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1. INTRODUCTION

Phytoplankton is of great ecological significance, comprising the major organisms involved in primary production. A knowledge of the distribution of the various phytoplankters in both space and time is essential for an understanding of a particular ecosystem. The seasonal succession of species has an effect on the components higher up the food chain and is consequently important for economic reasons.

In addition to their significance in the food chain, phytoplankton contain species recognised as potent toxin producers. These microalgal toxins have been classified as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP). The increase in reported cases of algal toxins has led to speculation that industrial pollutants, agricultural and wastewater runoffs may be to blame.

Besides knowing which species are present at different locations and seasons a knowledge of the total abundance of phytoplankton is necessary for a better understanding of the ecosystem and its dynamics. This can be measured and expressed as cell numbers in samples of sea water. Such a method of measuring phytoplankton populations is not without its problems as cell size can be variable not only interspecies but even intraspecies. The significance of cell size variability is that species which are unimportant numerically may be important in terms of biomass and such a numerical census may overestimate small cells and underestimate the contribution of large cells (Paasche 1960). Biomass can be measured from proximate analyses, typically chlorophyll or indirectly from the cell-volume characteristics of an enumerated population. For most purposes speed of analysis is essential for obtaining a good picture of a current situation.

Monitoring of marine phytoplankton is undertaken to comply with the Water Resources Act (1991) and EC Directives, to assess eutrophication and identify toxic and nuisance species. The relevant EC Directives are Urban Waste Water Treatment Directive [91/271/EEC], Bathing Water Directive, and Nitrate Pollution Directive [91/676/EEC]. There is also the North Sea Action Plan for which work has been incorporated into the National Marine Monitoring Programme (NMMP) for chlorophyll *a* analysis and phytoplankton sampling.

A degree of standardisation of methods is necessary to ensure the comparability of data. Regular quality control is required to ensure that results of acceptable quality are being obtained in a subject area which is renowned for the difficulties of analysis.

For EC UWWTD and the Nitrate Directive, in relation to eutrophication, it has been recommended "that a primarily observational approach is adopted, with the aim of establishing the occurrence of exceptional nuisance algal blooms, the duration of blooms, excessive macro-algal growths, or other evidence of undesirable disturbances" (NRA Programme for the monitoring of Water Quality, Nov 1994, Annex 6 UWWTD). This should be undertaken at existing monitoring points, notably those for bathing waters and shellfish waters.

All EC bathing waters are monitored weekly, May to September inclusive, as part of the National Marine Algal Bloom Monitoring Programme. On occasions where algal blooms are seen to be present, samples are taken to confirm the bloom. For sites where regular 'significant blooms' occur monthly summer sampling is recommended. Consideration should be given to remote sensing surveys, from aircraft, at such sites to determine spatial distribution of blooms and provide evidence of impact. (NRA Guidance Note on Information Gathering for Future Designation Reviews UWWTD August 1994. In: NRA Programme for the Monitoring of Water Quality Nov 1994, Annex 6).

2. DEFINITION

Plankton, as defined by Hensen in 1887, can be summarized as those organisms, both plant and animal, that 'drift' passively in water. Many of these organisms are able to swim but, because of their small size, progression through the water is slow and their distribution is often patchy and determined by the movement of currents and tides.

Phytoplankton is predominately unicellular algae which are either solitary or colonial. The main components of marine phytoplankton are diatoms (Bacillariophyta), dinoflagellates (Dinophyta or Pyrrophyta), coccolithophorids and chrysomonads (Haptophyta) and some other flagellates. Phytoplanktonic organisms are mainly autotrophic containing photosynthetic pigments such as chlorophylls and carotenoids. Some phytoplankton organisms, mainly dinoflagellates, can be heterotrophic, building up organic particulate matter from dissolved organic substances (osmotrophy) or even particulate organic matter (phagotrophy). Some taxa, such as *Protoperidinium* are classified as both phytoplankton and zooplankton (protozoa).

Autotrophic phytoplankton is most abundant in the euphotic zone, defined as the zone reaching from the sea surface to a depth where the production of organic matter by photosynthesis in an individual phytoplankton cell balances destruction by respiration.

Phytoplankton is not evenly distributed in the sea but is believed to occur in three-dimensional patches of various size.

Cell size and volume cover a wide range of *ca* 1-200μm. Picoplankton is the term used for the smallest cells of less than 2μm, nanoplankton (or nannoplankton) for those between 2 and 20μm, microplankton (or net-plankton) organisms are between 20 and 200 μm. Plankton nets can be used to concentrate the larger phytoplankton species, but nanoplankton and picoplankton normally passes through phytoplankton nets and sedimentation or filtration methods are used to collect these organisms. In general the use of plankton nets underestimates the total standing stock of phytoplankton.

3. PRINCIPLE OF METHOD

Phytoplankton is monitored for different reasons and the sampling method depends on the objectives of the study. Whatever the aims of the investigation the samples collected should be representative, as far as is possible, of the whole water body being studied. This implies a careful consideration of water flow and tidal state, particularly in estuarine and near-shore areas. In open sea areas provision should be made to allow for an adequate number of samples to be taken to allow for the possibility of patchiness in the occurrence of phytoplankton. Wherever possible a larger initial sample should be taken than needed and one or more subsamples taken from this after thorough mixing.

Chlorophyll analysis of sea water either from laboratory analysis of filtered samples, continuous flow fluorometry, or aerial surveillance can give valuable information, particularly when used in conjunction with species identification and other chemical analyses.

The quality of the results depends on the method of collection of samples, and their subsequent subsampling, their analysis and the skill of the operators at each stage. To ensure that these processes are carried out in the best cost-effective and reproducible manner requires quality evaluation at each stage.

4. CHEMICAL HAZARDS ASSOCIATED WITH COLLECTION AND ANALYSIS

4.1 Water

Localised water pollution from sewage or other discharges may pose a significant health hazard. The risks associated with Leptospirosis and other water born diseases must be appreciated.

Physical contact should be avoided by use of appropriate clothing including long PVC/rubber gauntlets and all wounds should be covered with waterproof dressing.

4.2 Other Chemicals

COSHH	No.	Nature of risk
Formaldehyde- Fixative/preservative	0106	Toxic, flammable
Lugol's Iodine- Fixative/preservative	0513	Toxic, corrosive
Acetone- Solvent	0009	Flammable, irritant
Ethanol- Solvent	0005	Flammable, irritant
Methanol- Solvent	4001	Toxic, flammable, irritant
Glutaraldehyde- Fixative	0508	Irritant, harmful
Glacial Acetic Acid- Preservative	0097	Irritant, corrosive, flammable
Hexamethylenetetramine- Fixative	0477	Irritant, flammable
Sodium Thiosulphate- Decolourant	0419	Harmful
HEPES- Buffer	0803	Harmful

4.3 COSHH ASSESSMENTS

Refer to the relevant COSHH assessments and safety manuals for detailed information on each of the chemicals listed above. For Lugol's Iodine and Formaldehyde also refer to Appendix 1 'Fixatives and Safety Precautions.

5. PHYSICAL HAZARDS

5.1. Procedures

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

Sampling should not be carried out alone; always work in pairs or groups. A procedure exists for recording the time that the biologist(s) leave for and are expected to return from fieldwork. Details of the route, locations and estimated times of departure and arrival should be recorded and left with a designated person. Any significant changes from the plan should be reported. When the work is completed the designated person should be informed. If staff have not reported in by the expected time (+ 1 hour) then the designated person will set the emergency search procedure in motion. The procedure will involve search parties who will check the areas intended to be visited.

Note: Further details on safety information are held in the laboratory and with your Safety Officer.

When intending to use boats at sea, inform the Coastguard/Harbour Authority of the route plan, estimated arrival times and details of the work to be undertaken.

Before use, the Leader or most experienced person must judge if the boat is safe and adequate for the job, and is not overfilled either by people or equipment. In some circumstances, a Leader may need to insist that all members of the party are competent swimmers.

5.2 Clothing

Suitable clothing for the worst potential weather should be worn. A dry-suit and suitable 'bear' is ideal for wet and/or cold boat work. In colder weather gloves and a hat or hood should be worn to prevent excessive heat loss. PVC/rubber gauntlets possibly worn over thermal gloves protect against the cold and allow most jobs to be carried out normally.

Footwear, normally waterproof, must be worn with adequate tread. Chest waders should be avoided because their buoyancy could hold the wearer in an inverted position if swept away, but where necessary only trained staff should wear them together with a life-jacket.

A life-jacket, such as an Environment Agency approved twin chamber automatic inflation - Crewsaver "Seafire Solas" and not 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 and life-jackets must be tested/serviced at least once a year.

5.3 Equipment

Each group should either know the tide times or carry a set of local tide-tables.

At sea, charts must be carried of the area of operation and local information obtained, if possible, of tidal conditions, races, rocks, wrecks and other hazards, and pencilled onto the chart.

Adequate food and drink should be taken for the duration of the collecting trip. Always ensure hands and forearms are clean by washing them before eating or drinking.

If possible a portable marine band VHF radio should be carried. Working in hazardous and dangerous areas at darker times of the year is discouraged. A torch and spare batteries must be taken. See Appendix II for International Distress Signals. In hazardous areas, each group should carry a whistle, a watch and a first aid kit, plus flares and a length of rope. On extensive shore surveys, each group should also carry a compass and maps.

All staff must wear life-jackets (with attached lights) when sampling or working on or near deep water; this includes water where the maximum depth exceeds knee height.

The leader of the group should determine from charts or local experts where there are special dangers, such as quicksand.

All members of a collecting party should be made aware of potential hazards before the sampling commences. On the sea shore work should be carried out on the ebb, working down the shore, and the return journey should begin, depending on the distance to be covered and the softness of the substrate, from one hour before to one hour after the predicted time of low water. If the substrate is particularly difficult or extensive then the aim should be to be clear of the shore before the tide turns.

Any strange looking objects on the shore or in the sea which could be a bomb or canister of dangerous chemical should be left untouched and the Police or Coastguard informed. If you see anyone in danger, or a flare out to sea, act at once giving or calling for assistance as appropriate, but do NOT take unjustified risks.

5.4 The Use of Boats

Only a brief outline of boat policy is given here; further information is contained in: NRA Code of Practice - Marine Activities:- Part I: Use of sea-going vessels, Part II: Use of Small Vessels; NRA Safety Guidelines for Water Quality Fieldwork, Aug 1995.

Boat users should be qualified to RYA Level I as a minimum and all visitors should ideally have some boat handling experience, however, if the ratio of trained to untrained crew is 2:1 then this requirement may be relaxed.

5.4.1. Checklist of Safety and Emergency Equipment

a) A suitable anchor and rope should be made fast to the vessel and be ready for use at all times.
b) A rope (painter) attached to the bow.
c) Alternative means of propulsion, such as a backup engine.
d) A knife, spike or pliers, in an appropriate case, for use in an emergency to cut moorings or to clear a fouled propeller.
e) Flares, as in Department of Transport Schedule, emergency foghorn.
f) A throw line.
g) A waterproof torch.
h) A bailer.
i) A compass.
j) A VHF radio.
k) Charts for the operational area, adjacent waters and coastline, preferably in a waterproof wallet.
l) First aid kit.
m) Waterproof watch.
n) Boat-hook.

6. SAMPLING

6.1. Introduction

The sampling method employed is determined by the objectives of the survey and the methodology of analysis. These objectives may be varied and include the production of a species list, an indication of abundance of certain species or for a calculation of chlorophyll present. The Environment Agency has a requirement to monitor marine plankton as a means of monitoring pollution and environmental quality. It is recommended that where possible a phytoplankton sampling programme is developed in conjunction with zooplankton sampling and chemical analysis. A more comprehensive understanding of the environment year on year is obtained if, for instance, analysis of chlorophyll and other chemicals in the water can be related to identified species and their abundance.

The nature of the distribution of phytoplankton, that is its patchiness, vertical differences and diurnal changes, poses problems for statistical analysis. Regular sampling and analysis throughout the year, but at more frequent intervals during the spring and autumn blooms is necessary for an understanding of the ecosystem. The sampling programme employed must be practical in terms of manpower available to analyse the samples and flexible enough to be able to adapt to changing conditions, such as the development of 'red tides'. Long-term studies are essential to illustrate trends in eutrophication, although the numbers of samples taken may be reduced with experience or where good correlations have been established. One possible example might be an aerial surveillance system superseding water sampling for pigment analysis although financial considerations might render this impractical. Similarly, if a good understanding of the species composition at different times of the year exists, automatic counting devices could be use to reduce the number of plankton samples counted manually.

6.1.1. Sampling strategy for different identified requirements

Before commencing a sampling programme it is essential to identify the exact purpose of the sampling; to develop the sampling strategy which will best fulfil the requirements; list equipment and manpower required to carry out the programme; draft the time schedule for obtaining samples and their subsequent treatment and analysis.

6.1.2. Treatment of Samples

It is normally desirable to examine fresh material, both for biological and chemical analysis, in addition to preserving material. Some species can be identified reliably only from live specimens. Ideally live samples should be examined on the day of collection. If this is impractical, they should be stored in the dark in a refrigerator or cool place (normally at sea temperature), then looked at within 24 hours. Fixation of samples not only distorts cell shape but reduces the size of cells. The very small flagellates frequently burst when a fixative is added and are lost for identification and analysis, resulting in an underestimate of biomass.

Fresh sea water samples for chlorophyll analysis should be kept cool and dark and sent to the laboratory for examination with the minimum delay. In estuarine waters chlorophyll concentrations vary with the stage of the tide so this should be borne in mind when samples are taken for comparative purposes. There are also diurnal variations to be considered (Yentsch and Scagel 1958).

6.1.3. The Importance of Salinity

Salinity has a considerable influence on what species are present and their distribution. Consequently, in estuaries and nearshore areas a decision needs to be taken whether to sample from fixed sites or, alternatively, in relation to salinity isohalines.

6.1.4. Quality Assurance

* The sampling strategy should meet the needs of the investigation. These may change over time to allow for altered circumstances. A clear understanding of the objectives is essential for the best sampling programme to be in operation.

* For long term studies sampling from as near as possible the same location is required. The marking of locations with buoys has been thought to influence the composition of the phytoplankton around it (Leger 1971 a, 1971 b).

6.2. Qualitative and Semi-quantitative Sampling

Sampling with phytoplankton nets provides useful material for qualitative purposes but because of their selective and unpredictable filtering properties nets should not normally be used for quantitative purposes (Braarud 1958). The main advantage of nets is the large quantity of water

which can be filtered for concentrating cells. A horizontal or vertical net haul (only possible in deep water) is a quick and effective method for collecting rarer larger species. The chief disadvantage is the distortion to species composition resulting from loss of very small cells. The filtering qualities of the net can be affected by the species composition of the plankton and chain-forming species, or those with spines or setae (e.g. *Chaetoceros* spp) can themselves form a network inside the gauze. Where there is a large quantity of suspended debris the net quickly becomes clogged.

6.2.1. Qualitative Sampling

Nets constructed for vertical hauls are weighted on the line before the mouth of the net. Modern nets are made of synthetic filaments such as polyamide or polyester. The finer mesh gauzes have low porosity, and so a compromise has to be reached between filtration and species concentration and the size of species collected.

For general purposes it is recommended that a net with meshes of 53μm be used. This is the standard mesh used in most commercially available phytoplankton nets.

6.2.2. Semi-quantitative Sampling

A semi-quantitative study can be achieved if there is consistency in sampling. For vertical hauls the net should be lowered to the same depth and raised at the same slow speed each time. If the weight is attached behind the tail of the net the net does not filter on the way down but only when being hauled up. Horizontal tows should be made at the same speed for the same length of time. A plankton sample from a particular depth is obtained by towing the net horizontally while a weight holds the net at the selected depth. Care should be exercised to limit speed when towing a net. For nets of about 50μm mesh this speed should not exceed 1 m. s^{-1} or 2 knots; nets with meshes below 20μm should be towed at speeds below 0.3m. s^{-1} or 0.5 knots. A flowmeter can be fitted in the mouth of the net to enable a calculation to be made of the amount of water passed through the net.

Although the collecting bucket at the end of the plankton net receives most of the plankton during towing some cells remain on the gauze and should be washed into the bucket at the end of each haul.

Net hauls should not be too dense, and they should be kept in a large amount of water (250 - 1000ml) if they are to be examined live. They should also be kept cool (2 - 5° C) to minimise bacterial growth. Dense samples from nets, as well as those from blooms decay rapidly and should either be diluted with fresh seawater to extend viability or fixed immediately after collection.

6.2.3. Quality Assurance

* Nets should be washed as soon as possible after use, and should not be allowed to dry before washing. They should be washed in tap water to remove salts. After washing the net should be air dried and stored in a dark, cool place. It should be inspected for damage before further use.

* Nylon nets can be occasionally cleaned by soaking in warm soap or detergent solution or an alkaline solution up to 15% strength. Polyester nets should be washed in dilute acids instead of alkaline solution but, in general, soap will be adequate for cleaning. After soaking, rinse thoroughly in clean freshwater before air drying and storing in a dark, cool place.

6.3. Quantitative Sampling

Water samplers should collect a known quantity of water from a specified depth and keep the sample free from contaminants.

6.3.1. Surface Samples

For surface water a container such as a bottle or bucket is lowered into the water and allowed to fill before recovery. To avoid contamination from the research vessel or from disturbed sediment when collecting from the shore the container may need to be weighted, have a surface float and line attached, and be tossed free of the collector, allowed to fill and then hauled back.

Most commercial bottles are now constructed from or coated with an inert material, such as teflon or PVC to eliminate chemical contamination. However, if physiological work is to be carried out it may be necessary to avoid rubber and plastic, which may be toxic to some phytoplankton species (Throndsen, 1970). Water bottles are available in sizes from 1-1000 litres, but most laboratories find that 1-3 litre bottles are adequate.

6.3.2. Tube Samples

For sampling the top 5 metres or less an integrated sample can be obtained with a length of hose or flexible tubing, which is attached to a line and weighted at the end that is lowered into the water to the desired depth. The surface end is then sealed with a clip or plug and the lower end raised to the surface with the line. A 2.5 cm internal diameter tube of 5m length gives a sample of over 2 litres. This quantity would be sufficient for chlorophyll analysis and microscopical work. (For further details see Lund et al 1958; NRA Standard Methodologies: Freshwater Phytoplankton. 1993)

6.3.3. Profile and Fixed Depth Samples

Samples from predetermined depths are taken with either a reversing-bottle of the Nansen type, a non-reversing bottle, Van Dorn type or a sampler which opens only at the required depth. Simultaneous sampling at several depths permits savings in time and effort over a single bottle lowered repeatedly to different depths.

6.3.4. Quality Assurance

* Sampling precision and accuracy should be evaluated regularly.

* All sampling bottles, hoses and nets should be rinsed after use to avoid contaminating samples.

* Sample bottles must be clearly labelled with waterproof marker. Where possible physical data such as temperature and salinity should also be marked on the bottle. Where wet conditions preclude the satisfactory use of a marker pen a paper label written with pencil should be inserted in the sample bottle.

* A record book stating the location and time of sample should be kept.

* Live samples should be kept cool using a cold box or portable refrigerator and transported with the minimum of delay to the laboratory. They should be kept in the dark.

* For comparative purposes there must be consistency in the sampling method. Personnel engaged in these activities should attend appropriate training sessions.

* Sufficient bottles for the sampling programme, plus a few extras to account for any losses or unexpected phenomena, such as red tides, should be taken.

6.4. Pump Sampling

Samples from selected depths may be obtained by pumps. Pumped water volumes can be accurately measured with a water meter for quantitative studies, however frictional resistance may inhibit the movement of some organisms. Samples can be concentrated by filtering through a plankton net on board ship. Pump systems are normally more expensive than nets and bottles but can prove cost effective when sampling for a variety of zoo- and phytoplankton taxa together with chemical analysis.

6.4.1. Quality Assurance

* Pumping systems must be tested for toxicity, particularly when the samples are used for live organism studies.

* The growth of organisms in the tubing can be reduced by washing with fresh water.

6.5. Samples for Chlorophyll Analysis

The methods used to obtained to obtain water samples are the same as those described above as for Quantitative samples (6.3.) or pumped samples (6.4.). Preferably samples should be filtered immediately as described in section 9.5. If this is not possible, they should be stored in sealed containers in the dark, and in a cool box, and transported to the laboratory as soon as possible.

6.6. Bathing Water Samples

Samples should be taken if there is any sign of an algal bloom, scum or if the water or beach surface is discoloured. This should be done with the minimum of disturbance to the site by gently lowering bottles to sample the surface water. Normally two samples are taken of at least 100 ml volume but preferably of 250 ml each, of which one is kept live and the other immediately fixed with Lugol's Iodine (see Fixation Methods: 7.3.). These are kept cool, packed in light proof containers and sent or taken to the laboratory for analysis. Following analysis a Toxic Algal Bloom Report should be completed and sent off according to The Marine Algal Monitoring Programme, V.2 1994 (Appendix V).

Samples of the substrate are taken whenever bloom deposits are found on a beach. With the aid of a flexible scraper a sample from at least 5 cm^2 should be taken for qualitative or quantitative studies. The sample must normally be kept cool and dark and examined microscopically as soon as possible.

Accumulations of floating scum should also be sampled where they are within safe sampling distance of the shore. Enough material should be taken to fill a sample tube and a duplicate should be fixed with Lugol's iodine if it is not possible to examine the sample within an hour or so of collection.

6.7 Subsampling in the Laboratory

The original sample should be thoroughly mixed before subsamples are taken. The size of the subsample depends on the material to be examined and the aim of the investigation. The sample container should be shaken or inverted a standard number of times so that the particulate matter is evenly dispersed. 20 to 30 times is generally sufficient. When subsamples are taken by pipette the tip should be sited as near the centre of the sample bottle as possible.

6.8 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 dominant taxa should be selected and enumerated to a 95% confidence limit $< \pm 25\%$. 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 in 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. Results should be kept in a log-book for future reference.

6.9. Summary of Sampling Procedures

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

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

- Quantitative samples are essential if accurate results are required.

- Use Lugol's Iodine to fix routine samples for cell enumeration.

- Samples intended for chlorophyll analysis must be treated particularly carefully (see Section 6.1.2.) to avoid breakdown of the pigments.

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

- All sampling equipment should be kept clean and free from contamination.
- Sampling precision and accuracy should be evaluated regularly.

7. FIXATION METHODS

7.1 Introduction

The handling of samples after collection is a critical stage in most phytoplankton work and it is important to minimise quantitative and qualitative changes in their composition. This is achieved by fixation of the sample(s) or by keeping them at a low activity rate. The requirement for live samples for identification of certain organisms, such as naked flagellates, should not be ignored.
No single fixation method can be expected to be suitable for the preservation of all types of phytoplankton.

7.2. Glutaralderhyde

When examining live samples a gentle "fixing" agent, such as glutaralderhyde may be used if swimming disturbs the examination/counting (Trondsen 1996). This fixative should also be used if later examination by a taxonomic expert who might need to use electron microscopy is contemplated (e.g. as in the case of a particularly unusual occurrence).

7.3. Lugol's Iodine

Fixation should take place immediately after sampling. The recommended fixative for routine sampling is Lugol's Iodine (100g potassium iodide dissolved in 1 litre of distilled water and then 50 g crystalline iodine dissolved and 100 ml glacial acetic acid added). Use the minimum required to give water samples a weak brown/straw colour, between 0.4 and 0.8 ml per 200 ml sample (Willén 1976), and shake well. Containers should be clear glass bottles, as coloured glass would make it difficult to gauge the correct amount of Lugols. Plastic bottles take up iodine from the solution and so when stored for a long period samples should be checked for iodine concentration. If this is low add more Lugol's. When there is much particulate matter present or the phytoplankton is abundant the iodine will be quickly absorbed. Samples should be inspected after 24 hours to see if more Lugol's needs to be added. Note: Light increases the rate of loss of molecular iodine from samples.

Lugol's has the advantage over formaldehyde of preserving the flagella on many flagellates. The cells stain brownish yellow aiding counting, when there is not too much particulate matter. The main disadvantage is that coccoliths and also silica will dissolve with long storage.

Note that this method is suitable only for water samples; net hauls being preserved better in formaldehyde.

7.4 Formaldehyde

The agent is a 20 % aqueous solution of formaldehyde neutralised with hexamethylenetetramine (100 g hexamethylenetetramine to 1 litre of the 20% solution). For water samples 2 ml of this solution is added to each 100 ml sample and the sample shaken gently immediately to facilitate fixation. For net hauls sufficient solution is added to the sample to make up about one quarter of the volume, in order to give a final concentration of 4-5% formaldehyde. Once preserved, the samples will keep for years if stored in a dark, cool, place.

A disadvantage of using formaldehyde as a fixative/preserving agent is that it distorts the cell shape of naked species and causes flagella to be thrown off in many flagellates. Bleaching of cell contents may occur rendering it difficult to distinguish between pigmented and non-pigmented cells.

8. ANALYSIS METHODS AND EQUIPMENT

The chosen method of analysis is determined by the aim of the study.

Cells may be counted with a Coulter counter or flow cytometer, although these methods are best suited for detritus free conditions. They have the disadvantage of not providing species identification, or evaluation of appearance such as colony formation or size. Use of a standard light microscope or inverted microscope such as the Zeiss Axiovert 10 is preferable. Where the population is composed of many species of different size and abundance a combination of

methods may be employed, such as settling chambers examined with an inverted microscope and counting slide on a standard light microscope.

8.1. Inverted Microscope (Utermöhl method)

An inverted microscope, such as the Zeiss Axiovert 10 or Wild M40, has the light source and condenser above the chamber and the objectives view the settled specimens from underneath. The microscope is fitted with a mechanical stage, with vernier scales. The stage is designed to take Hydro-Bios counting chambers of sizes from 2 to 10ml (Fig. 8.1). Ideally there should be three x10 eyepieces; one with a Whipple graticule and one with a cross-hair graticule (Fig. 8.2). The various squares of the Whipple graticule are calibrated by use of a micrometer slide and the results tabulated and kept with the microscope. The cross-hair graticule is calibrated by measuring the distance between the two side hairs so that when multiplied by the diameter of the sedimentation tube the area of one traverse can be calculated. Magnification is required at x6.3 or 10, x20 phase objectives, x40 phase and x100 phase water or oil immersion.

8.1.1. Counting Chambers

A number of 2 ml, 5 ml and 10 ml chambers should be available sufficient for the scope of work proposed. The Hydro-Bios type are recommended . Each should be indelibly marked with a reference number.

Hydro-Bios chambers consist of a threaded metal base plate with screw-in 'plexiglass' tubes which hold a glass cover-slip in place to provide the viewing window at the bottom of the chamber. If leakage occurs around the cover-slip it can be sealed with a smear of grease, such as Apiezon AP101, applied carefully to the base plate. This should then have the chamber screwed in and excess grease removed before dismantling and fitting the cover slip.

To carry out a primary sedimentation of volumes greater than 10 ml clean glass measuring cylinders should be used.

Judgement should be used to determine the ideal sedimentation volume. This should be determined by phytoplankton density, amount of detritus and purpose of the study. Experience will help with the determination of the volume from the colour of the sample, from previous records or from chlorophyll *a* data. For an initial study, settle samples in a range of chamber sizes to determine ease of counting. Whatever volume is selected clear records must be kept for each sample showing:

Sample location
Sample site
Date of sampling
Chamber number
Sedimentation volume
Sedimentation date
Sedimentation time

A sample with a sparse population and little detritus is best examined in a chamber with a bottom area relatively small compared to the volume. Samples with a high density of detritus, frequently found in estuarine and turbulent inshore waters, are more easily examined in a chamber with a large bottom area relative to the volume. It may also be necessary to dilute such samples.

Tangen (1976) found that an exactly horizontal table, fitted with a spirit level, assured a more homogeneous distribution of the sediment on the chamber floor, and reduced leakage.

The sedimentation time is determined by the fixative used, the volume of the sample in the chamber and the size of the organisms in the sample. The time taken to allow samples to settle is normally at least one hour per millilitre (Lund et al, 1958) for small chambers but 3 hours is often adequate for a 10 ml chamber. Providing that enough chambers are available, it is preferable to standardise on a longer time such as overnight (approx. 12 hours) for all samples. Lugol's fixed samples settle more quickly than formalin fixed samples since the iodine increases the density of the plankton cells.

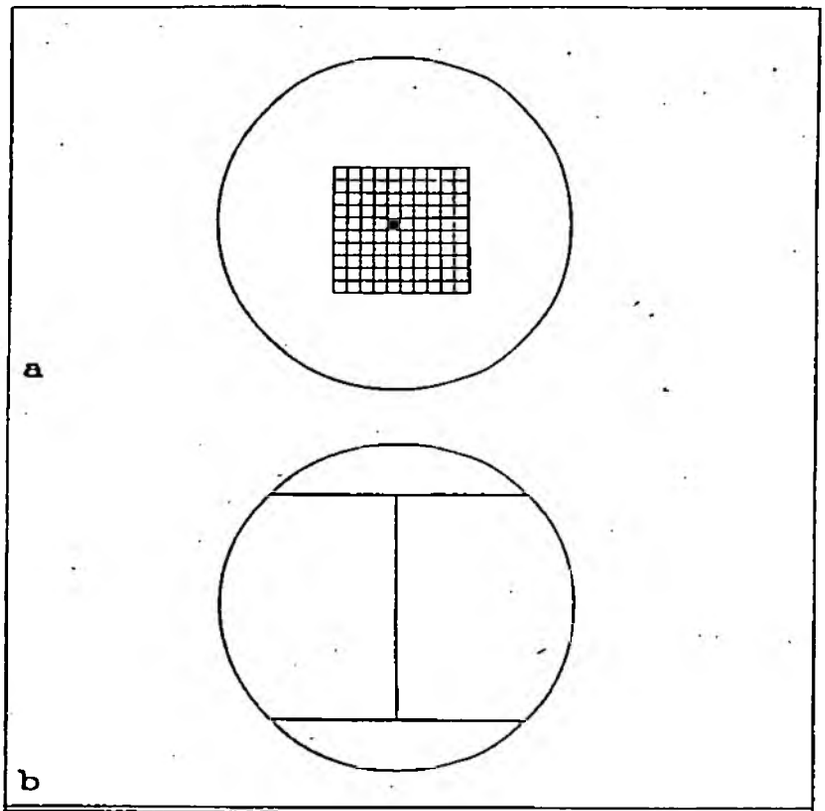


FIG: 8.2 (a) Whipple graticule.
(b) cross-hair graticule.

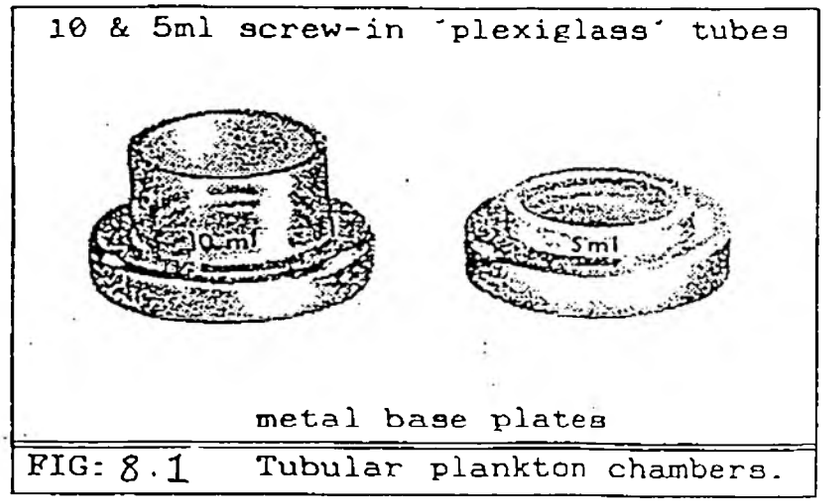


FIG: 8.1 Tubular plankton chambers.

8.1.2. Glass Cylinders

Glass cylinders, used for initial sedimentation, must not be filled above a height five times that of the cylinder's diameter, as convection currents can prevent some forms from sedimenting. Cylinders should be thoroughly washed immediately after use, and rinsed in filtered water.

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 sediment is then transferred to a 100 ml cylinder re-sedimented and the sediment finally transferred to a counting chamber.

8.1.3. Siphoning Cylinders

Some form of siphoning device is required for removing the supernatant with the minimum of disturbance. 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. 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.

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.

8.1.4. 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 greater than 90%, 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.

8.1.5. Quality Assurance

* The microscope should be kept clean and covered when not in use.

* Lenses should be cleaned with lens tissue.

* The microscope should be serviced annually.

* Samples should be at room temperature before filling the chamber to prevent air bubbles forming on the chamber walls.

* During the settling period the temperature should be fairly constant to prevent convection currents interfering with sedimentation. Similarly exposure to sunrays should be avoided.

* Chambers should be cleaned immediately after use with a jet of water from a wash bottle. They should be wiped dry with a medical wipe and stored inverted or covered.

* The glass cover-slips of chambers should be replaced regularly.

* Clear records must be kept with the chambers to avoid confusion.

8.2. Standard Microscope

8.2.1. Counting Slides

Living cells are best examined and identified with a conventional light microscope and are counted on a counting slide such as a Sedgwick-Rafter or Palmer-Maloney Counting Slide or, where the plankton consists of very small sized cells (less than 20μm), with a standard haemocytometer. When examining live samples a gentle "fixing" agent, such as glutaralderhyde or the short term fixative Uranyl acetate, may be used if swimming disturbs the examination/counting (Throndsen 1996). This method is not recommended for routine samples but only for *ad hoc* qualitative examination.

Any cells over stained by iodine, following Lugol's fixation, may be decolourised by adding one drop of a saturated solution of sodium thiosulphate.

Throndsen (1996) recommends Palmer-Maloney counting slides for routine surveys. He uses a standard microscope fitted with x10 x20 and x40 objectives and preferably with phase contrast optics. A x100 oil-immersion objective will be necessary for identification of the smaller organisms. A Whipple graticule should be fitted in the eyepiece, calibrated with a ruled micrometer slide. As with the chambers for the inverted microscope a settling time is necessary for counting slides. A Palmer-Maloney chamber holds 0.1 ml, whereas the Sedgwick-Rafter slide holds 1 ml. The microscope should have a mechanical stage. Haemocytometers come with grids marked on them and eyepiece grids are unnecessary.

Before counting, examine the chamber under low power to detect obviously unsatisfactory distributions of cells. Counts can be made of the whole chamber or in a given number of randomly selected Whipple fields or grids.

8.2.2. Quality Assurance

* Counting slides should be scrupulously clean. After the removal of sample with distilled water they can be cleaned with ethanol, 'denatured' alcohol or acetone and dried with a medical wipe.

8.3. Identification and Counting of Plankton in Water Samples With a High Detritus Content

These should be diluted as necessary and settled in 5 ml tubes in order that cells are visible. The degree of dilution depends on the amount of sediment in the sample but should be carefully recorded so that results can be related to the original sample size.

9. ENUMERATION TECHNIQUES

9.1. Introduction

Success of counting depends to a large extent on the chosen sampling programme. Normally counts are made from subsamples of the initial field sample. Phytoplankton cell concentrations can vary enormously and an individual species may be rare but of significance rendering its detection important. For this reason it may be necessary to concentrate the original sample.

In nature the actual numbers of phytoplankton species at any one time cannot be known, making testing of methodology for accuracy impossible. At each stage variability is introduced into the data. The nature of phytoplankton distribution rarely matches the conditions demanded by statistical methods but by careful sampling and analysis the best possible results can be obtained.
It is often preferable to make counts of several replicate samples rather than to rely on a count of a single sample.

Phytoplankton samples usually need to be examined at more than one magnification. Larger taxa, such as the dinoflagellate *Ceratium* and the diatom *Biddulphia* can be identified at low power and the whole chamber scanned in a few minutes by horizontal or vertical transects. Smaller taxa are identified at high magnification. If immersion objectives are required for identification it is often best to record the position of the cell and return to it after the entire count has been completed. Care must be taken to avoid counting the same cell twice when the

cell, for instance, overlaps the grid or threads in an eyepiece. 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. It is recommended that at least two replicate subsamples are counted to increase confidence limits.

9.2. Cell Counts

Laboratory counters with five or more keys are useful for counting predominant species. Depending on the purpose of the investigation all organisms encountered should be identified to species, group of species, genus or algal group.

Phytoplankton is counted in algal units i.e. an individual cell or a colony. The number of cells in a colony is also counted and recorded, although this can be done by counting a set number (10 or more) after completion of the scan if this is more convenient.

It is common practice in dense phytoplankton samples to count only a fraction of the sample and estimate the total aliquot count from this.

Whether the entire base of the chamber, alternate stripes (Utermöhl 1958), or one or more diameter transects are counted is a matter of judgement dependent on the nature of the sample. Diameter transects help to eliminate any bias caused by uneven settling in the chamber. The ratio of the whole bottom area of a chamber to one diameter transects is π n/4, where n is the ratio between diameter and width of the diameter transect. The total number of cells in the whole chamber is found by multiplying the cell numbers by this formula.

For very small organisms where high magnification is required, it is customary to count a number of Whipple fields and average the result. The number of fields counted should be adequate to give a total of at least 120 algal units.

9.2.1. Number of Cells to Count

Assuming a Poisson distribution, it is normally considered that a count of at least 100 cells should be made to give a reliable cell number estimate to 95 % confidence interval of the estimate within +/- 20% of the mean value. A count of 400 cells should give a precision of +/- 10% of the mean (Lund et al 1958).

There may be an advantage in accepting lower precision in order to reduce the counting time. One short-cut method involves recording the presence or absence of species in a set of random fields or subsamples (Legendre and Watt 1972). The method assumes a Poisson or binomial distribution of cells in the sample. The density of material or the size of each field should be regulated so that the most abundant species is present about 80 % of the time. It is usually sufficient to count 30 fields.

9.2.2. Enumeration Options

9.2.2A. Random Transects

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 (Figure 8.2). 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. 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, 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.

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.

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 achieved. 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.

9.2.2B. Random Fields

Nanoplankton 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 fields, 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.

9.2.2C. Placing Random Fields

A simple, but not entirely effective, way of assessing randomness is to conduct 'dummy' counts using specially modified cover-slips 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'.

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 Chi^2 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 and if randomness is not disproved, then move onto the edge/centre test. Another cover-slip could be made up. 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 major 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.

9.2.2D. 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.

9.2.3. 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, unimodal or bimodal in some colonial species. The cell numbers of other colonial algae have an inconsistent distribution which can vary from a few to tens of millions, eg as in *Phaeocystis*.

For low density colonial units it is most efficient to enumerate the cells 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.

Phaeocystis colonies are often too large to enumerate using sedimentation chambers, and an alternative method may be employed. 10ml of a sample containing *Phaeocystis* is placed in a petri dish, over suitable graph paper, and sub-samples (in the squares of the graph paper) counted using a binocular microscope. The calculation for colonies per volume of sea water is as follows:

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

9.3. Calculating Cells per ml or Litre

Cell numbers obtained from counts in sedimentation chambers or counting slides can be converted into cells per millilitre or litre:-

$$\text{Cells/ml} = \frac{\text{No. of units counted}}{\text{No. of fields}} \times \frac{\text{area of chamber/or fields}}{\text{sample volume in mls}}$$

9.4. Enumeration Methods

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. 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. 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.

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.

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.

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. 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.

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 $> \pm 50\%$ 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.

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.

9.5. 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 Procedures for Toxic Algae, see Appendix V. This lists abundance values relating to bloom levels for common bloom forming algae. If a bloom is identified then the relevant Quality Officer should be notified, as soon as possible by fax. A Significant Blooms Report should be completed for all samples.

analysed (see Appendix V), and copies sent to the relevant Catchment and Quality Officers, the Principal Biologist, and one retained on file.

9.6. Cell Size

Cell numbers alone may not give a totally accurate picture of the community, particularly as phytoplankton cell sizes can be between 1 and 2000 μm in diameter and their volume is even more diverse. Lohmann (1908) was the first investigator to recognise the need for plasma volumes for determining standing stock of phytoplankton. Cell size is used for taxonomic purposes as well as for biomass estimations. Cell size can vary according to the age of the organism and season (Margalef 1963). Large species may be less abundant than smaller species but their biomass may be higher. In general smaller forms have a more intensive metabolism and divide faster than larger forms; they may also be the main producers in a nutrient rich area.

The calculation of species cell volumes to estimate phytoplankton community biomass is complicated by the variations that can occur in both time and space and it is therefore unwise to rely on measurements taken elsewhere. When size/volume is thought to be important, a set number of cells in the sample should be measured and the figure applied to the cell counts. The effects of fixatives in shrinking cell size and cell vacuoles should also be borne in mind, for this can affect plasma volume.

9.7. Summary of Cell Enumeration Procedures

- Select the method most appropriate to the investigation in hand.
- Ensure that samples are well mixed before subsampling.
- Count an adequate number of cells to be statistically significant.
- Regularly check calculations to ensure that the correct cell concentration is being recorded.

10. Chlorophyll Analysis

10.1. Introduction

Chlorophyll *a* is widely used as a measure of phytoplankton, although it can be a poor estimate of biomass because it assumes algal cells to be homogeneous with respect to their chlorophyll concentration. The sensitivity of the method described here is adequate except where the chlorophyll content of the water is below 0.2 mg/m^3. Chlorophyll degradation products (phaeophytin or phaeophorbide) in sea water samples can be determined by chromatography, although this is a lengthy process and unsuitable for routine applications.

10.2. *In-vitro* Spectrophotometric Methods

Samples are best analysed on the day of collection, or at most after overnight storage in the dark in a refrigerator or cool (4° C) place. Samples should be kept out of strong light and not exposed to high temperatures in transit. If these criteria cannot be met samples may be frozen on filters, following the filtration step.

Chlorophylls are unstable compounds and very sensitive to light and air, undergoing isomerisation and oxidations. Samples therefore must be subject to minimum exposure to light and air. All work should be carried out in dim light and solutions should be kept in glassware, covered with a black cloth.

The volume of water to be analysed will be determined by the local situation and the expected chlorophyll concentration. In addition factors such as the amount of detritus (which can severely impede filtration) will affect the volume used.

Normally a one litre sample of sea water is filtered through a Millipore filter apparatus fitted with either a 47mm diameter Millipore AA filter or a 4.5 Whatman GF/C glass filter paper. Glass filters are cheaper and are recommended if a cell grinding stage is required, to give better extraction. A manostat must be used with glass papers to ensure that the suction never exceeds 0.25 - 0.33 atm as some pigment may then pass through the filter. It was previously standard practice to add magnesium carbonate at the filtering stage in order to prevent phytoplankton chlorophyll becoming acid and decomposing to give phaeophytin pigments. This step is now considered to be unnecessary. Care should always be taken to ensure that filtration equipment, including filters, centrifuge tubes and spectrophotometer cells are kept free from acid.

Note: if large zooplankters are present these are first removed by straining the sea water through a clean 0.3mm nylon mesh.

The filter is drained thoroughly under suction before removing it.

10.2.1. Chlorophyll Extraction Methods

Filters should be extracted as soon as possible, but when this is impractical they can be stored by folding in half, with the plankton inside, and placed in a specimen tube, in the dark, in a desiccator frozen to -20°C. Specimens will keep for a few weeks but such storage usually results in an underestimation of chlorophyll levels.

Ethanol is now the preferred solvent for total chlorophyll extraction, in line with European recommendations (see DIN 1986, DS 1986). Methanol is permitted as the second choice but since much greater care must be exercised in its use (COSHH) this will reduce the speed of analysis and it is consequently not recommended here. Acetone extraction is normally used for the separate measurement of chlorophylls *a*, *b* and *c* and other chloroplast pigments.

10.2.2. Ethanol Extraction

Filter a measured volume of sample through a glass-fibre filter clamped in a suitable holder. For most natural waters 1 litre is a suitable volume but this may need to be adjusted if the phytoplankton content is abnormally high or low.

After filtration is complete, reduce the residual water by allowing air to be drawn through for a short time, approx. 30 seconds.

Discard the filtrate.

Remove the filter from the holder and allow to dry in the dark.

Weigh the filter to determine the weight of residual water.

Fold the filter three times and transfer to a test tube.

Follow **one** of the following methods:-

a. Add a known volume of hot (70°C) ethanol, sufficient to cover the filter. This will usually be 15 or 20 ml. Carry this out in a fume cupboard.

b. Add a known volume of cold ethanol, usually 15 or 20 ml, sufficient to cover the filter, and heat to boiling by dipping the tube in a water bath held at a temperature just above the boiling point of ethanol (78.5° C). Boil for 2-3 seconds with the tube covered to prevent loss of solvent.

c. Add a known volume of cold ethanol, usually 15 or 20 ml, sufficient to cover the filter.

Stopper the tube and place in a dark, cold (4° C), place for 12 hours (e.g. overnight). Agitate the filter from time to time.

Add distilled water so that the final concentration is 10% water in ethanol. If 20 ml of 100% ethanol was used initially, a total of 2.2 ml of water will be needed to make the final solution 90%, but take into account the residual water in the filter, which should weigh between 0.5 - 0.7 g for a 9 cm filter, and adjust the water accordingly.

Operating in dim light, ensure that the sample is well mixed, using a vortex stirrer. With forceps, remove the filter paper from the ethanol and squeeze it against the side of the tube so that as much ethanol as possible drains back into the tube.

Either, centrifuge the extract in a stoppered tube to obtain a clear extract, or, filter the extract through a small GF/C filter into a clean tube. Retain the extract in a stoppered tube, in the dark, for absorbance measurements.

Fill a stoppered spectrophotometer cuvette (generally 10 mm or 40 mm pathlength) with the pigment extract. Let the pathlength used be d mm.

Use 90% v/v aqueous ethanol in the reference beam of the spectrophotometer. (Note that 10 mm pathway cuvettes require 3 ml of extract whereas 40 mm pathlength cuvettes require 10 ml.)

Measure the absorbance of the extract at wavelengths of 665 nm and 750 nm. Note that absorbance at 665 nm should fall within the range of 0.05 to 0.70 units, otherwise adjust either the volume of the sample, the volume of aqueous ethanol, or the pathlength of the cell, to meet

these criteria. Absorbance at 750 nm should not exceed 0.005 units per 10 mm of pathlength i.e. 0.02 units in a 40 mm pathlength cell.

Subtract the absorbance value obtained at 750 nm from that obtained at 665 nm and let this be A.

The chlorophyll *a* content of the sample expressed as $\mu g\ l^{-1}$ (= mg m^3) is obtained from the equation:-

$$\frac{12.2 \times A \times v}{d \times v}$$

Where A = net absorbance
v = volume of solvent in ml
V = volume of original filtered sample in litres
d = cell pathlength in cm.
12.2 = the reciprocal of the specific absorption coefficient at 665 nm for chlorophyll *a* in ethanol.

Note: this calculation makes no correction for the presence of degraded matter or other pigments.

10.2.3. Acetone Extraction

This method is only recommended when it is required to separate other pigments in addition to chlorophyll *a*.

Filter the sample as described above.

Place the filter in a 15ml stoppered graduated centrifuge tube. For Millipore filters add approximately 8ml of 90% acetone and shake the stoppered tube vigorously to dissolve the filter. If a glass paper was used add approximately 10ml of 90% acetone and shake the stoppered tube vigorously to disintegrate the paper. A "Potter" type grinder may be used to which the glass filter and 2ml of 90% acetone is added and the grinder run for 1-2 mins in subdued light. After use the pestle should be rinsed into the tube with a few millilitres of 90% acetone and the contents of the grinder tube transferred to a 15 ml centrifuge tube. The total volume in the centrifuge tube should not exceed 10 mls. Allow the pigments to be extracted by placing the tube in a refrigerator in complete darkness for about 20hr.

Remove the tubes and allow them to reach room temperature, in the dark. Add 90% acetone to make the extracts from Millipore filters up to exactly 10.0 ml and those from glass filters to exactly 12 ml, to allow for the solvent left in the pulp.

Centrifuge the content of the tubes, with plastic stoppers, for 5 - 10 minutes. In most centrifuges 3000 - 4000 rpm for about 10 minutes is satisfactory. When glass filters have been used tubes should be centrifuged for about 2 minutes and the tubes removed and flicked gently to remove any fibres adhering to the side of the tube. The tubes are then returned to the centrifuge and spun for a further 5 minutes.

Decant the supernatant liquid into a 10 cm path-length spectrophotometer cell designed to hold 10ml or less of liquid.

Without delay measure the extinction of the solution against a cell containing 90% acetone at 750nm and 665nm.

Calculation of amount of chlorophyll *a*.
Subtract the absorbance value obtained at 750nm from that at 665 and let this figure be A.
The chlorophyll *a* content of the sample, expressed as $\mu g\ l^{-1}$:-

$$\frac{11.9 \times A \times v}{d \times V}$$

Where A = net absorbance
v = volume of solvent in ml
V = volume of original sample in litres
d = cell pathlength in cm
11.9 = the reciprocal of the specific absorption coefficient at 665 nm for chlorophyll *a* in acetone.

10.2.4. Quality Assurance Procedures

It is recommended that tests to check sources of inaccuracy are made regularly.

* Always use matched spectrophotometer cuvettes

* Number these cuvettes on their base and always use them in the same location in the multiple cell holder and always in the same orientation.

* Before using, check the variations between cuvettes by measuring the absorbency at 750 and 665 nm, filled with the standard solvent in normal use. These values will give the extent of cuvette to cuvette differences and must be recorded in a log book. When used regularly, for example every month, changes will indicate deteriorating optical surfaces or accumulated deposits.

* Spectrophotometers nowadays have built-in wavelength checking mechanisms which are activated when they are powered up. Standard didymion filters, which have two sharp absorption maxima at 573 and 585 nm, can be used to check the wavelengths.

* Standard solutions of potassium dichromate ('Spectrosol' for calibration of spectrophotometers obtainable from Merck) can be used to check the absolute accuracy of the absorbency reading.

* The absorbency of the extract is partly controlled by the volume filtered, the volume of the extractant and the path length of the cuvette.

10.3. *In Vivo* Fluorescence

This method is recommended where sample volumes are restricted or very high inorganic concentrations prevent efficient filtration. Also it is the standard method where on-board pumped water systems are used in connection with an auto-analyser for continuous study of nutrients or pollutants. This method uses a commercially available system (e.g. Aquatracker II from Chelsea Instruments, London) which, when used in accordance with the manufacturers instructions, gives an estimate of chlorophyll *a* per volume of water.

Measurements of chlorophyll *a* fluorescence are made at 680 nm by excitation of the water sample by light of 430-450 nm.

It is essential that regular comparisons are made between the results obtained with this method and by means of spectrophotometry, as described above. Checks should also be made for background fluorescence which can interfere with the result for chlorophyll.

10.4. Aerial Surveillance

Aerial surveillance is achieved via satellite or aircraft mounted monitoring devices. These measure visible-wavelength light and can be used to generate coloured maps showing suspended

sediment distribution patterns and the concentration of the sediment. Multi-spectral scanners can be used for pollution monitoring, mapping red tides and can provide information of chlorophyll concentration. Chlorophyll absorption and fluorescence is between 650 and 700 nm, with a peak at 685 nm. Different pigments in some phytoplankton are detectable at other peaks. More work is required to evaluate aerial surveillance results with those obtained by analysing water samples in the laboratory by standard methods.

Further use should be made of spectral signatures for identifying organisms such as *Noctiluca* and some bloom-forming diatoms.

Further information regarding the availability of aerial surveillance may be obtained from:

Environment Agency
National Centre for Instrumentation & Marine Surveillance
Rivers House
Lower Bristol Road
Bath, BA2 9ES
(Tel: 01278 457333).

10.5. Chlorophyll *a* and Cell Counts

Comparisons and correlations between cell counts and chlorophyll *a* levels should be made to build up an understanding of the environment and for potential model building. It has been estimated that 4 μg of chlorophyll *a* are approximately equivalent to 1 cm^3 of cell volume. If the chlorophyll *a* result is significantly greater than the combined cell volumes an error exists. It should be borne in mind that computation of cell volumes is not without its difficulties and sources of error.

The dominant taxa responsible for chlorophyll *a* peaks can be determined by examination of cell counts only if straight water samples have been counted and that a bias has not been introduced, such as loss of smaller plankton by filtration or fixation.

11. DATA HANDLING AND ANALYSIS

11.1. Introduction

There is no internationally agreed format for the exchange of phytoplankton data, nor any agreed system of numerical labels for species or other taxonomic categories (Colebrook in Sournia 1978).

The objective of a data storage system is to facilitate retrieval and processing. The format and structure of the stored data is determined by the requirements of the retrieval procedures.

Computer database systems are now more user friendly and allow for simple inputting of data, and mathematical manipulation. Robson and Bailey-Watts (1978) describe a system for recording and sorting plankton data.

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.

11. 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, and the one 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 the phylum Cyanophyta, 0132?? for the genus Oscillatoria and 013208 for the species *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.

11.3. 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.

11.3.1. Basic Data Base

Figure 11.1 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 11. 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.

11.3.2. Support Data Base

Figure 11. 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.

11.4. 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 re-examined. 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.

12. REPORTING

12.1. Introduction

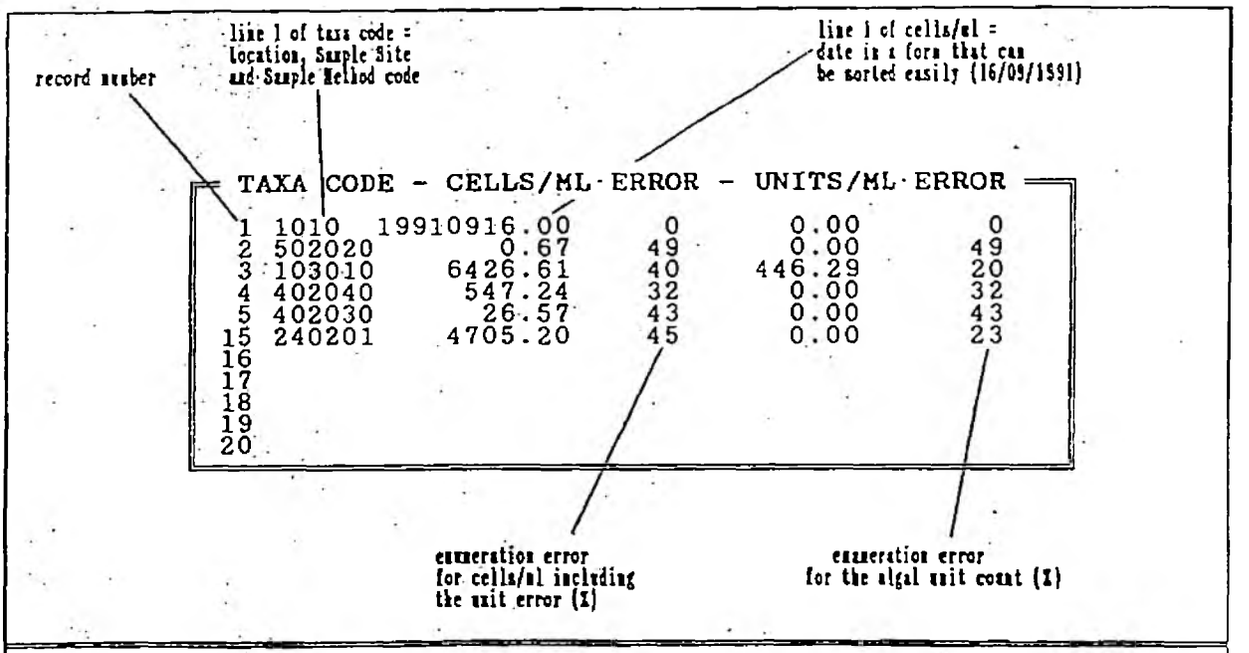
Standardisation of reporting, where possible, would simplify report writing, comparison and interpretation. Before writing a report it is important to identify the 'target', and pitch the report at an appropriate level. There is no point in producing a large scientific document if it is not going to 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.



	TAXA CODE	CELLS/ML	ERROR	UNITS/ML	ERROR
1	1010	19910916.00	0	0.00	0
2	502020	0.67	49	0.00	49
3	103010	6426.61	40	446.29	20
4	402040	547.24	32	0.00	32
5	402030	26.57	43	0.00	43
15	240201	4705.20	45	0.00	23
16					
17					
18					
19					
20					

FIGURE 11. 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.

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: RO2BA22H22
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 11. 2 Record sheet from support data base, see text.

12.2. Special Requirements

Reports on marine phytoplankton should include the following chemical and physical data, together with the phytoplankton results:

Chlorophyll *a*
Total phosphate
Ortho-phosphate
Total oxidised nitrogen
Ammonia
Silica
pH
Conductivity
Salinity
Dissolved oxygen
Temperature
Light penetration (Secchi disc and/or meter)

All the above should be shown graphically in the results section, with the 'x' axis as a time scale. Phytoplankton results should be plotted as natural logs. against time. Where possible, depth profiles should be obtained for dissolved oxygen, salinity and temperature. These are presented as isopleths.

13. TAXONOMY

13.1. 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 an 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.

13.2. Texts

Recommended literature, including taxonomic works, are listed in Appendix III. 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 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, 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.

Many of the standard algae texts 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.

It is a good idea to visit academic libraries to explore other material. Access to the libraries of the Freshwater Biological Association at Ferry House (FBA - Tel: 015394 42468) and Plymouth Marine Laboratory (PML - Tel: 01752 222772), are open to Environment Agency staff, for reference only, with prior permission from the relevant Librarian. Before visiting the library, clearance should be gained both from your line manager and the Head of Information Services at Ferry House or the Librarian at Plymouth.

13.3. 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 an established worker. In these cases particular attention should be paid to the taxonomy during the transition period.

13.4. Unidentified Algae

If an alga cannot be identified within a reasonable time period then it should be described as thoroughly as possible, along with a labelled drawing, and 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. 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 by centrifugation may help) then it may 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 Training Section 14 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. Batches of unidentified material could be taken to the Natural History Museum (London), where the facilities exist for examining their extensive collection of mounted material.

13.5. Examining Live Algae

The value of examining live algae should not be underestimated. In many groups colour, and type of movement provide major clues 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).

13.6. 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. The collection could be kept in a ring binder or on index cards.

13.7. 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'.

13.8. Taxonomic Audits

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 an 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.

13.8.1. 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 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.

13.8.1.(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, 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.

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

13.8.1.(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 (preferably a mixture of species), 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 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.

13.8.1.(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 13.5). Although a system would have to be devised, spot check audit results may lend themselves to a statistical quality control system, of some type.

13.8.2. External Taxonomic Audits

External taxonomic audits are a necessary component of overall taxonomic quality control, 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 13. 4. The results of all external audits should be made known, and this would be an important training aid.

14. TRAINING

Training is an important feature of a quality system. Not only is it essential for beginners, but it is necessary for established staff to be informed of new developments, both in their own region and in other regions of the Environment Agency and Europe.

Where possible, training should be given by experienced staff. Safety and quality of work should be given special attention. Identification of species requires an introduction to taxa, help when required and if possible reference slide, photographs and drawings of species likely to be encountered. If experienced inhouse staff are unavailable training days should be organised using outside experts. A list of marine phytoplankton specialists (with their particular area of expertise indicated) who could run such courses, is given below:

Diatoms
Cox, Dr E.
The Natural History Museum, Cromwell Road, London SW7 5BD
Tel 0171 938 9001.
Dinoflagellates: toxic algae
Dodge, Prof. J.D.
School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX.
Tel 01784 443774.

Lewis, Dr J.
School of Biological and Health Sciences, University of Westminster,115 New Cavendish Street, London W1M 8JS
Tel 0171 911 5000.

Haptophytes and Prasinophytes
Green, Dr J. C.
Plymouth Marine Laboratory, Citadel Hill, Plymouth, PL1 2PD.
Tel 01752 222772.

Cryptophytes
Lucas, Dr I.A.N.
School of Ocean Sciences, University of Wales, Menai Bridge, Gwynedd, LL59 5EY
Tel 01248 351151.

APPENDICES

Appendix I Fixatives and Safety Precautions

A. 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 I_2 are then added, followed by 10 ml of acetic acid. This should be carried out in a fume cupboard while wearing gloves. Stock solutions should be kept in a stoppered container, preferably thick polythene, and kept in a safety cupboard. A dropper bottle containing c 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 of m, 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

B. 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 of buffers 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 II International Distress Signals

[I] At Sea

a) Whistles and torches. Morse-code signal 'SOS' is 3 short blasts/flashes followed by 3 long blasts/flashes and 3 short blasts/flashes. The signal is repeated.

b) Red flares or orange smoke.

c) Outstretched arms, raised and lowered slowly and repeatedly.

d) an oar with a cloth tied to it, waved slowly from side to side.

[II] On Land

6 long flashes/blasts/shouts/waves in succession, repeated at one minute intervals.

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Appendix V. Monitoring Procedures for Toxic Algae

Chapter 7 NRA Monitoring Procedures

7.1 Introduction

Marine algal blooms most commonly cause aesthetic nuisance in bathing waters and on beaches when large accumulations, foams or scums are washed in shore. These may be present on the surface of the water and/or be deposited on beaches and can result in offensive smells as the algal material decays. Since the formation of the NRA, it has become an organisation which the public approach with any concerns about aquatic habitats. It is to be expected, therefore, that the NRA are very likely to receive reports and questions about exceptional algal blooms in coastal waters. The common public perception that they are some sort of pollution incident will reinforce the likelihood of information and action being sought from the NRA. Additionally, NRA staff regularly monitor designated bathing beaches (E.C. Directive 76/160), and thus has the infrastructure to monitor coastal waters for such exceptional events. In view of the increasing international concern over the effects of eutrophication of coastal waters and the apparent increase in incidents of algal blooms around the U.K. coastline, the Department of the Environment, via the Marine Pollution Monitoring Management Group (MPMMG) requested the NRA to implement some monitoring of algal blooms in coastal waters. It was proposed that the Marine Algal Monitoring Programme might be built on to the existing NRA Bathing Waters Monitoring Programme as an opportunistic and cost-effective means of obtaining this information at most relevant sites. The NRA implemented such a programme in 1991. Initially, the DOE had asked that instances of exceptional blooms be reported to them directly, although in the event no reporting mechanism was established. Subsequently, the Ministry of Agriculture, Fisheries and Food have had to implement the E.C. Shellfish Hygiene Directive which requires algal monitoring in shellfish production areas. Again, the network of NRA staff is more extensive than that of MAFF, and NRA monitoring can provide valuable extra information for MAFF with regard to potentially toxic algal species. Since 1995 close contact has been established, and regular exchange of information takes place. However, at present, there is no formal coordination of MAFF and NRA programmes.

Since 1993 the NRA has also undertaken a national baseline coastal surveillance survey. This consists of simultaneous boat and airborne surveys in spring, summer and autumn and a boat survey in winter. Various parameters are measured, but parameters of interest with regard to this report are chlorophyll *a* concentrations, various nutrient concentrations and various physical oceanographic measurements. Data from this survey is collated and stored at the NRA's National Centre for Instrumentation and Marine Surveillance.

7.2 NRA Marine Algal Monitoring Programme

A phased programme of marine algal bloom monitoring was introduced by the NRA in 1991. This proposal allowed for increased effort as resources and skills became available, and in the

light of experience within particular regions and their possible need for further, more detailed monitoring. The programme includes two levels of monitoring:

a) A "minimum effort" programme - which is incorporated within the E.C. Bathing Waters Monitoring Programme in all NRA maritime regions

b) a "best endeavours" programme to provide more information than the minimum effort programme but within the time and sampling restraints of the E.C. Bathing Waters Monitoring Programme.

The second level of monitoring has been applied in those regions that were adequately resourced and which perceived they had significant marine algal bloom problems. The "minimum effort" programme was implemented in all maritime regions during the 1991 season. Since that time, those regions with recognised microalgal problems, e.g., the Welsh and Northumbrian regions have managed to implement the "best endeavours" programme. However, for most others the "minimum effort" programme has sufficed. The detailed methodology for these programmes appears in Appendix C.

7.3 Reporting

7.3.1 Guidance on "Notifiable" Toxic Marine Algae Species

The following list of marine algae (Table 7.1) 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 list should be used as a guide as to when a toxic algal bloom report needs to be made (see Appendix C). Other species such as *Noctiluca scintillans, Phaeocystis pouchetii, 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. When toxic or potentially toxic algal blooms are present, the toxic algae bloom report form in Appendix C should be used for communicating the information to TAPS.

All completed forms should be sent to the toxic algae scientist, NRA, Anglian Region, Peterborough for collation and forwarding to the national network on toxic algae. If *Alexandrium, Dinophysis* or *Pseudo-nitzschia* are present at action levels (see Table 7.2), the report should be faxed (01733 464 270) and the toxic algae scientist will inform the MAFF.

Table 7.1 NRA Notifiable Toxic Marine Algae Species

CLASS	SPECIES	TOXIC EFFECT
Bacillariophyceae	*Pseudo-nitzschia* spp.	Amnesic shellfish poisoning (ASP)
Dinophyceae	*Alexandrium* spp. *Gymnodinium catenatum*	Paralytic shellfish poisoning (PSP)
	Dinophysis acuminata *D. acuta* *D. norvegica* *Prorocentrum lima*	Diarrhetic shellfish poisoning (DSP)
	Amphidinium carterae *Gyrodinium aureolum*	Icthyotoxic
Haptophyceae	*Chrysochromulina polylepis* *Prymnesium parvum*	Fish Kills
Raphidophyceae	*Fibrocapsa japonica* *Heterosigma akashiwo*	
Cyanophyceae	*Nodularia spumigera*	Contains hepato toxins

Table 7.2 Action Levels for Various Toxic Marine Microalgae

Species	Numbers
Dinophysis acuminata *Dinophysis acuta* *Dinophysis norvegica*	Present at a density of greater than 100 cells per litre
Alexandrium tamarense *Gymnodinium catenatum* *Prorocentrum lima*	Present at a density of greater than 100 cells per litre
Pseudo-nitzschia spp.	Present at a density of greater than 10^6 cells per litre

7.3.2 Problems of Identification

It is important that staff are cognisant of the limitations of their algal identification skills. Where there is uncertainty about identification, this must be expressly stated and efforts made to be as consistant as possible. Any significant incident should be adequately documented. Where possible, this might include photomicrographs of the species concerned or line drawings where photography is not available. In these cases it is important that a reference sample is kept which can be consulted at a later date if required. If the incident is particularly significant and a firm identification required, then Appendix D should be consulted. It will be important to try and recognise as many features as possible about species under consideration. In particular live observations are valuable in this context. Once such observations have been made, staff might consult the the policy document to obtain a list of scientists who have specific taxonomic expertise. To consult the relevant expert, it will probably be necessary to identify the organism at least to class. Staff are encouraged to attend training courses where these are available.

7.3.3 Responsibilities of Toxic Algae Scientist

On receipt of toxic algal bloom report, the Toxic Algae Scientist will contact the MAFF plankton laboratory (Chapter 6). The Ministry scientist will subsequently arrange any further plankton sampling required and shellfish flesh sampling as necessary directly with the local contact.

The Toxic Algae Scientist will also hold a list of active researchers, who would wish to be informed about bloom events in order to carry out research upon them. These researchers will be informed by fax/email of such exceptional events.

Routine and exceptional reports will be collated into an annual report by the TAPS National Centre. This Annual Report will be circulated to the national network on toxic algae; active researchers as identified above and also to the representative for England and Wales on the ICES Harmful Algal Blooms committee.

7.3.4 Further Sampling

In the case of a widespread exceptional algal bloom, it might be necessary to determine the extent of the bloom. In this case some aerial form of survey might be useful. The National Centre for Instrumentation and Marine Surveillance should be contacted in this case. They might have data relevant to the incident or may be able to arrange an appropriate survey. It will also be necessary to maintain a "watching brief". The level of such further sampling would be determined by the severity and significance of the event. If further sampling is required by MAFF or interested researchers, then they will request these from the regional contact directly. Where algae that are potentially toxic to humans are involved MAFF will inform the regional contact directly of the outcome of any shellfish testing. Where a toxic incident is involved, then it is anticipated that the Ministry will keep in close contact with the regional representative.

7.4 Advice to the Public

Where the NRA has been contacted directly by a member of the public, then after investigation of the incident, the enquirer should be informed as to the nature of the incident. This information might include the identities of the alga concerned and possible reasons for its occurrence. Any advice should be couched in reassuring terms. It would also be helpful to send them the NRA Marine Algae leaflet. This leaflet is reproduced in Appendix G. Where potentially toxic algae are involved, the advice should be cautious and not alarmist. In particular in shellfish production areas, advice to the public can only be given when the outcome of shellfish testing is known. In the event of a toxic incident, then the competent authorities will inform the industry, and it is only after this that general advice should be given to the public. Environmental Health Officers will, where appropriate, erect warning notices. Staff should be aware that serious economic consequences can result from toxic algal incidents, and as such must be backed by factual information on toxin levels. In these incidents the protection of public health is not in the NRA remit, and the public should be referred to the appropriate authorities. Advice on public health issues will be given by Environmental Health Officers. Advice on fisheries matters will be given by the MAFF headquarters.

GUIDANCE NOTES ON COMPLETION OF TOXIC ALGAL BLOOM REPORT

1. The 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 (e.g., it may become toxic)

2. All completed forms should be sent to the toxic algae scientist, NRA, Anglian Region, Peterborough for collation and forwarding to the national network on toxic algae. If *Alexandrium*, *Dinophysis* or *Pseudo-nitzschia* are present at action levels (see Table 7.2) the report should be faxed (01733 464 270) and the toxic algae scientist will inform the MAFF.

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 (e.g., 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.