths.

11. 3 Shallow Waters Not Liable to Stratify

Where possible reports on shallow water phytoplankton should include the following chemical and physical data, along with the phytoplankton results.

Chlorophyll *a*
Total phosphate
Ortho-phosphate
Total oxidised nitrogen
Silica
Ammonia
pH
Temperature
Conductivity
Salinity (where appropriate)
Light penetration (Secchi and/or Meter)

All the above should be shown graphically in the results section, with the 'x' axes as a time scale. Phytoplankton results should be plotted as natural logs against time,

11. 4 Marine/Saline Waters

Where possible reports on marine phytoplankton should include the following chemical and physical data, along with the phytoplankton results.

Chlorophyll *a*
Total phosphate
Ortho-phosphate
Total oxidised nitrogen
Silica
Ammonia
pH
Conductivity
Dissolved oxygen }
Salinity } profiles if possible
Temperature }
Light penetration (Secchi and/or Meter)

All the above (except profiles) should be shown graphically in the results section, with the 'x' axes as a time scale. Phytoplankton results should be plotted as natural logs against time. Profiles should be shown as isopleths.

12. TAXONOMY

12. 1 Introduction

Taxonomy is an integral component of enumeration, a taxon cannot be counted until it is identified as being discrete. This does not necessarily mean that each taxon encountered has to be given its full generic and specific name, but some sort of 'label' has to be applied. For example, if a unknown taxon is encountered then it is permissible to give it a temporary name (species 'x'- for example), and an attempt made to ascertain a better level of taxonomy later.

12. 2 Texts

Recommended literature, including taxonomic works, are listed in section 13. There is no one general recommended text. It is best to examine as many different keys and references as possible. Even when you find one you feel happy with, it should not be used exclusively. For example Bellinger, 'A Key to Common Algae' (fourth edition), is a useful text for beginners and established workers alike, but it should not be used on its own, always cross-reference as many texts as possible. When looking in detail at certain groups, such as blue-green algae or marine dinoflagellates, then there are some well established 'standard' works.

Many phytoplankton species are ubiquitous in their distribution, therefore taxonomic works aimed at particular geographic areas have some relevance everywhere. For example, Korshikov's 'The Freshwater Algae Of The Ukrainian SSR (in English)', is of similar value to British and Ukrainian algologists, alike.

Many of the standard algae texts (such as 'Die Binnengewasser Band') are printed in languages other than English. However, as taxonomic identification relies heavily on 'diagram and dimension comparisons' a text written in an unfamiliar language can still be of considerable value.

Several taxonomic works are listed in section 13, and many of these are held within the Region. It is a good idea to visit academic libraries to explore other material. The library at the Freshwater Biological Association (FBA), Ferry House Ambleside, is available for use by NRA staff, through the 'Technical Services' contract. Before visiting the library clearance should be gained both, from your line manager and the head of information services, at Ferry House (see section 13).

Further information on literature searches and information services available through the FBA and PML (Plymouth Marine Laboratory) are given in section 13.

12. 3 The Fritsch Collection of Freshwater Algal Illustrations

The collection was begun by Professor F E Fritsch in 1912 and given to the FBA in 1953. It currently contains about 500,000 illustrations, many with notes, drawn from published sources all over the world. NRA staff can use the collection, under the 'Technical Services' contract, but before visiting clearance should be gained both, from your line manager and the head of information services, at Ferry House (see section 13).

The Fritsch collection is also available on a series of microfiches. The microfiches consists of a basic collection (costing about £1,236) and five supplements, at a total cost of approximately £4,100. See appendix 1 - Suppliers). A copy of the Fritsch collection is held in the Region, at the Haddiscoe laboratory.

12. 4 Taxonomic Level

Where practicable all taxa, especially the dominant forms, should be identified to the lowest taxonomic level possible. However, much depends on the time available, type of sample and purpose of analysis, but as a general rule it is best to identify to a level which you feel most competent about. For example, it is better to identify to the generic level only, than to misidentify at the specific level.

With long term monitoring sites it is important to try and maintain and improve upon, if possible, the established taxonomic standard. This poses problems for inexperienced 'new starters' who take over from established worker. In these cases particular attention should be paid to the taxonomy during the transition period.

12. 5 Unidentified Algae

If an alga cannot be identified within a reasonable time period then firstly describe it as thoroughly as possible, along with a labelled drawing, with appropriate scale bars. If the equipment is available then a photograph should be taken. The use of a video system and 'colour video copy processor' is highly recommended, as this will give an instant photograph, which can be printed ready labelled (see appendix 1 - Suppliers). The sample should be circulated, along with the notes, diagrams/photographs to other laboratories, within the region.

Material circulated for taxonomic identification should be accompanied by as much information as possible. As follows.

Sample (concentrated if necessary)
Approximate abundance of unidentified taxon
Preservative Used
Diagram and Taxonomic Notes
Photograph(s)
Laboratory
Analyst
Location
Date Collected
Date Circulated
Recent Weather Conditions
Other Comments

If the circulated taxon is in low abundance (concentration in a centrifuge may help) then it will probably be very difficult for others to find it, and identification will rely on the drawings and notes etc., alone. If the unidentified alga is reasonably abundant then an attempt should be made to collect some live material (if not already available), as this may aid identification (see below).

If an internal circulation fails to yield a satisfactory identification then a suitable external authority should be approached (see External Audits - below).

Appropriate, documentation and procedures, requires drawing-up for internal and external circulation of material, for taxonomic identification. In practice it would probably be best to coordinate external circulation of material from one location, and material collected, and sent off in batches. Another possibility, for freshwater taxa, is for batches of unidentified material to be taken to the FBA laboratory, Ferry House, where the 'Fritsch', library and expert advice are all at hand. Likewise, batches of unidentified material could also be taken to the Natural History Museum (London), where the facilities exist for examining their extensive collection of mounted material.

12. 6 Examining Live Algae

The value of examining live algae should not be underestimated. In many groups colour, movement and type of movement are a major clue to identification. If the taxon under investigation is moving too rapidly to see properly, then the addition of dilute glycerol to the chamber will often create enough resistance to allow a fuller examination. Particular attention should be paid to the overall movement pattern and the positioning of flagella and their movement (where applicable). Even live observation of blue-green algae can help to distinguish *Oscillatoria* spp. from other similar but less motile forms.

12. 7 Reference Collection

Each laboratory should compile, as an ongoing project, a reference collection of algae, both common and rare, encountered through routine analysis. This can consist of drawings, photographs and mounted slides. The collection should be well indexed and contain as much relevant information as possible (see section 12. 5). The collection could be kept in a ring binder or on index cards.

12. 8 Non-Algal Material And Unfamiliar Algal Forms

Along with the collected phytoplankton there can also be a wide range of other material, which can lead to taxonomic confusion. For example, inshore coastal samples can contain large quantities of marine detritus, such as diatom frustules, phytoplankton and zooplankton resting stages. Some of the material which can cause problems is as follows, and should be kept in mind if taxonomic problems are encountered.

Pollen Grains
Terrestrial Spores
Zooplankton Fragments, Eggs and Resting Stages
Flagellate Protozoa
Ciliated Protozoa
Flagellate Bacteria
Macro-Bacteria
Bacterioplankton
Aquatic Hypomycetes
Yeast Cells

In addition to non-algal material, problems can be encountered with common taxa in unfamiliar forms, such as resting stages etc. For example swarming zoospores of common taxa are almost impossible to identify (even by experts), and at best are classed simply as 'small flagellates'.

12. 9 Taxonomic Audits

12. 9: 1 **Introduction**

Taxonomic audits are an integral component of quality control and training. Internal and external taxonomic audits, alike, will lead to higher standards throughout the Region and act as a important training aid. The results of all taxonomic audits should be made available to the algologist concerned along with a copy of all records held at Head Office.

12. 9: 2 **Internal Taxonomic Audits**

There are three main types of internal audit, on site, spot-check and circular/inter-laboratory audits. On site audits are more of an exchange of experience than an actual audit, and are therefore difficult to quantify. Whereas circular and spot check audits would be more rigorous and could possibly lead to some type of statistical quality control system.

12. 9: 2 (a) ***On-site Taxonomic Audits***

On-site taxonomic audits should take place at the same time as a general laboratory visit. Working with routine samples an individual operator and quality manager/operator from another laboratory (within the Region) would examine particular taxonomic problems currently being encountered.

In addition to general discussion, Whipple field comparisons should be carried out. Using a Whipple graticule, and a recording sheet - figure 12. 1 (A4 copy in appendix 3), both the resident and visiting operators take it in turns to select a field and identify the taxa present. This is done by marking each taxon's position on the record sheet along with the identification. This is carried out without the second operator knowing the identifications or positions. The sheet is then folded, to obscure the first operators identification, and the second person repeats the operation on the same field of view. Differences in identification are then discussed and experiences exchanged.

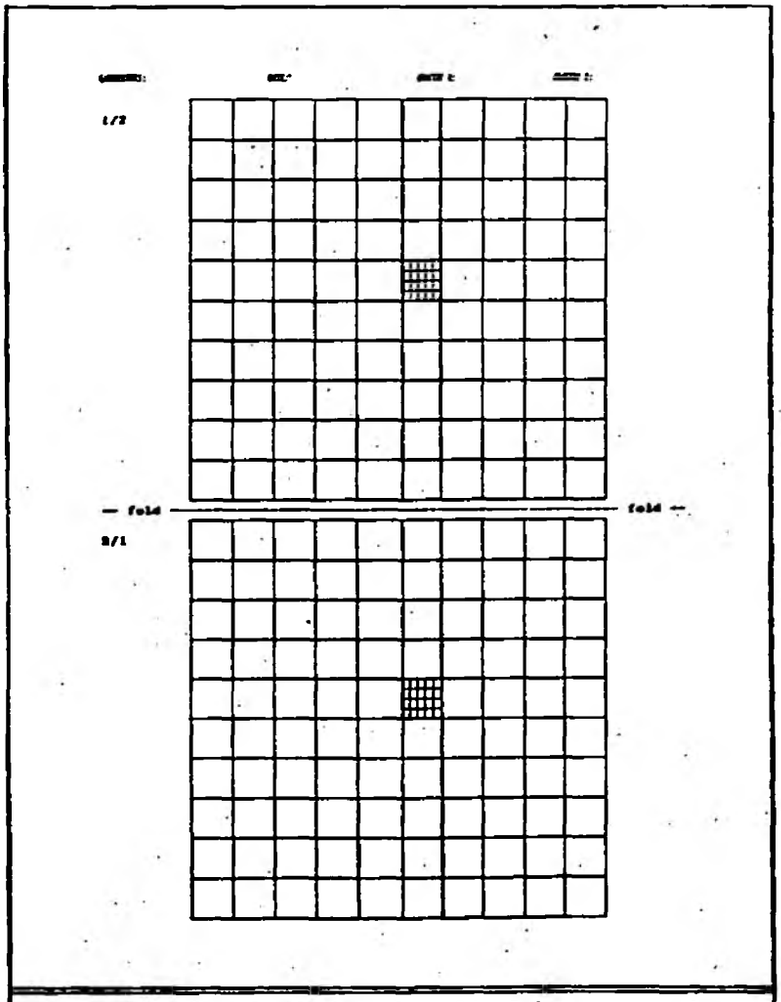


FIGURE 12. 1 Whipple field comparison record sheet, see text & appendix 3.

Although it is inappropriate to quantify Whipple field comparisons, it is an extremely useful method of identifying weak areas (in both operators), taxonomic training and improving overall taxonomic quality.

12. 9: 2 (b) ***Circular/Inter-laboratory Taxonomic Audits***

Circular taxonomic audits would be carried out simultaneously with enumeration circular audits. Either an actual sample, or a culture, would be circulated to all laboratories carrying out phytoplankton work (although a marine/freshwater distinction may have to be made) and the algae present identified to the lowest taxonomic level, within the capabilities of the individual operator.

In the case of mixed samples each taxon identified would have to be accompanied by a labelled drawing or photograph, so that the audit coordinator/quality manager was sure which names were being applied to each taxon.

If considerable differences were found in the identifications then this would 'trigger' an external audit, but if there was a general consensus of opinion this may be inappropriate. Although a system would have to be devised, circular audit results may lend themselves to a statistical quality control system, of some type. Circular audit results would rapidly identify weaknesses and allow early remedial action.

12. 9: 2 (c) ***Spot-Check Taxonomic Audits***

Spot-check taxonomic audits would take place on the same samples that were being enumeration spot-checked. Samples chosen at random would be requested for a spot check of taxonomic identification.
The sample would be sent to the 'checking laboratory' along with labelled drawings or photographs of the dominant taxa found (as section 12 .5). Although a system would have to be devised, spot check audit results may lend themselves to a statistical quality control system, of some type.

12. 9: 3 **External Taxonomic Audits**

External taxonomic audits are a necessary component of overall taxonomic quality, and could be used regularly to verify identifications of both spot-checks and circular audits. The sample for external audit would be sent off to an appropriate authority, along with labelled drawings/photograph and taxonomic notes etc., as listed in section 12. 5.

The results of all external audits should be made known, and this would be an important training aid. A list of possible external auditors is in table 12. 1.

NAME	LOCATION	TAXONOMY UNDERTAKEN
Bailey-Watts, A.E.	IFE Edinburgh Lab. Bush Estate, Pinicuik. Tel: 031 4454343	General Freshwater
Bellinger E.G.	Univ. Manchester. APEM Oxford Road Tel: 061 275384	General Freshwater (slow turn-over likely)
Dodge, J.D.	Univ. Of London, At Royal Holloway, Egham, Surrey. Tel: 0784 43551	Marine Dinoflagellates
Jaworski, G.H.	IFE The Ferry Ho. Ambleside, Cumbria. Tel: 053944 2468	General Freshwater
Dr D. John[a] and Dr I Cox[b]	Cromwell Road London. Tel: 071 9388781[a] Tel: 071 9389001[b]	Diatoms & Greens. Mainly benthic algae.
Lewis, J.	Univ. Westminster, 115 New Cavendish St, London. Tel: 071 9115000	General Marine
Plymouth Marine Laboratory (Possible Only)	Prospect Place, Plymouth. Tel: 0752 222772	General Marine Larger Taxa Only
Whitton. B.A.	Univ. Of Durham Science Labs. South Road. Tel: 091 3742000	Blue-green Algae. (Both Lugol's and formaldehyde samples required)

TABLE 12. 1. List of External Auditors, see text.

13. LITERATURE

3. 1 Library Access

Many graduates can remain external library readers of the university from which they graduated and thus, have access to a range of facilities. Access to academic libraries for non graduates, of a particular university, is often restricted. Therefore, to avoid a wasted journey, check access availability before visiting academic libraries. Access to the libraries of the Freshwater Biological Association (FBA) and Plymouth Marine Laboratory (PML), are open to NRA staff, for reference only, with prior permission from the relevant Librarian (PML - Tel: 0752 222772. FBA see below).

3. 2 Literature Searches And Services

Both FBA and PML libraries offer literature search facilities. The FBA library charges £45 per hour (extra for on-line searches), and the PML library charges £10 per hour for self-service CD searches, and £50 per hour for staff conducted CD searches. The main purpose of the FBA library search facility is to identify papers within their own library.

The FBA library also offers a Document Delivery Service. On request the FBA library staff will send photocopies of papers, conference proceedings, articles and chapters of books (only 10% of any monograph), held at their library. The request documents for using the delivery service are available from the Regional R&D coordinator and cost £3 per article (1 article = up to 20 A4 sheets - often 2 article pages can be copied onto 1 A4 sheet). The above, FBA services are available through the 'Technical Service' contract. Inform your line manager before using any of the above services and contact the head of library and information service, at the relevant library. The contact for FBA library is as follows, (see above for PML).

Head of Library and Information Services
Freshwater Biological Association
The Ferry House
Ambleside
Cumbria
LA22 OLP

Tel: 05394 42468
Fax: 05394 46914

13. 3 Recommended Literature (see Bibliography, also)

13. 3: 1 **General Text**

BELL, P & WOODCOCK, C. *The Diversity of Green Plants*. 3rd ed., Arnold 1983, Chapters 1 - 3.

BONY, A.D. *Phytoplankton.* E Arnold, 1988.

FITTER, R & MANUEL, R. *Freshwater Life.* Collins, 1986.

HARPER, D. *Eutrophication of Freshwaters - Principles, Problems and Restoration.* Chapman and Hall, 1992.

HUTCHINSON, Evelyn G. *Treatise on Limnology.* Volumes 1 & 2, Cloth Wily, 1975 & 1967.

LARSON, Jacob & MOESTRUP, Ojvind. *Guide to Toxic and Potentially Toxic Marine Algae.* University of Copenhagen, 1983.

BERRY, David R. *Biology of Yeast.* Arnold, 1982.

CURD, C.R. *An Illustrated Key to the British Freshwater Protozoa Commonly Found in Activated Sludge.* Water Pollution Research, Tech. Paper No. 12, HMSO 1969.

ERDTMAN, G. *An Introduction to Pollen Analysis.* Cronica Botanica Company, 1949.

FINLAY, J.B, ROGERSON, A & COWLING A.J. *A Beginners Guide to the Collection, Isolation, Culturing and Identification of Freshwater Protozoa.* CCAP (at FBA), 1988.

INGOLD, C.T. *Guide to Aquatic Hypomycetes.*
FBA. Publication No. 30, 1975.

MACKERETH, F.J.H, HERON, J & TALLING, J.F. *Water Analysis.*
FBA. Publication No. 21, 1989.

MUNDY, S.P. *A Key to British and European Freshwater Bryozoans.*
FBA. Publication No. 41, 1980.

SMITH, I.R. *Turbulence in Lakes and Rivers.*
FBA. Publication No. 29, 1975.

PONTIN, Rosalind M. *A Key to British Freshwater Planktonic Rotifera.*
FBA. Publication No. 38, 1978.

WHITTON, B.A, HOLMES, N.T.H & SINCLAIR, C. *A Coded List of 1000 Freshwater Algae of the British Isles.* Water Archive Manual Series No. 2. [DoE, Water Data Unit].

13. 3: 2 **Taxonomic Works**

BARBER, H.G. & HAWORTH, E.Y. *A Guide to the Morphology of the Diatom Frustule.*
FBA. Publication No. 44, 1981.

BELCHER, J & SWALE, E. *An Illustrated Guide To River Phytoplankton.*
HMSO, 1979.

BELCHER, J & SWALE, E. *A Beginners Guide to Freshwater Algae.*
HMSO, 1976.

BELLINGER. E.G. *A Key to Common Algae - Freshwater, Estuarine, and Some Coastal Species*. Fourth ed., IWEM, 1992.

BOURRELLY, P. *Les Algues D'aeu Douce*.
Three Volumes. Boudée et Cie, Paris, 1966-1973.

BROEN, W.C. *Algae of the Western Great Lakes Area - With an Illustrated Key to the Genera of Desmids and Freshwater Diatoms*. Dubuque, Iowa, 1962.

DESIKACHARY, T.V. *Cyanophyta.Indian Council of Agricultural research*,
New Delhi, 1958.

DODGE. *Marine Dinoflagellates of the British Isles*.
HMSO, 1982.

FRITSCH, R.E. *The Structure and Reproduction of the Algae*. Volumes I & II,
Cambridge , 1953.

FRÉMY, P. *Cyanophyées de Cotes d'Europe*.
Asher & Co, 1972.

HINDAK. *Kluc Na Urcovanie Uytrusnych Rastlin* [Czech. Flora].
I Diel, 1972. (Copy at FBA library).

KORSHICOV, A.W. *The Freshwater Algae of the Ukrainian SSR*. Volume V,
Koeltz Scientific Books, 1978.

LEBOUR, Maria V. *Planktonic Diatoms of Northern Seas*.
London - Printed for Ray Society, 1930.

LIND, Edna M & Brook, Allen J. *An Key to the Commoner Desmids of the English Lake District.* FBA. Publication No. 42, 1980.

PENTECOST, A. *Introduction to Freshwater Algae.*
Richmond Publishing Company, 1984.

SMITH, Gilbert M. *The Freshwater Algae of the United States.*
McGraw-Hill, 1933.

STARMACH, Karol. *Flora Slodkavodna Polski* [Polish Flora].Fourteen Volumes,
Polska Akademic Nauck, Warsaw, 1964-1980.
(Copy at FBA library).

SYKES, J.B. *An Illustrated Guide to the Diatoms of British Coastal Waters.*
Field Studies Council, 1981.

THIENEMANN, A. ed. *Die Binnengwasser Band.* Nine Volumes,
Schwizerbart's Stuttgart, 1938-1982.

TIKKINEN, Toini. *Kasviplanktoncpas.*
Helsinki, 1986. (Copy at FBA library).

VINYARD, William C. *Diatoms of North America.*
Mad River Press Inc., 1971.

WEBBER, Cornelius I. *A Guide to Common Diatoms at Water Pollution Surveillance System Stations.* U.S. Environmental Protection Agency, 1971.

WEST, G.S & FRITSCH, F.G. *A Treatise on the Freshwater Algae.*
Cambridge, 1927.

The majority of the above books are held at laboratories within the Region (although some may be personal copies) so phone around and ask. The books marked 'Copy at FBA library' does not mean that these are the only books on the list held at the FBA, but these books may be particularly difficult to locate elsewhere (photocopies of sections from Hindak are held in the Region). Several of the books listed above are out of print, but may be available from second-hand book dealers (some offer a search facility), see appendix 1.

13. 3: 4 Course Notes

Course notes are often more up to date than the literature and often contain very useful unpublished information. People who attend suitable training courses should make their notes available to others and distribute any useful information.

14. TRAINING

14. 1 Introduction

Training is an integral component of the Quality System. Without trained and experienced staff a Quality System, however elegant, will not run efficiently and effectively.

A suitable training programme can adequately be divided into three parts, as follows:

1. Initial Training - For 'New Starters'
2. Ongoing Training - To Maintain a Set Standard
3. Forward Training - To Improve and Update Standards

14. 2 Initial Training

Initial training is for 'new starters' both with or without previous experience. In addition to initial safety training, 'new starters' should be given induction training of a suitable duration, depending on previous experience. Where possible initial training should be carried out by experienced staff in the laboratory where the new operator is to be based. If, however, there are no suitably experienced staff to carry out the initial training, then the 'new starter' should receive their initial training at another operational laboratory where experienced staff are available. As well as the initial operational training a 'new starter' should be briefed by the technical manager on all aspects of the quality system. An initial training programme should be as follows.

1. Introduction to Sampling Methods
2. Literature Introduction (including this manual)
3. Taxonomic Introduction
4. Introduction to Analysis Methods
5. Hands-on Enumeration and Taxonomy Experience
6. Introduction to the Quality System

The time spent on each stage will depend on previous experience and speed of learning. The order in which the stages are carried out is not crucial and will depend on local requirements, but, if possible, start with sampling methods, as these are usually the easiest stage to understand. Hands-on enumeration and taxonomy experience should involve 'dummy' counts and identification, and can include time spent drawing and describing the taxa found.

When a 'new starter' begins actual analysis, someone should be on hand to help out during the initial period. If a new starter is based at a laboratory where there is no suitably trained help, then they should analyse their samples at another laboratory, where help is available, for the initial period.

14. 3 Ongoing Training

Ongoing training is all part and parcel of enumeration and taxonomic quality audits. On-site visits, spot-checks and circular audits should all feed-back on the operator and acts as training along with the quality checks.

14. 4 Forward Training

Forward training consists of improving standards through courses, workshops and information circulation.

Several external courses are available, and either run on a regular basis, or on demand. The main problem with general courses is they have to be pitched at a standard to suit all, and this is often too basic for the operational algologist. Additionally, most external courses are not open to temporary members of staff, and as the majority of algology within the Region is done by temporary staff, this makes external courses inaccessible to most operators (see appendix 6 - Training).

One solution to the above problems, specific training and training for temporary staff, is to run one or two day workshops within the Region. These workshops should be based somewhere central and with good communication links to all areas. The workshops should be devised to be of current operational value (eg. not too theoretical), and only attended by those people who are regularly involved in algology. A subject specialist should be approached and arrangements made for them to visit. Every effort should be made to ensure all operational algologists can attend. A list of specialist who are willing to run day workshops is shown in table 14. 1.

Another training possibility is for algologists to visit the FBA laboratory, at Ferry House. George Jaworski (see table below) is prepared to run short courses, to suit our purposes, at the FBA laboratory. The FBA course can cater for up to six people and cover any aspect of their work. In some cases, low cost, accommodation can be arranged, and the course fee (about £300 per day for all six people attending - 1992 price) can be paid for out of the 'Technical Services' contract. Although it may not be possible for temporary staff to visit the FBA laboratory, every effort should be made to see that they do, as the training and other facilities (Fritsch Collection, Library, etc) offered by the FBA are of great educational value.

NAME	CONTACT	SPECIALLY/SUBJECT
Bailey-Watts, A.E.	IFE Edinburgh Lab. Bush Estate, Pinicuik. Tel: 031 4454343	General Freshwater Identification and Methods
Bellinger E.G. (overnight stay necessary)	Univ. Manchester. APEM Oxford Road Tel: 061 275384	General Freshwater Identification and Methods
Dodge, J.D.	Univ. Of London, At Royal Holloway, Egham, Surrey. Tel: 0784 43551	Marine dinoflagellates/ nuisance algae
G. Jaworski, (may not be prepared to travel)	IFE The Ferry Ho. Ambleside, Cumbria. Tel: 053944 2468	General Freshwater Identification and Methods
Dr D. John[a] and Dr I Cox[b]	Cromwell Road London. Tel: 071 9388781[a] Tel: 071 9389001[b]	Diatoms & Greens. Mainly benthic algae.
Lewis, J.	Univ. Westminster, 115 New Cavendish St, London. Tel: 071 9115000	General Marine Identification and Methods
Whitton. B.A.	Univ. Of Durham Science Labs. South Road. Tel: 091 3742000	Blue-green Algae. Identification only

TABLE 14. 1 Specialists who will run short courses

Other forward training should consist of information and literature circulation. All relevant information, regarding methods, taxonomy or procedures, should be circulated to other laboratories.

15. QUALITY AUDITS

The following is a list of necessary quality audits, the recommended frequency which they should be carried out and the page where they are found in this manual. Until a sufficient data base of quality audits is established, only estimates of the necessary frequencies can be made.

AUDIT TYPE	REQUIRED FREQ.	PAGE
Sampling equipment and method	annual per eqip. type/samp. type	17
Filtered water	two months	25
Siphoning efficiency	100 siphons	26/7
Pipettes	100 deliveries	27
Microscope checks - centring pin, phase rings, etc (if applicable)	two months	20/38
Intra-chamber transect counts	four months	39
Intra-chamber field counts	two months	40
Randomness movements	two months	40/1
Chamber replication (if not done routinely)	two months	48
Data computation system, if applicable	twice/year	49/50
Enumeration/taxonomy spot checks	four/year/person	50/72
Enumeration/taxonomy circulations	four/year	50/72
Enumeration/taxonomy site checks	four/year	50/72
Data-base/raw data comparisons	annually	59
External taxonomic	every spot check and circulation/ + when necessary	72

Table 15. 1. Quality audits, their frequency and page reference

16. **BIBLIOGRAPHY** (See LITERATURE, also)

A.W.A (1979). *Report on the Comparative Exercise on Counting Methods to the Plankton Methodologies Working Group of the Reservoir Evaluation Committee.* Internal Report.

BELLINGER. E.G. *A Note on the Use of Algal Size in Estimates of Standing Crop.* Br.phycol.J. 9: 157-161, 1974.

CUNNINGHAM, C.R & PUREWAL, J.S. *A Microcomputer Based Aid for Counting Plankton Samples.* Journal of Plankton Research, Vol.5, 1983.

IRISH, A.E & CLARKE, R.T. *Sampling Design for the Estimation of Phytoplankton Abundance in Limnetic Environments.* Br. Phycol.J. 19:57-66, 1984.

DAVIS, Owen L. ed. *The Design and Analysis of Industrial Experiments.* Longman, 1956.

EDMONDSON, W.T & WINBERG, G.G *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters.* Blackwell, 1971.

ELLIOTT, J.M. *Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates.* FBA. Publication No. 25, 1977.

FURET, J.E & BENSON-EVENS, K. *An Evaluation of the Time Required to Obtain Complete Sedimentation of Fixed Particles Prior to Enumeration.* Br. Phycol.J. 17:252-258, 1982.

HMSO. *The Enumeration of Algae, Estimation of Cell Volume, and Use in Bioassays.* 1990.

JONES, J.G *A Guide to Methods of Estimating Microbial Numbers and Biomass in Freshwater.* FBA. Publication No. 39, 1979.

LUND, J.W.G. *A Simple Counting Chamber for Nano-plankton.Limnology and Oceanography*, Vol. IV, No.1 1959.

LUND, J.W.G, KIPLING, C & LE CREN, E.D. *The Inverted Microscope Method of Estimating Algal Numbers and the Statistical Basis of Estimation by Counting. Hydrobiologia*, Vol.11, No.2, p. 143-70, 1958.

LUND, J.W.G & TALLING, J.F. *Botanical Limnological Methods with Special Reference to the algae. Botanical Review*, Vol.23, 1957.

N.R.A. (1990). *Toxic Blue-green Algae.* Water Quality Series No. 2.

REYNOLDS, C.S & JAWORSKI, G.H.M. *Enumeration of Natural Microcystis Populations. Br. phycol.* J. 13:269-277, 1978.

ROBSON, P.G & BAILEY-WATTS, A.E. *A Computer System for Recording and Sorting Plankton Count Data.* Br. Phycol. J. 22, 1897.

SOURNIA, A. ed. *Phytoplankton Manual*
Unesco, 1981.

SUTHERLAND, T.F, LEONARD, C & TAYLOR, F.J.R. *A Segmented Pipe Sampler for Integrated Profiling of the Upper Water Column.* Journal of Plankton Research, Vol.14, 1992.

UTERMÖHL, H. *On the Perfecting of Quantitative Phytoplankton Methods. International Association of Theoretical and Applied Limnology* - Communication No. 9, 1958.

WATER DATA UNIT. (1980). *A Guide to the Use Of Algae for the Biological Surveillance of rivers*. Technical Memorandum No. 20.

WHITTON, B.A, DIAZ, B.A & HOLMES, N.T.H. *A Computer Orientated Numerical Coding System for Algae*. Br. Phycol. J. 14: 353-360 1979.

APPENDIX 1 - SUPPLIERS AND MANUFACTURES

Note: Where only one supplier is shown, per item, no other suppliers of that item is known, at the time of writing.

ACHRO OPTICS. Suppliers of Lund Chambers (with-out cover slips)

Drury Lane
Pondswood Ind. est.
St Leonards on Sea
East Sussex
TN38 9BN
Tel. 0429 429357

BDH and ABINGHURST. Suppliers of laboratory equipment.

Tel. 0604 581111
Fax. 0604 588150

CARL ZEISS (OBERKOCKEN) LTD. Suppliers of Axiovert 10 microscopes and equipment.

PO Box 78
Welwyn Garden City
Herts
AL7 1LU
Tel. 0707 331144
Fax. 0707 373210

DUNCAN & ASSOCIATES. Suppliers of plankton nets, sedimentation/combination chambers and universal sampler.

Beech Court
cart Lane
Grange-over-Sands
Cumbria
LA11 7AF
Tel. 05395 33857
Fax. 05395 34963

E.W.CLASSEY LTD. Second hand and Antiquarian Natural History Books

P.O Box 93
Faringdon
Oxon
SN7 7DR
Tel. 0367820 399
Fax. 0367820 429

FBA publication. Dept. DWS
Freshwater Biological Association
The Ferry House
Far Sawtry
Ambleside
Cumbria
LA22 0LP
Tel. 053944 2468
Fax. 053944 6914

P&R LABORATORY SUPPLIES LTD. Tel. 0744 819596
Fax. 0744 810885

FISONS Tel. 0509 231166
Fax. 0509 231 893

GB NETS. Suppliers of plankton nets, order 53um mesh.
Linden mill
Hebden Bridge
W.Yorks
HX7 7DP
Tel. 0422 845365

GRATICULES LTD. Suppliers of graticules. Standard and specially made graticules and optical aids - randomness test plates.
Morley Road
Tonbridge
Kent
TN9 1RN
Tel. 0732 359061
Fax. 0732 770217

INTER DOCUMENTATION COMPANY AG. Fritsch Collection on microfiche slide
Indusrtriestrasse 7
6300
Zug
Switzerland
Tel. 42 214974
Fax. 42 213187

LEICA UK LTD. Suppliers of video printers and video equipment
Davy Avenue
Knowhill
Milton Keynes
MK5 8L8
Tel. 0908 686663
Fax. 0908 609992/3

NIKON UK LTD. Suppliers of video printers and video equipment
Heybrook
Halesfield 9
Telford
Shropshire
TF7 4EW
Tel. 0952 587444
Fax. 0952 588009

SUBBUTEO. New and Second Hand Natural History Books
Treuddyn
Mold
Clwyd
CH7 4LD
Tel. 0352 770581
Fax. 0352 771590

APPENDIX 2 - FIXATIVES AND SAFETY PRECAUTIONS

A2. 1 Lugol's Iodine

The following notice should be displayed where Lugols Iodine is being prepared or used.

SAFETY PRECAUTIONS WITH LUGOLS IODINE

1: STOCK SOLUTION

When making up stock solution dissolve 95g of KI in 100 ml of de-ionized water, to which 14g of I2 are then added, followed by 10 ml of acetic acid.

This should be carried out in a fume cupboard while wearing gloves.

Stock solution should be kept in a stoppered container, preferably thick polythene, and kept in a safety cupboard.

A dropper bottle containing ca 10 ml can be available for additions to smaller samples.

2: USAGE

Avoid contact with skin and eyes. When dispensing large volumes ($>$ 5 ml) wear disposable gloves.

Avoid ingestion, NEVER MOUTH PIPETTE.

Use minimum volume. 1 - 5 drops for a sample $\leq$ 100ml, dispensed from a dropper bottle, is usually sufficient.

Cover vessels to be left for a day or more.

Spillage onto a person should be rinsed well with water and an eye wash used where necessary.

Spillage on equipment should be washed with water or potassium thiosulphate solution

A2. 2 Formalin

Despite the fact that formalin poses a health hazard (see chemical hazards) and sometimes causes changes in cell dimension, it is still a popular preservative with some workers. The recommended concentration range is between 2.5 and 4% formaldehyde. In general, the lower the concentration, the better the morphology is retained in the short term, but a higher concentration is needed for long term storage of organisms. The ideal concentration also depends on the amount of material in relation to volume of liquid.

Formaldehyde must be buffered, and a variety have been suggested. HEPES (N-2-hydroxymethylpiperazine-N'-sulphonic acid, COSHH Assessment 0803) is probably the best for freshwater, whereas, hexamethylenetetramine (0.4%, COSHH Assessment 0477) is recommended for seawater. Glycerol is also commonly added to samples for long term storage, as algae are readily damaged if the sample dries out completely.

Formalin Mixtures

The addition of copper nitrate, COSHH Assessment 4034 (c 1 g l-1) in a formalin solution helps to maintain the green appearance of chloroplasts of green algae.

FAA is a mixture of formaldehyde, alcohol and acetic acid (10ml 40% formaldehyde, 5ml glacial acetic acid, 50 ml 95% ethanol, 35ml water). This mixture is particularly useful for preventing flagella loss.

PROCEDURE FOR HANDLING FORMALDEHYDE (under review).

Principle

Formaldehyde, in the form of a solution of Formalin is used as a fixative agent prior to the preservation of biological material, or to prevent subsequent microbial growth. Formalin is a hazardous material and requires careful handling, reference must be made to the COSHH assessment 0106. Formalin should only be used where absolutely necessary.

The stock solution normally purchased is a 37-41% solution of the gas formaldehyde in water. This may be known as "100% formalin", "40% formaldehyde solution" or "concentrated formalin". The working dilution for algae fixation/preservation is up to a 1 in 10 dilution of this, giving 4% formaldehyde or 10% formalin.

Toxicity

Formalin is acutely toxic. There have been 13 deaths due to the ingestion of amounts estimated to be 100 ml (or a few drops in the case of a child).

Inhalation is the most likely hazard in the biology laboratory. The threshold for detecting an effect on the eyes has been claimed to be as low as 0.01 ppm, symptoms of mild throat irritation occur at about 0.5 ppm and it is intensely irritating to the eyes at about 4 ppm. Brief exposure to 50 ppm would cause very serious injury. There is some evidence that continued exposure can result in desensitisation to the irritant effect.

Splashes to the eye of 40% solution have resulted in permanent eye damage. Splashes of a 4% solution produce a strong irritant effect and visual disturbance for one day, after which the eye returned to normal.

Contact with the skin at concentrations greater than 2.5% may cause dermatitis. Skin sensitization and allergic contact dermatitis can occur.

Carcinogenicity

There is no evidence to suggest that exposure to formaldehyde has produced cancer in humans, nor is there acceptable evidence for any adverse effects on the reproductive system. However, formalin has been shown to be carcinogenic in laboratory animals and so a possible risk of cancer caused by chronic inhalation exists. Precautions are required when using formaldehyde solution.

First Aid - Standard Treatment:

Irrigate thoroughly with water for at least 10 minutes. OBTAIN MEDICAL ATTENTION.

Lungs: Remove casualty from exposure, rest and keep warm. In severe case or if exposure has been great OBTAIN MEDICAL ATTENTION.

Skin: Drench the skin with plenty of water. Remove contaminated clothing and wash before re-use. Unless contact has been slight OBTAIN MEDICAL ATTENTION.

Mouth: Wash out mouth thoroughly and give water to drink. OBTAIN MEDICAL ATTENTION. DO NOT INDUCE VOMITING.

Exposure limits: Long and short term exposure limit is 2 ppm or 2.5 mg m^3. This is well below the threshold of mild irritation and it is safe to assume that if Formalin cannot be detected in the laboratory it is below the MEL. Routine checks for Formaldehyde should be carried out using a suitable formaldemeter.

General precautions (see COSHH assessment 0106)

Clothing

When dealing with >500 ml of formaldehyde (COSHH regulation) and also formalin a PVC apron, Grade 2C plastic goggles/visor and appropriate gloves e.g. black chemical resistant heavyweight Marigold gloves, not disposable vinyl gloves, must be worn.

In situations of high formaldehyde vapour the use of an appropriate respirator is recommended, e.g. 3M formaldehyde respirators which protect up to the OEL. In addition, goggles which seal around the face, rather than eye shields should be used in such situations.

Spillage

In the event of a spill of more than 500 ml of 40% formalin the laboratory should be evacuated and assistance from the fire brigade requested. Use formalin neutraliser Chroma FNC for significant spillages. Smaller or more dilute spills can be handled.All sources of ignition should be shut off and the area evacuated - do not re-enter until ventilation has been achieved. Wearing a face-shield or goggles and gloves the formalin can be mopped up with plenty of water and run to waste, diluting greatly with water. The area should be well ventilated to evaporate remaining liquid and to dispel vapour.

Under no circumstances should formalin be disposed of down general laboratory sinks not designated for the purpose.

Formalin must not come into contact with hydrochloric acid to avoid the formation of Bis-chloromethyl ether (BCME) a known carcinogen.

APPENDIX 3. - DOCUMENTATION

This appendix contains the following documentation:

1. Inspection of Standing Waters
2. Table 1. Samples for toxin analysis
3. Equivalents Among Cyanobacteria
4. Significant Blooms Report & Instructions
5. Whipple Field Comparisons

NATIONAL RIVERS AUTHORITY
ANGLIAN REGION

AREA REF:

NRA

INSPECTION OF STANDING WATERS

SITE NAME

SAMPLE POINT CODE

SITE OWNER

ADDRESS

USES

FISHING

WATER CONTACT SPORTS

LIVESTOCK WATERING

PORTABLE WATER SUPPLY

NATURE RESERVE

TELEPHONE NO.

DISTRICT COUNCIL

NATIONAL GRID REF

CATCHMENT NORFOLK SUFFOLK N. ESSEX S. ESSEX

HISTORY OF PREVIOUS BLOOMS Y / N If yes and sample not taken please complete above details and send form to area office as usual

ALGAL SAMPLE TAKEN WATER SCUM **CHLOROPHYLL A SAMPLE TAKEN** Y / N

DATE SAMPLED

SAMPLERS INITIALS

WATER COLOUR CLEAR BROWN GREEN

ALGAL BLOOM (OPEN WATER): NONE SLIGHT MODERATE SUBSTANTIAL

% AREA AFFECTED

ALGA SCUM (SHORELINE): NONE SLIGHT MODERATE SUBSTANTIAL

% AREA AFFECTED

COMMENTS

WEATHER WHEN SAMPLE TAKEN

WEATHER PRIOR TO REPORT

ANALYST **DATE** **METHOD**

ALGAE PRESENT	Cells per ml	Filaments/Colonies per ml	Average Cells per fil or Average Colony diameter

CHLOROPHYLL A RESULT

Table 1. Samples for toxin analysis

This protocol applies to samples from fresh and brackish waters and from animal carcases. Quantities given are the minima necessary for comprehensive bioassay and physio-chemical analysis.

1. **Scum available.** Three samples to be taken at each site :

   a) **either** at least 50 ml **or** at least 5 grams wet weight of fresh, wet scum, in a clean plastic bottle or a fresh plastic bag, for toxin analysis;

   b) 1 to 2 ml scum, in a glass or plastic specimen tube, for strain isolation and cultivation;

   c) 1 to 2 ml scum, in a glass or plastic specimen tube, preserved in Lugol's iodine or other plankton preservative, for reference purposes.

   Toxin analysis, or despatch for analysis elsewhere, should be on the day of collection. Use an express service for overnight delivery. If same day analysis or despatch is impossible, store samples a) and b) overnight at 4°C. If longer delays are anticipated, store deep frozen at about minus 20°C.

2. **Algal crusts available.** It is assumed that fresh, wet scum is not available.

   One sample, 0.5 to 2.0 grams dry weight of material, scraped from beach, rocks, boats, etc, in a clean plastic bottle or a fresh plastic bag. Store at room temperature for same or next day analysis or despatch. If longer delays are anticipated, samples should be stored deep frozen at about minus 20°C.

3. **Blue-green algae in water.** It is assumed that fresh, wet scum is not available. Four samples to be taken at each site :

   a) 2 to 10 litres of water. If only the presence of algal toxins is required, a clean plastic container can be used. If precise analysis of toxins is required, clean glass bottles should be used. Leave container undisturbed in the laboratory for three to four hours and, if a scum develops, this should be drawn off and dealt with as in paragraph 1, above;

   b) 2 to 10 litres of water, in a plastic or glass container, as in a) above. Toxin analysis or despatch should be on the same day as sample collection, or store at 4°C overnight for next day processing, or store at minus 20°C. Note that separate determination of intra- and extra-cellular toxins is not possible on samples which have been frozen and thawed;

   c) 1 to 2 ml, in a glass or plastic specimen tube, stored at room temperature for same day or at 4°C for next day processing;

   d) 1 to 2 ml, in a glass or plastic specimen tube, preserved in Lugol's iodine or other plankton preservative.

4 Animal carcases

a) Fish. Moribund, rather than dead, fish are preferred. These should be killed and their livers excised immediately. One piece, about 1.5 to 2.0 cm^3, should be placed in a specimen tube and preserved. The preferred preservative is buffered neutral formalin, the recipe for which is 100 ml formalin, 6.5 grams anhydrous Na_2HPO_4, 4.0 grams $NaH_2PO_4H_2O$ and 900 ml distilled water. Less suitable is formol-saline, the recipe for which is 8.5 grams NaCl, 100 ml formalin and 900 ml distilled water. Stock solutions and dispensing should take place in a fume cupboard and rubber or plastic gloves should be worn. In the field, use screw top containers of pre-dispensed solution, only in a well-ventilated area. Disposable gloves and basic eye protection should also be worn. A similar volume of gill tissue should also be preserved separately. The remainder of the specimen should be deep frozen to minus 20°C. Two or three whole fish should be stored at minus 20°C until it is known whether toxin analysis is merited.

b) Mammals. Two samples of stomach or rumen contents should be taken from each carcase:

   i) 1 ml in a specimen tube, preserved in Lugol's iodine;

   ii) about 50 ml in a clean plastic or glass container. Analysis or despatch should be same day, otherwise store overnight at 4°C or at minus 20°C for later analysis.

The precautions identified in Paragraph 3.4 of the Procedure Manual should be followed when obtaining samples which could be toxic.

Table 3. Equivalence among Cyanobacteria

Taxon, with scale of potential scum production	Typical (μm^3) volume	Typical dry weight (μg)	Population (ml^{-1}) - 1 μg chlorophyll a l^{-1}	Assume "Units" are:	Unit (ml^{-1}) equivalent to "warning" threshold (5 μg l chl a)
Synechococcus sp.	1 - 5 $cell^{-1}$	0.4 - 2.2 $cells^{-1}$	50 - 250 x 10^3 cells	Unicellular	0.25 - 12.5 x 10^6 cells
Aphanothece/Aphanocapsa o	5 - 8 $cell^{-1}$	2.2 - 3.7 $cells^{-1}$	30 - 40 x 10^3 cells	40 μm colonies (= 100 cells) 80 μm colonies (= 1000 cells)	2 x 10^3 cols 0.12 x 10^3 cols
Microcystis aeruginosa ***	50 - 80 $cell^{-1}$	22 - 37 $cell^{-1}$	3 - 4 x 10^3 cells	90 μm colonies (=1000 cells) 200 μm colonies (= 10000 cells)	20 cols 1.5 cols
Gomphospaeria naegeliana **	40 - 60 $cell^{-1}$	18 - 27 $cell^{-1}$	4 - 5 x 10^3 cells	50 μm colonies (= 300 cells) 80 μm colonies (= 1000 cells)	80 cols 20 cols
Coelosphaerium kuzingianum **	20 - 40 $cell^{-1}$	9 - 18 $cell^{-1}$	6 - 11 x 10^3 cells	50 μm colonies (= 300 cells) 80 μm colonies (= 1000 cells)	175 cols 30 cols
Merismopedia sp. o	10 - 20 $cell^{-1}$	4 - 9 $cell^{-1}$	11 - 25 x 10^3 cells	30 μm^3 'plates' = 64 cells	1000 - 21000 plates
Pseudanabaena sp. *	11000 - 12000 (mm filament)$^{-1}$	5200 - 5600 mm^{-1}	20 mm	300 - μm filaments	300 - 350 filaments
Limnothrix redekei *Limnothrix limnetica* *	1800 - 7500 (mm filament)$^{-1}$	800 - 3500 mm^{-1}	40 - 100 mm	300 - μm filaments	650 - 1750 filaments
Planktothrix agardhii *	1200 - 28000 (mm filament)$^{-1}$	5600 - 13000 mm^{-1}	8 - 18 mm	300 - μm filaments	125 - 300 filaments
Planktothrix rubescens *	12000 - 38000 (mm filament)$^{-1}$	5600 - 18000 mm^{-1}	6 - 18 mm	1000 - μm filaments	60 - 180 filaments
Planktothrix mougeotii *	18000 - 71000 (mm filament)$^{-1}$	13000 - 33000 mm^{-1}	3 - 8 mm	1000 - μm filaments	30 - 80 filaments
Aphanizomenon flos - aquae **	8 - 30 $cell^{-1}$	3.8 - 14.2 $cells^{-1}$	7 - 25 x 10^3 cells	60 - cells filaments 60 - 70 filaments	600 - 2100 filaments 10 - 30 flakes
Anabaena flos - aquae **	35 - 110 $cell^{-1}$	16 - 52 $cell^{-1}$	2 - 6 x 10^3 cells	26 cells/gyre	380 - 1150 gyres
Anabaena circinalis *Anabaena spiroides f spiroides* **	90 - 120 $cell^{-1}$	41 - 56 $cell^{-1}$	1.8 - 2.5 x 10^3 cells	18 cells/gyre	500 - 700 gyres
Anabaena spiroides f crassa **	300 - 600 $cell^{-1}$	145 - 280 $cell^{-1}$	370 - 700 cells	50 cells/filament	35 - 40 filaments
Anabaena solitaria *	260 - 1100 $cell^{-1}$	120 - 520 $cell^{-1}$	200 - 800 cells	50 cells/filament	20 - 80 filaments
Nodularia spumigena ***	60 - 160 $cell^{-1}$	27 - 70 $cell^{-1}$	3.5 - 14 x 10^3 cells	15 cells/filament	1000 - 4000 filaments
Gloeotrichia echinulata ***	20000 - 40000 (mm filament)$^{-1}$	9 x 10^3 - 18 x 10^3 (mm filament)$^{-1}$	6 - 11 mm	500 μm filaments	60 - 110 filaments or 0.5 - 1.0 colonies

Scale of potential scum production

*** Rapid scum formation. Warning threshold can produce toxic hazard.

** Fairly rapid scum formation. Toxic hazard begins at 5 x warning level concentration.

* Slow scum formation. Toxic hazard begins at 20 x warning level concentration.

o Minimal risk of scum formation.

(*Limnothrix redekei* = *Oscillatoria redekei*; *L. limnetica* = *O. limnetica*; *Planktothrix agardhii* = *O. agardhii*; *P. rubescens* = *O. rubescens*; *P. mougeotii* = *O. isothrix and O. limnosa*. See Meffert, 1987)

MARINE ALGAE MONITORING

SAMPLING METHODS

1. MINIMUM EFFORT PROGRAMME

On all routine sampling visits to an EC Designated Bathing Water, the following procedure must be adopted in each maritime Region:-

i. Observe the strandline within 50m of the sampling transect, and also observe along the sampling transect, and record presence or absence of any evidence of a bloom deposited by the previous high tide, such as slime, scum, gelatinous sludge, localised dark patches on sand, or sulphurous smell.

ii. Observe the sea water at the waters edge for any evidence of an algal bloom - excessive foaming, colour change, smell, etc. Presence or absence of algal bloom must be recorded.

iii. Observe sea offshore for signs of any slicks, windrows, or foaming indicative of algal blooms, and record presence or absence.

iv. Any positive observations under 1 or 2 above should be supported by a 100ml (min. vol) sample for laboratory examination in order to confirm the bloom, and identify and enumerate, if possible, the dominant species. Samples must be kept cool and in the dark until returned to the laboratory.

v. Record water temperature, local wind direction at waters edge (compass points), speed (Beaufort Scale) and sea state (Beaufort Scale). This information is valuable to Pollution Control staff in dealing with public complaints and in assessing the likely movement and impact of blooms.

vi. At the laboratory the sample should either:-

a) be examined immediately, and the phytoplankton identified and enumerated;

or

b) be fixed immediately with Lugol's Iodine and stored in the dark for future NRA examination on a batch basis, (in house or external contract).

2. BEST ENDEAVOURS PROGRAMME

The monitoring protocol for the "Best Endeavours" Programme is based on the "Minimum Effort" protocol, with the following additions:-

i. On every occasion that routine EC bathing waters monitoring samples are taken, a separate sample for algal examination must be obtained, at the same location as the bathing water samples.

ii. Whenever bloom deposits are found on a beach, or accumulations of floating scum etc. are evident in bathing waters within a safe sampling distance of the waters edge, a sample must be taken for laboratory examination. ie. There must always be a sample associated with bathing waters samples, and an additional sample(s) of the bloom manifestation.

when present. The routine sample will always be examined to identify and enumerate algae present. Bloom samples will normally be examined, unless the nature and extent of the bloom is so extensive or uniform as to render the examination unnecessary.

iii. On receipt at the laboratory samples should ideally be identified and enumerated fresh. Failing that, they should be fixed on receipt (Lugol's Iodine), and stored in the dark for examination within one week.

NATIONAL RIVERS AUTHORITY

MARINE ALGAL MONITORING PROGRAMME

TOXIC ALGAL BLOOM REPORT

REGION:

BLOOM REPORT NO: 9 /

DIVISION/AREA:

BLOOM REPORT DATE:

LOCATION:

GRID REF:

SAMPLE POINT NO:

DATE BLOOM FIRST RECORDED:

BLOOM CONTINUING?: YES ☐ NO ☐

DATE SAMPLE TAKEN: STORED ☐ OR ANALYSED ☐

IF SAMPLE ANALYSED, LIST DOMINANT TAXA:

TAXA	ABUNDANCE	TAXA	ABUNDANCE

DESCRIBE APPEARANCE OF BLOOM:

EVIDENCE OF AESTHETIC IMPACT ON BEACH? YES ☐ NO ☐

EVIDENCE OF TOXIC EFFECT?: ☐ ☐

IF YES, LIST ORGANISMS AFFECTED:

COMMENTS:

REGIONAL CONTACT : NAME TEL NO:

SEE GUIDANCE NOTE OVERLEAF

GUIDANCE NOTES ON COMPLETION OF TOXIC ALGAL BLOOM REPORT

1. This report form is to be used for communicating information ONLY WHEN actual/or potentially toxic algal blooms are present, NOT for reporting of all algal monitoring data. Once a bloom has been notified for a location it should not be reported again unless there is evidence that the dominant alga or nature of the bloom has changed significantly (eg. it may become toxic).

2. All completed forms should be sent to Martin Mills, NRA Welsh Region in Cardiff for collation and forwarding to national network on toxic algae.

3. Reports should be made as soon as possible after a bloom is recorded and should be as complete as possible. At this stage it is not anticipated that duration of the bloom will be reported, but some indication of whether or not the bloom is persisting should be given under "Bloom Continuing"?

4. The location should include the official bathing beach name and the Town (if different from beach name) and County.

5. The bloom report number should begin with the year, followed by the number of the report, to be allocated consecutively on a Regional basis (eg. 95/1 ..... 95/n). The Bloom Report date is the date the report is completed.

6. Description of the bloom should include information on such factors as appearance, colour, smell, foaming etc.

7. The taxonomic level to be reported will depend on the expertise and experience of the analysts but species names should only be used where there is a degree of certainty about the identification. Abundance should be recorded as unit/cell counts per ml.

8. Aesthetic impact: although somewhat subjective, an aesthetic impact will generally give rise to public complaint.

9. Toxic algae: Care should be taken, whenever possible, to differentiate between the direct toxic effects of an algal bloom and the indirect effects of bloom material coming ashore (eg. smothering of intertidal fauna by decaying algal material). The latter type of event should <u>not be</u> notified using this procedure.

GUIDANCE ON "NOTIFIABLE" TOXIC MARINE ALGAE SPECIES

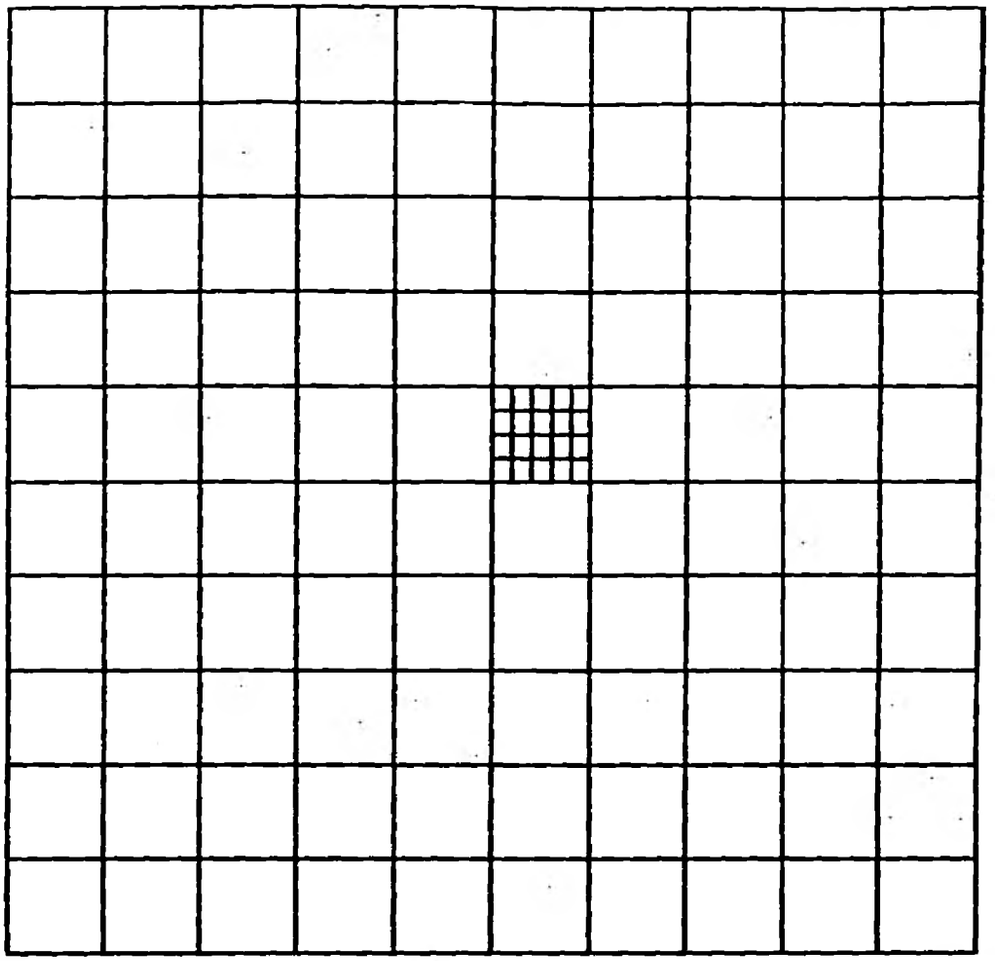
The following list of marine algae has been compiled from available information on recognised toxic marine algal species. It is reasonably comprehensive but not exhaustive as marine algal toxicity is a developing science. The list includes those species which may occur in British coastal waters and are known to produce toxins which may result in shellfish poisoning or direct toxicity of fish and/or invertebrates. This checklist should be used when completing the Toxic Algal Bloom Reports. Other species such as *Noctiluca scintillans, Phaeocystis pouchetti, Chaetoceros* spp. may result in mortalities of marine organisms, either by causing deoxygenation and/or high ammonia concentrations on bloom breakdown, or by clogging of gills which leads to asphyxiation. However, these algae are not considered to be "toxic" in the true sense of the term.

CLASS	SPECIES OR FAMILY	TOXIC EFFECT
Bacillariophyceae	*Nitzschia pungens*	Amnesic shellfish poisoning (ASP)
Dinophyceae *	*Alexandrium tamarense*	Paralytic shellfish poisoning (PSP)
	Pyrodinium bahamense	
	Gyrodinium aureolum	Fish Kill
	Gyrodinium cf. nagasakiense	
	Gymnodinium galatheanum	
	Dinophysis acuminata	Diarrhetic shellfish poisoning (DSP)
	D. acute	
	D. norvegica	
	Prorocentrum lima	
	Ptychodiscus brevis	Neurotoxic shellfish poisoning (NSP)
Haptophyceae	*Chrysochromulina polylepsis*	Fish Kills
	Prymnesium parvum	
Radiophyceae	*Chatonella antiqua*	
	Heterosigma akashiwo	
	Fibrocapsa japonica	
Chrysophyceae	*Dictyocha speculum*.	

* There are some 40 species of toxic dinoflagellates of which only the main problem species are given here.

LABORATORY: DATE: ANALYST 1: ANALYST 2:

1/2



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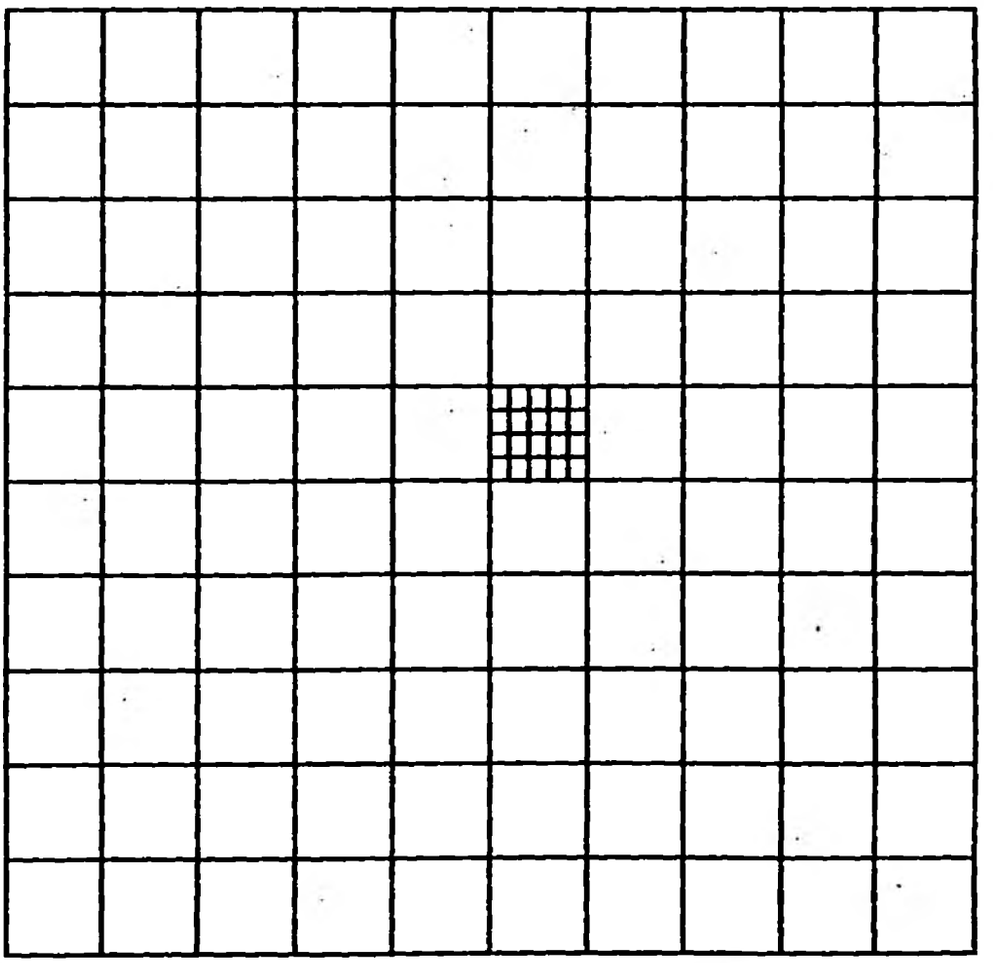
2/1



APPENDIX 4 - EQUIPMENT INSTRUCTIONS

AXIOVERT 10

Brightfield illumination and Long Distance Microscopy

1. Plug in the microscope and switch on the power.
2. Switch on the illumination power supply and adjust to 3-4
3. Place the specimen onto the stage. If mounted on a specimen slide, then the smaller, thinner glass cover must face down.
4. Turn the lowest objective X5 into position (red ring) on the nosepiece.
5. Move the condenser all the way down.
6. Close the diaphragm of the condenser to about half way.
7. Adjust both eyepieces to the photographic format reticle by turning the eyelens.
8. Focus on the specimen by using the coarse and fine focus controls.
9. Close the luminous field diaphragm moderately, it becomes unsharp in the image.
10. Focus the diaphragm image by slightly raising the condenser.
11. If not already, with the screws move the diaphragm image to the centre of the field of view.
12. Open the luminous field diaphragm until it just disappears from the field of view.
13. The microscope is now set up for brightfield illumination.
14. The contrast for each specimen can be adjusted by using the condenser diaphragm.
15. For long illumination distance microscopy ensure that the turret of the LD condenser is set of the two DIC positions.

Phase Contrast

Phase contrast is used mainly to enhance the contrast of unstained specimens.

Required Equipment

- 2 objectives designated PH, which are equally well suited for brightfield.
- For microscopy with long illumination distance the front lens 0.55 cannot be used in phase contrast. (see manual, pp34).
- LD condenser 0.3H, Ph 1, 2 or LD condenser 0.55H/Ph/DIC.
- Both condensers have turrets with phase contrast diaphragms.

The following additional adjustments are necessary in phase contrast

- The phase rings in the objectives have different sizes and are indicated on the objective by Ph 1, Ph 2, Ph 3. The ring size is indicated on the turret by the engraved numbers 1, 2, 3 for selection and combination with the objective.
- Perfect contrast is only achieved if the dark ring in the objective and bright ring in the condenser exactly coincide.
- This can be controlled after insertion of the centering telescope (see manual pp34). With the reducing ring and focusing by moving the eyelens of the centering telescope which must be held on its knurled ring.
- Without this attachment the control can be made without the eyepiece, like the condenser diaphragm control.

NOTE

Additional equipment is required in order to operate;

Differential Interference Contrast (DIC)
Fluorescence or Reflection - contrast microscopy.
Microscopy with high illuminating aperture.
Photomicrography.

APPENDIX 5 - HEALTH HAZARDS

Information regarding Leptospirosis (Weil's Disease) and Tetanus.(Southern NRA Safety Code of Practice - Field Sampling Activities)

Leptospirosis letero is a listed Industrial Disease under the R.I.D.D.O.R. Regulations 1985. This strain of the disease can be contracted through contact with material/water which has been contaminated with urine from infected rats.

The infection commences with high temperature and general muscle and joint pains. Medical advice must be sought immediately as the symptoms are similar to influenza, pneumonia, tonsillitis, rheumatic fever or nephritis and later catarrh, jaundice or gall stones. Show the doctor the Leptospiral Jaundice card issued to all NRA employees at risk, in addition, a letter should be kept with your medical records informing medical staff of your occupation and the risk of Weil's disease.

Tetanus is a reportable disease under R.I.D.D.O.R. Regulations 1985. It is a disorder of the nervous system, causing rigidity and spasms of the muscles. It is caused by a bacillus which inhabits soil and road dust.

It can be fatal, causing death through spasms, the loss of limbs has also been known.

The onset of the disease generally follows a wound contaminated with soil, especially deep puncture wounds and lacerations.

Symptoms usually appear 4 to 5 days after injury but can be delayed for 3 or 4 weeks. The first signs are usually muscle stiffness near the wound followed by stiffness in the jaw muscles.

Tetanus can be prevented by immunisation and persons exposed to soil and road dust in their work should have effective immunisation. Initial immunisation is achieved by a course of three injections with a booster every 5 years for those at risk. Precautions against infection.

After contacting sewage, water from a watercourse or cattle, wash hands and forearms with soap and water - even if gloves have been worn. It is especially important to do this prior to eating or drinking. If clothing or footwear becomes contaminated it should be thoroughly washed.

DO NOT WORK IN WATER OR SEWAGE WITH OPEN WOUNDS

Take care to wash and cleanse with antiseptic any cut, scratch or abrasion as soon as possible, whether caused at work or not. Keep any wound covered even when wearing gloves.

APPENDIX 6 - TRAINING

A6. 1 External Courses

Title: ALGAL IDENTIFICATION AND INTERPRETATION OF DATA
Run By: Water Training International
Course No: WS 18.
Cost: £730 (in 1992)
Duration: 1 Week - Monday - Friday
Location: Burn Hall, Yorkshire
Contact: Customer Information
Wti
Tadley Court
Tadley
Hampshire
RG26 6TT Tel: 0734 813011
Fax: 0734 817000

Title: NRA. ALGAL TRAINING COURSE
Run By: Department of Biological Science, University of Durham
Cost: £600 (in 1993)
Duration: 1 Week - Sunday - Friday
Location: Department of Biological Science, University of Durham
Contact: Dr Brian Whitton
Department of Biological Science
University of Durham
Durham
DH1 3LE Tel: 091 374 2427
Fax: 091 386 0619
or

Dr David John
Department or Botany
Natural History Museum
Cromwell Road
London
SW7 5BD Tel: 071 938 8781
Fax: 071 938 9260

Title: NRA. MARINE PHYTOPLANKTON TRAINING COURSE (Run on demand)
Run By: University of Westminster
Duration: 1 Week - Monday - Friday
Location: University of Westminster
Contact: Welsh Region NRA
Steve Knowles
Tel: 77 26 4604 (network access)
Tel: 01222 770088
Fax: 01222 798555

Title: ALGAL IDENTIFICATION AND/OR METHODS
Run By: Freshwater Biological association
Cost: About £300 per day, up to six people, plus accommodation Course fee could be paid for out of 'Technical Services'contract. Some 'student type' accommodation often available.
Duration: To suit
Location: FBA, Windermere
Contact: George Jawoski
Far Sawrey
Ambleside
Cumbria
LA22 OLP

Tel: 0153944 2468
Fax: 0153944 6914

A6. 3 Videos

MICROBIAL ENGINE - Algae and Protozoa Ecology and Bio-technology
Price: £40
Contact: The Administration Officer
The CCAP
IFE
The Windermere Laboratory
Far Sawrey
Ambleside
Cumbria
LA22 OLP

Tel: 0153944 2468
Fax: 0153944 6914

THE SAMPLING AND IDENTIFICATION OF HARMFUL PHYTOPLANKTON
Price: $40 (Canadian)
Contact: University of Columbia
Media Services
2206 East Hall
Vancouver
B.C.
Canada
V6T 1W5

Note: Specify UK 'VHS Systems' - Enquiry not answered. Copy at University of Westminster.

BLELHAM TUBES
Price: £130
Order: S326/02V
Contact: Open University
Educational Enterprises
Cofferidge Close
Stony Stratford
Milton Keynes
MK11 15Y

Tel: 01908 261 662