

The Direct Determination of Biomass of Aquatic Macrophytes and Measurement of Underwater Light 1985

Methods for the Examination of Waters and Associated Materials

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and Measurement of Underwater Light 1985**

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CORRECTIONS

Page 22, second line of caption *for "b)" read "a)"*

Page 32, para 4, line 7 *for "responses" read "responds"*

Page 32, para 8, after line 7 *insert the following "If relative
measurements are being made, the
transmission:"*

Department of the Environment

November, 1986

LONDON: HER MAJESTY'S STATIONERY OFFICE

Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

Lone working, whether in the laboratory or field, should be discouraged.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue

equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Service Monograph 6, HMSO, London.

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio-chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

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About this series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need for waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods and notes being issued when necessary.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality

Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

- 1.0 General principles of sampling and accuracy of results
- 3.0 Empirical and physical methods
- 4.0 Metals and metalloids
- 5.0 General nonmetallic substances
- 6.0 Organic impurities
- 7.0 Biological methods
- 9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee.

The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Secretary

1 July 1986

The Direct Determination of Biomass of Aquatic Macrophytes and Measurement of Underwater Light 1985

Methods for the Examination of Waters and Associated Materials

This booklet contains four methods for the direct determination of Biomass, with a method for locating position on a lake; and a series of methods for measurement of underwater light.

Contents

Warning to users	i	A Basic Guide to the Use of the Optical Square for Position Fixing	26
About this series	ii		
Disclaimer	2	Measurement of Underwater Light	29
Hazards	2	Symbols and Abbreviations	29
		1. Summary of Methods	30
The Direct Determination of Biomass of Aquatic Macrophytes	3	2. Introduction	31
Symbols and Abbreviations	3	3. Apparatus	33
1. Summary of Method	3	4. Procedure	34
2. Introduction	4	5. Calculations	35
3. Identifications	5	6. Interferences	36
4. Apparatus	5	7. Errors	36
5. Sampling Procedure	7	8. Calibration	37
6. Calculations	12	9. Addresses of Suppliers	37
7. Analyses	13	10. References	38
8. Estimation of the number of quadrats required for biomass estimates of selected precision using Method C	14	Address for Correspondence	44
9. Addresses of Suppliers	15	Membership of the Committee Responsible for these Methods	45
10. References	15		

Disclaimer

This booklet mentions suppliers of some of the more unusual equipment which may be required for these methods. This is in no way intended as an endorsement for any particular product and similar equipment with adequate performances would be equally acceptable.

Hazards

Lone working is discouraged. Always keep someone not with you informed of your itinerary and expected times of arrival and departure. Take note of expected weather conditions but allow for sudden changes which may occur at the sampling location at that time of year, dress accordingly, and have spare warm clothing available, especially in cold weather. If working on or near water wear a life jacket and a safety harness. Observe all safety rules prescribed for the equipment used and be thoroughly trained in its use beforehand. Ensure that equipment is expertly and regularly serviced and repaired (see also Section 4.1 in the Biomass method). It is strongly recommended that provision be made for summoning help immediately should an accident occur to the sampler, portable radios and telephones are useful for this.

Care needs to be taken when sampling for biomass determinations not to cause permanent denudation or erosion of a stretch of bed, disturb breeding fauna, and so cause what has aptly been named environmental monitoring pollution.

The Direct Determination of Biomass of Aquatic Macrophytes

D. F. Westlake, D. H. N. Spence and R. T. Clarke

Symbols Used

A	Whole area of site (m^2).
a	Area of weed-beds (m^2).
B	Mean biomass density of aquatic vegetation per unit <i>vegetated</i> area ($g\ m^{-2}$).
B_{max}	Maximum biomass per unit vegetated area (mean biomass at optimum depth).
b	Mean biomass density of aquatic vegetation per unit area of site (overall mean biomass).
c_r	'Cost' of sampling a quadrat (min or £) in stratum r .
CV	Coefficient of variation of biomass quadrats (i.e. containing plants; $CV = S/\bar{B}$).
CV_b	Coefficient of variation of <i>mean</i> biomass of whole site (see section 8. $CV_b = s/\bar{b}$).
L	Confidence limits of overall mean biomass, with a particular level of probability ($L = t_\alpha s$ with $\alpha\%$ probability).
λ	Highest error tolerated (with $\alpha\%$ probability), expressed as a proportion of the overall mean biomass; ($\lambda = t_\alpha s/b$ where $t_\alpha =$ Student's t value).
M_r	Total area of stratum r (m^2).
m_r	Area sampled in stratum r (m^2 ; area of quadrat \times no. of quadrats).
N	Total number of quadrats observed for presence of plants (presence/absence quadrats).
N_p	Number of presence/absence quadrats containing plants (vegetated quadrats).
n	Total number of quadrats sampled for biomass (biomass quadrats).
r	Stratum number.
p	Proportion of presence/absence quadrats containing plants (N_p/N).
S	Standard deviation of mean biomass (\bar{B}) per unit vegetated area.
S_r	Standard deviation of biomass samples in stratum r .
s	Standard deviation of the mean biomass density \bar{b} of the whole site (see section 8).
t	Student's- t (obtained from tables in statistical text-books).
v	Variance of mean biomass of site.
v_r	Variance of mean biomass of stratum r .
Z_c	Maximum depth (m) of plant growth.
α	Percentage probability of confidence limits. In $\alpha\%$ of cases the estimated mean will lie between the confidence limits.

1. Summary of method

1.1	Biota determined	Dry weight of macroscopic green plants.
1.2	Habitats sampled	Submerged or floating vegetation in flowing or static waters; emergent vegetation in water or on banks.
1.3	Type of sampler	Various forms of quadrats, corers or grabs.

1.4	Basis of operation	Samples of the vegetation are removed from known areas, weighed and their dry matter content determined. A range of modifications is given to suit vegetation, water depth and precision required; from wading to SCUBA diving.
1.5	Form of data	Quantitative; the precision depends mainly on the spatial variability of the stand and the number of samples; the accuracy depends mainly on interferences. Values in g dry wt per square metre of habitat.
1.6	Range of application	~100–10000 g m ⁻² . Not suitable for very swift or very turbid water or woody vegetation. Very poor precision if lack of time limits the number of samples taken.
1.7	Interferences	Adhering material such as epiphytic algae, detritus, calcium carbonate or water.
1.8	Errors	<ul style="list-style-type: none"> i. Errors due to spatial variability. ii. Errors of sampling technique, especially incomplete removal and edge errors. iii. Errors of determination, especially in washing, sub-sampling and drying.
1.9	Logistics	<ul style="list-style-type: none"> i. Sampling. Very dependent on water depth, vegetation and precision required. For 12 biomass samples, which may not be enough for good precision, from 6 to 50 man-hours, excluding time to design sampling programme and time to reach sites. For presence/absence quadrats the time needed will be almost entirely dependent on the time taken to locate the quadrats, which will depend on the size, depth and water movements of the water body. On average 15 to 30 man-hours for 100 presence/absence observations. Both figures exclude support and safety staff and must be nearly doubled for whole field teams. Sampling with grabs may reduce the time for 12 biomass samples to 10 man-hours but this excludes the essential calibrations. ii. Determination. Very dependent on need for washing and sorting as well as the number of samples. For 12 samples ~ 1 man-hour for weighing fresh, from 3 man-hours to 36 man-hours for washing, sub-sampling and sorting, 16 hours for drying and ~ 1 man-hour for weighing dry.

2. Introduction

Macrophytes are macroscopic green (or chlorophyll-containing) plants. They may grow out of the water, be completely submerged, or have leaves floating on the surface. Most are attached by roots in the sediment but a few are free-floating. Taxonomically they include angiosperms (flowering plants), pteridophytes (ferns, horsetails and quillworts), bryophytes (mosses and liverworts) and charophytes (stoneworts). The demarcation from larger green algae (or brown and red algae in the sea) is one of convenience; one of the common filamentous algae of freshwater (*Cladophora*) is dealt with in another issue of this series (1).

In lakes these plants may grow from the banks down to a maximum depth (Z_c) which usually depends on the transparency of the water, and may reach 15 m in clear UK waters. This depth, and the maximum biomass (B_{max}), found usually at some intermediate depth, are useful characteristics of lakes.

Under certain conditions the biomass data may be used as an estimate of productivity.

These methods are primarily intended for use in freshwaters, but most could be used at marine or estuarine sites.

3. Identifications

While identification of plants is not essential for this determination itself, to avoid problems and conservation of locally rare and of protected species (see section 3.4 below), identification is advisable and even essential in many localities. It is also useful simultaneously to assess the quality of the macrophytes present. For further information see the separate booklet in this series (22).

3.1 The following keys or texts are recommended:

Vascular plants

- In general — Clapham, Tutin & Warburg (2)
- Introduction to aquatic plants — Wade (3)
- Aquatic species — Haslam, Sinker & Wolseley; not all species included (4)
- *Ranunculus* spp. — Holmes (5)
- Grasses — Hubbard (6)
- Sedges — Jermy & Tutin (7)
- Introduced spp. — Stodola (8)

Mosses

- Smith (9)

Charophytes

- Allen (10)

3.2 Recording. The codes in Holmes, Whitton & Hargreaves (11) should be used (Whitton, Holmes & Sinclair (12) for charophytes). These manuals also give complete check lists and more information on identifications.

3.3 Preservation. Doubtful material should be mounted between blotting paper and pressed between newspaper, making several frequent changes over a few days until dry. It should then be labelled and filed for future reference. Mature, flowering specimens, including roots, are most useful.

3.4 Wildlife and Countryside Act 1981. Until case law is established it is difficult to be certain of the full effect of this legislation. However it seems that the majority of aquatic plants (non-scheduled) may be cut or uprooted provided that the owner or occupier of the land, or the relevant local authority, has authorised the operation. Scheduled plants must not be cut or uprooted. Also, in particular, if the sampling operation can be shown to be for scientific purposes or to prevent serious damage to crops or property, a licence may be obtained which will even allow cutting and uprooting of scheduled plants.* At present the only scheduled plants which might be found in water-bodies are: *Scirpus triquetrus*, *Ranunculus ophioglossifolius*, *Viola persicifolia* and *Alisma gramineum*, but others may well be added later.

Notwithstanding the legal requirements, quadrats containing, or near, any locally rare or visually attractive plants should be avoided if possible. Local Naturalists' Trusts will be able to list any such plants.

4. Apparatus

The equipment needed is dependent on the area and depth of the site, ease of access, nature of the substratum, the size and morphology of the dominant plants and the aims of the investigation. Fig. 1 outlines some of the major points requiring decisions and some suggested (not unique) solutions. Some general information on basic equipment may be found in Haslam & Wolseley (13).

4.1 Access options. These are mainly influenced by water depth. It is emphasised that direct sampling by diving nearly always achieves greater accuracy than any form of indirect, remote sampling.

* For information, consult The Nature Conservancy Council Regional Offices.

Essential safety precautions include notifying the base laboratory of your plans, wearing adequate clothing, including life-jackets when working in or near deep water, ensuring that boats are properly equipped (14), keeping watch for health hazards such as polluted outfalls, avoiding treacherous waves, currents, banks or mud and being accompanied whenever site conditions may be dangerous. Particular precautions must be taken when diving is used (15, 16). These are less stringent when work is limited to less than 1.5 m and certificates of exemption from certain regulations may be negotiated if the site depth does not exceed 50 m and no dive exceeds 20 m. A trained team must always be present.

- (i) **Thigh waders.** Only suitable for banks and water less than 30 cm deep — the length of hand plus arm is more relevant than the height of waders.
- (ii) **Wet or dry suit.** Very convenient for water down to about 60 cm and down to 1.5 m with the aid of a snorkel.
- (iii) **Wet or dry suit and self-contained breathing apparatus (SCUBA).** Used in water over 1.5 m.
- (iv) **Boat and remotely operated grab.** An inflatable boat is usually adequate, but some of the heavier grabs require something more stable such as a pontoon, fitted with a boom.

4.2 Sampling options. The most important decision is between direct or remote sampling, and then the nature of the plants and substratum must be considered.

- (i) **Metal quadrats.** These are laid on the soil and hence are suitable for bank vegetation without water, or for completely submerged vegetation. Ideally, the optimum size is determined by preliminary sampling (see 5.1) but 1 m² is often suitable. They are best made in four interlocking pieces so they can be slid into the vegetation and subsequently fitted together (e.g. two lengths of pipe with right angle female joints and two lengths with male joints to fit).
- (ii) **Wood or plastic quadrats.** These are designed to float so that the floating or emergent vegetation within the quadrat can be sampled. Again, they should be made in separate pieces and 1 m² is generally convenient. In flowing water anchoring weights or corner stakes will be needed.
- (iii) **Corer.** This is a cylinder made of sheet-metal, usually about 25 cm high and 20–40 cm in diameter, though these dimensions should be varied to suit the vegetation and the optimum sample size (see 5.1). The top rim is reinforced and the lower rim is sharpened (Fig. 2). The corer may be fitted with a nylon net with two arm holes, held on by an elasticated band (Fig. 2). In shallow water it is used without the net. It is lowered into the vegetation with as little disturbance as possible and pushed into the substratum with a rotating motion to sever trapped stems and underground parts. In tall vegetation it may be necessary to guide the stems growing within the sampled area into the corer, and exclude other stems. If found to be necessary in water deeper than the corer, especially if the water is flowing, the net is attached and the plants are handled through the arm holes.
- (iv) **Deep water corer.** A similar corer is hung on a rope tied to a rigging clamp. This rope then runs over a pulley attached to a group of small buoys and returns through the rigging clamp (Fig. 3). It can thus be towed out suspended from the buoys and lowered to the diver at the sampling area. By adjusting the length of rope between sampler and water surface the diver can move the sampler with minimal effort, place it precisely above the sample site and lower it slowly to include only the 'correct' shoots as for the shallow corer. The nylon net can then be fixed to the sampler to collect the material within.
- (v) **Grab.** When divers are not available, or time is short, and a low accuracy is tolerable, a suitable grab should be chosen from Table 1. They are usually operated by lowering from a boat until they rest in the bottom vegetation, when a remote release is operated and the sample can be hauled up. If possible their performance should be compared with samples taken in similar vegetation by a diver and a correction factor applied.
- (vi) **Underground corer.** The roots of many shallow-rooted plants may be adequately sampled using corers iii) or iv), but deep-rooted and rhizomatous emergent plants (e.g. reeds) require equipment such as the Schierup corer (17; Fig. 4). This is a PVC tube 150 × 20 cm, 6 mm thick with 5 cm sharpened teeth at the bottom. Two detachable iron handles (each made of 140 cm of 5 cm tubing) are screwed on near the top. Once the corer has been inserted, by pushing downwards and twisting, the top is

sealed with a plastic football before withdrawing it. Use of such corers is very hard work and should be avoided if underground parts are not essential. They are not suitable for sediments with gravel or much sand.

4.3 Accessory sampling equipment. Shears, pruners, knives, trowels, forks, spades etc may be needed to cut or remove the plants.

4.4 Sample collector. Plant material sampled underwater should be collected in the corer nets (or in a nylon shopping bag; or in shallow rivers, in a stop net downstream of a transect). It may then be washed by agitation *in situ*; or remote from the sampling site when diving, to avoid reducing visibility. For bulkier samples of emergent vegetation, plastic multi-holed containers (e.g. linen baskets) are useful. These need a metal reinforcement, with handles, around the top. It is often convenient if these collectors are a standard known weight.

4.5 Labelled polythene bags. Used to hold aerial material, washed samples and dried samples. Plastic garden labels are suitable for labelling. Bags should be kept closed, e.g. by twist ties. It is convenient if bag + label + tie is also a known standard weight.

4.6 Washing trough. A gently curved trough about 1 m × 40 cm supported at a convenient working height and with a coarse (4–10 mm mesh) net at the lower end. This is needed for vegetation with closely adhering detritus and algae and for underground samples with thick mud. The dirty material is placed in the trough and washed with a jet from a hose (the water pressure will need to be greater than 0.3 MPa or 40 p.s.i.), removing large rubbish by hand. The clean material is combined with the fragments collected in the net. Various refinements can be developed to improve the selection and correct for smaller fragments lost. An alternative is a large sieve.

4.7 Balances. A range of balances suitable for the weight and bulk of the fresh and dried samples, e.g.

- (i) Spring balance weighing up to 20 kg
- (ii) Self-indicating balance weighing up to 1 kg
- (iii) Small torsion balance weighing up to 100 g

4.8 Drying-oven. A forced-draught oven, with some change of air is recommended. The larger the oven the less sub-sampling is needed.* During drying, respiration, autolysis, decomposition, volatilization, charring and the hygroscopic nature of the material can cause errors. Some of these losses can be minimised by drying at a lower temperature, but this means that drying takes longer, which increases many of the other losses. For bulky samples, which will not be analysed for particular organic compounds, 105°C is the best compromise.

4.9 Computer. Computing facilities are often needed to design the most efficient sampling programme and analyse the results.

5.1 Sampling design. Ideally there should be preliminary trials to select the size and shape of quadrat and the pattern of sampling that give the least variance (18), and the number of samples that achieves the required precision.

Unless the weed bed is very even, or the quadrats very large, there will be quadrats (or grab samples) without plants, which will produce numerous zero values, and the distribution of biomass values resulting is difficult to treat statistically. If these empty quadrats are ignored the resulting mean biomass per unit vegetated (\bar{B}) area overestimates the true value for the whole area of the sampling site (\bar{b}). Therefore it is usually necessary to determine the proportion of vegetated quadrats, with plants (p , similar to % cover) and estimate \bar{b} as $\bar{B}p$. Unfortunately it is necessary to use a large number of quadrats to estimate this proportion accurately (usually >100; 18).

For the optimal estimation of biomass it is necessary to know how the proportion of quadrats with plants present (p) and the coefficient of variation of biomass ($CV = \text{standard deviation}/\text{mean}$, for vegetated quadrats) vary with size and shape of quadrat.

* A suitable large oven is supplied by Russell Lindsay Ltd.

5. Sampling procedure

Given this information, select a quadrat size and shape that is not too inconvenient, has a low probability of being empty and has a low CV . It is then possible to decide on the optimal combination of numbers of presence/absence quadrats (N) and biomass quadrats (n) to attain a required level of precision.

5.2 Numbers of quadrats required. If quadrats without plants are less than 10% of the total, the calculations and work are relatively simple, but once empty quadrats become more frequent the optimisation becomes complex and all solutions are laborious.

(a) **Whole area with more than 90% of quadrats with plants ($p > 0.9$).** No presence/absence quadrats are needed. The number of biomass quadrats required is approximately $n = (t^2 v) / (\lambda \bar{b})^2$, where v is the estimated variance, \bar{b} is the estimated mean of all biomass samples (including zeros) λ is the highest error tolerated, expressed as a proportion of the mean, and t selects the probability that the mean will be within the tolerated limits of error (for $n > 10$, 90% probability $t \sim 1.7$, 95% $t \sim 2$, 99% $t \sim 2.9^*$). For example, to obtain a probability of 95% that the mean is within 10% of the true mean when the mean is 1 and v is 0.09 (i.e. CV is 30%), biomass quadrats $n = (4 \times 0.09) / (0.1 \times 1)^2 = 36$.

(b) **Whole area with less than 90% of quadrats with plants ($p < 0.9$), but plants in few, well-defined, weed-beds.**

Restrict sampling to areas of weed-beds and use method (a) to determine the number of quadrats required. Survey and measure the areas of the weed bed a and the whole area of the site A (see 19) and calculate \bar{b} for the whole area as $\bar{B}(a/A)$. No presence/absence quadrats are needed.

(c) **Less than 90% of quadrats with plants ($p < 0.9$), plants scattered or in numerous, ill-defined, weed beds.**

Presence/absence and biomass quadrats must be used. The numbers required can be decided using the procedure outlined in Section 8, which gives equations and an algorithm. These can be readily converted to program code, including graphics appropriate to the computing system available. Given estimates of the proportion of quadrats with plants (p) and the coefficient of variation (CV) of biomass quadrats, plots can be produced of the numbers of presence/absence quadrats (N) and biomass quadrats (n) required for selected levels of precision. Fig. 5 illustrates a fairly typical case, where 60% of the quadrats contain plants and the CV is 20%. To estimate the average biomass with a 95% probability of being within 20% of the true mean, there is a range of suitable sampling combinations between $N = 333, n = 5$ and $N = 83, n = 20$. Since biomass sampling is often more expensive than recording biomass presence/absence, the former end of the range would be preferred; but note that if $n = 6$ rather than 5, N falls to only 200. The difference of 133 presence/absence observations would be worth more than one extra biomass quadrat in terms of work saved.

Plotting a variety of examples enables some rough generalisations to be made:

To use less than 200 P/A quadrats p must be greater than 40%; and the precision required must not be better than 20%, or CV must be less than 10%.

To achieve 10% with less than 500 P/A quadrats, p must exceed 50% and the CV must be less than 10%.

To use less than 10 biomass quadrats and achieve 10% precision the CV must be less than 10%.

If the CV is less than 25% and the precision required does not exceed 20% it will rarely be necessary to use more than 20 biomass quadrats.

If the first results indicate that sampling will be impossibly laborious, check that the tolerable error or the level of probability have not been set too high, check the possibility of using method (b), or try to find some stratification or size and shape of quadrat that will reduce CV .

* For especially precise investigations or when n is calculated as < 10 , t should be obtained from Student's t-tables and n recalculated.

(d) **Short cuts.** It may be necessary, at short notice or when low precision is adequate to make practical decisions, to design a sampling programme on the basis of judgement and experience without preliminary trials. In dense stands adequate estimates are often given by:

- about 15 one square metre quadrats for emergent vegetation;
 - 15, 30 cm diameter corer samples for submerged vegetation in lakes;
 - 3, 5 m transects across rivers;
- all taken according to a stratified random pattern (5.3).

If the successive values of 95% confidence limits, or even just the means, are calculated and plotted against the number of samples, as samples are taken, it will be possible to see where the curves begin to level out and undergo less oscillation. In extreme cases it may be sufficient to take samples from a few quadrats chosen by eye. Data from sites sampled in these ways cannot be used for quantitative comparisons with other sites and means will often be very inaccurate.

5.3 Sampling pattern. The samples may be taken at regular intervals, at random or in a stratified random pattern. There may be cases where regular sampling is easiest and most appropriate but the statistical interpretations of the results will always be suspect because the statistical methods used are based on an assumption of random sampling.

In many situations, especially where variation occurs along environmental gradients (e.g. from shallow to deep water, from SW to NE exposure), stratified random sampling will reduce the variance and hence the number of samples needed (19). In this type of sampling pattern the area to be sampled is divided into strata, i.e. areas that are seen or expected to be different. For example the strata from shallow to deep water could be between successive increases in depth of 1 m (Fig. 6a), or at a very varied site they could be the areas dominated by different species or areas with different sediments. The simplest application is to take equal numbers of random samples, not less than three, but usually many more, within each equal-sized stratum. If the strata are demarked by actual contours or boundaries the strata will often be of unequal sizes. For convenience equal numbers of random samples may still be taken in each stratum (Fig. 6b) and the individual stratum means for biomass may be weighted by the stratum areas to calculate the overall mean biomass (see 6.4b).

However, these simple approaches may not achieve the required level of precision of the overall mean biomass and are wasteful of sampling effort. The most straight-forward approach to selecting sample numbers, and achieving a certain level of precision for the biomass of each stratum, and overall, is to apply the methods described in 5.2 and Section 8, using preliminary observations for *each* stratum of the proportions of quadrats with plants present (p) and the coefficients of variation (CV). This gives the sampling requirements for each stratum and hence the whole site. This is an inefficient approach because it takes no account of the relative areas or costs of sampling of each stratum, and because large numbers of samples would be taken in strata that have low cover and biomass and a high variance, which contribute a negligible amount to the overall error of the mean biomass for all strata.

If no presence/absence quadrats are needed (methods 5.2a or 5.2b) the most cost-effective approach is to relate the numbers in each stratum to the area, variance of the biomass and cost per quadrat of sampling in that stratum, using:

$m_r \propto (M_r S_r) / c_r$, where m_r is the area sampled in stratum r (no. times area of quadrats), M_r is the total area of stratum r , S_r is the standard deviation of biomass samples in stratum r and c_r is the cost (per quadrat in time or money) of sampling in stratum r . Relatively larger numbers of samples are taken in strata that are unusually variable and smaller numbers in strata that are unusually expensive to sample.

The budget given for the project sets the maximum number of samples possible, which is divided up between the strata. Calculate the weighted variance of the biomass of the vegetated area (\bar{B}) as:

$$v = \sum_r \{ (M_r)^2 \cdot v_r \} / (\sum_r M_r)^2$$

where r 's are stratum numbers, M_r 's are stratum areas, and v_r 's are preliminary estimates of variance within each stratum. Then calculate approximate values of the relative limits

of error obtained (with 95% probability) from $\lambda = 2v^{1/2}/B$. If these confidence limits are unacceptable, other ways of reducing the variance must be sought, or the budget increased, or the project rethought.

It is not possible to give simple rules about the optimum allocation of sampling effort to each stratum when presence/absence quadrats are needed (method 5.2c), though the effectiveness of possible sampling schemes within the budget can be assessed by trial and error, using the procedure in Section 8 to calculate estimates of the variances of individual strata, and hence whole site means and approximate confidence limits, for each scheme.

In complex field situations strata in the other horizontal dimension may also be used. All these procedures enable the variance to be partitioned into components representing strata differences, within strata variation and sub-sampling errors. Further details of stratified sampling and methods for calculating standard errors (S.E.) of the overall means and for the analysis of variance of the results should be sought in statistical texts (e.g. 20, 21), or by obtaining statistical advice.

5.4 Location of quadrats. A preliminary survey (see 22) and a map of the lake vegetation should be used to prepare grids of potential quadrats over each area to be sampled, numbered individually or by rectangular coordinates (Fig. 6). Random number tables or a random number generator, are used to select the quadrats from each stratum to make the required total of P/A quadrats from the whole site (N , see 5.2; Fig. 7). A random sub-set of those which contain weed, within each stratum, is used for the actual biomass samples (n ; Fig. 7). In the field it is not necessary to use great accuracy (i.e. greater than 1% of the length of the whole site to be sampled) to locate these quadrats, as long as the procedure is consistent and this freedom is not exploited to select areas that look more interesting or less arduous. A rapid technique for locating positions in lakes is appended after this method.

All the quadrats (with a generous allocation of spares) should be selected before starting to sample, so that the most efficient and least damaging sequence of sampling can be worked out.

Sometimes it will be necessary to reject quadrats and replace them by another random selection; for example, when:

- (i) **Quadrats have been previously selected**, either for the current or on an earlier sampling occasion.
- (ii) **Quadrats are adjacent** (i.e. within $1\frac{1}{2}$ times the height of the vegetation) to quadrats used for earlier sampling occasions.
- (iii) **Quadrats are damaged** by reaching other quadrats during the current or earlier sampling.
- (iv) **Quadrats are impossible to reach**. This should be avoided if at all possible.
- (v) **In rivers, quadrats are directly downstream of a quadrat previously sampled**. The upstream quadrats often have weed trailing downstream for 1–3 m, which is lost when they are sampled. Also sampling exposes sediments which are then eroded for a similar distance downstream.
- (vi) **Quadrats contain rare species**.

Note that rejection of (vi) and possibly (iv) introduces a bias into the results and such rejection should be reported.

Quadrats occupied by bare rocks, structures etc. should be treated as 'zero' or 'absent' quadrats.

5.5 Recording percentage cover.* Presence or absence of plants is recorded for all the P/A quadrats or grab samples, so that N_p quadrats have plants present out of N total. It will be necessary to decide on a standard for 'presence' (e.g. at least 3 shoots m^{-2} ,

* This is not the most accurate method of determining the true percentage of the surface covered by plants, but it is the appropriate method for correcting the biomass data for the presence of quadrats without plants and will give comparative data. See 22 for other percentage cover methods.

>30 g fr. wt m⁻², >0.05 of mean biomass), though this need only be a visual assessment. The percentage cover, $100 N_p/N_t$, may be used directly as a crude measure of abundance, or as a good measure of the visual impact of the plants. However the biomass corresponding to a particular cover value may vary by a factor of three and the hydraulic effects of the plants will be even less correlated.

5.6 Collection of biomass samples. To obtain accurate measurements of biomass it is necessary to remove all the plant material associated with the area of the biomass quadrats and no more. To reduce edge errors a convention appropriate to the vegetation and the sampler should be established, e.g. include only shoots *rooted* in the quadrat, or include only material within the *upward* projection of the quadrat or, include all material taken by the standard method of operating the sampler. In rivers, with plants trailing downstream, cut first at the downstream edge and let the severed material go; then cut from the upstream edge, and collect the material. It may be necessary to loosen hard or compact substrata (e.g. with a small fork) to include the roots. To reduce subsequent sub-sampling errors, particularly if the plant material from one quadrat has to be divided between more than one sample collector, it is advisable to remove whole shoots (rather than place the upper parts in one collector and the lower parts in another). When taking direct samples which will need to be sorted into species it is often easier to place the species into separate collectors rather than sort later (Fig. 7). In most cases it is sufficient to separate the dominant species from the others in every third sample (see below).

With these precautions in mind the plant material is pulled or raised and placed in a sample collector.

5.7 Site records. For each site record the depth and any other features likely to be relevant to the investigation, e.g. exposure, shade, flow, sediment, grazing intensity, recent management, depth at lower limit of plants (Z_c). Separate records for each quadrat may be needed.

5.8 Initial washing, draining and wet weights. Most washing is conveniently carried out by agitation in the sample collector at a field base. Washing at the sampling site, especially in lakes, will obscure the operator's vision. The material is then drained for a standard time (e.g. 15 min.), weighed and placed in a closed, labelled polythene bag. Samples should be kept under water, or in polythene bags in a cool place, until returned to the laboratory where they should be placed, still in polythene bags, in a cold store (<5°C).

5.9 Estimation of water content by sub-sampling. If sufficient oven capacity is available it is best to dry all the samples, but it is often necessary to dry only sub-samples. If the field draining procedure is well standardised and each sample is representative of its quadrat vegetation, it is sufficient to take every third sample, or even every tenth if samples are very numerous, and homogenous (Fig. 7).

If any further washing or sorting is needed at the laboratory the samples must be redrained and reweighed, and if only a few samples have been sorted a 'rubbish' correction will have to be applied to all the others (Fig. 7) by assuming foreign matter is proportional to total weight of sample.

5.10 Drying. The plant material is spread thinly in trays lined with greaseproof paper and dried at 105°C in a forced-draught oven for about 15 hr. It should be checked that the procedure and equipment used achieves a constant weight after this time. Then the hot, dried material is placed in sealed polythene bags (e.g. with twist-ties, or using a vacuum heat sealer), with as little air as possible, and allowed to cool before weighing. (Dried plant material will take up about 10% of its weight from moist air.)

Any foreign matter removed during sorting should also be dried and weighed.

5.11 Further sub-sampling. If analyses are to be carried out small sub-samples of dried material will be needed. To reduce the number of analyses dried material may be pooled (e.g. all samples from one stratum, Fig. 7). Crush 50–100 g of the dried material (e.g. within a stout polythene bag, using a rolling-pin) immediately after weighing, pool and mix thoroughly. Replicate sub-samples are then taken by distributing all the material over a large surface with a small sample collector set flush in the centre, using numerous passes in several directions. Repeat the whole process for each replicate. Thus the samples receive both fine and coarse particles.

The replicates are ground in a star-beater mill to pass a 1 mm sieve. It is very important to clean out the mill completely and to thoroughly mix all the ground material before analysis.

If further foreign matter is found (e.g. small stones) further corrections to the dry weights may be needed.

See also the Ministry of Agriculture, Fisheries and Food publication (23).

6. Calculations

6.1 Dry weights. Use the dry weights determined, and the corresponding wet weights of species and total quadrat samples (or sub-samples) to calculate mean dry matter contents (mg g^{-1}). Use these to convert all the wet weights to dry weights. When species have been sorted separately, combine their dry weights to give total dry weights for each quadrat (g).

6.2 Proportions of species. From the total dry weight of all the sorted quadrats, and the total dry weights of each of the separated species, calculate the proportions of each species present (parts of 1 or %).

6.3 Biomass of vegetated areas. Divide the wet weight of each sample (summing the separate species if they have been sorted) by the area of the sampler or quadrat (in m^2) to convert to biomass per sq. metre, and use the mean dry matter content to convert these to mean dry weights (\bar{B}) and 95% confidence limits. Often biomasses are not normally distributed and it is better to use a logarithmic transformation before calculating statistics (24).

6.4 Biomass of site.

(a) Non-stratified sampling.

If only biomass quadrats have been used and the whole site has been randomly sampled (method 5.2a), the mean biomass for the whole site is the simple mean of the biomass for all the quadrats:

$$\bar{b} = \Sigma B/n.$$

If only biomass quadrats have been used and only vegetated areas have been sampled (method 5.2b) the mean biomass for the vegetated areas is the simple mean of the biomass for all the quadrats:

$$\bar{B} = \Sigma B/n.$$

Then multiply this by the ratio of vegetated area (a) to total area (A) to obtain the biomass for the whole site:

$$\bar{b} = \bar{B}(a/A).$$

If both biomass and presence/absence quadrats have been used (method 5.2c), find the proportion of the total number of quadrats observed that have plants present ($p = N_p/N$). Then the biomass of the whole site: (\bar{b}) is $\bar{B}p$ (g m^{-2}).

(b) Stratified sampling.

If stratified sampling has been used the weighted mean for the site is the sum of stratum means multiplied by their areas, divided by the total area of all strata:

$$\bar{b} = \Sigma_r (\bar{b}_r \cdot M_r) / \Sigma_r (M_r)$$

6.5 Confidence limits of means

(a) Non-stratified sampling.

For methods 5.2a and 5.2b the confidence limits of \bar{b} can be calculated directly from the individual estimates of variance in the normal way (assuming that the errors of measuring areas are negligible). See Section 8 for details of the calculation of confidence limits for \bar{b} when method 5.2c has been used.

(b) Stratified sampling.

The variance of the mean for an individual stratum (v_r) and confidence limits may be calculated using the methods from 6.5a above. The variance of the estimate of the site mean is the sum of stratum variances (v_r) multiplied by the stratum areas (M_r) squared, and divided by the total area of all strata squared:

$$v = \Sigma_r \{ (M_r)^2 \cdot v_r \} / (\Sigma_r M_r)^2.$$

Accurate calculations of confidence limits, especially when unequal numbers and areas, and presence/absence quadrats have been used, involve careful consideration of the appropriate degrees of freedom, and are beyond the scope of this manual. Statistical advice should be sought. For most practical purposes the overall mean \pm twice its standard error can be used as an approximation to 95% confidence limits.

6.6 Analysis of variance This may be used to apportion the total variance between strata, replication and sub-sampling errors, but the details are beyond the scope of this manual. Statistical texts (e.g. 20, 21) or a statistician should be consulted.

6.7 Productivity. Biomass data may be used as an estimate of annual production only if:

- (a) the growth pattern of the whole community has a single maximum seasonal biomass and the samples are taken at the time of the maximum;
- (b) no losses of biomass (by mortality, grazing or damage) occur before the maximum biomass;
- (c) at the time of the maximum no material from the previous year is included;
- (d) the entire plant (above- and under-ground material) is sampled.

It is difficult to be certain that these conditions are met without considerable experience or study and many of the procedures needed to confirm their validity, or to correct the results if they are not met, are very time-consuming (25). True annual production may be between 0.5 and 3.5 times the seasonal maximum biomass and this ratio varies with species, site and year (26). It is therefore difficult to use standard ratios, but there are times when they may be useful (see 26 to select appropriate ratios).

In the absence of losses, the productivity of short periods (one to four weeks) is close to the rate of change of biomass or, if translocation is not occurring, the rate of change of standing crop (above-ground) may be used. However, it may be difficult to determine biomass with sufficient precision to obtain significant differences over short periods.

7. Analyses

All analyses, except for chlorophyll, are carried out on dried sub-samples. If accurate determinations of minor constituents are planned, it is better to take and prepare samples specifically for that purpose (27).

7.1 Chlorophyll. This must be determined on fresh material, which is most conveniently collected by taking extra sub-samples. For suitable procedures see the IBP Manual of Methods for Primary Production (25), see also the booklet in this series (40).

7.2 Other analyses must be carried out on material re-dried, cooled in a desiccator and weighed immediately before use.

7.3 Organic weight. Dry water plants usually contain about 200 mg g⁻¹ ash, but samples may contain over 500 mg g⁻¹ when external carbonates are present that do not wash off. It is usual to determine the organic weight as dry weight minus the ash weight and to express other constituents in terms of the organic weight.

Combust weighed replicates at 550°C in a muffle furnace until only ash remains. The loss in weight is assumed to be the organic weight. (On the rare occasions when magnesium carbonate is a significant component this assumption is not valid because it starts to decompose at lower temperatures.)

7.4 Major components. Standard methods to determine organic carbon and nitrogen, crude protein, carbohydrate and fat may be used (23, 28). High amounts of carbonate may interfere with organic carbon determinations and should be removed by a preliminary treatment with dilute acetic acid or sulphur dioxide vapour applied to moistened material.

7.5 Oxygen demand. For some purposes estimates of the oxygen demand of the dead plant material are required. The total potential demand, equivalent to the carbon content of the vegetation, is $\sim 1 \text{ gO}_2 (\text{g dry wt})^{-1}$, or more accurately $2.67 \text{ gO}_2 (\text{g carbon})^{-1}$. The cumulative oxygen demand expected in the six days after death is much less:

$$1.5 + 4.5 \bar{B}, \text{ expressed as } \text{gO}_2 \text{ m}^{-2}, \text{ when } \bar{B} \text{ is the biomass in kg wet wt m}^{-2} \text{ (29).}$$

7.6 Metal content. Suitable techniques are described or listed in (30).

8. Estimation of the number of quadrats required for biomass estimates of selected precision, using the Method c. (Section 5.2).

Assumed sampling procedure and definitions:

N random presence/absence quadrats of which N_p are vegetated.

n random biomass quadrats are randomly selected from the N_p vegetated quadrats.

\bar{B} & S are the mean and standard deviation of the n biomass quadrats.

$$CV = S/\bar{B}$$

$$p = N_p/N$$

N.B. $n \leq p.N$ because there are only N_p known quadrats with biomass.

The overall biomass density (gm m^{-2}), $b = B.p$

An unbiased estimate of the variance of \bar{b} is:

$$(s)^2 = \left[\frac{N_p (N_p - 1) S^2}{N (N - 1) n} \right] + \left[\frac{N_p (N - N_p) B^2}{N^2 (N - 1)} \right] \quad \text{Eq. 1}$$

The coefficient of variation CV_b of b is approximately given by:

$$(CV_b)^2 = \frac{CV^2}{n} + \frac{(1-p)}{p.N} \quad \text{Eq. 2}$$

Approximate $\alpha\%$ confidence limits for the overall mean biomass density are $b \pm L$, where:

$$L = t_\alpha \cdot s \quad \text{Eq. 3}$$

$t_\alpha \sim 1.7$ for $\alpha = 90\%$, $t_\alpha \sim 2.0$ for $\alpha = 95\%$ and $t_\alpha = 2.9$ for $\alpha = 99\%$ and s is the standard deviation of b (from Eq. 1).

The following algorithm uses estimates of CV and p (as obtained in preliminary trials) to calculate the combinations of N and n quadrat numbers which will give a required level of precision to the confidence limits of \bar{b} ;

- (a) Decide the level of precision required for estimates of the overall biomass \bar{b} with $\alpha\%$ confidence limits within true mean $\pm \lambda.b$. Enter λ , CV , p , and t value corresponding to α .
- (b) Let $n = 1$
- (c) Let $C = (\lambda^2/t^2) - (CV^2/n)$
- (d) If $C < 0$ go to (j)
- (e) Let $N = [(1/p) - 1] \vee C$
- (f) If $N < n/p$ go to (j)
- (g) n and N are a valid combination of sample sizes — print-out, or plot on graph of n vs $\log N$, one curve for each combination of α and λ tested.
- (h) Let $n = n + 1$
- (i) Go to (c)
- (j) Stop (or go to (a))

Often CV and p will be known only very approximately. To see how this might affect your decisions CV and p can be varied over their likely range and the valid (N,n) quadrat numbers plotted (Fig. 8).

The final choice of quadrat numbers (N,n) will usually be a compromise between the cost of sampling the optimal combination of biomass and presence/absence quadrats and the precision and probability of the biomass estimate calculated from Eq. 3.

9. Addresses of Suppliers

Russell Lindsay Ltd, 60–62 Constitution Hill, Birmingham 19, England.
Ramsden Scientific Instruments Ltd, Carlone House, Ramsden Bellhouse, Church Road, Billericay, Essex. CM11 1RR, England.

10. References

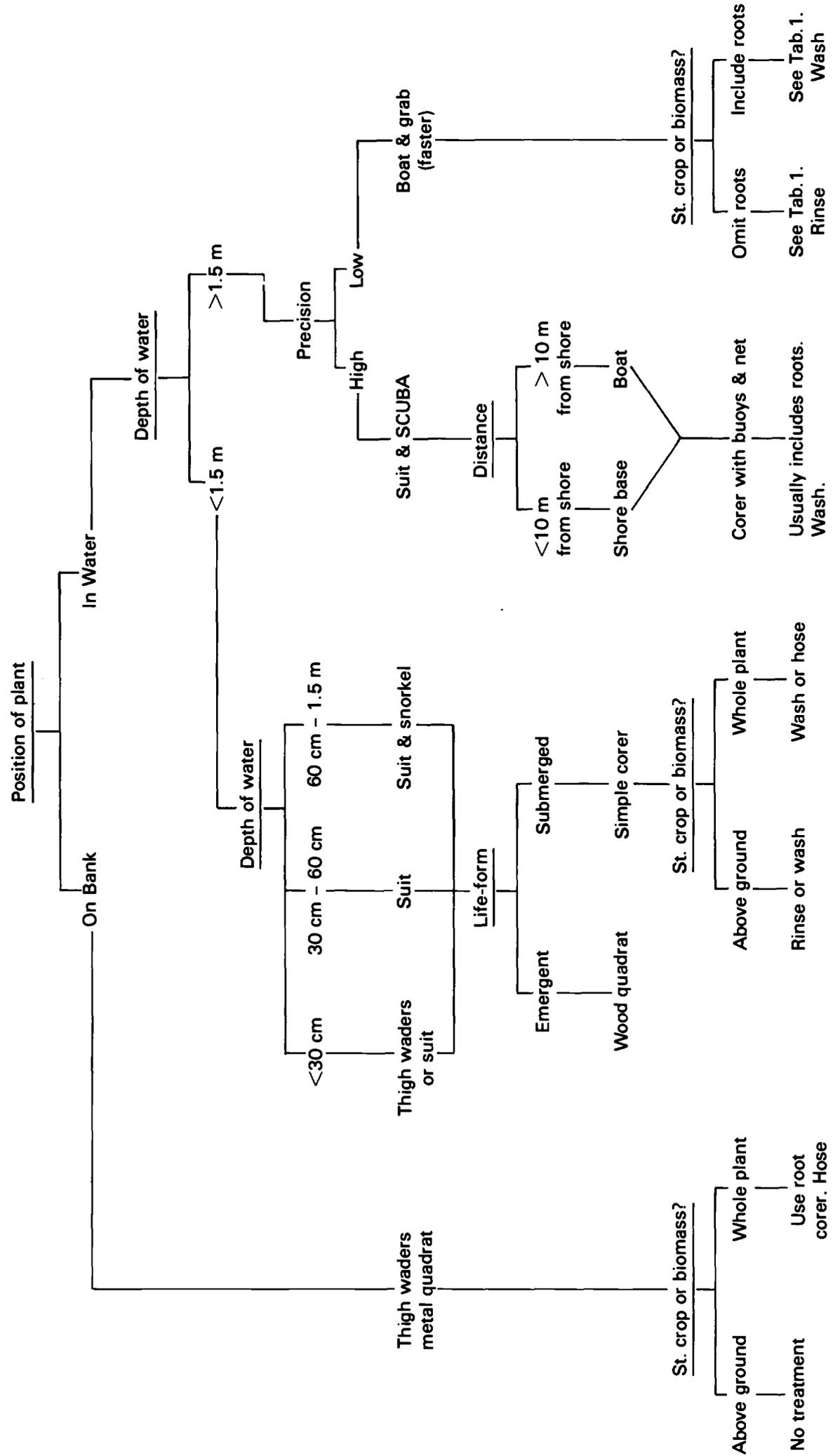
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Table 1. Remote sampling apparatus for macrophytes

Type of apparatus	Suggested applications and limitations	Reference
Grapnel	uniform cross-section, low biomass; small root systems	31
Pronged grab	luxuriant vegetation; roots only from soft sediments	32
Ekman grab	short plants, soft sediments; small root systems	33
Petersen grab (modified)	short plant, hard bottom; small root systems	34
Rotary sampler	tall upright vegetation; small root systems; <3m	35
Quadrat frame sampler	medium height, soft sediments; small root systems	36
Closing cylindrical sampler	upright plants <40 cm; damaging, small root systems; <2 m	37
Toothed grab	most stands, fairly soft sediments; not very dense stands, large root systems; heavy	38
Box sampler	medium height, soft sediments; small root systems, <2.5 m	39

Fig. 1 Diagram of some of the possible variants of the method



(N.B. the decisions suggested here are merely those that are most likely to be made; they are not obligatory and should be taken with the circumstances of each particular investigation in mind.)

Fig 2 Simple corer

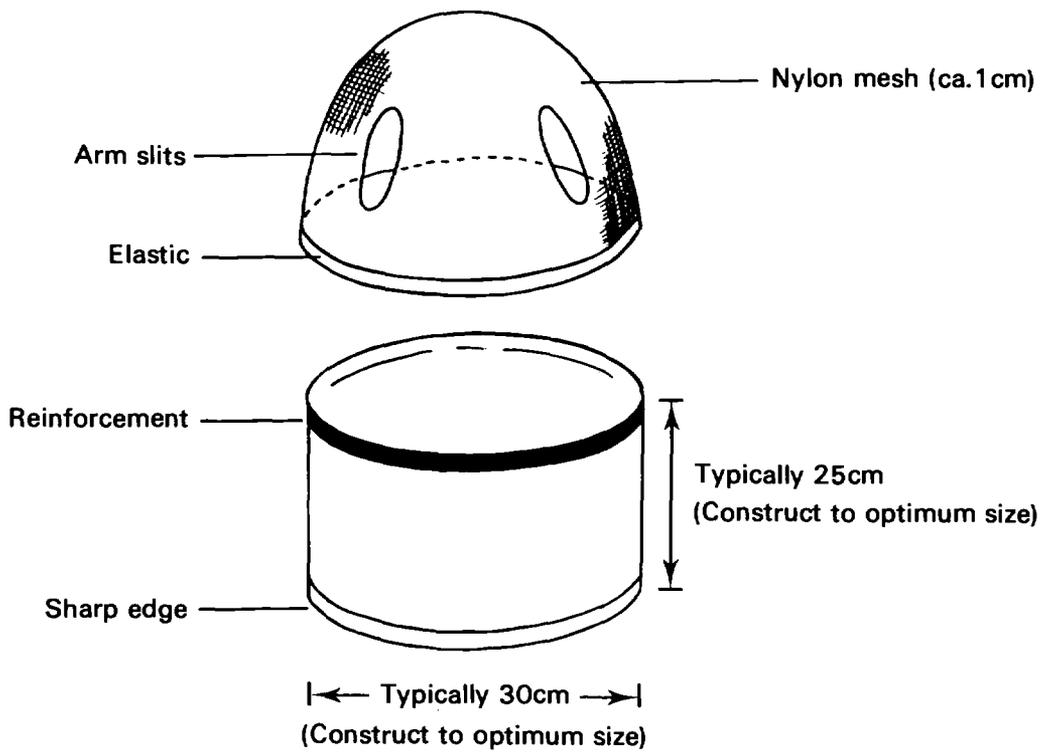


Fig 3 Corer suspension

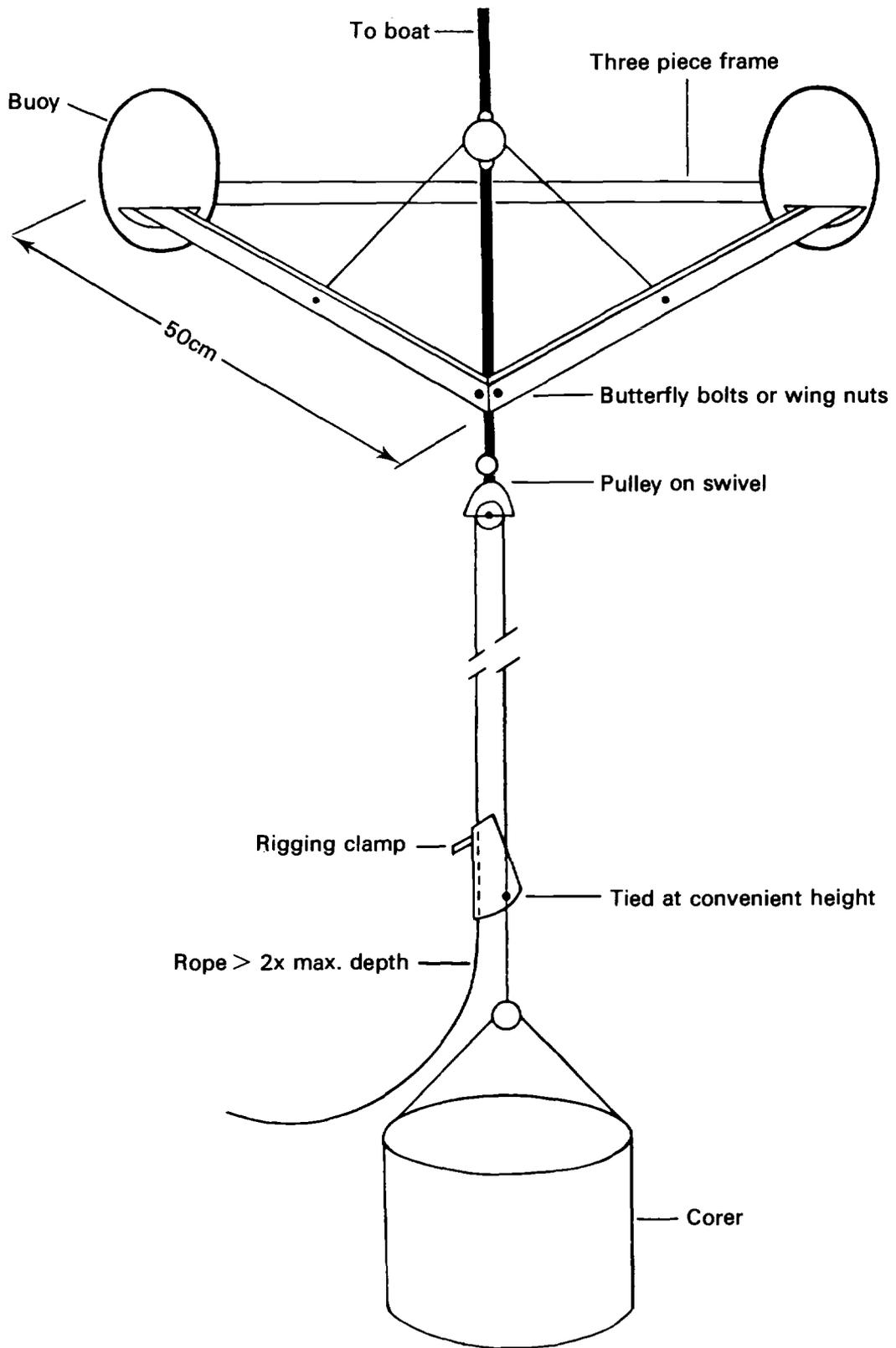


Fig 4 Schierup corer for underground parts (17)

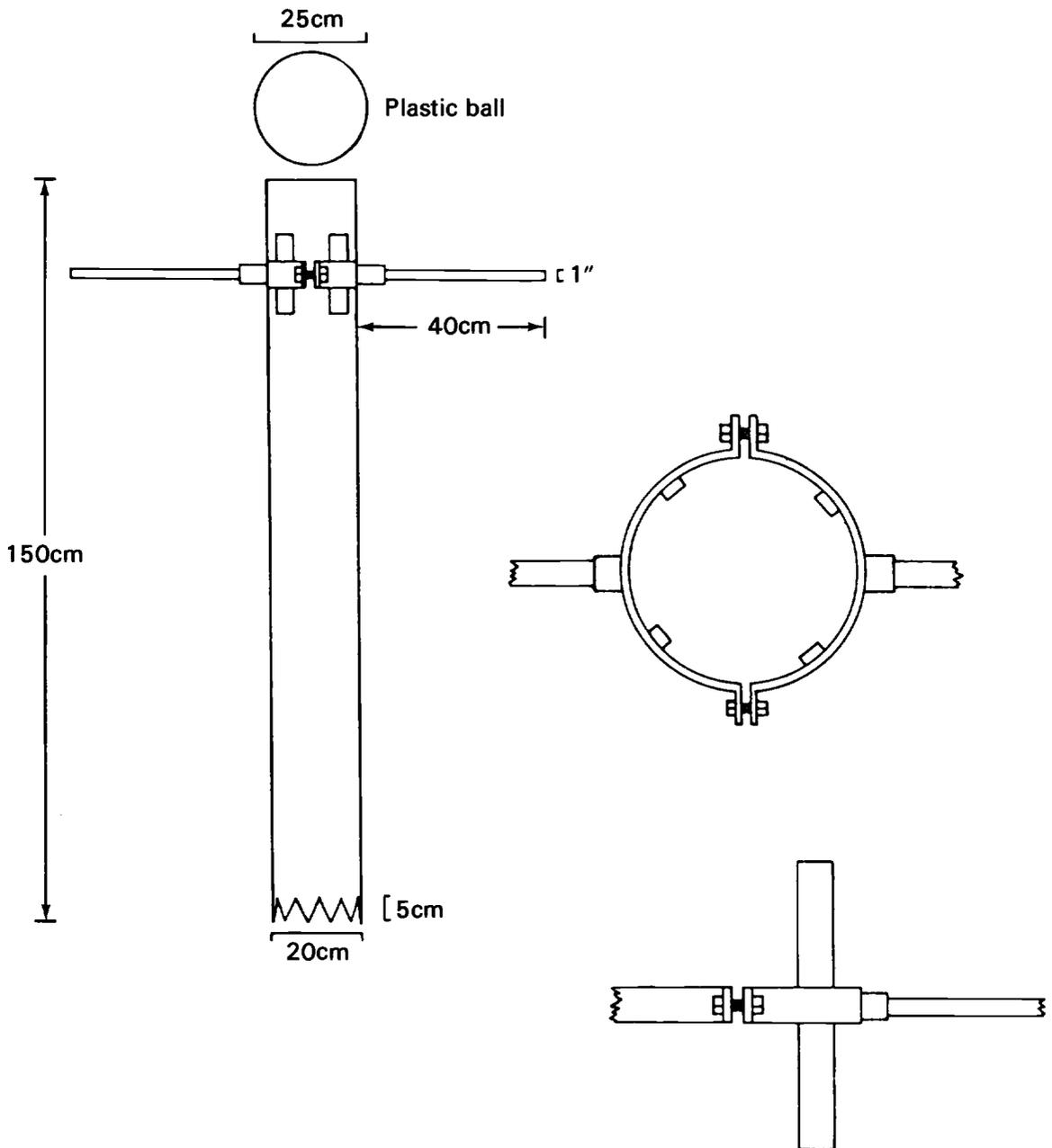
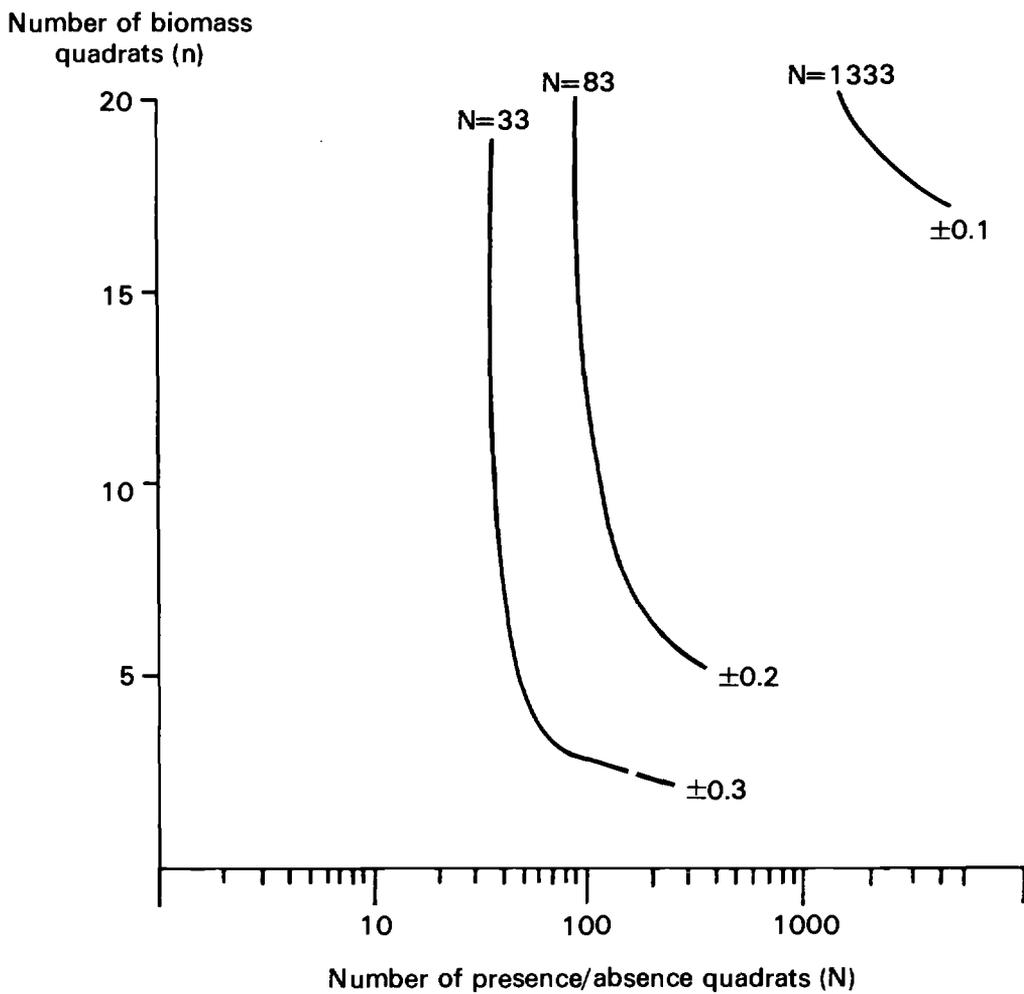


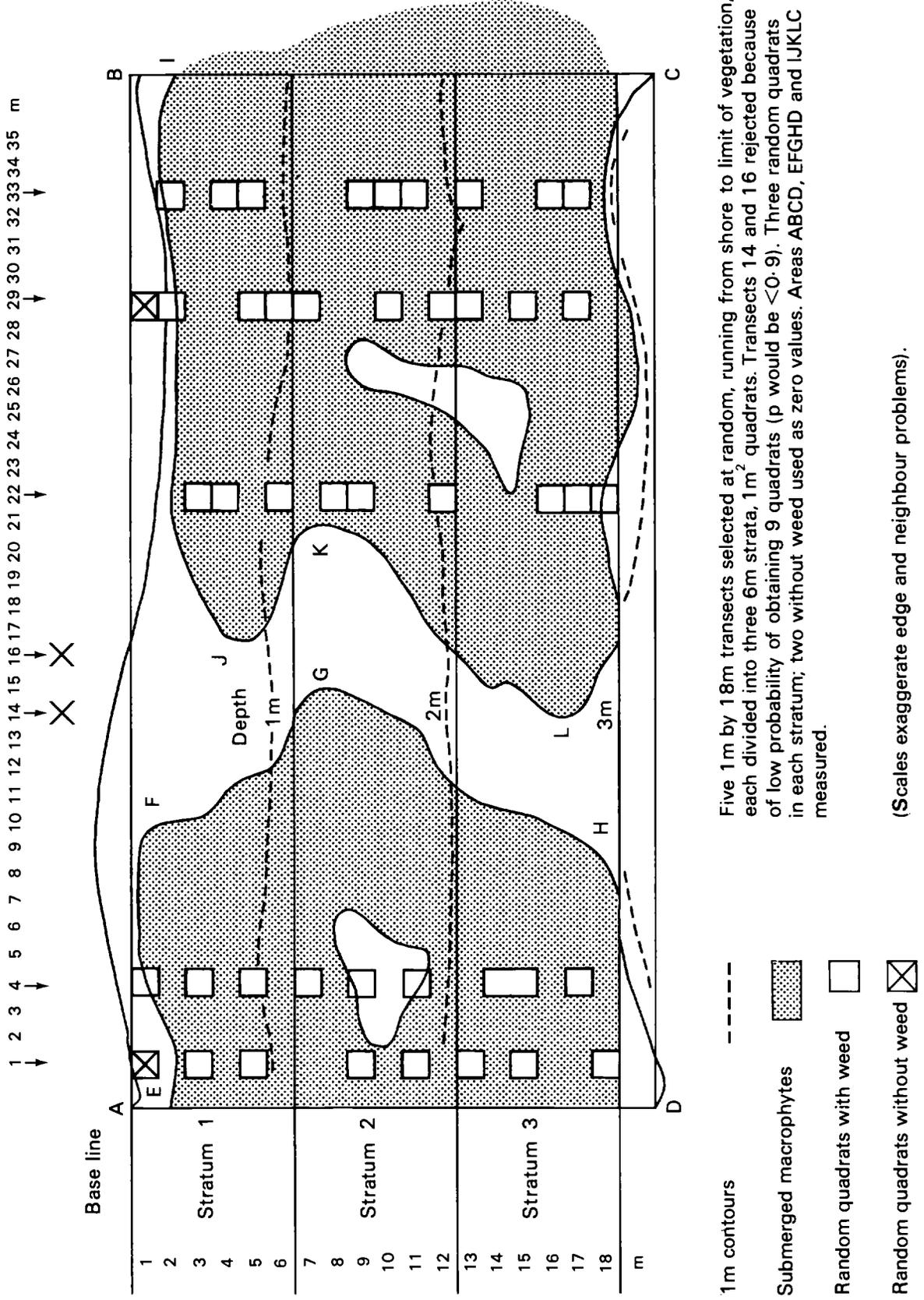
Fig 5 Possible combinations of numbers of presence/absence and biomass quadrats to achieve 95% probability of the true mean being within certain limits when 60% of the quadrats contain plants and the coefficient of variation of biomass in occupied quadrats is 20%



± X Limits of precision required, as a proportion of the mean biomass.

Fig 6 Simplified examples of stratified biomass sampling

b) On a coast with even, well-defined weed-beds suitable for method b (see 5.2b).

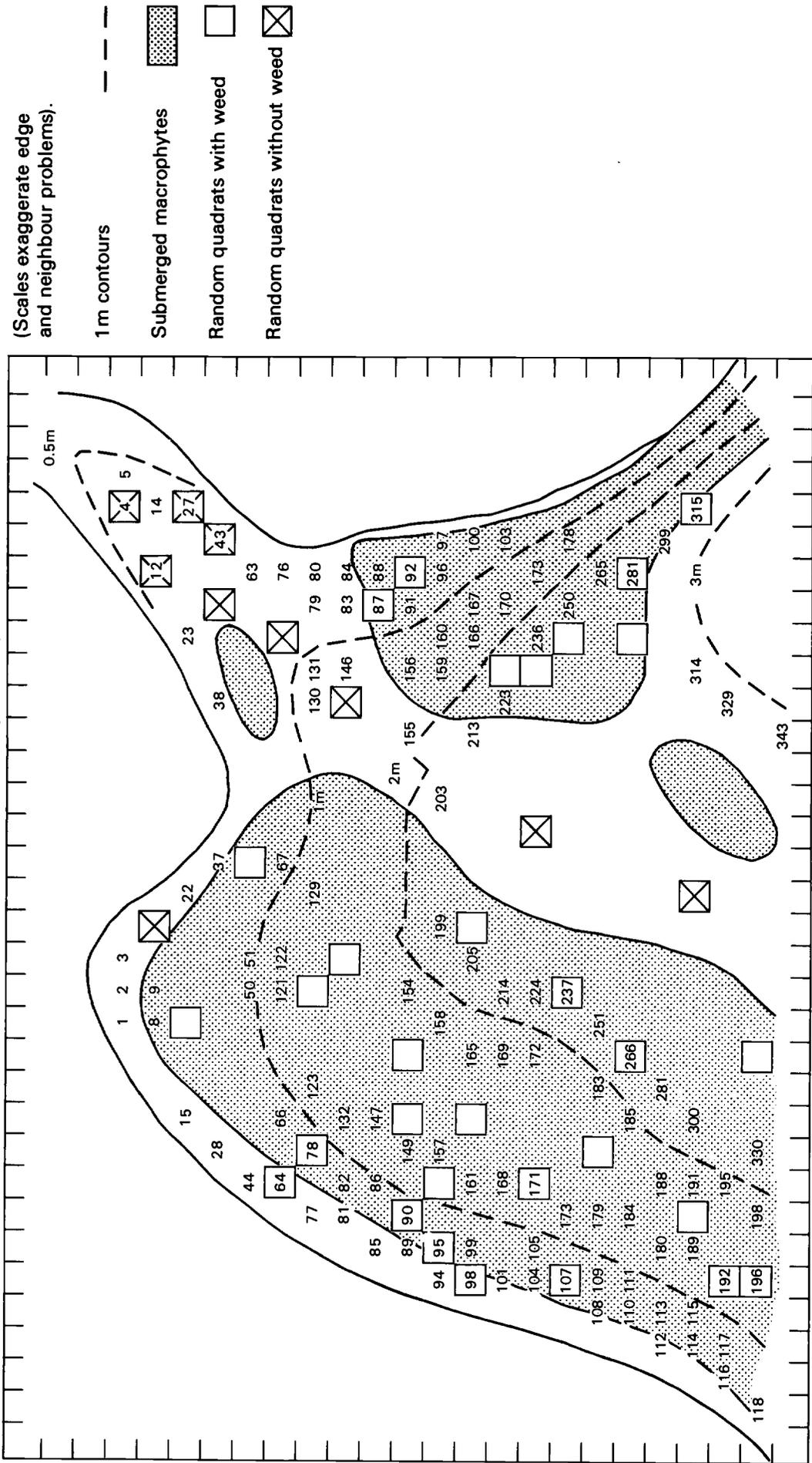


Five 1 m by 18 m transects selected at random, running from shore to limit of vegetation, each divided into three 6 m strata, 1 m² quadrats. Transects 14 and 16 rejected because of low probability of obtaining 9 quadrats (p would be <0.9). Three random quadrats in each stratum; two without weed used as zero values. Areas ABCD, EFGHD and IJKLC measured.

(Scales exaggerate edge and neighbour problems).

Fig 6 Simplified examples of stratified biomass sampling

b) In a bay, mapping not feasible, method c used (see 5.2c).



A grid of 1 m squares is superimposed (shown only at margins) and all quadrats within the areas to be sampled are numbered (shown only at edges of strata).

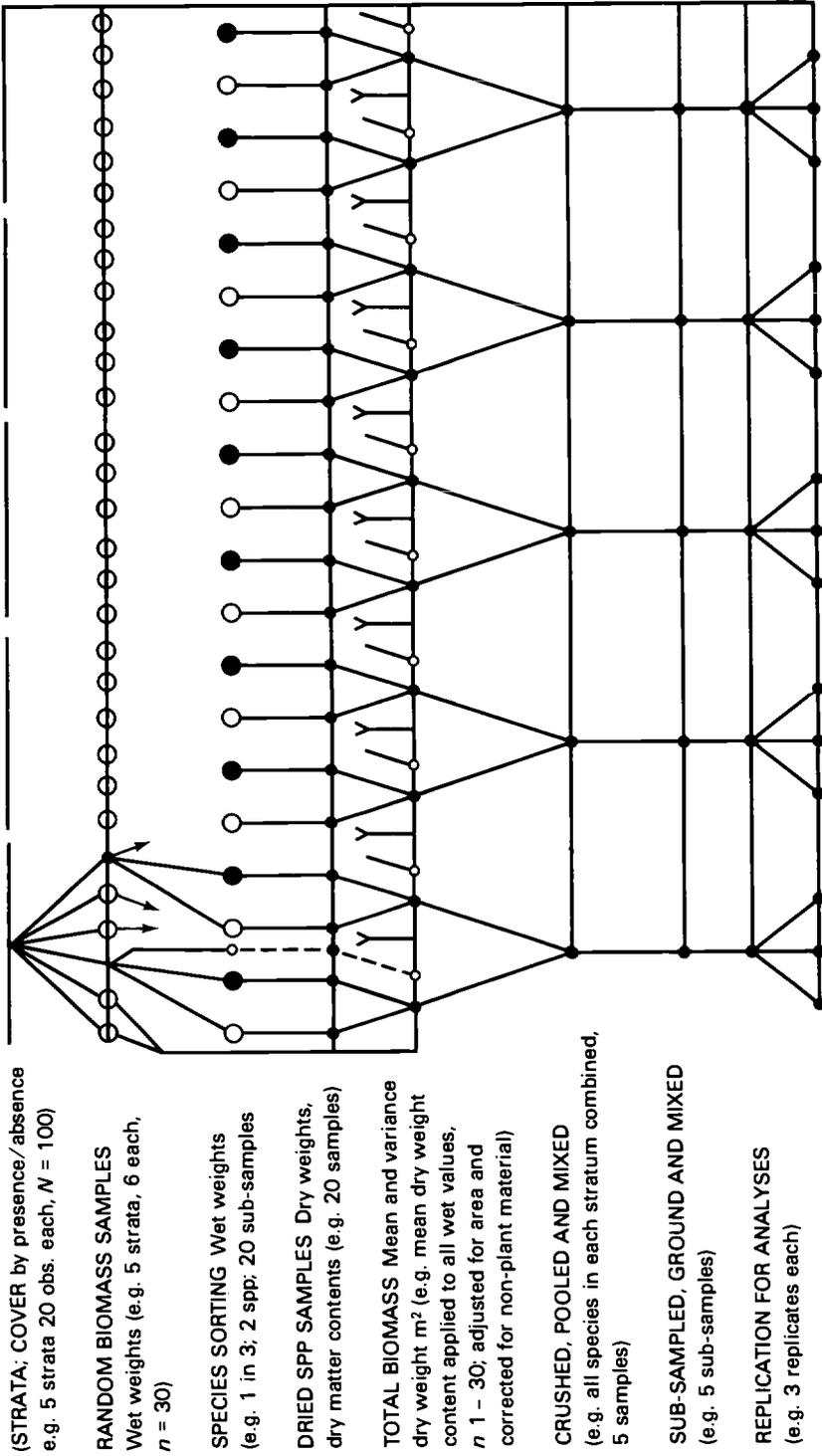
0-1 m, quadrats 1-120; of the first 17 random selections 7 contain no weed and are used for presence/absence only (4, 11, 12, 27, 41, 43, 74); 10 are used as biomass quadrats as well as presence/absence quadrats (18, 54, 64, 78, 87, 90, 92, 95, 98, 107).

1-2 m, quadrats 121-198; 1 of the first 11 random selections contains no weed (145), 10 are biomass and P/A quadrats (125, 137, 149, 151, 156, 163, 171, 190, 192, 196).

2-3 m, quadrats 199-343; 2 of the first 12 contain no weed (229, 307), 10 are biomass and P/A quadrats (206, 224, 235, 237, 249, 266, 279, 281, 315, 333). Random sampling would continue until the required total number of presence/absence quadrats had been observed.

Fig 7 Diagram of a sampling and sub-sampling design

(suitable for accurate determination of total dry biomass and major chemical components; and for estimation of species composition by dry weight).



○ Wet weights (mixed or dominant spp.)

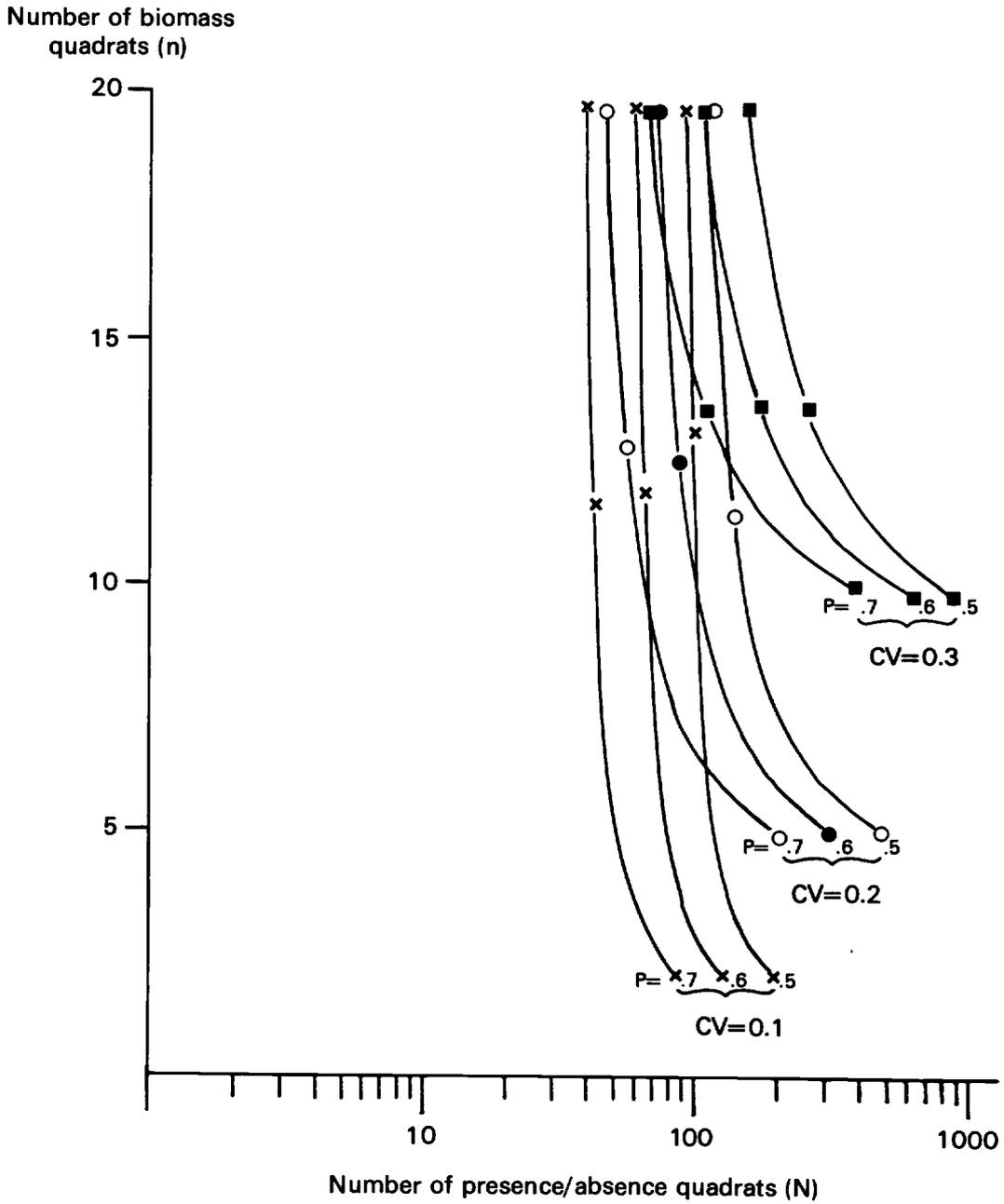
● Wet weight (2nd sp.)

○ Rubbish

● Dry weights

Y Pooling (of materials or data)

Fig 8 Influence of proportion of occupied quadrats (p) and coefficient of variation for biomass quadrats (CV) on the numbers of presence/absence and biomass quadrats required to achieve 95% probability of the true mean being within ± 0.2 of the estimated mean



Line ● — ● is the ± 0.2 line of Fig. 5.

A Basic Guide to the Use of the Optical Square for Position Fixing

These notes are meant to be a simple guide to the use of the optical square for position fixing, with a view to facilitating vegetation survey. It is not a guide to its use in formal surveying practice; if such highly accurate results are required, then reference must be made to Ordnance Survey literature.

The optical square is a hand held device with three prisms which are set so as to give three images, one of an object straight ahead and the others of objects at ninety degrees to either side. The three images are formed one above the other so that alignments can be very rapidly checked. It is particularly useful in transect work, since it enables both end markers of a transect line to be viewed simultaneously, thus giving a check on any drifting off the line.

By reference to the appropriate O.S. map (of the largest available scale) a suitable transect line is defined between two markers which are obvious both on the map and on site. Suitable markers would be corners of buildings, boathouses, fence or wall ends etc., in fact any permanent structure unaffected by change in water level. (In this context a tangent to the shore of an island would be suitable if the shore sloped steeply, a gently sloping shore would be affected too much by fluctuations in water level).

Having established a transect line, intermediate stations are then fixed at intervals along its length by means of intersections. Three intersecting lines are recommended, i.e. two plus the transect line to establish an intermediate station. The intersections are produced in the same way as the transect line by alignment through permanent markers; to minimise errors in this operation, each intersection should ideally be about 45° from the transect line. When surveying a very large water body it may be necessary to use the facility of throwing a right angle from the intersection line to a shore mark to give the third point of reference.

Having established intermediate stations, it is necessary to mark them; poles are preferred for this, but in deep water buoys may have to be used. In this event it must be remembered that a buoy tends to drift about its theoretical position due to the influence of wind and water movement.

The intermediate stations are then regarded as fixed points, set at convenient distance (30–60 metres) apart, and points along the transect line can then be located by reference to the fixed points either by direct measurement (tape or range finder) or by estimation.

An intermediate station can also be used as a centre for secondary transects consisting of a series of lines radiating from the station to shore marks.

Figure 9 demonstrates the methods in graphical form.

It is good practice to confirm that shore features being used as markers correspond to their positions as indicated on the map, since it occasionally happens that a building may have been rebuilt or a fence moved since the previous O.S. survey.

It must also be emphasised that good survey practice ideally requires three straight line intersections to establish an intermediate station, though two may be acceptable. The right angle facility should only be used as a check of say estimated distance along a transect line. Referring to the sketch map, points B and D are well established, C is acceptable and A is acceptable to confirm a measured or estimated distance from B.

Known suppliers of optical squares in Britain

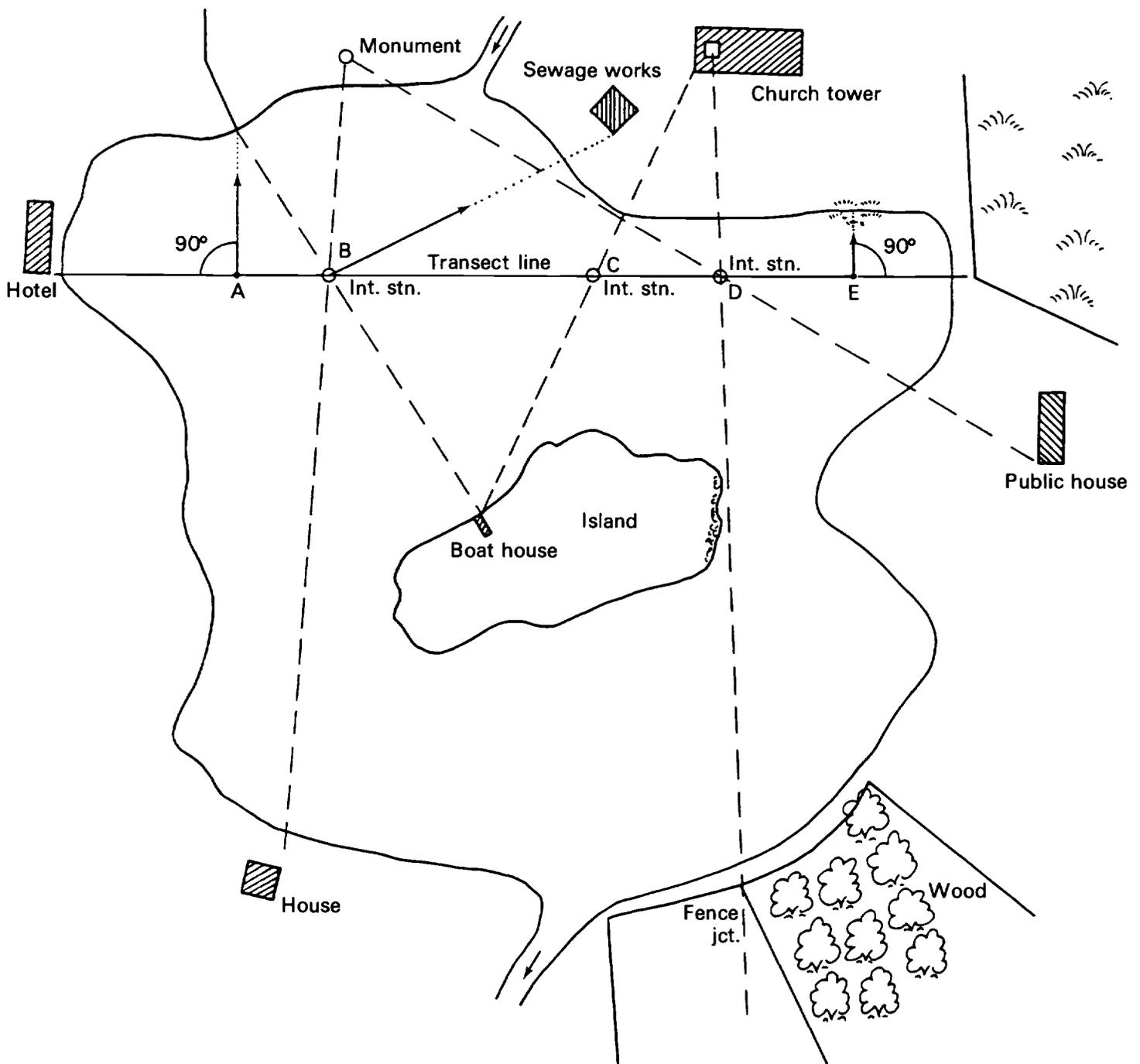
Clarkson & Co Ltd, 1 Brixton Hill Place, London SW2 1HL
Telephone 01 671 5454, telex 947618

Hall & Watts Ltd, 266 Hatfield Road, St Albans, Herts AL1 4UN

Wild Heerbrugg (UK) Ltd, Revenge Road, Lordswood, Chatham, Kent ME5 8TE
Telephone Medway 64471/5

W.F. Stanley & Co Ltd, 33 Avery Hill Road, New Eltham, London SE9 2BW
Telephone 01 850 5551, telex 896414

Fig 9 Use of optical square to fix positions for sampling vegetation



Transect Line fixed by sighting from Hotel corner to fence corner.

- A. Point fixed by 90° sighting from transect line to fence end.
- B. Intermediate station fixed by intersections:-
 - (i) Boathouse to fence end.
 - (ii) Monument to house corner.
 - (iii) Transect Line.
- C. Intermediate station fixed by intersection:-
 - (i) Boathouse to Church tower.
 - (ii) Transect Line.
- D. Intermediate station fixed by intersection:-
 - (i) Church Tower to Fence Junction tangential to island shore.
 - (ii) Monument to Public House.
 - (iii) Transect Line.
- E. Marginal vegetation clump located by 90° projection from point on transect line measured from Intermediate Station D.
- F. Secondary Transect from Intermediate Station B to shore mark (sewage works building).

Measurement of Underwater Light

D.F. Westlake

Symbols and abbreviations

Throughout 'water' means the whole water, i.e. the pure water itself plus any dissolved or suspended materials.

- A — Attenuance of PAR for whole water between depths z_1 and $z_2 = \epsilon(z_2 - z_1)$, analogous to absorbance. See Sections 2.6 and 3.2.
- A_z — Attenuance of water column between 0.1 m depth and z m depth. See Section 4.4.
- E — Immersion error correction factor, true sub-surface transmission divided by observed sub-surface transmission = $(100-R)/U$. See Section 7.
- e — Base of natural logarithms.
- H — Height of sun above horizon in degrees. See Tab. 1.
- i — Meter readings of irradiance uncorrected for scale and immersion error. See Sections 4.2 and 5.2.
- I — Insolation = Total irradiance received on a horizontal surface from sun and sky at an unshaded site; $J\ m^{-2}\ s^{-1}$ (or $\mu E\ m^{-2}\ s^{-1}$). See Sections 2.6 and 5.5.
- I_o — PAR irradiance received on the water surface. See Sections 2.5 and 2.6.
- ${}_1I_o, {}_2I_o$ — PAR irradiances at the water surface before and after a corresponding reading of I_z . See Section 5.2.
- I'_o — PAR irradiance immediately below the water surface (after reflection losses). See Section 2.6.
- I_z — PAR irradiance received on a horizontal surface at depth z . See Sections 2.6 and 5.5.
- K — Linear scale factor on irradiance meters. See Sections 3.2 and 5.2.
- k — Amplification factor. See Fig. 1.
- λ — Local shade factor I_o/I . See Sections 5.2 and 5.5.
- \ln — Natural logarithm.
- PAR — Photosynthetically available radiation, between 400 and 700 nm. See Section 2.4.
- R — Reflection, % of incident irradiance reflected at the surface = $100.I'_o/I_o$. See Sections 4.3, 5.4, 6 and 7. and Tab. 1.
- S — Secchi depth, limit of visibility of standard disc. See Sections 3.1, 4.1 and 5.1, and also Fig. 5.
- t_z — Reading of % transmission to depth z on transmission meter. May need correction for accurate measurements of transmission. See Section 5.3.
- T_z — % transmission to depth $z = 100.I_z/I_o$. Includes surface reflection loss. See Sections 2.6, 3.2, 4.3 and 5.5.
- T_{zo} — % transmission immediately under the surface, $100-R$. See Section 5.4.
- U — The apparent % transmission immediately under the water surface uncorrected for immersion error. See Section 7.1b.
- V_s — Voltage produced by surface sensor
- V_u — Voltage produced by underwater sensor
- z — Depth below water surface. See Section 2.6.
- } See Fig. 1.

z_1	— Shallower of two depths	} See Sections 2.6 and 5.4.
z_2	— Deeper of two depths	
z_o	— Subscript indicating a measurement immediately under the water surface. See Section 5.4.	
ε	— The effective vertical attenuation coefficient for a defined waveband (usually PAR) $\varepsilon = [\ln(I_{z_1}/I_{z_2})]/(z_2 - z_1)$. See Section 2.4.	
ε_{min}	— The vertical attenuation coefficient for the most penetrating wavelength. See Sections 2.4 (iii) and 5.4.	

1. Summary of methods

1.1 Properties determined

- (i) Limit of visibility of Secchi disc.
- (ii) The absolute irradiance on a horizontal surface at a depth, usually as photosynthetically active radiation between 400 & 700 nm (PAR).
- (iii) The PAR at a depth relative to the incident PAR (percentage transmission).
- (iv) The effective 'absorbance' (strictly, the vertical attenuation coefficient, see Section 2.6) of the whole water (for PAR).
- (v) The effective 'absorbance' in defined wavebands.
- (vi) The illumination at a depth.

1.2 Habitats sampled. Most static or flowing inland or coastal waters.

1.3 Basis of method

- (i) The depth of the limit of visibility of a standard Secchi disc is determined. Very approximate conversions to vertical attenuation coefficients may be made, or readings may be calibrated for an individual water body against photometer readings.
- (ii) The determination of the irradiance at the surface and at a series of depths using a sensor weighted for the selected 'target' spectral response. The signal may be measured as mV or μ A according to the circuitry.

1.4 Form of data. Quantitative. The precision of sensor measurements depends mainly on the variability of the sub-surface irradiance or, under good conditions, on the instrumentation (1% of full scale deflection). The accuracy, depending mainly on the calibration and the fit of the sensor to the target, is usually about $\pm 5\%$. If the absolute calibration is suspect, accurate relative data can be obtained provided the cells are matched.

1.5 Units

- (i) Depth: m.
- (ii) PAR (400 – 700 nm); $\text{J m}^{-2} \text{s}^{-1}$ (or $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ see 2.4).
- (iii) Transmission: % surface irradiance.
- (iv) Vertical attenuation coefficient (400 – 700 nm): in reciprocal metres (m^{-1}) and expressed to the base of natural logarithms (e).
- (v) Vertical attenuation coefficients for narrow wavebands, e.g. ε_{min} , red (~600–700 nm), green (~470–610 nm) and blue (~360–500 nm) irradiance, are also in m^{-1} and should be quoted with appropriate wavebands.
- (vi) Illumination: klux.

1.6 Range of application. From above the maximum PAR irradiance that may occur in the British Isles ($\sim 500 \text{ J PAR m}^{-2} \text{ s}^{-1}$, 120 klux), through normal sunny noon values ($\sim 350 \text{ J m}^{-2} \text{ s}^{-1}$, 80 klux), down to $0.003 \text{ J m}^{-2} \text{ s}^{-1}$ or 0.3 lux (the lux instruments are more sensitive than the energy instruments). Underwater, down to the irradiance at the limit of the cable (usually 30 m), or to 1×10^{-5} of summer noon daylight. This corresponds to an attenuation coefficient of 12 in 1 m or 0.4 in 30 m.

1.7 Interferences. Shadows, reflections, unwanted wave-bands, local turbidity.

1.8 Errors. Surface and submerged readings not simultaneous, optical immersion and cosine errors, spectral shifts, water columns with layers of varying attenuation, natural variability. Observer differences when using Secchi disc.

1.9 Logistics. In good conditions the attenuation coefficient can be measured in 30 min in shallow water, rather longer in deep water. Measurements may take much longer when the surface irradiance is changing rapidly. (Not including access time.) Secchi disc readings are much quicker.

2. Introduction

2.1 Further information on light may be found in the book by Kirk (1).

2.2 Standards and conventions. Detailed decisions on the exact aims of the measurements are needed if the most precise and appropriate method of measuring irradiance is to be selected. In practice various standards and conventions are adopted which allow some relaxations of these requirements. The emphasis in these recommendations is on the measurement of irradiance for general ecological purposes including the optical classification of waters, the efficiency of energy fixation, the photosynthesis of phytoplankton, periphytic algae and macrophytes, the transpiration and net radiation balance of plant stands and the illumination of underwater objects. However, it is often possible, without great expense or inconvenience, to select a modification for a specific purpose.

2.3 Quantitative response. Ideally, the response curve of the sensor should match the response curve of the organism (the target), including any initial negative or threshold features, the region where increasing light increases response, the region of light saturation and any region of inhibition. For ecological applications a compromise is necessary because many different responses and many species and individuals (adapted to different light climates, with different threshold, slope and saturation parameters for their response curves) are involved. Also a non-linear response is useless for measurements of optical properties. Usually a linear instrument response to all levels of irradiance is used, and the significance of the levels observed is assessed by comparison with the response curves of organisms.

2.4 Spectral response. Again, ideally, the spectral response of the sensor should be the same as the spectral response of the organism (the target) and a compromise is necessary because many species and individuals, with differing spectral sensitivities, are involved. There are several possible targets, all of which limit the response to photosynthetically available radiation (PAR; by convention 400 – 700 nm*), which includes the wavelengths that are most directly useful to most aquatic organisms (ultraviolet wavebands are usually damaging, infrared usually act indirectly as heat and both are rapidly absorbed underwater).

- (i) **Equal quantum response, 400–700 nm.** This is based on the fact that (within this waveband) quanta (photons) *absorbed* by the photopigment may be equally active in producing photochemical action, whatever their wavelength (e.g. 8 quanta of red light or 8 quanta of blue light will produce 1 molecule of carbohydrate during light-limited photosynthesis). Also spectral shift errors will be avoided (see section 7.4). Against these advantages, the absorption varies greatly with wavelength, light saturation negates the equivalence and underwater values of photon flux density ('irradiance') are not readily and accurately converted into energy values if these are needed. This response is most useful for biophysical investigations. Results are in $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$.
- (ii) **Equal energy response, 400–700 nm.** This is preferred for general ecological work because spectral responses in the field, including the effects of light saturation and absorption by pigments and cell components, are often more level when expressed in energy than in quanta. Also production biology and discussions of the efficiency of use and distribution of incident energy require energy values which (apart from white light) cannot be easily obtained from

* For ecological purposes there is some disagreement about the conventional limits. Most commercially available apparatus limits the response to 400–700 nm, but some conventions extend the range down to 350 nm and up to 720 nm.

quanta values. The spectral shift error may become important but this is really more of an inconvenience than an error (see section 7.4). Results are in $\text{J m}^{-2} \text{s}^{-1}$.

- (iii) **Most penetrating wavelength response.** In most waters this is in the green waveband (550–600 nm). This is close to the peak sensitivity of unmodified selenium photocells which used to be used as sensors and are still useful as the basis for cheap and simple instruments. Spectral shift errors are reduced (section 7.4). An approximate conversion factor is available for conversions to PAR (energy) but such sensors are usually used for relative measurements such as % transmission.
- (iv) **Red, green, blue or narrow waveband responses.** These enable the spectral attenuances of different waters to be compared (water colour), and overcome spectral shift errors (Section 7.4) and some immersion error (Section 7.1).
- (v) **Action spectrum response.** The spectral response of such sensors follows the spectral sensitivity of the selected response of the selected organism (e.g. photosynthesis by green plants) taking absorption and energy transfers between pigments ('enhancement') into account. There are such photosynthesis sensors available but they are not suitable for other purposes. Even for photosynthetic measurements the action spectrum of plants is not constant, is only known well for a few common plants, and it is assumed that photosynthesis responses linearly to irradiance throughout all values.
- (vi) **Illumination response.** This is an international standard for the response of the average human eye, used *only* where light needs to be measured for human activities. It is a special application of an action spectrum, but takes no account of the adaptability of human vision to levels of irradiance. Results are in klux.

2.5 Angular response. Similarly the ideal angular response would be the same as the organism's, and, again, a compromise is necessary for general purposes. A scalar (4π) collector is often used for work on phytoplankton (e.g. 2, 3). This is equally sensitive to all angles of incidence and would be appropriate for a spherical organism. However few phytoplankton organisms are spherical and it is even less suitable for macrophytes or periphytic algae. A common convention is to use an upward-facing horizontal surface as a collector, designed to ensure that the output of the sensor is proportional to the cosine of the angle of incidence (of rays of equal intensity). This means that light reaching the sensor at a low angle appears less bright than light near the vertical, but is not totally lost by reflections. Various special shapes and materials are used to construct such collectors. If necessary the sensor can be inverted to check the significance of up-welling radiation (arising from scattering or reflection off a light-coloured sediment).

2.6 Penetration of light through water. Radiant energy from the sun and sky reaches the water surface where about 10% is reflected, depending on sun angle, cloud cover and disturbance of the water surface (see Table 1). Within a homogenous water column the irradiance measured on a horizontal surface (I_z) decreases logarithmically with depth. The relationship is approximately:

$$I_z = I_o e^{-\epsilon z}$$

where I_z is the irradiance at depth z (m), I_o is the irradiance immediately under the surface and ϵ is the effective vertical attenuation coefficient ($\epsilon = [\ln(I_o/I_z)]/z$).

Sometimes the upper reading is taken below the surface and z is then the vertical difference in depths $z_2 - z_1$. (Such a Bouger-Beer law is strictly true only for parallel radiation of a single wavelength in non-turbid water.) The vertical attenuation coefficient is a property of the whole water, including pure water, solutes and suspended material such as clay, tripton and plankton which both absorb and scatter radiation. Hence 'absorbance', which refers only to the absorption of energy, is not appropriate, and attenuance is preferred. The attenuance between two depths (A) is $\epsilon(z_2 - z_1)$ or $\ln(I_{z_1}/I_{z_2})$.

$$T_z = 100 I_z/I_o$$

(N.B. I_o is the *surface* irradiance.)

2.7 Summary of recommended method. Horizontal, cosine-correct sensors with an equal energy response are used to measure the incident irradiance and the

irradiance at a series of depths. The signals are usually measured on a calibrated meter equipped with switches to select either the surface or the underwater sensor, and a suitable range of sensitivity.

If considered necessary, sensors with other spectral or angular responses may be fitted, which usually means that a different calibration is needed.

3. Apparatus

3.1 Secchi disc. A 20 cm weighted disc with alternating black and white quadrants, hung on a graduated rope. (See the booklet on colour and turbidity in this series for more details, 4. See also Fig 5.)

3.2 Sensors and meters. Commercially available systems† usually use silicon photodiodes with a cosine-correct collector and an appropriate filter system, to produce the angular and spectral response targets. Quantum, equal energy and illumination sensors are usually available and the meters are provided with a selector and calibrated scales. *PAR meters* have scales reading in absolute values for quanta or energy between 400 and 700 nm. There is also a switch to select different linear ranges, usually from $\times 0.1$ to $\times 10^4$ (K).

It is usual to have separate surface and underwater sensors, and a changeover switch, so that relative readings can be made quickly, when the irradiance is varying rapidly. Some commercial *transmission meters* read out in percentage transmission directly*. Some information is lost but measurements can be made much more quickly. It is easy to design *attenuance meters* with further refinements which integrate and read out attenuance between two depths directly ($A = \epsilon [z_2 - z_1]$; (Fig. 1)†). This also enables more precise measurements at low irradiances. Both transmission and attenuance meters may have a time constant setting which is used to reduce fluctuations caused by small rapid changes in irradiance.

Colour meters measuring PAR within selected wavebands may have just a pair of sensors with exchangeable colour filters** or several sensors each with a different colour filter††. Exchangeable filters are very inconvenient because they must be fitted underneath the cosine collector. Also, with both exchangeable filters and exchangeable sensors, a series of readings with depth must be taken after each change.

Inexpensive sensors can be constructed using selenium photocells and transmission filters (Fig. 2). Selenium photocells with neutral transmission filters may be used for measurement of the most penetrating waveband. For optimum sensitivity and linearity it is best to measure the signal from selenium photocells as voltage, with a low variable resistance in series to keep the output within the linear range (~ 40 mV), and a digital meter in parallel (Fig. 3) (5). Colour sensors can be made using 2 mm glass colour filters over selenium photocells and underneath the cosine collector and neutral filter (6). The minimum standard set is: red RG2, green UG9, blue BG12 or equivalents by other manufacturers§. A colour meter using narrow wavebands (30–40 nm) can be constructed by mounting an interference wedge filter^o in a waterproof casing over a selenium photocell and underneath a moveable brass strip with a slit 1 mm wide that defines the waveband (7). The opal cosine collector is fixed above the brass strip. As the voltage output from a narrow waveband is small an amplifier* is built into the housing. All such

† For example: Ramsden Sci. Inst. Underwater Light Meter, LI-COR Underwater Light Meter, Macam Photometrics Underwater Light Meter; with the appropriate sensors Skye Instruments Ltd, will supply a data-logger system for PAR irradiance designed for use with these methods. Biospherical Inc. have a Profiling Scalar System with only quantum and 4π sensors.

* For example: Skye Instruments Ltd Ratio Meter (quantum sensor), and will supply a data-logger system. Ramsden Sci. Inst. Ltd., Macam Photometrics Ltd and Newland-Jennings Electronics market meters for terrestrial applications but they may be able to supply meters suitable for use with underwater sensors.

† Skye Instruments Ltd, data-logger system.

** Montedoro-Whitney, Solar 'Illuminance' Meter.

†† E.g. Kahlsico Universal Radiometric Submarine Photometer; Biospherical Instruments Spectroradiometer.

§ Schott u. Gen.; Chance-Pilkington OR2, OGr1, OB10.

^o Schott u. Gen., Veril B-60.

^o Precision Monolithics, OP-20GJ.

systems use two matched cells to give relative values and are not calibrated in absolute units.

3.3 Support. It is usually necessary to have supports to hold the sensors horizontal at the surface, or at the required depth, without casting a shadow across them.

- (i) Surface sensor. In inland waters it is usually sufficient to mount this on a small raft. For use in rough seas gimbals will be needed for accurate work and for swift rivers a bank site with similar exposure may be used.
- (ii) 0 – 2 m. A graduated pole with a 1 m boom at right-angles at the bottom can be used (e.g. Fig. 4a) (8). With a plumb-line attached to the pole, the boom can be kept horizontal, and depth markings on the brace will help in shallow water.
- (iii) Deeper waters. The sensor is fixed to a block held horizontally on a graduated cable by three thin arms (to keep shading to a minimum) (Fig. 4b). Such a spider is usually supplied with underwater meters. The cable should be lowered from a boom to keep the shadow of the boat off the sensor. Where there is a strong current arrange for the mounted sensor to be able to slide up and down a cable held vertically between a weight and the boat.

4. Procedure

Check that the required sensors are attached, the corresponding calibration is selected, the collectors are clean, the surface sensor is dry and the batteries are sound. The operator should normally work on calm days with his shadow behind him, at a site where open water (free from weeds etc.) extends for at least a 2 m radius.

Record the time, cloud cover (0 – 10/10) and surface conditions (and repeat these records as they change).

4.1 Secchi disc. See (4). Repeat readings at least three times.

4.2 PAR meters (and colour meters). Lower the underwater sensor to at least 10 cm, check that it is horizontal, and take readings (*i*) from surface and underwater sensors alternately, using the range scales that give at least half full scale deflection and recording both *i* and the range scale factor (*K*). Compare the surface readings before and after each underwater reading and, if these differ by less than 10%, accept the corresponding underwater reading. Where greater precision is needed, a higher standard should be adopted. Continue taking readings at the same depth until three underwater readings are accepted. Continue readings at depth intervals of say 10% of the maximum depth.

Using colour meters with multiple sensors, take similar readings at each depth from each sensor. Using meters with exchangeable filters or moveable slits, change the waveband after each complete depth series (ensuring no water enters the mount) and repeat the whole sequence.

4.3 Transmission meter. If fitted, adjust the variable time constant until steady underwater readings are obtained under the prevailing conditions. Then use the balance control to match the surface and submerged sensors or to provide an appropriate offset. The normal procedure is to hold the surface sensor above the water and the underwater sensor 10 cm under the surface, and adjust the offset until the meter reads $(100 - R)$, where the percent surface reflection *R* is found from Tab. 1. This matches the sensors, corrects for the immersion error and allows for surface reflection*. For studies requiring greater accuracy, determine the immersion error as described in 7.1 and adjust the balance setting with both sensors exposed, *dry*, side-by-side until the meter reads 100. Then hold the submerged sensor 10 cm under the water and note the reading, which is used for subsequent checks on the balance. In both cases keep the surface sensor above the water and take three readings at each depth when the meter is horizontal and the reading is steady. The first method gives transmissions (T_z) directly, or they can be calculated from the readings (t_z) given by the second method (5.3 ii). Check the balance setting every 12–24 readings.

* There is a small error arising from the attenuation of 10 cm of water, but unless the water is very turbid or deeply coloured, it is negligible compared with the error in estimating *R*.

4.4 Attenuance meters. Increase the time constant on the integrator until steady readings are obtained under the prevailing conditions. Hold both sensors at 10 cm under the water surface and adjust the setting until the attenuation is zero. Keep one sensor at 10 cm and lower the other sensor to take at least three readings of attenuation (A_z) at each depth. To determine the vertical attenuation coefficient (ϵ) directly, ensure that readings are made at 1.1 m (i.e. 1.0 m between the two sensors).

5. Calculations

5.1 The Secchi depth S is the mean of the depth of disappearance and re-appearance and is primarily a measure of visibility (see 4). It may be converted to an attenuation coefficient, $\epsilon \sim S/2.2$, but the denominator varies, with light and water conditions and the observer, between 1.4 and 3.

5.2 Absolute values of PAR. These are very dependent on the weather, time of day and season and hence are of little value as an indication of water quality.

- (i) **Using PAR meters.** Values of I_z are obtained directly from the meter readings i_z after application of range factors (K) and the immersion correction (E) (see 7.1) as necessary, e.g. $I_z = i_z K E$
- (ii) **Using transmission meters.** If the insolation I is known from a surface solarimeter calibrated in total irradiance then $I_z = \lambda I T_z / 100$, (λ is the local shade factor, surface PAR irradiance over total insolation; I_o/I).

5.3 Transmission (including surface reflection).

- (i) **Using PAR meters or colour meters.** Calculate I_o & I_z by correcting for range factor and immersion error as in 5.2. Then $T_z = 100 I / [(I_o + I_z) / 2]$, where I and I_o are the corrected surface readings before and after each underwater reading. Find the mean for the three accepted readings at each depth.
- (ii) **Using transmission meters.** The normal procedure gives $T\%$ directly and only the mean of the three readings has to be calculated. For more accurate work the immersion error E must be known from laboratory determinations (see 7.1) and then:
 $T_z = t_z E$ where t_z are readings obtained after the meter has been set to 100% with both sensors dry.
- (iii) **Using attenuance meters:**
 $T_z = (100 - R) / \{ \text{antilog} [z A_z / (z - 0.1)] \}$, where A_z is the attenuance measured between 0.1 & z m and R is estimated from Tab. 1. Calculate the mean of three values.

5.4 Vertical attenuation coefficient.

- (i) & (ii) **Using PAR meters, colour meters or transmission meters.** Calculate mean transmission T_z for a range of depths as above (5.2 or 5.3), ensuring that the immersion error is corrected. Plot T_z on semi-logarithmic paper against depth. Normally this will be close to a straight line for homogenous waters (but see 7.4), and anomalous values or stratification can easily be seen. Fit a straight line and read off T_{z_1} and T_{z_2} over a suitable range of depths. Then the attenuation coefficient: $\epsilon = [\ln (T_{z_1} / T_{z_2})] / (z_2 - z_1)$, where z_1 and z_2 are the upper and lower depths respectively. Extrapolation of the line to the surface will give an estimate of R as $100 - T_{z_0}$. If there is a spectral shift error, or the water column is not homogenous, a straight line over the whole depth range will not be obtained. A line should then be used over as wide a range of depths as possible within the depths that are of most interest or a narrow waveband meter should be used.
- (iii) **Using attenuance meters.** Attenuance A is obtained directly, and if readings are taken at 0.1 and 1.1 m, the vertical attenuation coefficient is obtained directly. Otherwise:
 $\epsilon = A / (z_2 - z_1)$, where z_1 and z_2 are the depths of the upper and lower sensors respectively. Take the mean of these determinations.
- (iv) **Using meters measuring the most penetrating waveband (ϵ_{min}).** Then $\epsilon_{PAR} \sim 1.33 \epsilon_{min}$ (see 9).
- (v) After using colour meters plot the values of the vertical attenuation coefficients against wavelength and join the points with a smooth curve (assuming attenuance increases towards the ultra-violet and infra-red). This gives a visual representation of the hue and colour saturation of the water.

5.5 Light climate. If continuous records of total insolation I are available these may be converted into underwater PAR irradiance data:

$$I_z = I \lambda T_z / 100$$

where λ is the local shade factor I_o/I and T_z is the mean percentage transmission to depth z . Both λ and T_z are likely to vary with the individual site studied, and with time, so calculations of average light climates can be very complex and approximate. Using observed values of transmission for each depth minimises spectral shift errors.

6. Interferences

The most likely interferences are shadows cast by the operator, the supports, vegetation in and around the water body and banks. Interference from surface reflection is treated as discussed above. In some situations the irradiance received by an organism will be strongly augmented by reflection from below from a light-coloured substratum, which will not be measured by these sensors.

Readings taken after disturbance of sediments, close to the shore, or during spates will not be representative of normal conditions in the whole water body.

7. Errors

The instruction manuals supplied with commercial meters describe the instrumental errors and their determination in detail.

7.1 Immersion errors. The optical properties of the collecting surface are different at an air-collector interface and at a water-collector interface, so that a given irradiance incident on a sensor in the air will give a different reading on the same sensor underwater (10, 11) even if water surface reflection is allowed for. This is accentuated in the first 10 cm of water by multiple reflections between the sensor and the water surface. The collector can be designed to minimise the immersion error, but the constraints applied by the need for the collectors to be cosine-corrected, similar in air and water, and to have a common linear calibration, usually enforce a compromise design. Typically, but by no means invariably, underwater readings need to be multiplied by about 1.3 to allow for this. The error varies slightly with spectral energy distribution (colour), but is usually determined in white light.

The error can be determined in two ways under constant insolation (e.g. a clear day). The two sensors are first matched for relative sensitivity with both collectors dry. Then:

- (a) **Laboratory method.** A tank ~2 m in diameter with dark sides is exposed to daylight, well away from shade. It is filled with water of known attenuation (ϵ for distilled water is ~0.08 which is almost negligible) and the underwater sensor is immersed to 10 cm. The expected transmission to 10 cm can be calculated from the attenuation and the surface reflection (from Tab. 1 for the prevailing conditions) and compared with the apparent transmission given by the two sensors (corrected for their dry difference in sensitivity if necessary).
- (b) **Field method.** Underwater readings are taken in open water in the field on a calm day, over a range of depths greater than 10 cm, and these are expressed as percent transmission relative to the surface readings (corrected for their dry difference in sensitivity if necessary). Or transmission values are taken using a transmission meter, set to 100% with both cells above the water. The transmission values are then plotted on semilogarithmic paper, against depth on the normal scale, and the resulting line or curve is extrapolated to zero depth. This intercept ($U\%$) represents the immersion error plus the surface reflection ($R\%$) which can be estimated from Tab. 1. Then the immersion correction factor $E = (100 - R)/U$.

It should be noted that the values and procedures for the immersion error that are supplied with commercial equipment do not always correspond with those recommended here. For example the immersion error may be taken to include surface reflection, so that $T = I_z/I_o$ and not I_z/I_o .

7.2 Cosine errors. These usually increase at low sun angles and are therefore worst early and late in the day. Commercial underwater sensors (the air sensors are better) usually have an error smaller than -7% for sun angles more than 38°, i.e. satisfactory for normal working hours between March and September; and for relative results the error will be less. Full error curves are usually provided, but it is difficult to apply corrections under field conditions where there is a mixture of direct and diffused light.

When constructing equipment it is important to keep exactly to the dimensions and materials recommended for the collectors.

7.3 Spectral response errors. In all designs the spectral response curves diverge from the target, especially close to 400 and 700 nm. These errors are usually less than 5% over three-quarters of the range. They are only serious if the radiation happens to be particularly rich in a waveband where the response is exceptionally divergent. If spectral errors are thought to be important narrow wavebands may be selected by using colour sensors.

7.4 Spectral shift errors. As the irradiance passes through the water the most readily attenuated wavelengths become a smaller proportion of the total energy and correspondingly the wavelengths with lower attenuation coefficients come to predominate, especially in water that is coloured rather than turbid. The attenuation coefficient therefore drifts with depth towards that for the most penetrating waveband (7). This only produces errors if attempts are made to derive a single attenuation coefficient from a wide range of depths, or if an attenuation coefficient from one depth is misapplied to another depth. If such errors are thought to be important narrow wavebands may be selected by using colour sensors.

7.5 Aging and calibration. The transducers and circuits change with time and recalibrations are necessary. Apparatus with silicon photodiodes is expected to need calibration only every two or three years but it has been found that meters may differ by up to 35% within a year of their calibration (7). If absolute values are important calibrations should be checked initially and at frequent intervals. Apparatus with selenium barrier-layer cells must be rematched frequently and checked for linearity every six months.

7.6 Variability of insolation, local shade, reflection and attenuation. All these components vary with time and changes in the environment, particularly near vegetation, in rivers, and with time of day. While spot measurements may be made quite accurately, long-term data on the light climate can be estimated only approximately unless continuous records are undertaken.

8. Calibrations

These are usually carried out by the manufacturers.

8.1 If a suitable standard lamp is available, with known emission under standard conditions, the calibration factor for the sensors can be determined. The scales can then be reset following instructions provided with the meter.

8.2 A rough calibration check can be made by exposing the sensor to sunlight beside a calibrated pyranometer (e.g. Kipp & Zonen), and assuming PAR is 0.48 of the total irradiance.

8.3 If only relative values are being measured it is sufficient to expose the sensors side by side in daylight to determine a matching factor.

9. Addresses of Suppliers

- (1) Ramsden Scientific Instrument Co. Ltd., Carlone House, Church Road, Ramsden Bellhouse, Billericay, Essex. CM11 1RR, England.
- (2) ICI PLC. Petroplastics Division, Squiresgate Industrial Estate, Squiresgate Lane, Blackpool, FY4 3RN, England.
- (3) Kahl Scientific Instrument Corp. PO Box 1166, El Cajon, California 92022, USA.
- (4) Kipp & Zonen, Mercuriusweg 1, P.O. Box 507, Delft, Holland. (UK agents Enraf-Nonius Ltd, Highview House, 165/7 Station Road, Edgware, Middx, HA8 7JU, England.
- (5) Lambda Instruments Corporation, 4421 Superior Street, P.O. Box 4425, Lincoln, Nebraska 68504, USA.
- (6) Macam Photometrics Ltd, 10 Kelvin Square, Livingston, EH54 5DG, Scotland.
- (7) Megatron Ltd, 105 Marlborough Road, London NE19 4NE, England.

- (8) Montedoro-Whitney Corp. 274IE, McMillan Road, San Luis Obispo, California 93401, USA. (UK agents Exploration Technology Ltd, Ocean House, 13 Warwick Road, Ashford, Middx, TW15 3PQ, England.
- (9) Precision Monolithics Inc., Hodford House, 17/27 High Street, Hounslow, Middx, TW3 1TE, England.
- (10) Rank-Strand Electric, P.O. Box 51, Great West Road, Brentford, Middx, TW8 9HR, England.
- (11) Skye Instruments Ltd, The Old Manse, Skeaboat Bridge, by Portsee, Isle of Skye, IV51 9XE, Scotland.

10. References

- (1) Kirk T.O., *Light and Photosynthesis in Aquatic Ecosystems* 1983, Cambridge Univ. Press.
- (2) Hojerslev N., *Limnol. Oceanogr.* 1975, **20**, 1024.
- (3) Williams P.J. le B. & Jenkinson N.W., *Freshwat. Biol.* 1980, **10**, 491.
- (4) *Colour and Turbidity of Waters 1981* Methods for the Examination of Waters and Associated Materials, HMSO, London (in this series).
- (5) Dawson F.H., *Hydrobiologia* 1981, **77**, 71.
- (6) Vollenweider R.A., In: *IBP Handbook No. 12, A Manual on Methods for Measuring Primary Production in Aquatic Environments* (2nd ed.) 1974, 158. Blackwells Sci. Publs, Oxford.
- (7) Jewson D.H., Talling J.F., Dring M.J., Tilzer M.M., Heany S.I. & Cunningham C. *J. plank. Res.* 1984, **6**, 259.
- (8) Westlake D.F., In: *Brit. Ecol. Soc. Symp. No. 6, Light as an Ecological Factor* 1966, 99.
- (9) Tilzer M.M., In: *IBP 22, The Functioning of Freshwater Ecosystems* 1980, 153. Cambridge Univ. Press.
- (10) Westlake D.F., *Photochem. Photobiol.* 1965, **4**, 849.
- (11) Smith R.C., *J. mar. Res.* 1969, **27**, 341.
- (12) Anderson E.R., *Tech. Dept Circ. U.S. Geol. Surv.* 1952, **229**, 71.

Table 1. Surface reflectance for calm water (12)

Values of a and b for substitution in the equations

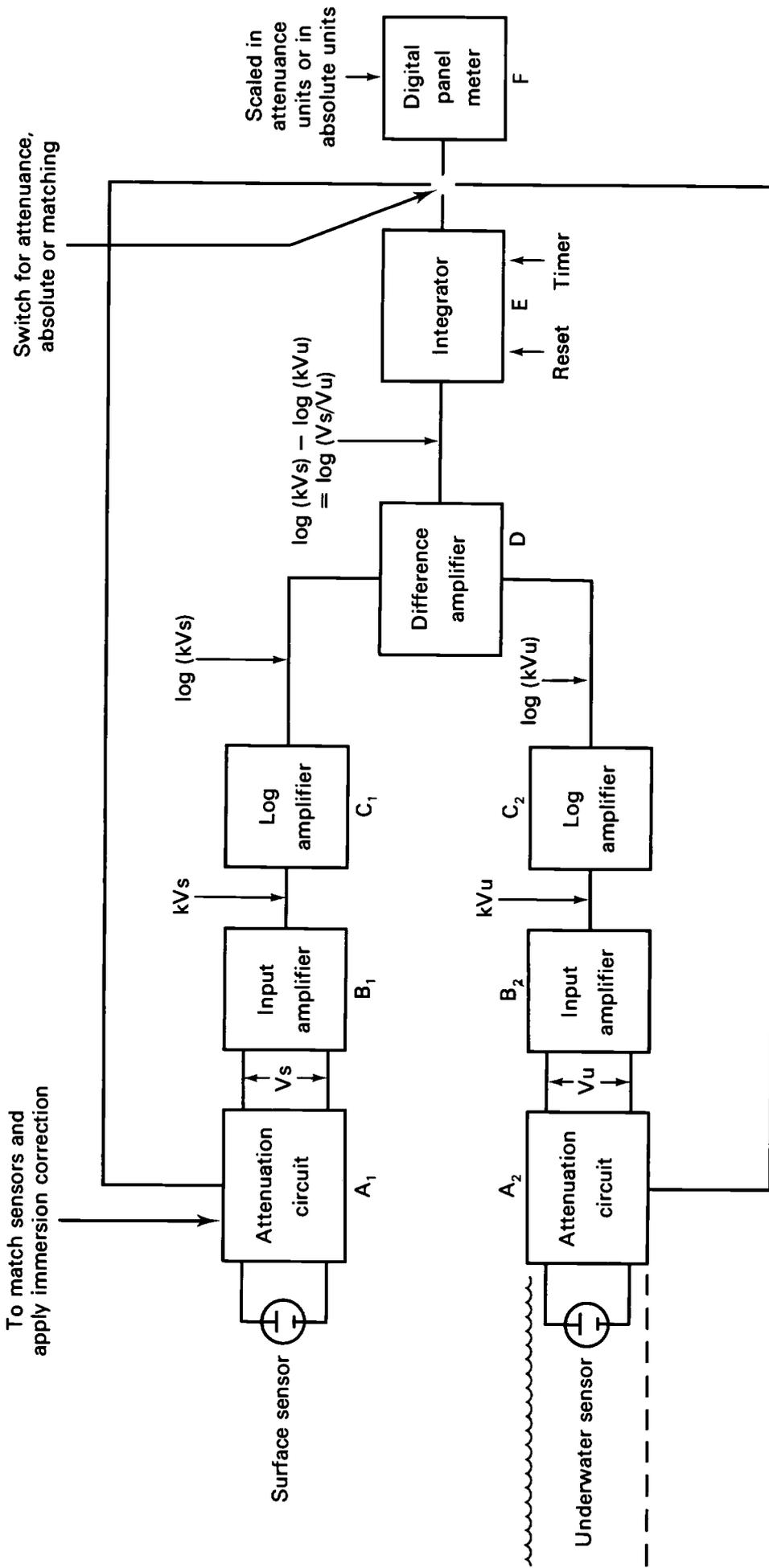
$$R = aH^b$$

$$R = \text{reflectance} = I_o/I_o$$

H = height of sun = degrees above horizon

	clear	low cloud <10,000 ft			high cloud >10,000 ft		
	sky	1-5/10	6-9/10	10/10	1-5/10	6-9/10	10/10
a	1.18	2.17	0.78	0.20	2.20	1.14	0.51
b	-0.77	-0.96	-0.68	-0.30	-0.98	-0.68	-0.58

Fig 1 Block diagram of attenuance meter



Based on equipment used by Westlake, Dawson and Williams (see 5); further details are not given because better components frequently become available. (See also 3).

Fig 2a PAR sensor using selenium photocell
(from 5)

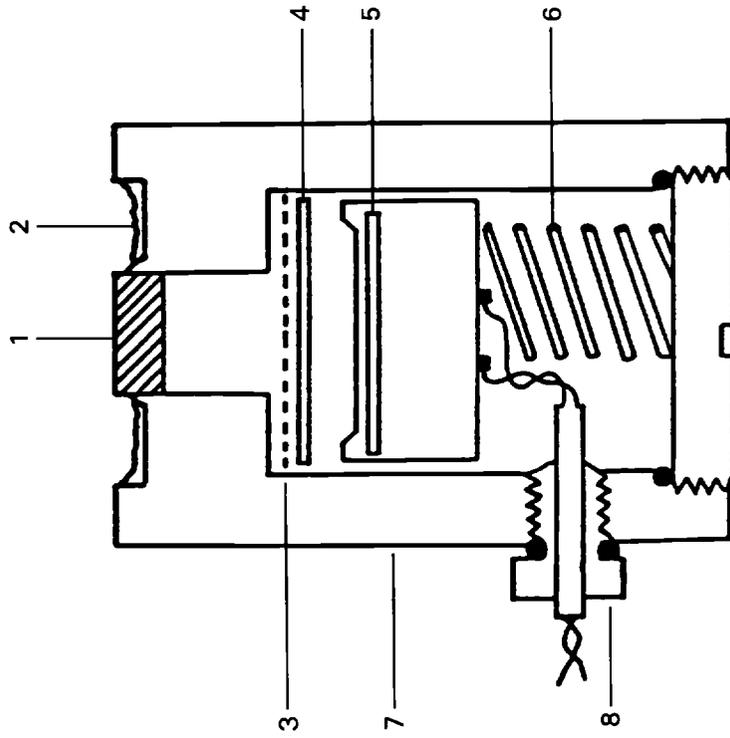
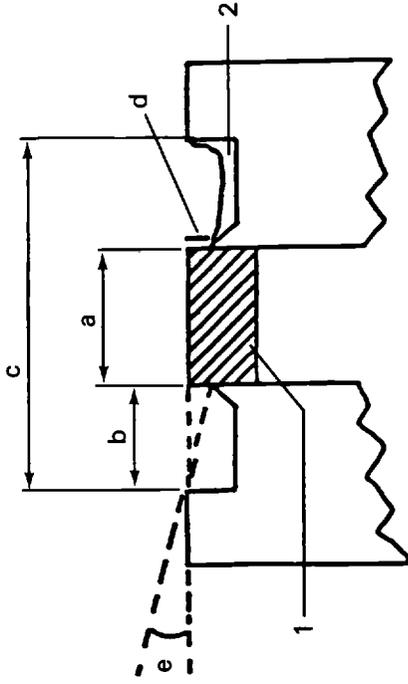


Fig 2b Dimensions of cosine -correct collector



1. 2.5mm ICI "Perspex", opal, grade O28, upper surface matt
2. Matt black paint and sealant
3. "Tyvar" cloth neutral filter (if needed to achieve linearity up to full sunlight)
4. "Cinemoid" filters, Rank Strand No.17 steel blue and No. 53 pale salmon (OR glass filters for colour sensors)
5. 16mm Megatron mounted and potted Type B Se photocell
6. Spring to support cell
7. Aluminium case
8. Watertight gland

- a. Diameter of opal
 - b. Width of annulus
 - c. Inner diameter of annular wall
 - d. Height of opal clear of sealant and paint
 - e. Angle between base of d. and top surface of annular wall; $\sim 10^\circ$
- $a/c \sim 1/2.5$
 $b/d \sim 6/1$

Fig 3 Block diagram of selenium photocell meter

(from 5)

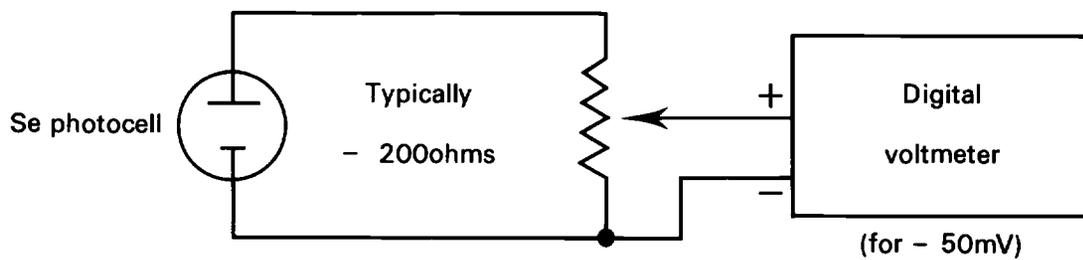


Fig. 4b Sensor support for deep water

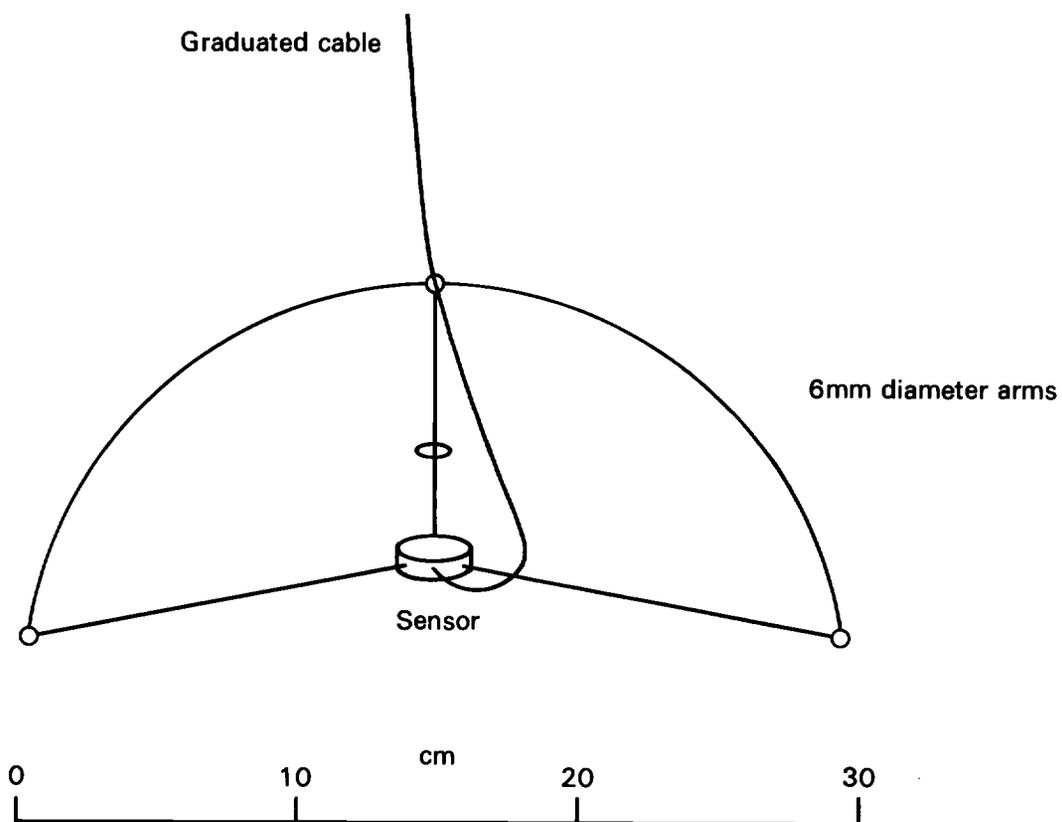


Fig. 4a Sensor support for shallow water

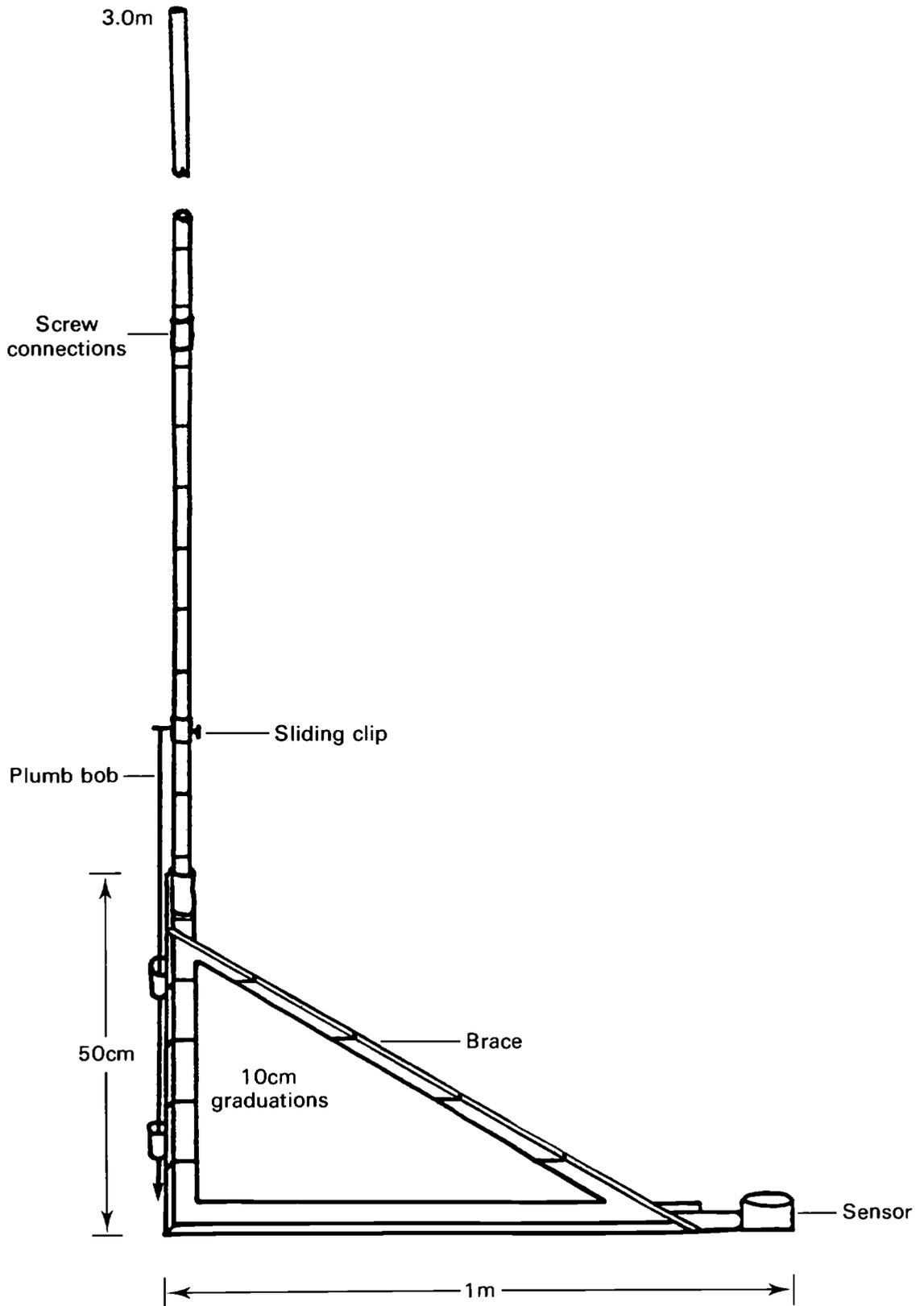
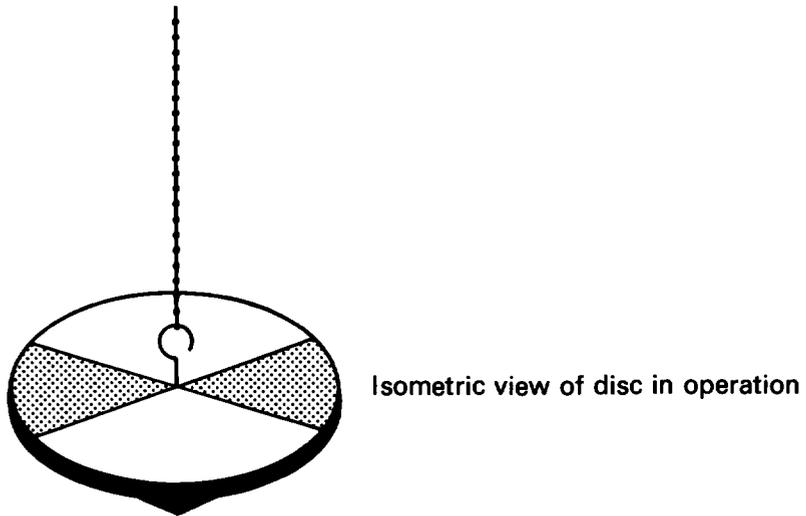
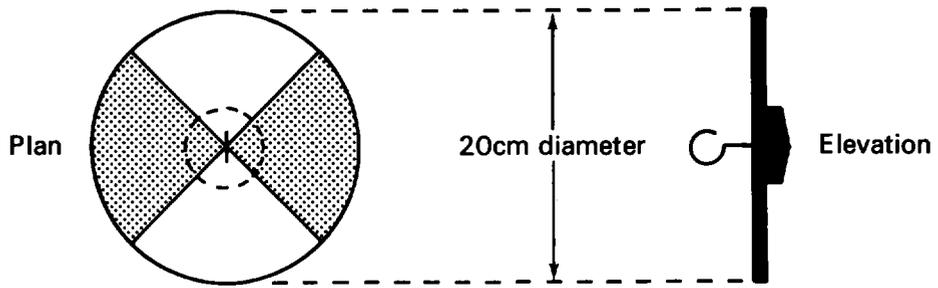


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