METHODS OF EXPLORATION AND ANALYSIS OF THE ENVIRONMENT OF AQUATIC VEGETATION

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INTRODUCTION

Aquatic plants are found wherever there is liquid water and at least a modest flux of light. Sometimes they are conspicuous: 700 km^2 of head-high *Typha dominguensis* around the African L. Chilwa or 5000 km^2 of equally tall *Typha* and *Phragmites* in the Danube delta or the even larger Nile Sudd. Many freshwaters appear, at first sight, to lack plants. A filter and microscope will rapidly show that such waters usually do contain large populations of microscopic algae. If the populations are large enough they give the water colour. Even in the unpromising conditions of Antarctica there are seasonal lakes, frozen solid for most of the year, which contain an astonishingly active micro- and macro-flora. In contrast there are peat bogs, which consist of large masses of water 'gelled' by about 10% of organic matter. In the latter case the plants are so conspicuous that the system is rarely considered to be (what it actually is) a body of freshwater.

Macroscopic water plants may be grouped in several ways. A convenient one is to distinguish emergent taxa (such as *Phragmites* and *Typha*), floating-leaved but rooted ones (*Nuphar* and *Nymphaea*), free-floating ones with unattached roots (*Lemna, Eichhornia, Salvinia*) submerged rooted ones (*Myriophyllum, Isoetes*) and, lastly, the free floating rootless mosses (*Fontinalis*). Submerged rooted plants are sometimes called rhizophytes. Another group — haptophytes — is submerged and attached by modified organs other than roots. Macrophytes differ in conspicuousness: some form large floating mats (*Eichhornia*); others occur as scattered individuals of small stature in deep water and are therefore inconspicuous. One important feature of all but the emergent macrophytes is that the water supports most of the plant weight. Gas spaces may enhance this effect. It may well be that the structure of such plants is determined mainly by the need to transport gases and to photosynthesize, and only slightly by the need for structural support which is so important in terrestrial plants. Reviews of

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these and many other features of water plants are given by *inter alia* Sculthorpe (1971), Denny (1980) and Best (1982a).

The species-abundance and changes in most aquatic vegetation types are to a large extent controlled by chemical, physical and biotic factors. Aquatic vegetation can sometimes be a substantial nuisance — *Eichhornia, Salvinia* and *Elodea canadensis* are examples. The plants often decline as rapidly as they became abundant, affected by several, sometimes interacting factors.

In many cases the particular requirement of individual plant species for light, nutrients, substrate, a particular range of the temperature etc. in conjunction produces a distinct zonation.

Several examples illustrate some of the complexities.

The macrophytic vegetation of an upland stream flowing from a granitic catchment is almost completely different from that in a lowland chalk stream, which in turn is different from that fringing a large lowland lake in a catchment on soft sedimentary rocks. Water movement, water depth, and water clarity may partly explain the difference, but water and sediment chemistry are probably more important. Some macrophytes — *Lemna* is an obvious example — absorb solutes solely from the water in which they float. Others, such as *Typha* and *Phragmites australis*, have a large rhizome and root system in the sediment. They absorb solutes from the interstitial water mainly through their roots. Taxa such as *Vallisneria, Ceratophyllum demersum* and *Potamogeton* are intermediate in character (Denny 1972). The range of tolerance of chemical differences is enormous. Some species are restricted in their occurrence: *Isoetes* and *Lobelia* to oligotrophic, usually acid, water for example, while *Myriophyllum spicatum* is found in relatively eutrophic water. Others, such as *Phragmites australis*, have a wider range. One must, however, be cautious of interpreting such observations in terms of nutrient supply alone. For example, oligotrophic waters are usually clear whilst eutrophic waters are often turbid: the water types differ in light climate as well as in chemistry. In water rich in detritus or silt, plants with finely dissected leaves may be smothered by deposited particles. A dense growth of periphyton may have the same effect. *Phragmites australis*, which ranges in height from a few centimetres in acid nutrient-poor water to 7 metres or more in the Rhone and Danube deltas, is known to consist of a polyploid series. Other examples are given by Denny (1980). In general it is the combined effects of genotype and environment which control the occurrence of individual species.

In some cases a small difference in water chemistry is correlated with a large change in the macrophytic vegetation: Lambert (1951) noted that in tidal (but fresh) water *Scirpus lacustris* rather than *Typha angustifolia* was favoured. When long-lived perennials are involved one

must beware of attributing the present distribution to present environmental conditions: Chapman (1964) records the occurrence of very small *Phragmites australis* plants at fairly high density at one small part of Coom Rigg bog. Examination of the peat showed that these were the last survivors of a reed swamp which surrounded a small lake 5000 years ago. A similar example is noted by Summerfied (1972).

Broad generalisations of these kinds are often based on observation of obvious factors such as water velocity, or less obvious but easily measured ones, such as pH, temperature, and electrical conductance of the water. But some very important factors, such as sediment chemistry, are not easily measured.

Finally, the strong correlation between the effects of concentrations of different substances and water movements, water depth (with its effect on light flux) and competition, prevent exact interpretation of the effects of single chemical factors on macrophyte growth. Such differences can only be made from controlled experiments — if at all.

Obviously, it is essential to define the objective(s) of work before measurements or experiments are begun. In the following we consider, therefore, a few of the consequent problems of sampling and analysis.

GETTING TO SAMPLING SITES

Wading is possible for short distances in waters up to 1 m depth. In temperate regions low temperatures during winter and in the tropics water-borne diseases will set constraints. Care should be taken not to sample where the sediments are disturbed by the sampler. Carrying equipment may be difficult. Collapsible (portable) rowing boats, of which the pneumatic type is probably the commonest, may be a good solution. They permit the transport of electrical measuring equipment for pH, oxygen concentration, conductivity and temperature, so that the variables can be measured *in situ* and often continuously. There is no practical limit to the number of samples than can be transported in a boat, although transport on land and the time required for the analyses will set limits.

With increasing site dimensions the boats have to be bigger and for large lakes large boats are needed for safety and speed. It must be realized that shallow lakes may have deeper parts and limnologists may drown just as easily as statisticians in a lake of average depth 10 cm. Safety measures are mandatory, particularly with small boats: life belts or jackets, somebody on the shore aware of the estimated time of return and so on. Radio contact is desirable.

A 'moon pool' i.e. a central hole (with sides of course) in the boat or

raft makes the handling of sampling equipment far easier and the craft more stable.

Running waters can be sampled for dissolved compounds from the shore or from bridges. Small rivers can be waded. For large ones a boat with a powerful and reliable motor is essential.

MORPHOMETRIC AND HYDROLOGICAL VARIABLES

Water flow

An important factor for vegetation studies in rivers is water flow. The following variables and their relations must be considered:

Water height	(h; normally in m);
Profile	(P; normally in m^2);
Velocity	(v; normally in m sec^{-1});
Flow rate	(Q; normally in m^3 sec^{-2});

The profile (or cross-section at a given place) depends on the physical form of the river and water height. In hydrology water height is often called stage. Stage is the variable which controls the period and intensity of immersion of the vegetation. Velocity — or, for a given profile, flow rate — will control which vegetation may develop (Wetzel 1975, Dawson 1978). Measurement of these variables is often a specialized job; pitfalls are numerous. Often data can be obtained from hydrological surveys. Very often — especially for smaller streams — data must be obtained by the botanist himself. Some indications and guidelines follow here; for more details see Ven Te Chow (1964).

Stage

The term stage refers to the water-surface height at a point along the stream, measured above an arbitrarily fixed point. Readings are either taken at intervals by an observer or are recorded continuously. In use are staff, chain, tape and wire gauges plus pressure devices or transmitters and crest-stage indicators. A staff gauge is a graduated scale set in a stream and fastened to a wall, pier, bridge etc. The level is read with proper allowance for fluctuations and meniscus. In chain, tape and wire stages the stage is determined by lowering a weight to the water surface. The distance that the weight descends before touching the water is measured from a graduated scale, a reference bead etc. These gauges are easily installed and safe from damage by boats, flood-transported debris etc. It is often difficult to observe the moment of contact. The weight can be made a part of an electric circuit with the

return through the water. The deflection of a galvanometer needle or an audible signal can be used as indicator of contact. In a float gauge a float is fastened to one end of a tape. The other end is mounted on a wheel and may drive the recorder directly. The stage may be converted to pressure by means of a cylinder and flexible diaphragm (pressure transmitter), however, the device is temperature-sensitive and needs careful calibration. The automatic instruments are based on floating gauges fitted to recorders, automatic printers etc. They give continuous records but their disadvantage is that they need protection from water-borne debris.

Velocity

Stream velocity can be measured by rotation or deflection of a mechanical device by floats or by chemical methods. Rotating current meters may be vertical or horizontal. Rotation around the vertical axle takes place by means of vanes or cups; around the horizontal axle by means of screw- or propeller-shaped blades. Examples of the first type are the Price (and derived) meters and of the second the Neyrpic (France) and Ott (F.R.G.) meters. Calibration is necessary and should be repeated, especially when they are wearing out. In humus-rich agressive waters and other agressive waters even stainless steel meters may corrode in a period as short as 6 months. Problems can arise if the current can reverse in the course of time, as may happen in artificial waterways.

Different floats can be used with different degrees of sophistication. They are discussed here in order of increasing reliability.

(1) Any object (cork, wood) floating on the water (but in good contact with the water) may be used ('Pooh Sticks', Milne 1928). Distance should be marked on the shoreline and the time between passing two marks should be noted. The float may easily be obstructed by branches, by bends in the river, or be deflected by wind.
(2) The object may be made to float at given depth by attaching a heavy weight to a cork by means of specific length of cable. These devices measure velocity at a single depth only — at the surface or at the depth of the weight respectively.
(3) Some sort of integration can be made by using a long rod (e.g. electric installation tube) closed at one end by a heavy stopper. If the lower end is near the bottom of the waterway the float probably approaches mean velocity. Floats are best in long and straight channels.

If the stream is not too large the sudden input of e.g. NaCl may be used to mark and define a certain water mass. At a fixed distance the

passage of the salt can be detected by measuring the hump in a record of conductivity or of chloride concentration (measured with a silver electrode immersed in the river). Salt velocity or dilution may be measured. The same can be done with fluorescein; the method is extremely sensitive if a fluorescence meter is available, but a spectrophotometer may be used instead, though with reduced sensitivity. A preliminary test has to be carried out to estimate the amount to be introduced. Chemical-electric methods include oxygen polarography, the hot-wire anemometer, electro-voltage generation and supersonic wave. They require skilled operators.

Especially for low current velocities a new method 'Laser Doppler Velocimetry' is coming into operation. In Laser Doppler Velocimetry particles moving with the fluid (either natural or added) are illuminated with a focused Laser beam and become sources of scattered light. The velocity of these particles is determined from the change in frequency (Doppler shift) of the scattered light due to the movement of the particles when observed by a stationary detector. From the particles velocity the fluid velocity is inferred. A laser is used as a light source because it is easily focused and is coherent. Some effects of low current velocities are described by Westlake (1967) and Madsen & Sondergaard (1983). For methodological details see Durrani & Greated (1977) and Madsen & Warncke (1983).

Depth

Depth can be measured by means of a wading rod (small streams) cable and weight (larger streams, either from a boat or bridge — care should be taken to make sure that the line is vertical) or with an echo sounder. The last solution is the best for larger waters as it gives a continuous record and can be used to survey a whole lake quickly. Cheap equipment is nowadays available or can easily be borrowed. Normally the size of the stream determines what kind of equipment is needed.

Discharge

Discharge can be calculated as velocity times profile ($v \times P$: units $m^3\,s^{-1}$). When stream velocity at a given observation point is measured sufficiently often, the stage-discharge relation can be found. This is a complex relation, which may be permanent, if channel characteristics are not changing; otherwise it is a varying relation which has to be recalibrated often (shifting control).

REMOTE SENSING (TELEDETECTION)

Information obtained from a distance is available in two forms: aerial photographs and digital data from satellites.

Panchromatic 'black and white' prints are commonest. They have relatively high resolution (often better than 1 m) and in many cases the same area has been recorded at several times over several decades. Individual prints are not expensive. Color prints are often made nowadays, but they are more expensive and may lack the resolving power of monochrome prints. But the color contrast may more than compensate for the disadvantages. 'False color' prints, of which those rendering the infrared visible are the most common, may be particularly valuable once the initially confusing change of color conventions is mastered. Such prints give information not observable at all by the unaided human eye.

The first non-military satellite designed to observe the Earth's surface — Landsat 1 — was put into orbit by the U.S. in 1972. Others followed and will continue to follow. The information provided by a satellite is not a (virtually instantaneous) photograph but a sequence of irradiance measurements at several wavelengths as the satellite scans the Earth's surface. It is thus more closely related to the production of a television picture by a scanning spot than to a photograph. The information received on Earth is stored on magnetic tape and it is these tapes which, after some editing, are copied and are the primary source that is sold. Coverage of the whole of the Earth's surface is available, repeated every few weeks, though cloud cover makes many of the scenes useless. The spatial resolution (pixel = smallest area analyzed) is about 80×80 m in Landsat images, and is thus very much less than that of most aerial photographs. The resolution of more recent satellites is better, but the limits are set more by political acceptability than by technology. The magnetic tapes can be read only by computer but it is also possible to obtain false color prints. They are produced by converting irradiance values into corresponding colors or shades of grey. The tape of a Landsat 'scene', or the corresponding print, is more expensive than a single aerial photograph and is of much lower resolution. But it does cover a much larger area, and the tape may be processed in a variety of ways to enhance or highlight chosen sorts of features. Aerial photographs and satellite images are, therefore, to a large extent complementary and not competitors.

Water quality and chlorophyll

Remote sensing of inland waters allows a synoptic view unobtainable by other means, and the first applications were in this field. Most of the

work, however, has been applied to the seas and oceans. Several satellites have been designed primarily for this work. Observation of the way in which the flux of light at different wavelengths changes across a tract of water allows deductions to be made about the nature and concentration of the elements contained therein. The concentration of suspended matter can be estimated with mediocre accuracy, whilst for chlorophyll the problem is more complex. The following are some of the problems.

(1) The relation between reflectance and the concentration of suspended matter appears to be generally curvilinear (Munday & Alfoldi 1979). The turbidity of the water is caused by attenuation of light by both organic and inorganic components of the suspended matter. The relation between concentration and reflectance depends on the nature and amount of the surfaces of the suspended matter. The results from one site cannot, therefore, be generalised to all others.

(2) The measurement of chlorophyll requires that the sensor has precise performance in the following ways.

First, the chlorophyll 'particles' are usually less abundant but individually darker than the non-chlorophyll particles in the same environment. Precise results can be obtained only if the detector has a high resolution.

Secondly, chlorophyll absorbs light strongly over a narrow part of the spectrum only, while the rest of the suspended matter absorbs over a broad band of the spectrum. The detector response should be limited to the narrow band in which chlorophyll absorbs light.

Thirdly, the spatial variation of chlorophyll is at a smaller scale than that of suspended matter in general, so the detector must have a higher resolution than it needs for suspended matter in general.

These three constraints are at least additive and result in a relatively low signal : noise ratio for chlorophyll. Only high concentrations can be estimated with any certainty.

In spite of these restrictions some interesting results have been obtained for continental waters. In S.E. Australia the concentration of chlorophyll in lakes has been estimated, using multiple regression analysis (Carpenter & Carpenter 1983). The variables in their model were the Landsat spectral windows 4 (green) and 5 (red), the solar elevation, and the time at which the satellite passed. With logarithmically transformed pigment concentration as the dependent variable the square of the multiple regression coefficient was 0.9. The same article gives an extensive bibliography of the use of remote sensing in water quality studies. A critical account, based on the chromatic properties of

water, is given by Bukata *et al.* (1983). These uses of remote sensing are still at the exploratory stage, but they do show great promise.

Wetlands, including marshes

Remote sensing is already in common use for the mapping and inventory of wetlands, though most published work deals with coastal wetlands. Soil type, sensed in this way, has been used as the basis for an inventory (Anderson *et al.* 1976) but only at a rather crude level. A more refined classification, specially designed for wetlands, has been used with some success in the U.S. (Cowardin *et al.* 1979).

False-color infrared aerial photographs have been used for an initial survey — reconnaissance — of emerging and floating vegetation (Carter 1977, 1982). But, for submerged vegetation, infrared sensing is not usually suitable because infrared radiation is so strongly absorbed by water that it penetrates only to a very shallow depth. Color aerial survey is probably best, but even this is seriously hindered by even small amounts of suspended matter. If only one survey is possible then it is probably best made in the autumn (if in temperate regions). Repetition of the survey at different seasons is much to be preferred as it improves discrimination and increases the reliability of identifications. With cover repeated over many years it is possible to follow the vegetation changes over large areas and long times, as has been done for the Great Dismal Swamp in Virginia, USA, between 1938 and 1952 (Garrett & Carter 1977).

The use of satellite data for mapping and inventory is still at the research stage. There is not, as yet, any nation-wide study of proven validity. For example, Gammon *et al.* (1979) compared the results obtained by aerial survey and by Landsat imaging of the Great Dismal Swamp. The comparison was disappointing: the proportion of correct classifications was low because of the small scale mixture of vegetation types, the relatively large pixel size, and the close spectral resemblance between different plant species. Considerably greater success was achieved in the mapping and inventory of peat covered wetlands in Ontario, Canada (Pala 1984) but it is still the case that satellite images allow the identification of wetlands only if they are of reasonable extent and homogeneity. Sensing from more spectral bands may improve our ability to discriminate between similar vegetation types. Narrow linear belts of vegetation, such as riverine and lake-shore zones cannot be distiguished on 80×80 m pixels. Only improved resolution will solve this problem. For most vegetation survey purposes aerial photographs, particularly infrared false-color ones, are still the most useful source of information about wetlands. This is likely to remain true for some years yet: low cost, high resolution, and the ability to choose the time and

scale at which the survey is made will remain advantages of this method.

But satellite sensing is barely twenty years old. There are immense possibilities for development and these are being eagerly exploited. Newer satellites — Thematic Mappers — record in 7 spectral bands, rather than the 4 bands of the Landsat series. The pixel of the French satellite Spot is 10×10 m — a sixty-four-fold improvement on the Landsat pixel. Finally, the digital nature of the recordings allows and encourages computer-processing which can be used to enhance boundaries, to choose the best criteria for classification, to selectively enhance particular features, and to automate inventory. We may reasonably expect enormous improvements in satellite sensing, but aerial survey should retain its complementary place.

PLANT SAMPLING UNDER WATER

Rapid estimation of the distribution and abundance of submerged macrophytes has been limited for a long time by the available sampling methods. Many different overboard samplers have been developed, their success being dependent upon exhaustive sampling and homogeneous macrophyte growth patterns (Forsberg 1959). Conventional limnological methods are laborious in the study of water bodies with heterogeneous populations of submerged macrophytes. Sampling by Ekman dredge, grapnel or other overboard sampling methods in sparsely populated regions yields misleading results due to the distances between neighbouring plants.

However, although at present SCUBA diving is increasingly used for research on submerged plants, its application depends strongly on the transparency within the water body concerned and thus on the visibility for the SCUBA diver. For instance, in the sea and in oligotrophic freshwaters its use has obvious merits. In detritus-rich systems, such as estuaries or peaty, eutrophic lakes with consequent bad visibility, the opportunities for SCUBA diving are much more limited.

If the lake is small enough then orientation can be made on landmarks along the shoreline only. In larger lakes it may be necessary to use these in combination with aerial photography and/or topographical maps (Wile 1973). Aerial photography itself is intricate when used for submerged plants, and gives only limited information; it should always be backed up by groundtruth, preferably verification by SCUBA diving (Long 1979, Lachavanne & Wattenhofer 1975).

For insight into the species diversity and population density, the use of a transect based on depth with variable distance can be adopted. At each station SCUBA diver and boat operator choose a transect

perpendicular to an innermost point on the shore. Orientation to this point is made using landmarks. Distance is estimated using topographic maps. The course of the transect can accurately be followed by aid of a diving compass and depth gauge. Depth and population density of each species are recorded on plastic sheet with a pencil. For the estimation of biomass of each species from density observations, the mean value for each population density index is multiplied by the average mature dry weight of an individual shoot. In choosing this method it should be borne in mind that it requires special expertise not only in the skills of SCUBA diving, but in taxonomy as well. Before population density can be estimated for a particular aquatic ecosystem, species diversity must be determined and the diver must be familiar with most of the species concerned and be able to make rapid taxonomic decisions. The few unknown species can easily be gathered for identification later on.

Relatively recently a recording fathometer has been used for the mapping of vegetation in the USA. Its use is limited to waters with good visibility and it has proved to give reliable information on plant biomass provided the species already has been identified (Maceina & Shireman 1980, Maceina *et al.* 1984).

In using the population density for biomass estimates as described, several factors should be taken into account, particularly the differences in seasonal growth patterns of the species, underground biomass and depth-dependent production of biomass.

The methods described are largely non-destructive. However, destructive methods are usually used. These include harvesting quadrats of different dimensions within macrophytic vegetation types. For the estimation of species diversity, random quadrat sampling has been shown by Sheldon & Boylen (1978) to be only about 60% as effective as SCUBA diving in enumerating species presence. On comparing data on population density collected by random quadrat sampling *vs* density-based estimates by SCUBA diving, they found that in general there was a highly significant correlation. However, a greater number of samples had to be taken in the random quadrat sampling method to give a set precision. Exceptions to this rule were densities lower than 1 plant m^{-2}, when random quadrat sampling yielded in general lower results compared with SCUBA diving, possibly due to the limited number of samples taken in the quadrat sampling method (Wood 1975, Sheldon & Boylen 1978; Wade & Bowles 1981). For the determination of growth rates of submerged plants, whole plants or parts of plants are tagged *in situ* at the onset of the growth season, and the tagged plants are hand-collected later on. This work can only be done by SCUBA diving (Jacobs 1979, Best 1982b).

When the aim of the study is to investigate the species distribution in relation not only to depth but also to type of substrate, then visual

observation or photography of type and homogeneity of the sediments often combined with accurate coring of plant-colonized substrates is useful (Patriquin 1975). Concurrent with this, diver-operated transplantation of the plants can be carried out (Phillips 1976). Underwater photography has been used for investigations on the growth of submerged plants in general, and in particular on effects of pollution, recreation etc. (Zieman 1976).

Submerged plants often form a substantial substrate for periphyton and invertebrates. From above, quantitative sampling is virtually impossible, since part of the periphyton is usually loosely attached and many of the invertebrates flee from the sampler. For quantitative sampling of these groups of organisms, which live in close association with submerged macrophytes though with their own specific habitats, a large variety of methods has been devised by divers (Fager *et al.* 1966, Drew *et al.* 1976). The most generally useful is a diver-operated net with a large square or hoop-like opening at one side, the opening being large enough to easily enclose the target part of the vegetation. The choice of mesh-size depends on the size of the objects to be sampled. After the sample has been netted the open side of the net is closed by a drawstring previously threaded through an extra layer of gauze around the opening. The net is brought to the surface and emptied into a bucket filled with water. Animals and plants are subsequently separated by sieving. This apparatus and procedure is better than using a plastic bag or tube because the net causes much less water movement than the bag or tube. The animals may be identified and counted, and the activity of the periphyton may be measured. Sometimes those tasks are performed in the field, but it is more usual to do this in the laboratory. The activity of the macrofauna living on the macrophytes is nearly always measured in the laboratory because intricate handling is required.

By their presence and physiological activity, submerged plants affect their physical and chemical environment. Measurements of rate of photosynthesis, nutrient uptake and similar variables are often made in the laboratory, but the meaning of these data for field situations is often questionable. It is preferable to measure these processes *in situ* in enclosures or by installing monitors within the vegetation; this equipment is largely diver-operated (Lindeboom & De Bree 1982, Best & Dassen 1987).

As with terrestrial communities, there are several approaches to floristic description. Phytosociological systems have been devised (e.g. Den Hartog & Segal 1964). A more adaptable system such as that of Tansley (1964) may be preferred, or a simple annotated abundance list may be used (Best 1982b). More details are given in the chapter 'The Phytosociological Approach to the Description and Classification of aquatic macrophytic Vegetation (Best 1988).

CHEMICAL SAMPLING

Details of methods of chemical analysis of freshwater are given in the IBP manual No. 8 (Golterman, Clymo & Ohnstad 1978). A similar manual exists for the analysis of sediments and suspended matter (Golterman *et al.* 1983).

When trying to describe the chemical and physical environment it is first necessary to define the purpose of the study. Subsequently the number, frequency and location of the samples should be determined. Two extreme cases serve as examples.

(a) A process or a phenomenon is being studied. In this case it is essential that the chosen process is occurring at the sample location, even if that location is in other respects 'atypical' of the area as a whole. The results will be valid for the process under study and its influencing factors, but extrapolation to the whole lake is not possible.

(b) A lake or a given area must be studied. Now for each typical sub-area sufficient samples must be taken, the number of which depends on the desired precision and the variability of the site. In the first place, therefore, a map must be prepared to recognize how many different zones can be distinguished. Aerial photographs may be useful but if not available a first reconnaissance must be made by whatever means are available. The desired precision will then determine how many samples must be taken. For initial study of stable variables three samples in nominally identical locations may be sufficient; the mean can be calculated and the difference between highest and lowest value gives some idea about the precision. When 10 samples are taken a standard deviation or standard error can be calculated and statistical methods may be applied. Experience shows that it is often — but not always — the case that increasing from 5 to 10 samples gives little extra knowledge and that more than 10 samples is a waste of time and money.

The decision about the frequency of sampling is also difficult to make. The chemical variability during maximum plant density can often be assessed from samples at two or three different times, but to get a measurement exactly at the time of maximum growth rate will need far more samples. The timing of these is likely to be more efficient if prior knowledge of the pattern of variation exists. Sampling should now probably not be at regular intervals but timed to coincide with biomass formation. (Compare the sampling of suspended matter in rivers where the frequency should be greatest during periods of high flow rate.) For the chemical factors in the plant environment the frequency depends

largely on the problem under study. To characterize the major elements or constituents four samples per year may often be sufficient — unless very rapid changes occur, e.g. as in tidal areas — while to study nutrient variations (N, P, Si) samples at 7 to 14 day intervals are indicated.

A first step in any detailed study should be an investigation of spatial and temporal variability. As a bare minimum three or more samples from different places taken as nearly as possible at the same moment and samples from one place at different moments (e.g. early morning, midday and sunset or late in the afternoon) are needed. Inadequate, invalid or even non-existent replication is one of the commonest deficiencies of published work.

Samples can be taken in one of three main ways.

(a) Cylinder samplers consist of a cylinder with caps which are held open while the cylinder is lowered to the desired depth. A 'messenger' weight is then slid down the supporting cable and causes the lids to spring closed. The sample thus trapped is then hauled to the surface. The commonest patterns are the Kemmerer, Ruttner and Friedinger types. The last is probably the best because the end caps are held open parallel to the length of the cylinder thus allowing water to flow through while the cylinder is being lowered.

(b) Gas-filled or evacuated flask samplers are sealed and then lowered to the desired depth. A 'messenger' then breaks the vacuum (Watt sampler) or a tug on a control string allows gas to escape (Dussart and Valas samplers). In all cases the flask fills with water from the sampling depth. These samplers are particularly useful for bacteriological and *in situ* work with tracers.

(c) Peristaltic pump samplers have the advantages that they cause little disturbance of stratification (if there is any) and produce large samples. They are particularly suitable for taking a "mixed" ("weighted") sample from different layers. The collecting flasks may be marked in advance for this purpose, the successive volumes in the flask being proportional to the corresponding volumes of the sampled layers in the lake.

Further details are given in Golterman, Clymo & Ohnstad (1978).

CORERS AND DREDGES

Corers are used to obtain sediment samples in which stratification is preserved and the sediment/water interface is relatively undisturbed. For sediment layers in shallow waters the 'push type' tube sampler is ideal, unless the sediment is too coarse. It consists of a Plexiglass

(Perspex) tube with an outlet valve at the top (any kind of valve will do) which is closed after penetration in the soil. The Jenkin sampler is the ideal sampler for sediment-surface sampling in deeper waters. The device allows plastic tubes to be driven into the sediment layer; the lids remain attached to the tube, so that one tube after another may be filled and brought to the laboratory. The Mackereth corer, driven by compressed air and controlled from the lake surface, allows long cores to be taken. It is a relatively expensive device and cannot take cores which are much shorter than its designed length. Cheaper and more flexible is the Livingstone corer in which a tube is driven by rods past a piston, held fixed at the top of the core by a wire attached to the boat.

Dredges or grab samplers (e.g. the Ekman or the Peterson Dredge) are used to sample the top few centimetres of bottom deposits. The samples are disturbed and the precise representation of depth in the sample is unknown. They provide a rapid means of characterizing the 'average' sediment composition over a limited range or depth — the so-called 'bulk sample'. A detailed critical evaluation of about 10 corers is given in Golterman *et al.* (1983) and of several sediment samplers, including the air-lift pump (which gives a very disturbed but large sample) by Elliott & Tullett (1978, 1983).

PHYSICAL VARIABLES

Temperature and light

Light and temperature are of interest in all vegetation studies and in the interpretation of, for example, pH measurements and solubility products. They are often measured at the same time that the water is sampled for chemical analysis. Both variables must be measured *in situ*; the methods are obvious and usually simple. Difficulties will be met when deciding what to measure, where to measure it, and when and what equipment to use. Both the light flux on the surface and its attenuation below the surface may be important, particularly for studies concerned with the growth of phytoplankton and submerged macrophytes.

Temperature

There are four types of apparatus in use.

(1) (*Reversing*) *thermometer.* If the water can be directly reached with a normal laboratory thermometer or if there is no serious delay in placing the thermometer in the sample itself (in the outlet tube of the pump for example), a normal laboratory thermometer is the

most common and simple instrument to use. Precision of 0.1 or 0.05 °C can easily be obtained. If direct access is impossible then a reversing thermometer may be used. After the thermometer has reached equilibrium at the required depth a messenger weight is sent down the cable and causes the thermometer to invert in its frame and separate the mercury thread from that in the main bulb, thus preserving the reading while the bottle is hauled up. A precision of 0.01 °C may be obtained.

(2) *Thermocouples.* These produce an electrical potential difference which is proportional to the difference in temperature between a reference junction and the sensing junction. The voltage is small and not easily amplified, though the difficulties are much smaller than they were before integrated circuits became common and cheap. They need a reference junction at known temperature.

(3) *Thermistors.* These are based on material which has a large negative temperature coefficient of resistance. They are relatively cheap. A precision of 0.1 °C is easily obtained.

(4) *Nickel or platinum resistance elements.* They are also based on a change in resistance. Compared with (3) they are more expensive, more accurate and more stable. A precision of 0.01 °C is possible.

The choice of equipment depends on purpose and availability. For general purposes a frequently calibrated thermistor is probably the most useful. If occasional more accurate measurements are needed a reversing thermometer will do very well. For the most accurate work — measurement of precise stratification for example — a nickel wire thermometer may be preferred.

Apparatus such as many O_2 electrodes includes a thermistor so that temperature is measured as well. In these cases it is necessary to make sure that the temperature of the water is being measured and not that of the cell.

Light and its attenuation

Measurement of light normally involves two aspects, the measurement of the total quantity and its spectral distribution.

Light, which is that part of the electromagnetic spectrum visible to the human eye, falls within the approximate limits 400 to 700 nm. This range is also roughly that of 'photosynthetically active (or available) radiation' or 'PAR', which falls between 350 or 390 and 700 nm depending on the definition used. Halldall (1967) has shown that light down to 310 nm may be active in photosynthesis in algae, but light of this short wavelength is rapidly attenuated by water.

Measuring light therefore implies integration over a particular range of the spectrum and often over time. The level of integration must be

carefully chosen. Collecting complete spectra at several depths at frequent intervals is very expensive and for vegetation studies rarely necessary. Integration can best be done automatically by the sensor. The time integral of electric signals may be obtained electronically or by allowing the current to remove and deposit metal on the plate of an electrolytic cell (voltameter; see Westlake & Dawson 1965).

There are three types of target for measurements: incident quantity (irradiance and photon flux density), absorbed quantities (such as absorption spectra) and effective quantities (photosynthesis and illuminance). The choice of the target depends on the purpose of the work. If the problem requires an energy balance, irradiance will be needed. Irradiance in the PAR range may be best for production studies, as the units in these studies are normally those of energy flux.

Photochemical reactions (among which is photosynthesis) depend in nearly all cases on the number of quanta absorbed. The quantum of light is called a photon. It is therefore useful in some cases to measure not irradiance but photon flux density (PFD). There is a close physical relation between irradiance and PFD because the energy of a photon is inversely proportional to its wavelength. One chemical mole of molecules, ions or atoms contains about 6.02×10^{23} particles, so PFD is conveniently expressed in mol m^{-2} s^{-1}, sometimes called Einstein m^{-2} s^{-1} = E m^{-2} s^{-1}. Some authors prefer quanta m^{-2} s^{-1}. Figure 1 shows the spectral curves for irradiance and PFD, and the Table 1 shows some equivalences and typical values. Incident quantities such as irradiance and PFD are well-defined and unique; absorbed quantities, such as the absorption spectrum of chlorophyll *a* or of a whole leaf, are less well-defined.

Table 1. Some light quantities and units.

Quantity	Irradiance (= Irradiant flux density)	Photon flux density	*Illuminance
Units	$W\ m^{-2} = 0.1\ mW\ cm^{-2}$	$\mu mol\ m^{-2}\ s^{-1}$ = μEinstein $m^{-2}\ s^{-1}$ = 6.02×10^{17} quantum $m^{-2}\ s^{-1}$	$lx = lm\ m^{-2}$
Obsolete	$cal\ cm^{-2}\ min^{-1}$		foot candle = lumen ft^{-2}
Units	$692 = \frac{cal\ cm^{-2}\ min^{-1}}{W\ m^{-2}}$		$10.76 = \frac{ft\ candle}{lux}$
At 555 nm	$1.0\ W\ m^{-2}$	= $4.15\ \mu mol\ m^{-2}\ s^{-1}$	= 680 lx

* Illuminance units are given because they have been widely used but illuminance cannot be recommended as a measure in limnology.

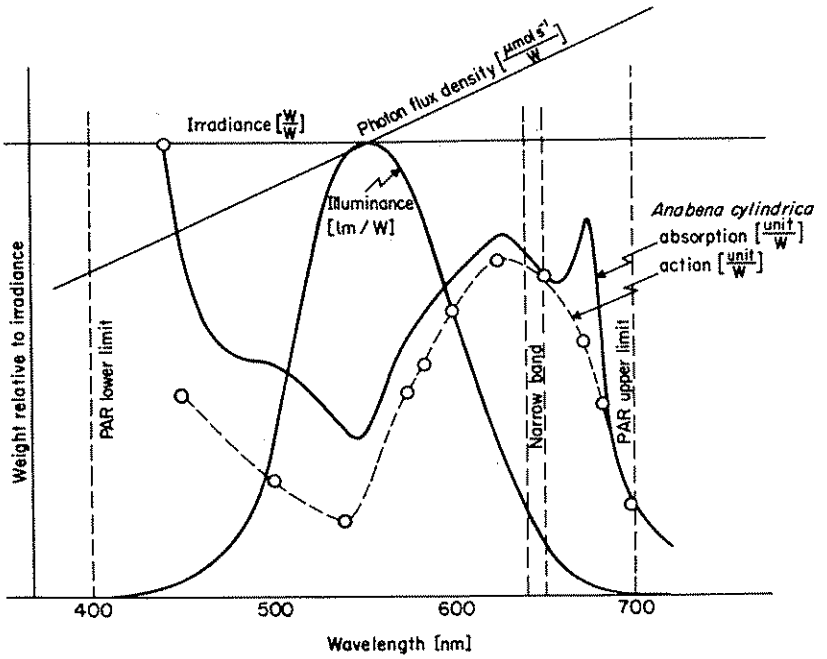

Fig. 1. 'Targets' for measurement: (a) incident (b) absorbed (c) effective. (a) Relative weight given to energy flux density as a function of wavelength for irradiance and photon flux density. (b) Absorption spectrum of whole *Anabaena cylindrica*. (c i) O_2 evolution (photosynthesis) action spectrum of *Anabaena cylindrica* for equal irradiance. (c ii) Illuminance: response of the light adapted young adult human eye. Both (b) and (c) are illustrative examples of whole classes of possible targets. Targets may be given specific spectral limits. For illustration a narrow band in the red and the broader 'photosynthetically active (or available) radiation', PAR, are shown. The definition of PAR is not agreed (see text). (From Golterman *et al.* 1978).

Measurement of total incident radiation

A series of thermocouple junctions alternate between the black and white areas of the sensor. As the black area becomes hotter, a combined potential difference is produced (typically between 5 and 15 mV in bright sunlight). This may be recorded by a potentiometer or it may be used to drive a sensitive current meter. A well-designed sensor has an almost level response between 400 and 2700 nm if glass-covered. In daylight PAR can be estimated as 0.50 times this irradiance, but it is preferable to use two instruments one of which has a filter which cuts out all wavelengths below 700 nm. PAR is then calulated from the difference. Better still is to cover the sensor with filters which have, in combination, a level and small absorption within the PAR range and a sharp change to almost complete absorption outside it.

Other light sensors are the photomultiplier tube, the selenium barrier

layer cell, the photodiode, silicon cell, and the CdS photoresistor. The energy-sensitivity of detectors other than thermopiles is markedly wavelength-dependent: Figure 2 shows examples. The selenium barrier layer photocell, which is the most commonly used cell, has a response fairly close to that needed for illuminance measurements, but this is of little use to the limnologist. On the other hand the electrical characteristics of these detectors are more favourable than are those of the thermopiles. Two solutions are possible.

(1) Use a filter or combination of filters which converts the spectral response to that needed for irradiance or photon flux density. The steps in the design are illustrated in Figure 3. Other solutions are given by Jerlov & Nygard (1969) and by Uphoff & Hergenrader (1976).
(2) Use filters which isolate narrow spectral bands and measure and calibrate with each separately. The filters are delicate. Evans (1969) gives details.

The CdS detector differs from others in that the property which varies with irradiance is the electrical conductance. Its spectral response is not easily transformed to irradiance or PFD.

The Si photocell is potentially useful; if the output is fed through a low (10—50 ohm) external resistor the response is linear, but if fed through a high resistor the response is logarithmic.

Photomultiplier tubes, which are in use in oceanography, are the

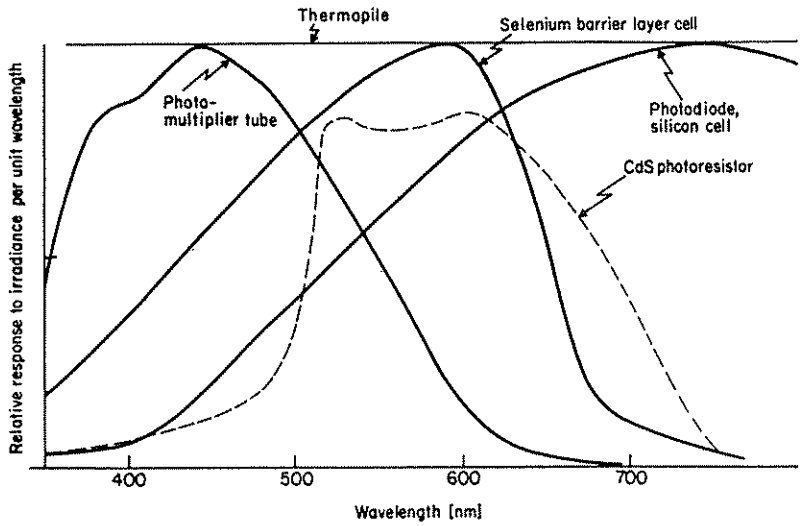

Fig. 2. 'Detectors': spectral sensitivity of various light detectors. These are typical for the type but *there is variation within types* i.e. do not assume that the curve here refers to your detector of this type. This is particularly true for photomultiplier tubes. The units are not comparable between detectors.

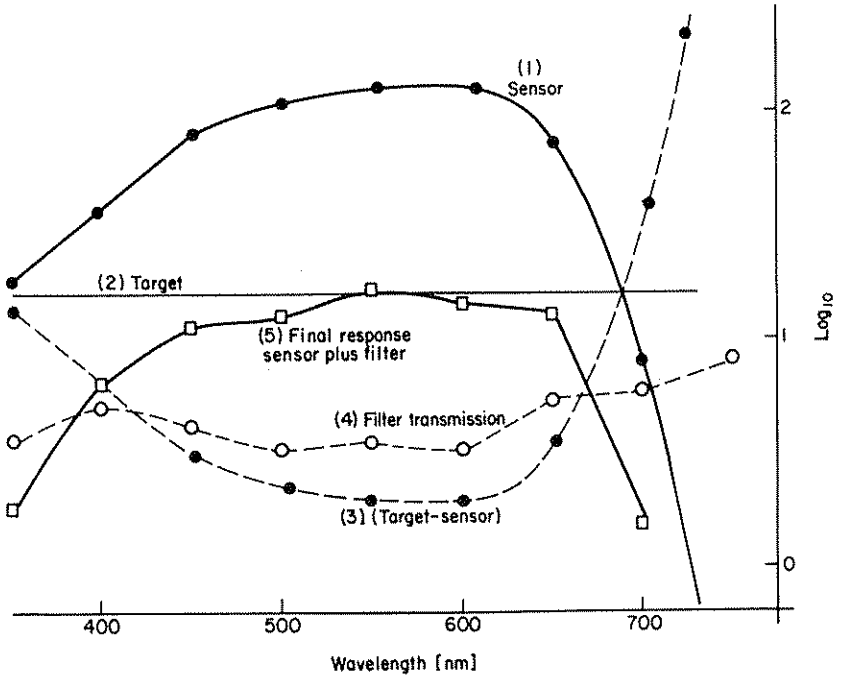

Fig. 3. Designing a sensor to measure irradiance in the PAR range. The example is that of Powell & Heath (1964) modified by Westlake & Dawson (1975), though the logarithmic plot used here is not theirs. First the sensor — a selenium barrier layer photocell (Megatron B) — is chosen and the spectral response (1) graphed with a logarithmic scale. Next the target — irradiance (2) — is graphed, again on a logarithmic scale. The vertical position is unimportant. Next the vertical distance (3) between target and sensor curves is plotted. This distance, a difference of logarithms, is the ratio target/sensor response and is the curve which must be *paralleled* by the filter transmission, again on a log scale. The filter curve (4) is for the combination of Cinemoid 'Steel Blue 17' and 'Pale Salmon 53' filters (Strand Electric). The overall response of sensor and filters is shown as (5), which has been shifted vertically to aid comparison with the target (2).

most sensitive of all detectors. They need high voltages and retain a memory of saturation, which occurs at very low irradiance. They are not a practical proposition for the non-specialist.

For detailed work on the photosynthesis of plants (or bacteria) absorption targets are attractive. Interactions with other chlorophylls and accessory pigments (including the photosynthetically inactive ones) may complicate matters and an action spectrum may be indicated. Photosynthesis action spectra in light limiting conditions are usually more level than absorption spectra, but are rarely known and are not measured *in situ*. The action spectra may well depend on other undefined physiological and environmental variables as well. Further-

more, near the surface of a water body during summer light is often non-limiting for photosynthesis so the target would be non-linear, and to different degrees for different plants. The effective target cannot in practice be defined for general limnological work when there are varying proportions of algae of different groups or a variety of vascular plants and varying environmental conditions.

It may be important to measure the amount of light absorbed by plants, particularly by phytoplankton, if primary production is to be calculated using a model. Examples for algae are given by Kirk (1976, 1980, 1981); for periphyton by Meulemans (1985) and Sand-Jensen & Sondergaard (1981); and for macrophytes by Best & Dassen (1987).

One effective target which is well defined is the response of the average photopic (which light adapted) young adult human eye. This is internationally agreed (Commission de l'Eclairage, 1924) and has recognised units. The target quantity is called illuminance, the units are lumen $m^{-2} = lx$ (lx is the symbol for the unit 'lux'). Intensity, which is the property of the light source, and illuminance are not among the effective quantities and are not usually of interest to the limnologist. They are not commonly measured either, though the terms are commonly mistakenly used for irradiance or illuminance or some other measure similar to illuminance. Illuminance as a target can only be justified where the problem involves the human ability to see under water. Even then it is probably incorrect because the sensitivity of the dark-adapted (scotopic) eye differs from the photopic one used in the definition of illuminance. The sensitivity of the fish eye is considerably different from that of the human eye.

Though illuminance is indefensible as a limnological quantity many published works mention it or contain illuminance units measured with a sensor which does not have the illuminance spectral weighting. To complete the confusion this is often called 'light intensity'. The reason is that the selenium barrier layer photocell with which most of these measurements have been made, is cheap, robust and gives a large current, which can drive a meter directly. It can be calibrated in illuminance or even irradiance units for a source *with a particular spectral composition*, but the readings will be incorrect if the spectral composition of light falling on the detector changes, as it does if the detector is lowered deeper into water or amongst aquatic vegetation. This may lead to very large error: Tyler (1973) estimated that 600 or 700% is not unlikely. Table 2.3 of the IBP Manual gives some guidance.

For some purposes — calculation of heating and evaporation for example — absolute measurements are necessary. For productivity studies relative measurements may often suffice. Sometimes most measurements are relative but one of them is absolute; this one will at the same time be used to calibrate the whole set. This is often the case

when spatial (horizontal or vertical) distribution must be measured. In limnological work light attenuation under water is often an important target. It can best be measured by lowering a sensor step by step. But because it is unlikely that during a series of measurements the light climate at the surface will remain constant, two matched sensors must be used one of which is kept in a standard position and the other moved to measurement positions. The meter may be switched rapidly between the two sensors and the movable sensor's reading recorded as a quotient of that of the fixed one. This may nowadays be done electronically with ease. In this way light attenuation with depth can easily be recorded; it must be kept in mind, however, that with increasing lake depth the distribution over the different parts of the spectra will change.

Light attenuation in water is caused by reflection at the surface both back into the air (albedo) and from below back into the waterbody, by scattering by particles and by absorption by the water itself and by the dissolved substances.

If no proper meter is available light attenuation can be estimated by recording the depth at which a white disk (Secchi disk, 25—50 cm diameter) just disappears. Measurements should be made preferably during the middle of a sunny day. It is usual to take the average of the depth at which the disk just disappears and just reappears. The results will be more reproducible, though different, if diving glasses or an observation box, both dipping just under water, are used.

In favorable conditions — calm water, bright light — irradiance at the Secchi disk depth is about 15% of that just below the surface. From this it follows, that the depth at which irradiance falls to 1% of that just below the surface is 2.5 times the Secchi disk depth and that the vertical attenuation coefficient ε_v is roughly 1.9/Secchi disk depth. These calculations assume however that the water column is homogeneous, and that the Secchi disk depth is related to irradiance. As there is little agreement about the size and pattern of markings on the disks, results are even more difficult to compare than need be.

The calculations should therefore be treated as rule of thumb estimates subject to errors of at least a factor 2. The method is much better than nothing however.

THE CHEMICAL VARIABLES

Chemical substances in the water can be divided into suspended material (or sediments) and dissolved compounds. The latter can be divided into gases, major, minor and trace elements or constituents, and organic compounds (Table 2). Of the gases, O_2 and CO_2 are measured

Table 2. Major, minor, trace elements, organic compounds, and gases normally found in natural waters.

Major elements		*Minor elements*	*Trace elements and organic compounds*	*gases*
Ca^{2+}	HCO_3^-	N (as NO_3^- or NH_4^+)	Fe, Cu, Co, Mo, Mn, Zn, B, V	O_2
Mg^{2+}	SO_4^{2-}	P (as HPO_4^{2-} or $H_2PO_4^-$)		CO_2
Na^+	Cl^-	Si (as SiO_2 or $HSiO_3^-$)		N_2
K^+	F^-		organic compounds such as humic compounds, excretion products, vitamins, other metabolites	
H^+				
(Fe^{2+})				
(NH_4^+)				
Concentrations usually found				
0.1—10 meq. l^{-1} (mean 2.4)		(< 1 mg l^{-1}) (Si sometimes higher)	$\mu g\, l^{-1}$ (trace elements)	

in most pollution and photosynthesis studies (including eutrophication studies). Nitrogen gas is seldom measured.

'Major' ions are generally present in the water and in living materials in relatively high concentration. They can normally be determined by classical titrations or precipitation reactions. Their sum largely determines the electrical conductivity. The 'minor' elements are often important determinants of the quantities of the selected biological species which an ecosystem can produce. They are normally measured by colorimetric procedures.

Titrations and precipitation procedures are normally specific or can easily be made to be so. Their calibration is absolute: e.g. 1 mg NH_3-N as base will neutralize 3.57 ml of 0.02M H^+.

Colorimetric reactions are normally not specific and interferences are to be expected and must be avoided by special measures which may differ from lake to lake. The extent of bias in the results will depend very much on knowledge of the possible presence of interfering substances.

It is common usage to speak about colorimetric methods if a filter colorimeter is being used and to use the term spectrophotometric procedures if a spectrophotometer is being used. This distinction is one of degree however: the same chemical reactions are involved in both cases.

Colorimetric and spectrophotometric methods depend on a calibration curve which gives the relation between quantities used and color developed. The relation is not necessarily a straight line, but it is convenient if it is. The final precision (and accuracy) of the method depends as much on the care with which the calibration curve has been prepared as on the care given to the sample.

If one studies a new environment, the following variables are normally the first to be measured: temperature, oxygen, conductivity, redox potential (pE) and pH. The information provided by these five variables is very valuable, especially when the variation in time and space are being assessed.

Conductivity gives information about the total salt content, the so-called salinity. It can normally be assumed that for freshwater with a pH in the range 4.5—10.5 then

$$C \simeq 0.01\,k \quad \text{and} \quad C' \simeq 0.75\,k$$

where: k = conductivity (μS cm^{-1}); C = sum of concentration of positive and negative ions (mmol l^{-1}); and C' = total salinity (mg l^{-1}).

Conductivity gives no information about which salts are present e.g. $Ca(HCO_3)_2$ or NaCl. In combination with the pH, however, a good guess at the nature of the ions can usually be made; waters containing $Ca(HCO_3)_2$ and in equilibrium with air usually have a pH greater than 8. A chloride titration (even a very simple one) may be extremely useful for a first classification of the chemical composition.

The redox potential gives a good description of the oxidation state of the water or bottom. It is a purely descriptive measure and it should not be given too much weight. Thermodynamically it is a well defined measure based on a reversible electrochemical reaction; in nature this is never the case. It is derived from unknown chemical reactions which essentially are not reversible. For detailed discussion see Golterman (1975) and Golterman *et al.* (1978). Temperature, oxygen, conductivity, pH and pE can be measured with reliable field equipment, but often not with great precision though sufficient for a first descriptive reconnaissance. The standard deviations are (roughly): temperature 0.1 °C; O_2 0.2 mg l^{-1}; conductivity 5 μS cm^{-1}; pH 0.1 unit.

Laboratory instruments provide a much smaller standard deviation: O_2 0.02 mg l^{-1} (Winkler titration); conductivity 0.5 μS cm^{-1}; pH 0.01 unit.

The equipment is described in Golterman *et al.* (1978, procedures 3.1, 3.2, 3.3 and 8.1). A few precautions should be noted however.

All these field instruments and instruments which are used for monitoring in the field should be inspected and cleaned regularly lest their response is changed by fouling and 'Aufwuchs'. It is essential that they are calibrated by independent means or equipment. O_2 probes, for example, should be calibrated regularly against the Winkler titration.

All these instruments have an analogue voltage and, increasingly commonly, a digital output. This may be recorded in the field or transmitted by telemetry. Such transmission of results by radio either continuously or on command may be useful for monitoring lakes at great distances or in relatively inaccessible areas. These signals can warn of sudden changes, but may be less suitable for recording small

concentration variations, which for biological observations may be much more useful or important.

For several major elements (calcium, sodium, potassium, chloride) specific ion electrodes are available. They are similar to glass electrodes in that they produce a voltage proportional to the logarithm of the activity. Monovalent ions produce about 58 mV for a ten-fold change in concentration; divalent ions produce about 27 mV for the same change. A reliable high-impedance voltmeter is as necessary for such measurements as it is for pH measurements. A pH meter may be suitable, though calibration for divalent ions will be different from that marked on the scale. Sometimes it is necessary to add specific reagents to the sample, and for this reason such methods are often used in the laboratory only. Specific electrodes such as those for calcium and chloride may be useful for quick survey, provided that high accuracy is not desired.

The major elements

All major elements can be measured by classical titration or precipitation reactions. Calcium, for example can be estimated with the classical oxalate-precipitation reaction; the method needs a lot of practice but gives excellent precision and accuracy. Easier — but not more precise — are the procedures shown in Table 3, which are commonly in use:

Table 3. Common procedures for determining major elements.

Element	Method	IBP No. 8 procedure
Ca^{2+}	AAFS	4.3.4
	EDTA titration	4.3.1—4.3.3
Mg^{2+}	AAFS	4.3.4
	EDTA titration	4.3.1—4.3.3
Na^{+}	flame emission	5.7
K^{+}	flame emission	5.7
Cl^{-}	volumetric with Ag^{+}	4.6
	with potentiometric endpoint	4.6.3
	with indicator endpoint	4.6.2
	with conductometric endpoint	4.6.4
SO_4^{2-}	volumetric EDTA	
	indicator endpoint	4.7.3
	potentiometric endpoint	4.7.4
	turbidimetric	4.7.1
Total alkalinity (OH^{-}, HCO_3^{-} and CO_3^{2-})	acidimetric	3.4.2 or 3.4.3
Alkalinity (OH^{-}, CO_3^{2-})	acidimetric i.e. phenolphthalein	*idem*
Acidity	titration with $Ba(OH)_2$	3.6

The AAFS (absorption spectrophotometry) method requires expensive equipment, which demands proper maintenance and calibration. All acid-base titrations can be made with a suitable indicator — which must be selected carefully — or with a pH meter endpoint. Some chemical knowledge is needed; the HCO_3^- titration has a precise endpoint which depends on the concentration of the HCO_3^- present. If the full titration curve is being made, this information is provided automatically, but preset endpoints may be dangerous if a high precision is desired.

The minor elements

The minor elements occur in soluble form (NH_4^+, NO_3^-, *o*-phosphate, hydrolyzable phosphate i.e. organic- and polyphosphates, and silicate) and in particulate form (inorganic and organic particulate phosphate, organic nitrogen and inorganic silicate). The separation is achieved by filtration, normally through a 0.5 μm filter. This pore-size is arbitrary, but practical. For special cases 0.2 μm is sometimes used. The particulate forms are made soluble by one of the following digestions: hydrolysis in strong acid medium, with or without oxidation, or by fusion with alkali.

For the minor elements the following reactions are suggested.

Phosphate is measured as the blue color formed after complex-formation with molybdate (in the presence of antimony) and subsequent reduction, both in about 0.4M H^+. Ammonia is measured either as the yellow color formed with Nessler's reagent (the active component of which is mercury ion), or as indophenol blue. The first method can only be carried out after a distillation of the ammonia from an alkaline medium. This distillation is time consuming and may hydrolyze weakly bound organic nitrogen; it can be argued that such ammonia compounds are also easily hydrolyzable in water and are thus biologically available. The indophenol blue method is based on the blue complex formed by ammonia and phenol which are coupled under oxidation, e.g. with alkaline chlorine. The method is not yet standardized, many different modifications are still in use and interferences in natural waters are not yet well studied. Some organic nitrogen may react. Nitrate must first be reduced, either to NO_2^- or to NH_4^+. The former reaction is often effected with cadmium in some special form. Organic compounds may poison the reductant; the addition of internal standards is still necessary. Most organic compounds may be destroyed by boiling with $K_2S_2O_8$, but then some organic nitrogen may be oxidized to nitrate. Normally the precision is 2—3%, but with the different steps needed to achieve the reduction, it may easily increase to 5%. Silicate is measured, like phosphate, with molybdate; the molybdate complex is

reduced again. The first step — the formation of the yellow molybdate complex — occurs in a weakly acid medium; the reduction step is carried out in strong acid medium or with specific organic acids in order to prevent the reduction of the phosphate complex. Arsenic interferes both in the phosphate and silicate procedures, but the concentrations are usually sufficiently low to be ignored. In special cases attention should be given to this interference.

The IBP Manual No. 8 gives for the three minor elements a choice of methods, often dependent on concentrations present, availability of equipment and complexity of the method itself.

Trace elements

The trace elements can be classified in one of two groups: those elements which occur naturally in water and are generally plant nutrients, and those which are potentially toxic even in very low concentrations and which have been widely distributed as a result of human activities. The elements of the first group are normally measured to obtain insight into the factors controlling biological characteristics, while elements in the second group are often monitored to detect threats to the health of human and other living things. Copper and zinc are examples of elements which may belong to both groups: they occur naturally and are plant nutrients, but the present concern is mainly related to their possible toxicity. Because of their low concentrations, analysis of these trace elements presents problems, which normally require special equipment and techniques. Contamination or losses can occur easily; dust in the laboratory is a serious problem because this interference is never reproduced in the blank. Standard additions and distilled water blanks should always be used. The validity of each method should be checked for each particular situation.

The trace elements normally occur as dissolved (both ionic and chelated), colloidal and suspended matter adsorbed to silt or as heavy metal oxides. The most common analytical method is atomic absorption flame spectrophotometry (AAFS), normally after a solvent extraction and chelation. AAFS methods do have some interferences. A development of flame emission spectrophotometry but at a temperature in the range 5000—10000 °C (produced by ICP — inductively coupled plasma) has even fewer interferences than AAFS and may prove useful because many elements can be measured simultaneously (or effectively so). The equipment is very expensive however. In the case of sediment and suspended matter, whatever the analytical method, the metals must first be extracted. For this purpose a destruction with several mineral acids is usually employed (Golterman *et al.* 1983). In specialized laboratories polarography (classical, cathode ray and anodic stripping

voltammetry) is useful. There has been some discussion about which forms of the element can be measured using this method (Golterman *et al.* 1978).

A few colorimetric methods are in use (e.g. copper, iron and manganese) but normally the concentrations in the aquatic habitat are too low.

Typical sensitivities (mg l^{-1}) for some elements measured by AAFS are: Al 0.05—1.0; Cd, Co, Cu, Mn, Zn 0.001—0.01; Fe, Pb 0.001—0.05; Cr, Mo 0.0002—0.01.

Sampling, storage and sample digestion need some special attention. Completely non-metallic samplers should be used. Rubber parts should be avoided as rubber may contain considerable amounts of zinc.

Precision, bias, accuracy, sensitivity

All measurements are susceptible to errors. These may be erratic mistakes, systematic errors, and random errors. Mistakes may be instrumental or human. Sometimes they can be identified: for example a wrong flask number or a wrong pipette. It is wise to keep the equipment including flasks available on the laboratory bench until the results have been definitively calculated.

Systematic errors are related to the concept of bias; random errors are related to precision, reproducibility and dispersion. In rifle shooting it is possible to get several shots grouped close together, but a long way from the target. Such a group has a high precision or reproducibility (small dispersion) with small random errors. The group has however, a large bias. High precision may be accompanied by large bias if, for instance, a calibration curve is not properly made. Low precision with small bias may also occur, but is less dangerous: it is more obviously unsatisfactory. One may define $\text{accuracy}^2 = \text{bias}^2 + \text{precision}^2$ so that accuracy is the hypotenuse of a right triangle whose sides are bias and precision. The same accuracy may be obtained by small bias and large imprecision or by large bias and small imprecision.

Sensitivity has many definitions. One is the concentration giving a value three times that of the blank. In atomic absorption flame spectrophotometry sensitivity is often defined as twice the background noise (manufacturers of AAFS are tending to define sensitivity in terms of standard error or standard deviation of repeated blanks); the same must be done with high blanks in colorimetry (e.g. Nessler). The IBP manual No. 8 gives a more extensive discussion of these problems.

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