Environment Agency
Anglian Region

Development of Marine Phytoplankton Methodology and Quality Assurance Procedures

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EA-Water Quality

CONTENTS

1. INTRODUCTION

Professor J.D. Dodge of Royal Holloway, University of London / Algal Associates was commissioned to write this report in October 1995.

The objectives of the project were to:

- Assess and develop an approach to marine phytoplankton sampling and analysis for a variety of needs.

- Identify and compare the available sampling and analytical techniques, assess their efficiency and comparability. This will enable the selection of suitable methods for use in the Environment Agency Anglian Region for the production of robust data using standard, tested methods.

- Develop suitable quality control procedures for all aspects so that the data will be checked giving increased confidence in the findings of the surveys.

The methodology was to include discussions with key Agency staff and a review of the available literature. Staff and others contacted are listed in Appendix I.

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 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 sizes.

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

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 waste water run-offs may be to blame (Anderson, 1984; Hallegraeff, 1993). Toxicity of phytoplankton can be transferred to suspension feeders rendering shellfish grown in red tide areas suspected of being dangerous for human consumption. The toxicity associated with red tides may also be felt in the effects of sea spray on man and in fish mortality rates.

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.

A degree of standardisation of methods is necessary to obtain the best use of results. 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 should be taken for cell counts and chlorophyll *a* concentration. 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. REQUIREMENTS

2.1 Introduction

Monitoring of marine phytoplankton by the NRA/Agency is undertaken to comply with the Water Resources Act (1991), and EC Directives, to assess eutrophication,and to identify toxic and nuisance species.

2.2 UK Legislation

The NRA was brought into existence by the enactment of the Water Act 1989, later superseded by the Water Resources Act 1991. The Water Resources Act requires the NRA to "monitor the state of pollution in *controlled waters*". Controlled waters comprise surface freshwaters, groundwaters, estuaries and coastal waters to the three-mile limit, in England and Wales. The Water Resources Act (1991) states that water samples should be taken throughout the period 1 May to 30 September, with additional samples taken if the water is or is likely to deteriorate.

The Environment Act 1995 establishes the Environment Agency, due to come into existence in April 1996, which will take over the functions of the NRA, Her Majesty's Inspectorate of Pollution and the waste regulation responsibilities of local authorities, to provide an integrated pollution control regulation in England and Wales.

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.

2.3 EC Legislation

The relevant EC Directives are:

- Urban Waste Water Treatment Directive [91/271/EEC],
- Bathing Water Directive [76/160/EEC]
- Nitrate Pollution Directive [91/676/EEC].
- Shellfish Waters Directive [79/659/EEC]
- Shellfish Hygiene Directive [91/492/EEC]

The Urban Waste Water Treatment Directive was introduced to protect waters from the adverse effects of waste water from sewage treatment works, and certain sectors of industry. It specifies secondary treatment for all discharges serving population equivalents greater than 2,000 to inland waters and estuaries, and 10,000 to coastal waters. It lays down minimum standards for Phosphorus and Nitrogen sewage discharges to "*sensitive areas*", that is, areas such as estuaries and coastal waters "which are found to be eutrophic, or which in the near future may become eutrophicated if no protective action is taken". The Agency's role is to gather data in support of proposed *"sensitive area"* designation. A major limitation of the directive is the definition of "eutrophication". The directive defines it as " the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of the organisms present in the water and to the quality of the water concerned". Anglian Region has no designated coastal nitrogen limited Sensitive Areas.

There is a stated requirement for good international laboratory practices to be employed, aimed at minimizing the degradation of samples between collection and analysis. The Directive does not detail the methods to be employed for sampling or analysing phytoplankton, in the way in which chemical analyses methodologies, for oxygen or suspended solids, are outlined.

The **Bathing Waters Directive** sets a range of standards which must be achieved in designated bathing waters. During the season May to September there is a requirement for weekly sampling of water to measure total and faecal coliforms per 100 ml, faecal Streptococci, Salmonella, Enterovirus, pH, colour, mineral oils, surface active substances reacting with methylene blue, phenols, transparency, dissolved oxygen, tarry residues, plastic bottles etc, ammonia, Kjeldahl nitrogen, pesticides, heavy metals, cyanides and nitrates.

Under the terms of the **Nitrate Pollution Directive** surface water is to be sampled and the eutrophic state of fresh surface waters, estuarial and coastal waters reviewed every four years.

The **Shellfish Waters Directive** is concerned with the microbiological quality of the waters from which shellfish are harvested. There is currently no requirement for NRA/Agency in this respect although some operational monitoring is carried out.

Shellfish Hygiene Directive. There is no requirement for NRA/Agency monitoring under this directive as it is carried out by the Environmental Health Departments of Local Authorities.

In 1992 there was a Council Resolution on the future Community Policy concerning the European coastal zone. This recognised the fragile nature of the European coastal zone and said it was "essential that its biological diversity, ecological quality and capacity to sustain life, health, social activities and social well-being are safeguarded". Clearly, this implies that some form of monitoring should be undertaken so that changes, which may result from natural or artificial (eg. pollution, land-use, exploitation) causes, can be noted and action taken if necessary.

Table 1: Summary of NRA/Agency Requirements for phytoplankton and algal monitoring

	Requirement	Monitoring
Urban Waste Water Directive (UWWTD)	Primarily an observational approach to assess for eutrophication by the presence of exceptional nuisance algal blooms, and their duration.	Undertake at existing monitoring points for Bathing Waters and Shellfish Waters. Some special survey work undertaken.
Nitrate Pollution Directive	As above.	As above.
Bathing Waters Directive	Observation for algal blooms, scums and discoloured water.	Weekly, May-Sept. Samples taken for cell counts and chlorophyll when blooms, scums or coloured water observed. Monthly sampling at sites where regular significant blooms occur.
Shellfish Hygiene Directive (91/492/EEC) and Shellfish Waters Directive (79/659/EEC)	No NRA/Agency requirement	Operational monitoring only.
National Coastal Baseline Survey and National Marine Monitoring Program (NMMP)	To establish the natural changes in the coastal ecosystem on a seasonal basis.	Spot samples taken approx. every 15 km along the coastline for chlorophyll *a* and cell enumeration (to combine with airborne surveys carried out by the National Remote Sensing Centre)

2.4 Phytoplankton Monitoring

Phytoplankton is, therefore, 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 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 and most cost-effective manner requires quality evaluation at each stage.

3. SAMPLING

3.1 Introduction

The sampling method employed is determined by the objectives of the survey and to some extent the proposed 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. In order to determine cell numbers or biomass it is necessary to collect the abundant forms of all sizes as well as the rarer large cells, which because of their size may be an important constituent of the biomass.

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'.

The concepts of accuracy and precision are distinct and not necessarily synonymous (Venrick, 1978). Net tows, for example, may give precise estimates of abundance but have poor accuracy because they underestimate the true abundance of phytoplankton as the smaller cells are only partially retained by the mesh and others may be destroyed during the fixation process.

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 used to reduce the number of plankton samples counted manually. Such an approach might be particularly appropriate to a sheltered bay or sea loch, for example.

Experience gained from sampling sites over several seasons is important for sampling programme design, especially in estuarine situations, where a marked change in chlorophyll concentrations near the halocline or thermocline can be expected (Yentsch, 1965) as well as at different stages of the tide (Welch and Isaac 1967).

3.1.1. 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) and 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 sent to the laboratory for examination with minimum delay. Preferably they should be processed on the day of collection, having been maintained in cool conditions and out of strong light between collection and analysis. Alternatively, samples may be stored, at most overnight, at < 10 °C in the dark (Herve and Heinonen, 1984).

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).

3.1.2 The Importance of Salinity

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

3.1.3 Sampling Strategies

A purely descriptive programme does not have the stringent requirements of a statistical one. Any quantitative programme must have:

(i) a rigorous statement of the objectives and

(ii) a definition of the target population (Cochran, 1963).

In reality, the conditions required by classical statistical methods are rarely, if ever, realised in the planktonic environment (Ibanez, 1978). The three dimensional nature of marine phytoplankton has been referred to above and in addition the difficulties of executing a specific sampling strategy on an invisible, motile population are huge. It is preferable to plan for long-term studies as they can show sustained trends due to climatic change and the effects of eutrophication or other cultural effects (Margalef, 1978). Eutrophication is defined in the UWWT Directive (91/271/EEC) as "the enrichment of water by nutrients, especially compounds of nitrogen and /or phosphorus, causing accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned".

3.1.4 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 (Léger 1971 a, 1971 b).

- Sampling precision and accuracy should be evaluated regularly.

- All sampling equipment should be kept clean.

3.2 Sampling Methods currently used by the NRA/Agency (Anglian Region)

As part of the National Coastal Surveillance Programme, initiated by the NRA's National Centre for Marine Surveillance at Twerton, Bath, the Region has a purpose-designed coastal survey vessel the *Sea Vigil*. Surface water (0-27 m depending on location) samples are collected from 34 sites with data collected at approximately monthly intervals. Samples are immediately filtered and analysed on-board for nitrate, ammonia, phosphate, silicate and nitrite, using an auto-analyser. The methods used contain built-in Analytical Quality Control procedures and have passed special marine AQC exercises, particularly the stringent ones prepared for ICES (International Council for the Exploration of the Seas). Measurements of temperature, salinity and dissolved oxygen are also made. Chlorophyll *a* is determined by flow-through analysis using a fluorimeter. In addition, water samples are filtered on-board, preserved and sent for analysis by spectrophotometry. There is a lack of historical data to make comparisons with but as the programme continues trends will become discernible.

The NRA/Agency employs a variety of sampling methods throughout its regions. Water samples fixed in Lugol's Iodine is the most used sampling method in all three areas. Unfixed water samples are less commonly taken. Net samples are taken by the Agency, Ipswich (Eastern Area). Intertidal substrate sampling is undertaken by Eastern Area.

Water samples for chlorophyll analysis are taken. For the Northern area these are sent to the Spalding Laboratory where chlorophyll is extracted by the hot methanol method. Central Area sends samples to the National Laboratory Service for analysis by the methanol method, and Eastern Area analyses samples in the Ipswich Laboratory using the cold acetone extraction method.

The integrated monitoring and logging system developed by the NRA's National Centre in Bath uses a QUBIT TRAC V Navigation package on its marine survey vessels. The Centre sets a common specification for all four coastal survey vessels, ensuring that all data can be integrated into a national classification programme. The parameters measured include:

- Conductivity, Temperature and Depth
- Salinity
- Dissolved Oxygen
- pH
- Transmission
- Fluorescence
- Plankton Sampler
- Saline Nutrients

Table 2: Phytoplankton sampling in Anglian Region NRA/Agency 1995-96.

	Reasons for sampling	Locations	Sample types
Region	NMP	Coastal samples	Chlorophyll *a* (optional samples for phytoplankton enumeration)
All Areas	Bathing Waters	Minimum Effort or Best Endeavours	Chlorophyll *a* and cell enumeration in 1996
Northern Area	UWWTD	Estuaries of the Rivers Nene, Welland and Witham	Chlorophyll *a*, cell enumeration, occasional love samples, net samples from seaward sites.
Central Area	UWWTD	Estuary of the Great Ouse	Chlorophyll *a* and cell enumeration
Eastern Area	UWWTD	Estuaries of the Rivers Ore/Alde, Deben, Stour, Colne. Offshore coastline from Hunstanton to Shoeburyness	Chlorophyll *a* and cell enumeration

3.3 SAMPLING TECHNIQUES

3.3.1 Water Samplers

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

3.3.2 Surface Samples

For surface water, 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 bottle may need to be weighted, have a surface float attached and also a line 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, including rust and paint flakes. 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, 1970b). Water bottles are available in sizes from 1-1000 litres, but most laboratories have found 1-3 litre bottles are adequate.

Samples for chlorophyll analysis should be filtered immediately but if this is not possible they should be stored in sealed containers in the dark, kept cool (in a cool box or refrigerator), and transported to the laboratory as soon as possible.

3.3.3 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 5 m 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.)

3.3.4 Profile and Fixed Depth Samplers

The NRA/Agency takes water samples which are then fixed with Lugol's. They use 25 ml, 150 ml or 1 litre samples.

Samples from predetermined depths are taken with either a reversing-bottle of the Nansen type (Helland-Hansen and Nansen, 1925), a non-reversing bottle, Van Dorn (Van Dorn, 1957) 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. The design most frequently used is a plastic or metal cylinder which is lowered to the required depth with both ends open. Closure is usually triggered mechanically by a messenger. In reversing bottles, such as the Nansen bottle the messenger strikes and releases the upper clamp, allowing the bottle to swing 180° about the lower clamp. This reversal closes valves in the top and bottom of the bottle. In non-reversing bottles, such as the Van Dorn the top and bottom caps are held open by a clamp against the tension of a spring or rubber connecting them through the bottle. The action of the messenger releases the clamp, and the caps are pulled into position, closing off the top and bottom of the bottle (Venrick, 1978).

There are other water bottles which are designed to reach the desired depth in the closed position and are then opened. These have been produced primarily to avoid contamination from the surface layer and descriptions of their use are in Aabye Jensen and Steemann Nielsen, 1953, Niskin, 1962 and Throndsen, 1970b.

Horizontal samplers, designed to sample a narrow stratum are useful in very shallow water, or for epibenthic samples (Joeris, 1964; Ottman, 1965)

3.3.5 Quality Assurance

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

- Samples 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 this a paper label written in 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, in 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 if possible.

- 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.

3.4 Nets

Sampling with phytoplankton nets provides useful material for qualitative purposes but because of their selective and unpredictable filtering properties they should not 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. The chief disadvantage is the distortion of the 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. Chain-forming species, or those with spines or setae (e.g. *Chaetoceros* spp.) can themselves form a network inside the gauze. A vertical or horizontal net haul is a quick and effective method for collecting rarer, larger species. Where there is a large quantity of suspended debris the net quickly becomes clogged.

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. Fine mesh gauzes have low porosity and so a compromise has to be reached between filtration and species concentration and 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 phytoplankton nets, and has relatively good porosity (See Table 3 below). Nybolt is a monofilament nylon fabric, which although having limited resistance to acid shows no evidence of toxicity, is stable to ultraviolet radiation (there is some loss in tensile strength after long time exposure), and is resistant to decay and bacteria.

Table 3 Porosities of some Nybolt monofilament nylon gauzes

Mesh size μm	Thread diameter (warp/weft) μm	Porosity (free sifting surface) %
100	43	49
80	43	42
60	37	39
55	37	34
53	30	35
50	39	27
48	35	31
40	30	30
30	30	23
25	39	14
20	30	12
15	30	10
11	30	4
5	35	1

Source: Plastok Associates Ltd., Birkenhead, UK Supplier of Schweizerische, Seidengazefabrik, AG Zurich, Switzerland, and manufacturer of bespoke plankton nets.

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 time. A plankton sample from a particular depth is obtained by towing the net horizontally while the weight holds the net at the selected depth. Care should be exercised to limit speed when towing a net. For nets with a mesh of about 50μm, this speed should not exceed 1 m. s^{-1} or 2 knots; nets with meshes below 20μm should be towed at speeds below 0.3 m. 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. Nets which are equipped with a non-filtering cone in the front or long nets may be towed at slightly higher speeds than the standard net of the same porosity; the low net-mouth/gauze-area ratio in these nets results in reduced pressure on the gauze (Tangen,1978).

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 with seawater. Filtered seawater should be used to wash the gauze when the net has been used for a deep-water tow using an opening-closing net to avoid contamination from surface organisms.

Net hauls should not be too dense, and they should be kept in a large amount of water (250 - 1000 ml) if they are to be examined live. They should 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.

3.4.1 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 cleaned occasionally 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 although a solvent, eg. acetone, may be necessary to remove extensive clogging by organic compounds such as oil. After soaking rinse thoroughly in clean water before air drying and storing in a dark, cool place.

3.5 Pumps

The use of pumps for phytoplankton studies can be traced back to Hensen (1887). Pumping of water as a means of continuous sampling for biological (e.g. chlorophyll *a* by *in vivo* fluorometry; Lorenzen, 1966) and chemical (e.g. inorganic nutrients; Strickland and Parsons, 1972) parameters is now widespread. Samples for the study of phytoplankton population abundance and composition are often taken from pumped water.

A pumping system consists of the pump itself, tubing to take the water to and from the pump, valves for regulating the water flow, meters for measuring the volume of water sampled and a sample collecting unit.

Centrifugal pumps are among the most commonly used (Cassie, 1958; O'Connell and Leong, 1963; Beers *et al.*, 1967; Kuwahara *et al.*, 1973). In a centrifugal pump the water enters near the axis of an impeller and is thrust radially outward into the casing. The energy imparted is dependent upon such factors as the design of the impeller and casing, the rotational speed of the impeller, and the number of impeller stages employed (Beers, 1978). Other pumps in use for phytoplankton work are rotary pumps, which move seawater through the action of gears, lobes, vanes, screws or flexible impellers; reciprocating pumps, which displace seawater by changing the volume of the pump by use of either pistons or diaphragm units; the 'air-lift' pump which uses compressed air to force the movement of seawater (Bernard and Rampi, 1966) and the pump developed by Lenz (1972) which draws water by use of a vacuum without it having to pass through any moving parts.

It has been reported that pumps using diaphragms cause minimal damage to phytoplankton

(Beers,1978). Centrifugal pumps have the disadvantages of potentially causing damage to phytoplankers and requiring priming (that is the displacement of air in the lines and pump prior to the commencement of pumping). Diaphragm and other positive displacement pumps do not need priming.

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. Plastic tubing is recommended in preference to rubber and other natural products because its non-porous surface gives relatively little frictional resistance to water flow and is relatively lightweight and long lasting. In general, it is preferable to use the largest diameter tubing practical to deliver the desired volume of water per unit time, since a slower flow rate through a larger tube has less total frictional loss than a higher flow rate through a smaller tube. 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.

3.5.1 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.

3.6 Bathing Water Samples

As a minimum 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. Normally two samples are taken of 250 ml each, of which one is kept live and the other fixed with Lugol's Iodine. 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. The sample is taken with a flexible scraper from at least 5 cm^2. A measured sample can be taken in this way for quantitative studies. The sample should be kept cool and dark and examined microscopically as soon as possible.

Accumulations of floating scum should be sampled where they are within safe sampling distance of the shore. Enough material should be taken to fill a sample 250 ml sample bottle and a duplicate should be fixed with Lugol's Iodine if it is not possible to examine the sample within an hour of collection.

3.7 Subsampling

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. Hasle, (In: Sournia 1978) suggests shaking a fixed sample 100 to 200 times by hand to loosen organisms attached to the bottom of a storage bottle before subsampling. For fresh samples and those with little detritus, it should be sufficient to shake or invert the bottle 20 to 30 times to disperse particulate matter evenly. When subsamples are taken by pipette the tip should be sited as near the centre of the sample bottle as possible.

3.8 Recommendations for sampling

The NRA/Agency has a requirement to monitor marine plankton as a means of monitoring pollution and environmental quality (See Table: Section 2.4). It is recommended that where possible a phytoplankton sampling programme is developed in conjunction with zooplankton sampling and chemical analysis programme. A more comprehensive understanding of the environment year on year is possible if, for instance, analysis of chlorophyll and other chemicals in the water can be related to identified species and their abundance.

Live samples are often necessary for accurate identification, particularly for non-thecate organisms. Where fixatives are to be used these should be added as soon as possible after collection.

Samples for chlorophyll analysis must be treated carefully to avoid breakdown of the pigments. They should be analysed on the day of collection if possible and kept cool (4°C) and out of strong light.

4. FIXATION METHODS

4.1 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 the 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.

There is no single fixation method which is suitable for the preservation of all types of phytoplankton (naked algae, armoured dinoflagellates and the calcareous organisms). Pico- and nano-plankton are now recognised as being an important constituent of biomass (Hallegraeff, 1981; Booth *et al*, 1982; Furuya and Marumo, 1983; Murphy and Haugen, 1985; Davis *et al*, 1985). Most fixation methods normally used for phytoplankton distort, shrink or destroy these delicate organisms. Measurements made of different nanoplankton species live and after fixation have been made by Booth (1987). He showed that cells preserved with Lugol's were the closest in size and shape to live cells, whereas those preserved with formalin were the most distorted and shrunk, with many cells bursting. There was significant shrinkage (between 2 and 36%, average 15.7%) in cell length in cells fixed with glutaraldehyde.

The effect of fixation and preservation should be checked by comparing counts of preserved subsamples with those of live subsamples, or with the results of autoradiography (Magure and Neill, 1972; Watt, 1971). Comparisons of biomass estimates from cell counts with photosynthetic rates in other subsamples are also useful to test results.

4.2 Glutaralderhyde

When examining live samples a gentle "fixing" agent, such as glutaralderhyde may be used if swimming disturbs the examination/counting (Throndsen 1996). This fixative should also be used if later examination by a taxonomic expert, possibly using electron microscopy, is being contemplated (e.g. as in the case of a particularly unusual occurrence), or for cryo-preservation (Booth, 1987).

4.3 Lugol's Iodine

Fixation should take place immediately after sampling. The recommended fixative for routine sampling is Lugol's Iodine (100 g 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 Lugol's. 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 abundant detritus or phytoplankton the Lugol's is absorbed quickly. Samples should be inspected after 24 hours to see if more Lugol's is required. Note: Light increases the 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.

4.4 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 somewhat difficult to distinguish between pigmented and non-pigmented cells.

4.5. Cryo-preservation

This method has been described as being useful for storing samples for later use of epifluorescence microscopy (Booth, 1987). It is not recommended for routine analysis.

For water samples containing < 1 mg chlorophyll *a* m^{-3} 50 ml aliquots of sample are fixed (glutaraldehyde, 2.5% final concentration) in a filter funnel over a 0.4 μm Nuclepore filter stained with Irgalan Black, for 20 minutes. After filtering the filter is mounted on a drop of immersion oil (Cargille Type B, non-fluorescent) and another drop added on top of the filter and a coverslip put on top. The filters are stored in the dark at 4°C for a few days while the water is replaced with oil and then stored in a freezer (-20°C). Samples stored in this way will keep for two years or more without significant loss of cells.

5. ANALYSIS: METHODS AND EQUIPMENT

5.1 Introduction

The chosen method of analysis is determined by the aim of the study. Samples collected for quantitative examination can be grouped into those used for estimates of cell numbers or biomass and those which will be used for assessments of all cells and species.

Cells may be counted with a Coulter counter or flow cytometer, although these methods are best suited to 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.

5.2 Inverted Microscope (Utermöhl method)

The Utermöhl technique (Utermöhl, 1931, 1958), or as it is referred to sometimes less correctly, the sedimentation method, is the preferred method for identification and enumeration of phytoplankton (for species which are identifiable in preserved condition), particularly for cell concentrations in the $10\text{-}10^6$ cells l^{-1} range (Sakshaug, 1980). For higher concentrations samples can either be diluted or an alternative method employed, such as Palmer-Maloney counting chambers or haemacytometers particularly when cells are very small.

The method uses an inverted microscope, such as the Zeiss Axiovert 10 or Wild M40, which has the light source and condenser above the chamber and objectives which 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 10 ml. Ideally there should be three x10 eyepieces; one with a Whipple graticule and one with a cross-hair graticule. The various squares of the Whipple graticule are calibrated using 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.

5.2.1 Counting Chambers

The number of 2 ml, 5 ml and 10 ml chambers available should be sufficient for the scope of the work proposed, taking into consideration the time taken to settle samples, which can be as long as 24 hours. The Hydro-Bios type are recommended. Each should be indelibly marked with a reference number. Some workers have devised chambers with a smaller floor area than the standard in order to concentrate the sample (Throndsen, 1970a). These are

useful for studies where the whole floor area is examined and when small organisms are to be enumerated. In coastal and estuarine areas with a high quantity of suspended particles a wider surface area is preferred.

Combination plates, with a detachable sedimentation cylinder fitted into a bottom plate which holds the settled sample, have been developed to allow a relatively large sample to settle, without rendering the use of a condenser impossible. (This had been the disadvantage of Utermöhl's original design). The top cylinder holds 10, 50, or 100 ml and after sedimentation it is removed. The bottom-plate chamber consists of a rectangular perspex plate, a ring and a circular base plate of coverslip thickness. The plate fits into the mechanical stage of an inverted microscope. Combination plates have the disadvantage of being expensive, unreliable and difficult to use, although they can save time by settling a larger quantity of water. In coastal and estuarine waters with large quantities of detritus this advantage is of no value. Their use is not recommended for routine work.

Hydro-Bios chambers consist of a threaded metal base plate with screw-in 'plexiglass' tubes, which hold a glass cover-slip, through which the sample is viewed, in place. If leakage occurs through 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.

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, colour of the sample, previous records or 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 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 volume. It may also be necessary to dilute such samples.

Tangen (1976) found that an exactly horizontal table, one 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 sufficient chambers are available, it is preferable to standardise settling on a longer time, such as overnight (approximately 12 hours) for all samples.

Incomplete sedimentation can result when cells become attached to the chamber walls, convection currents occur in tall chambers, or because cells do not gain sufficient weight when preserved to settle. Lugol's fixed samples settle quicker than formalin fixed samples since the iodine increases the density of the plankton cells; this method does however destroy coccolithophorids. Hasle (1978) has reported that some species, such as *Chaetoceros* sp. are not distributed randomly on the bottom of the chamber, but tend to accumulate around the periphery. Care must be taken to ensure that when counting only a part of the bottom area this is taken into account.

5.2.2 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 sunlight 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.

5.3 Standard Light Microscope

5.3.1 Counting Slides

Living cells are best identified and counted with a light microscope and examined on a counting slide such as a Sedgwick-Rafter or Palmer-Maloney Counting Slide or, where the plankton consists of very small sized cells ($<20\mu m$), with a standard haemacytometer. 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 overstained by iodine, following the addition of Lugol's, may be cleared by adding a drop of saturated solution of sodium thiosulphate.

Throndsen (1996) recommends Palmer-Maloney counting slides for routine surveys. He recommends 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 microscope 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. Haemacytometers come with grids marked on them and so ocular grids are unnecessary. The microscope should have a mechanical stage.

Before counting, examine the chamber under low magnification 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.

5.3.2 Quality Assurance

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

5.4 Identification and counting of plankton in water samples with a high detritus content

These should be diluted 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 recorded so that results can be related to the original sample size.

6 ANALYSIS: ENUMERATION TECHNIQUES

6.1 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 than to rely on a count of a single sample. It is recommended that at least two replicate subsamples are counted to increase confidence limits.

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.

Various methods have been devised to estimate the abundance of nanoplanktonic cells, but none are universally applicable. Picoplankton cannot always be identified with light microscopy, even when living (the problems associated with fixation have been discussed above). The available methods are described by Thomsen (1986) as:

a) Counts of living cells with a haemacytometer chamber (Guillard, 1978; Booth *et al,* 1982). The advantages are that cells are seen in perfect shape, but the disadvantage is that they cannot survive for long.

b) Counts of cells using an inverted microscope on preserved water samples (Utermöhl, 1958). The disadvantage is that the fixation can distort or even destroy cells.

c) Counts of cells using SEM (scanning electron microscopy), critical point dried or air-dried. Photosynthetic and non-photosynthetic cells cannot be distinguished, neither can dead cells (eg. empty diatom frustules) be distinguished from those that were alive.

d) Estimates of cell abundances from serial dilutions (Throndsen, 1978, 1996). This may underestimate cell densities and favour certain groups of organisms, depending on the culture medium used.

Butterwick *et al* (1982) compared eight methods for estimating the biomass and growth of planktonic algae using the freshwater colonial diatom *Asterionella formosa* Hass., the centric diatom *Cyclotella pseudostelligera* Hustedt and the cryptomonad *Rhodomonas minuta* var. *nannoplanctica* Skuja (*R. lacustris* var. *nannoplanctica* (Skuja) Javornický). The eight methods were:
1) Inverted microscope counts (Utermöhl method, 1958);
2) Electronic Coulter counter;
3) Nephelometry, using a nephelometer to measure amount of light scattered at 90°;
4) *In vivo* attenuance, measuring attenuance at 680 nm in a double-beam spectrophotometer;
5) *In vivo* fluorescence of chlorophyll *a*;
6) Reducing capacity;
7) Extracted chlorophyll *a* by spectrophotometry using hot 90% methanol for extraction and

8) Extracted chlorophyll *a* by fluorometry using hot 90% methanol for extraction. Direct counting with an inverted microscope was adopted as a standard method by which the others were compared. The results obtained showed that the chemical methods were all relatively slow, taking 50 minutes to 210 minutes. Both the spectrophotometric and fluorometric produced similar results. The inverted microscope had insufficient resolution for the smaller organisms but was satisfactory for *Asterionella*. The authors conclude that whatever method is adopted for determining algal biomass some degree of compromise has to be accepted regarding specificity, manipulative ease, precision and limit of detection. They believe qualitative microscopical observations should supplement any chosen method to indicate cell 'condition' and to detect any contamination.

6.2 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, 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 transect 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.

6.2.1 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 $\pm$20% of the mean value and 400 cells for a precision of $\pm$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 number of random transects of the counting chamber. For this method it is generally necessary to count a minimum of 250 cells. A third method is to count 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. Which of the above methods is chosen will depend on a number of factors such as the concentration of cells, the size of cells, the time available for the analysis and the accuracy required (see § 6.6).

6.3 Calculating cells per ml or l

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

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

6.4 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 2,000μm in diameter and their volume 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.

6.5 Serial Dilution Culture (SDC) method

The main purpose of the serial dilution-culture method is to provide material for identification and enumeration of the non-preservable fraction of the phytoplankton (the delicate flagellates). It is based on the assumption that cells will grow under culture conditions and that cells are evenly distributed in the sample. From the presence or absence of a species in different amounts of water the most probable number (MPN) per unit can be determined statistically. The principle of the method is to dilute the sample until only one specimen is left in the subsample. The number and amount of dilutions enable estimates of the original cell number to be made.

The technique has been used infrequently (Knight-Jones, 1951; Throndsen, 1969). Although it is generally agreed that quantitative estimates obtained by this method are too low it is a practical technique for a quantitative and qualitative survey of the fragile 'naked' flagellates.

The disadvantage of the SDC is its selectivity and dependence on growth conditions; the advantage is that it provides material of delicate organisms, sufficient for identification (Throndsen, 1978, 1996).

6.6 Recommendations for Enumeration

It is important to select an appropriate method for the investigation in hand. The accuracy of any results is dependent on the sampling and subsequent treatment of those samples.

Table: Accuracy of counting methods

Method	Cells to count	Accuracy
Whole chamber	Minimum of 100 cells	± 20 %
	" 400 cells	± 10 %
Random transects	Minimum of 250 cells (5-9 transects)	± 20 %
Random fields	100 fields /120 cells	± 20 %
	50 fields / 100 cells	± 25 %
	50 fields / 200 cells	± 20 %

Quality Control and Quality Assurance relating to enumeration methods is recommended as described in: NRA Standard Methodologies: Freshwater Phytoplankton. 1993.

7. CHLOROPHYLL ANALYSIS

7.1 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 variability in chlorophyll content in various species is shown by Madgwick (1966). Chlorophyll degradation products (phaeophytin or phaeophorbide) in sea water samples can be determined by chromatography (Jeffrey, 1974), although this is a lengthy process and unsuitable for routine applications.

The choice of method used for determination of chlorophyll *a* depends to some extent on the level of accuracy required, particularly in respect of degradation products, which have absorption maxima near that of chlorophyll *a* (Yentsch, 1965). In field samples a known volume of water is filtered and the pigments extracted from the filter. The results obtained will be on a volume rather than on a cell basis.

For many years extraction with 90% acetone, or 100% acetone (Hallegraeff, 1981) was considered satisfactory, although it was recognised that results were low in many instances because of the presence of plant cells that were not fully extracted (Madgwick, 1966; Strickland and Parsons, 1972) and because extraction efficiency varies between, and even within, different algal groups (Holm-Hansen and Riemann, 1978). It has been suggested that variability in chlorophyll measurements is because chlorophyllase is not completely inactivated in 90% acetone (Riper *et al,* 1979). Shoaf and Lium (1976) found that they achieved more complete extractions of chlorophyll in green algae with a 1:1 mixture of acetone (90%) and dimethyl sulphoxide (DMSO). This mixture was also found to be best suited to estimating chlorophyll in diatoms and particularly Chlorophyta.

Other workers (Holm-Hansen and Riemann, 1978; Marker, 1972) have obtained better extractions using methanol. Stauffer *et al* (1979) compared results from four solvents and found extraction to be 2.5 % - 6 % greater in 90 % acetone compared with methanol and 2.5 % greater in a 1:1 mixture of methanol and dimethyl formamide (DMF) compared with methanol on its own. The results also varied with the main species being extracted and, for example, three solvents (Methanol, Methanol:DMF, Acetone:DMSO) gave identical results with certain blue green algae but 90 % acetone gave a 29 % lower figure for the amount of chlorophyll. They concluded that a 1:1 mixture of acetone and dimethyl sulphoxide was best for routine chlorophyll extractions of diatoms and green algae, and the same solvent or methanol for blue green algae.

Absolute methanol was found to extract pigments from phytoplankton faster (one hour for methanol, 20 hours for acetone) and more completely than acetone (Holm-Hansen and Riemann, 1978).

In spite of the merits of the various solvents discussed above, ethanol is now the preferred solvent for chlorophyll extraction, in line with European recommendations (see DIN 1986, DS 1986; Jespersen and Christoffersen, 1987; Marker, 1994). Methanol is permitted as the second choice but requires greater care in its use (COSHH), reducing the speed of analysis, and is not recommended here. 100 % acetone extraction is normally used when it is necessary to make separate measurements of chlorophylls *a*, *b* and *c* and other chloroplast pigments.

When changing to what would be a new technique for the Environment Agency, it is important that parallel analyses are made using both the particular original method used and the ethanol method. This should be carried on until either it can be seen that there is no significant difference between the results obtained by the two methods or, alternatively, until a correction factor can be derived to enable direct comparisons between results obtained by the two methods.

The sensitivity of the method described here is adequate except where the chlorophyll content of the water is below 0.2mg/m^3.

7.2 *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 (Herve and Heinonen 1984). If these criteria cannot be met, samples may be frozen on filters, following the filtration step (see below). Araujo and Brereton (1995) analysed the molar absorption coefficients at 665 nm of chlorophyll *a* dissolved in acetone and showed that there was no significant difference when the solutions were kept for 84 days in the dark in the refrigerator and at room temperature.

Chlorophylls are unstable compounds and very sensitive to light and air, undergoing isomerisations and oxidations. Samples therefore must be subjected to the minimum exposure of 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. As much as 10 litres of seawater can be filtered (Hallegraeff, 1981).

Normally a 1 litre sample of sea water is filtered through a Millipore filter fitted with either a 47 mm 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 this could cause some pigment to 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 (Lenz and Fritsch 1980, Lloyd and Tucker 1988). 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 0.3 mm nylon mesh.

The filter is drained thoroughly under suction before removing it and if necessary cutting away the unstained membrane with clean scissors.

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 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 (Strickland and Parsons, 1972; Sand-Jensen, 1976). Holm-Hansen and Riemann (1978), however, tested the effect of storage at -20°C over different periods of time, of samples filtered onto glass fibre filters and extracted with acetone or methanol. They found there there was neither loss of pigment nor any significant change in the chlorophyll/phaeopigment ratio up to 560 hrs (23 days). These workers also reported no detectable differences in the amount of chlorophyll when filters were extracted

in absolute methanol after three weeks storage at -20°C.

In some species half or more of the pigments were left behind in the cell and the use of a sonic disintegrator was suggested to resolve this problem, although the results in improvement do not merit the application of such equipment on a routine basis (Strickland and Parsons, 1972). Holm-Hansen and Rieman (1978) found that grinding may sometimes be necessary when extracting with acetone, but is superfluous when using methanol. Yentsch and Menzel (1963) recommended the use of a tissue grinder, which is relatively convenient but can fail to give complete extraction in a reasonable time. Other workers have developed specially designed spectrophotometers to record the diffuse transmittance spectra of phytoplankton assemblages concentrated on glass-fibre filters (Faust and Norris, 1985). This method has the advantage of being quick to perform, the authors claim analysis takes about 2 minutes per sample, but comparisons with other methodologies would be required to assess accuracy and practicality.

7.2.1 Recommended Procedure: Ethanol Extraction

(i) 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.

(ii) After filtration is complete, reduce the residual water by allowing air to be drawn through for a short time, approximately 30 seconds. Discard the filtrate.

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

(iv) Weigh the filter to determine the weight of residual water.

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

(vi) Follow one of the following methods (a - c):

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

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 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.

(vii) 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.

(viii) 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.

(ix) 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.

(x) 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.

(xi) 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.

(xii) 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 $\mu g\ l^{-1}$ ($\equiv mg\ m^3$) 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.

7.2.2 Acetone Extraction

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

(i) Filter the sample as in 7.2.1 above.

(ii) Place the filter in a 15 ml stoppered graduated centrifuge tube. For Millipore filters add approximately 8 ml of 90% acetone and shake the stoppered tube vigorously to dissolve the filter. If 0a glass paper was used add approximately 10 ml 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 2 ml 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.

(iii) 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.

(iv) 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.

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

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

(viii) Calculation of amount of chlorophyll *a*. Subtract the absorbance value obtained at 750nm from that at 665nm and let this figure be A.

The chlorophyll *a* content of the sample, expressed as $\mu m\ l^{-1}$:-

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

Where

A = net absorbance
v = volume of solvent in ml
V = volume of original filtered sample in litres
d = cell pathlength in cm
11.9 = represents a factor derived from the specific absorption coefficient at 665 nm of chlorophyll *a* in acetone.

7.2.3 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.

7.3 *In Vivo* Fluorescence

Chlorophyll concentration can also be measured with a fluorometer. The method has a number of advantages over the spectrophotometric technique, such as having a higher sensitivity, requiring less concentrated samples, being fast and direct. A fluorometer emits short wavelength light that is absorbed by chlorophyll molecules and re-emitted (fluoresced) at a longer wavelength. The intensity of the fluorescence is measured by the fluorometer, and this reading can be used to calculate the pigment concentration. Among eucaryotic algae there is considerable difference in the wavelengths of excitation for chlorophyll *a* fluorescence between organisms with a carotenoid-protein-complex and those without (Yentsch and Yentsch, 1979). The major difference is that the presence of fucoxanthin in diatoms or peridinin in dinoflagellates allows chlorophyll *a* to be excited efficiently at wavelengths of 525-530 nm in addition to wavelengths of 450 nm.

The method for measuring chlorophyll *a* and phaeopigment by fluorescence is normally that of Lorenzen (1966). Solutions of pure chlorophyll *a* (obtainable from Sigma Chemical Co.) are used to calibrate the fluorometers.

The use of fluorescence allows continuous monitoring and has been used for remotely sensing algal blooms by multiwavelength laser induced fluorescence (Mumola *et al.*, 1975). This method is recommended where sample volumes are restricted or very high inorganic concentrations prevent efficient filtration. It is also the standard method where on-board pumped water systems are used in connection with an auto-analyser for continuous study of nutrients or pollutants. There are commercially available systems (e.g. Aquatracker II from Chelsea Instruments, London, which, when used in accordance with the manufacturer's 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 (Heaney, 1978). Checks should also be made for background fluorescence which can interfere with the result for chlorophyll.

A recent comparison of chlorophyll and phaeophytin measurements on field and laboratory cultures of algae using fluorometry and the standard spectrophotometric method (Axler and Owen, 1994) showed that there was little difference in the results obtained over a wide range of chlorophyll and phaeophytin values. These authors showed that differences in filtration and extraction technique and in frozen storage time of the samples did not result in significant changes in pigment estimates.

A recent development of remotely sited flourosensors (McStay *et al.* 1995) uses a twin optical fibre to convey signals between an offshore sensor and a shore-based facility. One optical fibre conveys a white excitation light to the remote sensor through which water containing plankton passes. The second (detector) fibre collects fluorescence from the sampler and transmits it to a photo diode from which an amplified signal is sent to a

computer for analysis. This method has given encouraging results for both recording changes in chlorophyll levels and in having the potential to differentiate between various groups of phytoplankton.

7.4 Aerial Surveillance

Mapping of phytoplankton biomass by aircraft (including fixed wing, helicopter and balloon) and satellite sensors has become an important tool for studying marine biology in recent years (Gower and Borstad, 1990). Algal chlorophyll and suspended sediments are two of the major optically active factors affecting surface water quality (Richie *et al.* 1990). The spectral signature of chlorophyll in water is well documented and remote sensing is an established technology for assessing the variable spatial and temporal densities of chlorophyll and estimating biomass (Eppley *et al.*, 1985; Gordon and Morel, 1983; Khorram and Cheshire, 1985; Sathyendranath, 1986; Platt and Sathyendranath, 1988; Campbell and Aarup, 1965; Campbell and O'Reilly, 1988; Sousa and Bricaud, 1992; Lavery *et al.*, 1993). There have been studies on the effects of varying suspended sediment concentrations on the signature of chlorophyll (Quibell, 1991; Han *et al*, 1994).

Aerial surveillance is achieved via satellite or aircraft mounted monitoring devices. Sunlight penetrating the upper layers of the water is changed in its intensity and spectral composition by absorption and scattering caused by dissolved and suspended matter and the water molecule itself. Increasing concentrations of chlorophyll in water result in a progressively greener colouration due to a combination of this absorption and increased backscatter at 500 to 600 nm. Remote measurements of the fluorescence of chlorophyll pigments are seen over a narrow spectral range centred on 685nm.This visible-wavelength light and can be used to generate coloured maps showing suspended sediment distribution patterns and the concentration of the sediment. The horizontal dimensions and temporal changes in blooms lend themselves to aerial surveillance and are easier to spot and map than from on board ship.

One satellite is the Nimbus-7, launched by NASA in November 1978 which carries a Coastal Zone Color Scanner (CZCS). This is an experimental radiometer designed to examine the bio-optical properties of the surface layers of the ocean by measuring reflectances for specific wavebands in the visible part of the spectrum. Large spatial scale blooms have been monitored very effectively by this satellite (Holligan *et al.* 1983a). Scanners are unable to assess the vertical distribution of chlorophyll but this is compensated for by the valuable spatial and temporal distributions of surface distributions that they can provide, and the additional information showing, for instance, sea-surface temperatures.

Multi-spectral scanners can be used for pollution monitoring, mapping red tides and can provide information on 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 different peaks. Yentsch and Phinney (1990) found that there are three main fluorescence bands associated with marine micro-organisms:

(i) A broad band emission centred around 470-490 nm and extending to around

600 nm. Heterotrophic dinoflagellates have been shown to exhibit such blue-green fluorescence (Mazel, 1990).

(ii) An emission band centred around 570-585 nm due to phycoerythrin (Yentsch and Phinney, 1990).

(iii) The 685 nm emission of chlorophyll *a*.

An enhanced understanding of the results obtained from aerial surveillance is possible with the examination of water samples taken from the scanned area at the same time. High reflectance detected by the CZCS on Nimbus 7 along the continental shelf edge was found, on examination of water samples, to be due to a coccolithophorid bloom (Holligan *et al.* 1983b). Comparisons between chlorophyll levels measured on board ship and by satellite have been reported by several other authors (Alfonin *el al*, 1992; Gordon *et al*, 1980, 1983; Smith and Baker, 1982; Peláez and Guan, 1982 and Guan *et al*, 1985). They have shown than under cloud-free though generally hazy conditions, an elaborately processed CZCS image can measure the distribution of upper-layer chlorophyll for pigment concentrations ranging from 0.05 to 5.0 mg pigment m^{-3} with an error of 40% or less.

Further investigations should be made of spectral signatures for identifying organisms such as *Noctiluca* (Dinophyta), Coccolithophorids and some bloom-forming diatoms. This could be based on the spectral values of a range of species described by Sathyendranath *et al.* (1987) and the specific values for various bloom species which have been established (see section 7.4.1 below).

7.4.1 NRA/Environment Agency's Use of Aerial Surveillance

The NRA/Agency group responsible for aerial surveillance is based at the National Centre for Instrumentation and Marine Surveillance, Bath. The National Centre has developed an aerial survey system utilising the Compact Airborne Spectrographic Image (CASI). The twin engined aircraft used is located at an airfield in Coventry and carries out approximately three complete flights around the British coast each year. In addition to being fitted with a CASI, the aircraft has a thermal scanner, colour video and stills camera. To ensure accurate position referencing and geo-correction of images, a satellite navigation package has been integrated into the system. The advantages of CASI over satellites are:

- Resolution
- Adaptability
- Avoidance of cloud cover
- Cost and controllability

This aerial survey provides:

- Monitoring and pollution detection
- Estimates of chlorophyll *a* for use in eutrophication studies
- Estimates of suspended solids concentration and changes in coastal morphology
- Mapping of mixing zones, outfalls and rivers
- Identification and tracking of dissimilar bodies of water
- Land classification

A major limiting factor in the use of aerial surveillance for phytoplankton monitoring is the weather.

The aerial surveys conducted by the National Centre are linked to the National Coastal Baseline Survey. This latter programme detects the major natural changes in the coastal ecosystem on a seasonal basis. The key elements are:

- Spot samples about every 15 Km
- Continuous monitoring
- Entire coverage with airborne imagery
- Co-ordination of survey between aircraft and boats
- Standardised methodologies and chemical analysis

It is recommended that there is greater liaison between the aerial surveillance team and the shore/ship based sampling teams. This would assist both sides to obtain the maximum information from their efforts and help the planning of future sampling strategies.

Further work should be carried out to assess the value of certain bandwidths to enable specific identifications of bloom algae. Some have been measured, for example, the planktonic blue green alga *Trichodesmium* (Subramanian and Carpenter, 1994) and the dinoflagellate *Noctiluca scintillans* (Macartney) Ehrenb. (Holligan *et al.*, 1983a; Balch and Haxo, 1984), in the aerial surveillance data. Because of the high concentration of the brown carotenoid fucoxanthin in diatoms it should be possible to identify blooms of these organisms from their spectral signatures. Similarly, the blooms of some of the red-tide dinoflagellates should be identifiable.

The National Centre is the Agency's specialist point for all remotely sensed applications, including satellite data. 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
Telephone: 01278 457333

7.5 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 4μg of chlorophyll *a* are approximately equivalent to 1 cm^3 of cell volume (Bailey-Watts quoted in NRA Freshwater Phytoplankton Standard Methodologies, 1993). 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.

8. DATA HANDLING AND ANALYSIS

8.1 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.

The sampling and subsampling design, including numbers and volumes of each subsampling level, should be recorded so that comparison with data from other designs can be made.

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 it should interface with an entry program which permits simultaneous data entry to both systems.

Each database file should hold enough records to facilitate the maximum number of taxa encountered per sample.

Whatever database design is adopted it should always be possible to work back 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. The requirement for adequate backup of data cannot be overstressed.

Reference should be made to the NRA Freshwater Phytoplankton manual, pp 64-67, for details of the system in use by the NRA/Agency for freshwater algae, which is entirely appropriate to be extended for use with marine phytoplankton.

8.2 Data Handling by the Agency

Phytoplankton data and some chlorophyll data are generally held in spreadsheets or on paper records. Routine chlorophyll *a* data are held in the Region's Laboratory Information System (LIMS).

Algal Image Database. A software package has been developed for Anglian Region by Unicomarine to take and store video images of phytoplankton. Images can be retrieved using the taxonomy or by physical attributes. This system can be very important as a training aid and also to maintain consistent identification and exchange of information.

Marine Algal Database. Software has been developed to hold national marine phytoplankton data. The programme is not yet finalised and needs more development work.

The Marine Section of the Agency's Anglian Region produces coastal reports from the water quality monitoring of its vessel 'Sea Vigil'. Field data, information on nutrients gathered from the on-board autoanalyser are entered onto the laboratory database at the Anglian Region's Headquarters in Peterborough. The data is presented as a mixture of graphs and charts, by each survey showing:

- Table of Data
- Chart of Sample Programme, showing Order of Sampling
- Graph of Nutrient Data Values against Site
- Contour Plot of Ammonia Data
- Contour Plot of Phosphate Data
- Contour Plot of Silicate Data
- Contour Plot of Nitrate Data
- Contour Plot of Nitrite Data
- Contour Plot of Chlorophyll *a* Data
- Contour Plot of Temperature Data
- Contour Plot of Salinity Data
- Contour Plot of Dissolved Oxygen Data.

The ecological value of being able to add phytoplankton and zooplankton data to the above information would be immense.

The National Centre for Instrumentation and Marine Surveillance operates data-handling systems. The data collected by the CASI and thermal scanner is rapidly processed to a very high standard using image processing techniques. These images can be fitted to a map so that they can be cross indexed and calibrated with data gathered from the ground or water. Images are processed to give valuable results such as chlorophyll quantities and suspended sediments.

The survey data in all its forms is collated into a series of archives and databases. The databases are tailored to each data type, allowing rapid searching and retrieval of data into either electronic or hard copy. Routine outputs from the surveys are sent to the regions, as well as national assessments to the Head Office.

8.3 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, with the possible upgrading from a six to eight digit code to accommodate extra taxa, in most cases it can be used without modification.

The system uses a six digit number, in which the first two are the Phylum Pair, the second two the Genus Pair and the remaining two the Species Pair. This makes it possible to search at different taxonomic levels. 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 system many more species have been described within some genera, and the present species pairs will probably require expansion to a four

digit field to accommodate them.

9. TAXONOMY

A list of references for taxonomic purposes is contained in Appendix III and training in this area is covered in the Training Section below.

Taxonomy is an integral component of enumeration; a taxon cannot be counted until it is identified as being discrete. This does not mean that each taxon encountered has to be given its full generic and specific name, but some sort of 'label' must be given to it for numerical purposes. If the unidentified taxon constitutes a significant part of the phytoplankton population it may be necessary to have it identified by an expert, either internal or external, for instance at the Natural History Museum, (London), which has an extensive collection of mounted material. A careful description should be made, including a labelled drawing and if the equipment is available, a photograph should be taken.

Some species which are known to cause nuisance or blooms are listed below.

Gyrodinium aureolum Hulburt - produces blooms which are important for their primary productivity (Holligan, 1981) and can cause extensive animal mortalities (Tangen, 1977; Ottway *et al*, 1979; Forster, 1979)

Noctiluca - can be concentrated on the surface in calm weather conditions, forming a reddish scum. This causes problems of anaerobiosis during the breakup and decay of the bloom.

Phaeocystis - forms gelatinous brown colonies which are harmful to fish. When abundant in breaking waves it is responsible for foaming which can be washed onto beaches causing unsightly windrows.

Alexandrium tamarense - one of several dinoflagellates responsible for producing toxins which enter the food-chain via shellfish. The main cause of Paralytic Shellfish Poisoning (PSP) around the British Isles.

10. 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 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 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 and nuisance algae
Dodge, Prof. J.D.
Royal Holloway College, Egham, Surrey, TW20 0EX
Tel 01784 443774

Lewis, Dr. Jane
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

11. RECOMMENDATIONS

It is recommended that procedures are monitored and adapted, if necessary, to accommodate new technologies, and changes in ecological conditions.

The recommended methods for marine phytoplankton are detailed in the Biology Laboratory Procedures Manual, Marine Phytoplankton Methodology, Environment Agency, Anglian Region, 1996.

APPENDICES

Appendix I

Experts contacted in connection with this report
(All located at Kingfisher House, Peterborough unless stated otherwise)

Chris Ashcroft, Senior Marine Survey Officer

Dave Balbi, Biology Technician (Northern Area), Spalding

Brian Barnett, Marine Scientist (Northern Area), Lincoln

Robin Chatterjee, Eutrophication Scientist, Toxic and Persistent Substances Centre (TAPS)

Alison Frogley, Toxic Algae Scientist (TAPS)

Terry Clough, Senior Biologist (Central Area), Brampton

Alastair Ferguson,Regional Biologist

David Foster, Directives Scientist

Barrie Harbott, Regional Water Quality Planner

Mark Johnson, Marine Scientist (Eastern Area), Ipswich

Richard Saull, Data Manager, Environment Agency,Twerton

Robert Davidson, Environment Agency,Twerton

John Nicols, MAFF, Lowestoft

Richard Thompson, Port Erin Marine Laboratory, Isle of Man

Dr E. Cox (Diatoms), The Natural History Museum, London

Dr. J.C. Green (Haptophytes and Prasinophytes), Plymouth Marine Laboratory

Dr Jane Lewis (Dinoflagellates) University of Westminster

Appendix II

General References

AABYE JENSEN, E; STEEMANN NIELSEN, E. 1953. A water-sampler for biological purposes. *J. Cons. CIEM,* vol. 18,no. 3, p.296-9.

ALFONIN, E.I.; MISHONOV, A.V. and YUNEV, O.A. 1992. Estimation of marine-phytoplankton parameters based on remote measurements of the water mass color properties (in Russian). *Okeanologiya,* vol. 32, p 1059-1067.

ANDERSON, D.M. 1984. Shellfish toxicity and dormant cysts in toxic dinoflagellate blooms. In: *Seafood Toxins* Ed. E.P Ragelis, American Chemical Society, Washington DC. p. 125-138.

ARAUJO, P.W. and BRERETON, R.G. 1995. Stability studies of chlorophyll-*a* using different storage systems. *Analyt. Proceedings,* vol. 32, no. 10, p. 415-417.

AXLER, R.P. and OWEN, C.J. 1994. Measuring chlorophyll and phaeophytin: Whom should you believe? *Lake Reservoir Management,* vol. 8, no. 2, p. 143-151.

BALCH, W.M. and HAXO, F.T. 1984. Spectral properties of *Noctiluca miliaris* Suriray, a hetertrophic dinoflagellate. *J. Plankton Res.*, vol. 6, no. 3, p. 515-525.

BEERS, J.R. 1978. Pump Sampling. In, *Phytoplankton Manual*. (U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6,), ed. by A. Sournia, U.N.E.S.C.O. Paris, p. 41- 49.

BEERS, J.R.; STEWART,G.L.; STRICKLAND, J.D.H. 1967. A pumping system for sampling small plankton. *J. Fish. Res. Board Can.*, vol. 24, no. 8, p. 1811-1118.

BERNHARD, M.; RAMPI, L. 1966. Horizontal microdistribution of marine in the Ligurian sea. *Bot. Gothoburg.*, vol. 3, p. 13-24. (Proc. 5th mar. Biol. Symp., Göteborg, 1965.)

BOOTH, B.C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. *Bot. Marina,* vol. 30, p. 101-108.

BOOTH, B.C.; LEWIN, J. and NORRIS, R.E. 1982. Nanoplankton species predominant in the subarctic Pacific in May and June 1978. *Deep-Sea Res.*, vol. 29, p. 185-200.

BRAARUD, T. 1958. Counting methods for determination of the standing crop of phytoplankton. *Rapp. et Proc.-Verv, vol. 144, Cons. Int. Explor. Mer.* p. 17-19.

BUTTERWICK, C.; HEANEY, S.I. and TALLING, J.F. 1982. A comparison of eight methods for estimating the biomass and growth of planktonic algae. *Br. Phycol. J.*, vol. 17, p. 69-79.

CAMPBELL, J.W. and AARUP, T. 1992. New production in the North Atlantic derived from seasonal patterns of surface chlorophyll. *Deep-Sea Research,* 39, No. 10, pp. 1669-1694.

CAMPBELL, J.W. and O'REILLY, J.E. 1988. Role of satellites in estimating primary productivity on the northwest Atlantic continental shelf. *Continental Shelf Research,* vol. 8,179-204.

CASSIE, R.M. 1958. Apparatus for investigating spatial distribution of plankton. *N.Z. J. Sci.,* vol. 1, no. 3, p. 436-448.

COCHRAN, W.G. 1963. Some consequences when the assumptions for the analysis of variance are not satisfied. *Biometrics*, vol. 3, no. 1, p. 22-38.

CUNNINGHAM, C.R. and PUREWAL, J.S. 1983. A microcomputer based aid for counting plankton samples. *J. Plankton . Res.*, vol. 5, p. 783-786.

DAVIS, P.G.; CARON, D.A.; JOHNSON, P.W. and SIEBUTH, J. McN. 1985. Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical, seasonal and diel distributions. *Mar. Ecol. Prog Ser. 21.* p. 15-26.

DIN, 1986. Bestimmung des Chlorophyll *a* Gehaltes von Oberfläschenwasser (L16). *DIN*, 38,p. 412.

EPPLEY, R.W.; STEWARD, M.R.; ABBOTT, M.R. and HEYMAN, U. 1985. Estimating ocean primary production from satellite chlorophyll: introduction to regional differences and statistics for the Southern California Bight. *J. Plankton Research,* 7, pp. 57-70.

FAUST, M.A. and NORRIS, K.H. 1985. In vivo spectrophotometric analysis of photosynthetic pigments in natural populations of phytoplankton. *Limnol. Oceanogr.*, vol. 30, no. 6, p. 1316-1322.

FORSTER, G.R. 1979. Mortality of the bottom fauna and fish in St Austell Bay and neighbouring areas. *Journal of the Marine Biological Association of the United Kingdom,* vol. 59, p. 517-520.

FURUYA, K. and MARUMO, R. 1983. The structure of the phytoplankton community in the subsurface chlorophyll maxima in the eastern North Pacific Ocean. *J.Plank. Res.* vol. 5, p. 393-406.

GORDON, H.R.; CLARK, D.K.; MÜLLER, J.L. and HOVIS, W.A. 1980. Phytoplankton pigments from NIMBUS 7 Coastal Zone Scanner: comparisons with surface measurements. *Science,* vol. 210, p. 63-66.

GORDON, H.R. and MOREL, A.Y. 1983. Remote assessment of Ocean Color for Interpretation of Satellite Visible Imagery. *Springer Verlag, Berlin.*

GOWER, J.F.R. and BORSTAD, G.A. 1990. Mapping of phytoplankton by solar-stimulated fluorescence using an imaging spectrometer. *Int. J. Remote Sensing,* Vol. 11, No. 2, 313-320.

GUAN, F., PELAEZ, J. and STEWART,R.H. 1985. The atmospheric correction and measurement of chlorophyll concentration using the coastal zone color scanner. *Limnol. Oceanogr.,* vol. 30(2), p. 273-285.

GUILLARD, R.L. 1978. Separating phytoplankton components. In, *Phytoplankton Manual.* (U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6,), ed. by A. Sournia, U.N.E.S.C.O. Paris, p. 129-131.

HALLEGRAEFF, G.M. 1981. Seasonal study of phytoplankton pigments and species at a coastal station off Sydney: Importance of diatoms and the nanoplankton. *Mar. Biol.* vol. 61, p. 107-118.

HALLEGRAEFF, G.M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia,* vol. 32, p. 79-99.

HAN, L.; RUNDQUIST, D.C.; LIU, L.L. and FRASER, R.N. 1994. The spectral responses of algal chlorophyll in water with varying levels of suspended sediment. *Int. J. Remote Sensing,* vol. 18, p. 3707-3718.

HASLE, G.R. 1978. Handling diatoms. In: *Phytoplankton Manual.* Ed. by A. Sournia, U.N.E.S.C.O, Paris.

HEANEY, S.I. 1978. Some observation on the use of the *in vivo* fluorescence technique to determine chlorophyll-*a* in natural populations and cultures of freshwater phytoplankton. *Freshwat. Biol.* vol. 8, p. 115-126.

HELLAND-HANSEN, B.; NANSEN, F. 1925. The eastern North Atlantic, *Geofys. publr,* vol. 4 [1927], p. 1-76.

HENSEN, V. 1887. Über die Bestimmung des Planktons oder des im Meere treibenden Materials an Pflanzen und Tieren. *Ber. Komm. wiss. Untersuch. Kiel,* vol. 5, p. 1-108.

HERVE, S. and HEINONEN, P. 1984. Factors affecting the chlorophyll *a* assay of phytoplankton samples during transport and analysis. *Annales Botanica Fennica* vol. 21, 17-21.

HOLLIGAN, P.M. 1981. Biological implications of fronts on the northwest European continental shelf. *Philosophical Transactions of the Royal Society of London,* vol. A302, p. 547-562.

HOLLIGAN, P.M., VIOLLIER, M., DUPOUY, C., and AIKEN,J. 1983a. Satellite studies on the distributions of chlorophyll and dinoflagellate blooms in the western English Channel. *Continental Shelf Research,* Vol. 2, Nos 2/3, p. 81-96.

HOLLIGAN, P.M., VIOLLIER, M., HARBOUR, D.S., CAMUS, P., and CHAMPAGNE-PHILIPPE, M. 1983b. Satellite and ship studies of coccolithophore production along a continental shelf edge. *Nature*, 304, p.339-342.

HOLM-HANSEN, O. and RIEMAN, B. 1978. Chlorophyll *a* determination: improvements in methodology. *OIKOS*, VOL. 30, P. 438-447.

IBANEZ, F. 1976. Contribution à l'analyse mathématique des événements en écologie planctonique. Optimisations méthodologiques; étude expérimentale en continu à petite échelle de l'hétérogénéité du plancton côtier. *Bull. Inst. océanogr., Monaco,* vol. 72, no. 1431, p. 217-29.

JEFFREY, S.W. 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. *Marine Biol.* vol. 26, p. 101-110.

JESPERSEN, A-M. AND CHRISTOFFERSEN, K. 1987. Measurements of chlorophyll-a from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie,* vol. 109, 445-454.

JOERIS, L.S. 1964. A horizontal sampler for collection of water samples near the bottom. *Limnol. Oceanogr.,* vol. 9, no. 4, p. 595-8.

KHORRAM, S. and CHESHIRE, H.M. 1985. Remote sensing of water quality in the Neuse River Estuary, North Carolina. *Photogrammetric Engineering and Remote Sensing*, vol. 54, p.329-341.

KNIGHT-JONES, E.W. 1951. Preliminary studies of nannoplankton and ultraplankton systematics and abundance by a quantitative culture method. *J. Cons. CIEM,* vol. 17, no. 2, p. 140-155.

KUWAHARA, A.; ABE, T.; SHIMONO, M.; KASHIWAI, M.; SINODA, M. 1973. A pump-sampler as a method of collecting plankton and its preliminary operation. *J. oceanogr. Soc. Jap.*, vol. 29, no. 3, p. 106-112.

LAVERY, P.; PATTIARATCHI, C.; WYLLIE, A. and HICK, P. 1993. Water quality monitoring in estuarine waters using the Landsat Thematic Mapper. *Remote Sens. Environ.* vol. 46, p. 268-280.

LEGENDRE, L; WATT,W.D. 1972. On a rapid technique for plankton enumeration. *Ann. Inst. océanogr. Paris (nouv. sér.)*, vol. 48, no. 2,
p. 173-7.

LÉGER, G. 1971a. Les populations phytoplanctoniques au point θ = 42°47'N, G=7°29'E Greenwich,bouée-laboratoire du C.O.M.E.X./C.N.E.X.O., A. Géneralités et premier séjour (21-27 fevrier 1964). *Bull. Inst. océanogr. Monaco,* vol. 69, no. 1412a, p. 1-42, 1 pl.; 1412b, tabl.

LÉGER, G. 1971b. Les populations phytoplanctoniques...., B. Deuxième séjour (17-30 juillet 1964). *Bull. Inst. océanogr. Monaco,* vol. 70, no. 1413a, p. 1-41; 1413b, tabl.

LENZ, J. 1972. A new type of plankton pump on the vacuum principle. *Deep-sea Res. oceangr. Abstr.,* vol. 19, no. 6, p.453-459.

LENZ, J. and FRITSCHE, P. 1980. The estimation of chlorophyll *a* in water samples: a comparative study on retention in a glass-fibre and membrane filter and on the reliability of two storage methods. *Ergebnisse der Limnologie* vol. 14, 46-51.

LLOYD, S.W. and TUCKER, C.S. 1988. Comparison of three solvent systems for extracting chlorophyll *a* from fish pond phytoplankton communities. *Journal of the World Aquaculture Society* vol 19, 36-40.

LOHMANN, H. 1908. Untersuchungen zur Festellung des vollständigen Gehaltes des Meeres an Plankton. *Wiss. Meeresuntersuch. Kiel*, N. F., vol. 10, p. 129-370.

LORENZEN, C.J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. *Deep Sea Research,* vol. 13, p. 223-227.

LUND, J.W.G., KIPLING, C. and LE CREN, E.D. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimation by counting. *Hydrobiologia*, vol. 11, no. 2, p. 143-70.

MADGWICK, J.C. 1966. Chromatographic determination of chlorophylls in algal cultures and phytoplankton. *Deep-Sea Res.* vol. 13, no. 3, p. 459-466.

MAGUIRE, B., Jr. and NEILL, W.E. 1972. Species and individual productivity in phytoplankton communities. *Ecology*, vol. 52, p. 903-907.

MARGALEF, R. 1978. Some examples of sampling design. In, *Phytoplankton Manual.* (U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6,), ed. by A. Sournia, U.N.E.S.C.O. Paris, p. 17-31.

MARGALEF,R. 1963. Succession in marine population. In: R. Vira (ed.), *Advancing frontiers of plant science*, vol. 2, p. 137-88. New Delhi, Inst Adv. Scient. Cult.

MARKER, A.F. 1972. The use of acetone and methanol in the estimation of chlorophyll in the presence of pheophytin. *Freshwat. Biol.* vol. 2, p. 361-385.

MARKER, A.F. 1994. Chlorophyll *a* - SCA Method Revision. Report to NRA, PR 395/3/A.

MAZEL, C. 1990. Spectral transformation of downwelling radiation by autofluorescent organisms in the sea. *Proceedings of Ocean Optics X*, vol. 1302, p. 320-327.

McSTAY, D.; MILNE, R.; WRIGHT, G.G. and DUNN, J. 1995. A single probe fibre optic fluorosensor for marine and freshwater measurements of phytoplankton populations. *International Journal for Remote Sensing* vol. 16, 957-965.

MUMOLA, P.B.; JARRETT, Jr.,O. and BROWN, C. 1975. Multiwavelength LIDAR for remote sensing of chlorophyll in algae and phytoplankton. The use of Lasers for hydrographic studies. *NASA SP-375 Symposium*. H.H. Kim and P.T. Ryan, eds, 207pp.

MURPHY, L.S. and HAUGEN, E.M. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. *Limnol. Oceanogr.*, vol. 30, p. 47-58.

NISKIN, S.J. 1962. A water sampler for microbiological studies. *Deep-sea Research Oceanogr. Abstr.* vol 9, 501-503.

O'CONNELL, C.P.; LEONG, R.J.H. 1963. A towed pump and shipboard filtering system for sampling small zooplankters. *Spec. scient. Rep. U.S. Fish. Wildl. Serv., Fisheries,* vol. 452, p. 1-19.

OTTMAN, F. 1965. Une nouvelle bouteille horizontale. *Mar. Geol.,* vol. 3, p. 223-6.

OTTWAY, B; PARKER, M; McGRATH, D and CROWLEY, M. 1979. Observations of a bloom of *Gyrodinium aureolum* Hulburt on the south coast of Ireland, summer 1976, associated with mortalities of littoral and sub-littoral organisms. *Irish Fisheries Investigations Series B,* vol. 18, p. 1-9.

PAASCHE,E. 1960. On the relationship between primary production and standing crop of phytoplankton. *J. Cons. int. Explor. Mer.*, vol. 26, no.1, p. 33-48.

PELÁEZ,J. and GUAN, F. 1982. California Current chlorophyll measurements from satellite data. *Calif. Coop. Oceanic Fish. Invest. Rep.,* vol. 23, p. 212-225.

PLATT, T. and SATHYENDRANATH, S. 1988. Oceanic primary production: estimation by remote sensing at regional and larger scales. *Science (Washington, D.C.),* vol. 241, 1613-1620.

QUIBELL,G. 1991. The effect of suspended sediment on reflectance from freshwater algae. *Int. J. Remote Sensing,* vol. 12, p. 177-182.

RICHIE, J.C.; COPPER, C.M. and SCHIEBE, F.R. 1990. The relationship of MSS and TM data with suspended sediments, chlorophyll, and temperature in Moon Lake,Mississippi. *Remote Sensing of the Environment,* vol. 33,p. 137-148.

RIPER, D.M., OWENS, T.G. and FALKOWSKI, P.G. 1979. Chlorophyll turnover in *Skeletonema costatum,* a marine plankton diatom. *Plant physiology,* vol. 64, p. 49-54.

ROBSON, P.G. and BAILEY-WATTS, A.E. 1978. A computer system for recording and sorting plankton count data. *Br. Phycol. J.* vol. 22, p. 261-167.

SAKSHAUG, E. 1980. Problems in the methodology of studying phytoplankton. In *The Physiological Ecology of Phytoplankton,* ed. by I. Morris, Blackwell, Oxford, p. 57-91.

SAND-JENSEN, K. 1976. A comparison of chlorophyll *a* determinations of unstored and stored plankton filters extracted by methanol and acetone. *Vatten* vol. 1976, 337-341.

SATHYENDRANATH, S. 1986. Remote sensing of phytoplankton: A review with special reference to Picoplankton. In *Photosynthetic Picoplankton. Can. Bull. Fish. and Aquatic Sci.,* p. 214.

SATHYENDRANATH, S.; LAZZARA, L. and PRIEUR, L. 1987. Variations in the spectral values of specific absorption of phytoplankton. *Limnology and Oceanography* vol. 32, 92-98.

SHOAF, W.T. and LIUM, B.W. 1976. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. *Limnol. Oceanogr.* vol. 21, p. 926-928.

SMITH, R.C. and BAKER, K.S. 1982. Oceanic chlorophyll concentrations as determined by satellite (Nimbus-7 Coastal Zone Color Scanner). *Mar. Biol.* vol. 66, p. 269-279.

SOURNIA, A. ed. 1978. *Phytoplankton Manual.* U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6, Paris.

SOUSA, F.M. and BRICAUD,A. 1992. Satellite-derived phytoplankton pigment structures in the Portuguese upwelling area. *J. Geophys. Res. (C. Oceans),* vol. 97, p. 11343-11356.

STAUFFER, R.E., LEE, G.F. and ARMSTRONG, D.E. 1979. Estimating chlorophyll extraction biases. *J. Fish. Res. Board Can.* vol. 36, p. 152-157.

STRICKLAND, J.D.H. and PARSONS, T.R. 1972. *A practical Handbook of Seawater Analysis.* Fish. Res. Bd. Canada. Bull. 167.

SUBRAMANIAN, A. and CARPENTER, E.J. 1994. An empirically derived protocol for the detection of blooms of the marine cyanobacterium *Trichodesmium* using CZCS imagery. *Int. J. Remote Sensing* vol. 15, 1559-1569.

TANGEN,K. 1976. A device for safe sedimentation using the Utermöhl technique. *J. Cons. CIEM*, vol. 36, no. 3, p. 282-4.

TANGEN, K. 1977. Blooms of *Gyrodinium aureolum* (Dinophyceae) in North European waters, accompanied by mortality in marine organisms. *Sarsia* vol. 63, p. 123-133.

TANGEN, K. 1978. Nets. In, *Phytoplankton Manual.* (U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6,), ed. by A. Sournia, U.N.E.S.C.O. Paris, p. 50-58.

THOMSEN, H.A. 1986. A survey of the smallest eucaryotic organisms of marine phytoplankton. In *Photosynthetic Picoplankton. Can. Bull. Fish. and Aquatic Sci.*, p. 121-158.

THRONDSEN, J. 1969. Flagellates of Norwegian coastal waters. *Nytt. Mag. Bot.*, vol. 16, no. 3-4, p. 161-216.

THRONDSEN, J. 1970a. A small sedimentation chamber for use on the Wild M40 and the Zeiss plankton microscopes. *J. Cons. Int. Explor. Mer.*, vol. 33, no. 2, p. 297-98.

THRONDSEN, J. 1970b. A non-toxic water sampler for shallow waters (0-50m). *J. Cons. Int. Explor. Mer.*, vol. 33, no. 2, p. 298-300.

THRONDSEN, J. 1978. The dilution-culture method. In, *Phytoplankton Manual.* (U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6,), ed. by A. Sournia, U.N.E.S.C.O. Paris, p. 218-224.

THRONDSEN, J. 1996. Enumeration Methods. In, *A Manual on Harmful Marine Phytoplankton.* Ed. Hallegraeff, Anderson and Cembella. In press.

UTERMÖHL, H. 1931. Neue Wege in der quantitativen Erfassung des Planktons. *Verh. int. Ver. Limnol.*, vol. 5, p. 567-95.

UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. int. Ver.theor. angew. Limnol.*, vol. 9, p. 1-38, pl. 1.

VAN DORN, W.G. 1957. Large-volume water sampler. *Trans. am. geophys. Un.*, vol. 37,no. 6, p. 682-4.

VENRICK, E.L. 1978. Sampling strategies. In, *Phytoplankton Manual.* (U.N.E.S.C.O. Monographs on Oceanographic Methodology, no. 6,), ed. by A. Sournia, U.N.E.S.C.O. Paris, p. 7-16.

WATT, W.D. 1971. Measuring the primary production rate of individual phytoplankton species in natural mixed populations. *Deep Sea Res.*, vol. 18, p. 329-339.

WELCH, E.B. and ISAAC,G.W. 1967.Chlorophyll variations with tide and with plankton productivity in an estuary. *J. Water Poll. Control Fed.*, vol. 39, no. 3, p. 360-366.

WHITTON, B.A.; DIAZ, B.A. and HOLMES, N.T.H. 1979. A computer orientated numerical coding system for algae. *Brit. Phycol. J.*, vol. 14, p. 353-360.

WILLÉN, T. 1976. A simplified method of phytoplankton counting. *Brit. Phycol. J.*,vol. 11, no.3, p. 265-78.

YENTSCH, C.S. 1965. The relationship between chlorophyll and photosynthetic carbon production with reference to the measurement of decomposition products of chloroplastic pigments, p.323-346. In C.R. Goldman [ed.], Primary Productivity in aquatic environments. *Mem. Int Ital. Idrobiol.*, 18 Suppl., Univ. Calif. Press, Berkeley.

YENTSCH, C.S. and MENZEL,D.W. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin. *Deep-Sea Res.* vol. 10, p. 221-231.

YENTSCH, C.S. and PHINNEY, D.A. 1990. Autofluorescence and Raman scattering in the marine underwater environment. *Proceedings of the International Society for Optical Engineering, Proceedings of Ocean Optics X,* vol. 1302, p. 328-333.

YENTSCH, C.S. and SCAGEL, R.F. 1958. Diurnal study of phytoplankton pigments an *in situ* study in East Sound, Washington. *J. Mar, Res.*, vol. 17, p.567-583.

YENTSCH, C.S. and YENTSCH, C.M. 1979. Fluorescence spectral signatures: The characterization of phytoplankton populations by the use of excitation and emission spectra. *J. Mar, Res.*, vol. 37, p. 471-483.

APPENDIX III

Taxonomic References

General

DREBES, G. 1974. *Marines Phytoplankton.* Thieme, Stuttgart.

PARKE, M.; DIXON, P.S. 1976. Check-list of British marine algae - third revision. *J. Mar. Biol. Assoc. U.K.*, vol. 56, no. 3, p. 527-94.

TOMAS, C.R. (Ed.) 1993. *Marine Phytoplankton: A Guide to Naked Flagellates and Coccolithophorids.* Academic Press, San Diego. 263pp.

TOMAS, C.R. (Ed.) 1996. *Identifying Marine Diatoms and Dinoflagellates.* Academic Press, San Diego. 584pp.

Coccolithophorids

DEFLANDRE, G. 1952. Classe des Coccolithophorides. In: P.-P. Grasse, (ed.), *Traité de Zoologie* (Paris, Masson.), vol. I, no.1, p. 439-70.

DEFLANDRE, G.; FERT, C. 1954. Observations sur les Coccolithophoidés actuels et fossiles en microscopie ordinaire et électronique. *Ann. Paléontol.*, vol 40, p. 115-76, pl. 1-15.

OKADA, H.; MCINTYRE, A. 1977. Modern coccolithophores of the Pacific and North Atlantic oceans. *Micropaleontology*, vol.23, no. 1, p. 1-55, incl. pl. 1-13.

SCHILLER, J. 1930. Coccolithineae. In: Rabenhorst, *Kryptogamen-Flora* (Leipzig, Akad. Verlag), vol. 10 (2), p. 89-273.

Diatoms

ANON. 1975. Proposals for a standardization of diatom terminology and diagnoses. (In: R. Simonsen (ed.), *Third Symp. Recent and Fossil Mar. Diatoms.* Kiel, 1974.). *Nova Hedwigia (Beih.)*, vol. 53, p. 323-54.

HENDEY, N.I. 1964. An introductory account of the smaller algae of British coastal waters. Pt. 5: Bacillariophyceae (Diatoms). *Fish. Invest. Lond.* ser. IV, 317pp.

LEBOUR, M.V. 1930. *The Planktonic Diatoms of Northern Seas.* London, Ray Society. 244 p., 4 pl.

ROUND, F.E.; CRAWFORD, R.M.; MANN, D.G. 1990. *The Diatoms: Biology and Morphology of the Genera.* Cambridge University Press.

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

Dinoflagellates

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

SCHILLER, J. 1931-37. Dinoflagellatae (Peridineae) in monographischer Behandlung. In: Rabenhorst, *Kryptogamen-Flora* (Leipzig, Akad. Verlag), vol.10 (3): Teil 1, p. 1-617.

Other flagellates

BUTCHER, R.W. 1959. An introductory account of the smaller algae of British coastal waters, I. Introduction and Chlorophyceae. *Fish. Invest. Lond.*, ser. 4, p. 1-74, pl.1-14.

BUTCHER, R.W. 1961. An introductory account of the smaller algae of British coastal waters, VIII. Euglenophyceae (Eugleninae). *Fish. Invest. Lond.*, ser. 4, p. 1-17, pl.1-3.

BUTCHER, R.W. 1967. An introductory account of the smaller algae of British coastal waters, IV. Cryptophyceae. *Fish. Invest. Lond.*, ser. 4, p. 1-54, pl.1-20.

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