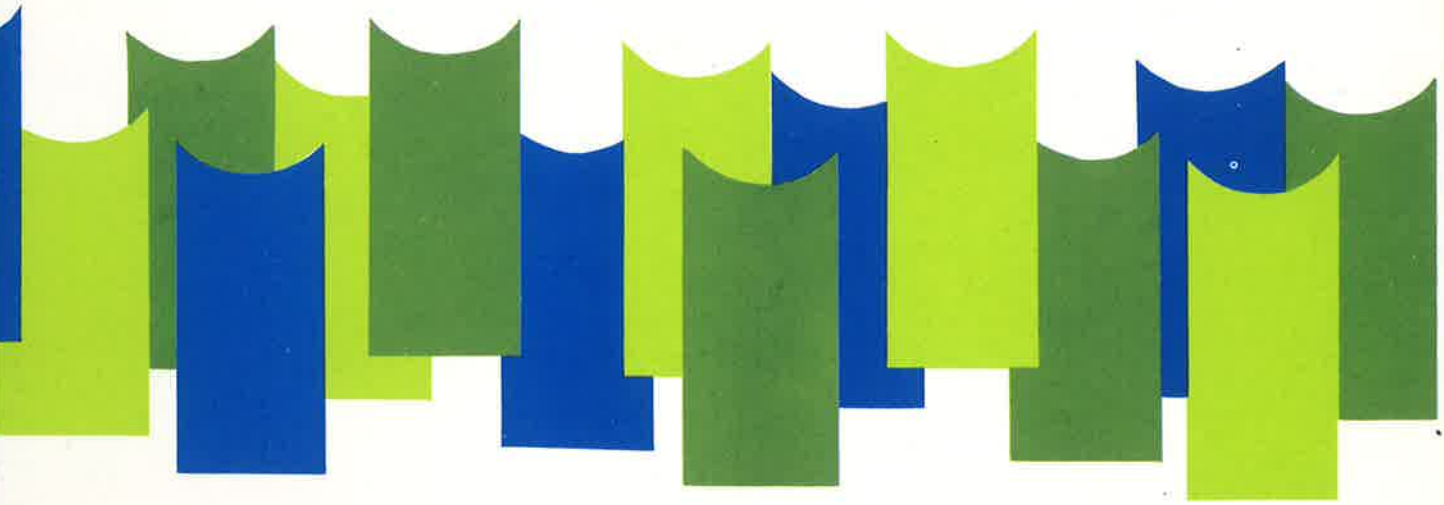
WATER & SOIL

TECHNICAL PUBLICATION

NO. 18

A REVIEW OF SOME BIOLOGICAL METHODS FOR THE ASSESSMENT OF WATER QUALITY WITH SPECIAL REFERENCE TO NEW ZEALAND



NATIONAL WATER AND SOIL CONSERVATION ORGANISATION

ISSN 0110-4144

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A REVIEW OF SOME BIOLOGICAL METHODS FOR THE ASSESSMENT OF WATER QUALITY WITH SPECIAL REFERENCE TO NEW ZEALAND

Standing Biological Working Party
of the
Water Resources Council

Wellington 1979

A review of some biological methods for the assessment of water quality with special reference to New Zealand

Standing Biological Working Party of the Water Resources Council

Water & Soil Technical Publication No. 18. 1979. 46p.

ISSN 0110-4144

Some biological methods for the assessment of water quality are briefly reviewed. Following discussuion and evaluation of some methods presently being used in New Zealand are more detailed sections on algae, bacteria, aquatic macrophytes and methods for assessing the toxicity of pollutants to aquatic animals.

Recommendations on the future development and use of many of the techniques are also included.

National Library of New Zealand
Cataloguing-in-Publication data

NEW ZEALAND. Water Resources Council. Standing Biological Working Party.
A review of some biological methods for the assessment of water quality with special reference to New Zealand / Standing Biological Working Party of the Water Resources Council. — Wellington : Water and Soil Division Ministry of Works and Development for National Water and Soil Conservation Organisation, 1979. — 1v. — (Water & soil technical publication, ISSN 0110-4144 ; no.18)

628.161
1. Water—Analysis. I. Title. II. Series.

Published for the National Water and Soil Conservation Organisation by the Water and Soil Division, Ministry of Works and Development, P.O. Box 12-041, Wellington, New Zealand.

Contents

Foreword

In 1975 the New Zealand Water Resources Council issued a statement on its aims for water quality to more than one hundred agencies and organisations for comment. The Council subsequently set up two committees, one to investigate biological and the other chemical problems of water quality. These committees became known as the Standing Biological/Chemical Working Parties. Their terms of reference were to consider and report upon the techniques used to assess quality of waters in and around New Zealand, to report on new developments in water quality assessment, and to recommend areas for further enquiry, and draw attention to techniques that could be used in regulations governing water quality.

The Standing Biological Working Party decided that a survey of the state of the art would be the best way to find out the criteria that were at present being used in water quality assessment and the suitability of these criteria for New Zealand conditions. To this end, the members of the Working Party have prepared a series of papers reviewing the field, as a basis for identifying areas that require further investigation.

Legislation introduced over the past decade has lead to improvements in certain aspects of water quality. This has been, for the most part, due to the better treatment of point sources of discharge which mainly consist of sewage treatment plant effluents and industrial discharges. However treatment of effluents from farming operations, especially cowsheds and piggeries, has also been upgraded in many areas. This improvement in water quality has also been assisted by a smoothing out of the process of granting water rights due to a better understanding of the administrative processes involved by all concerned.

These modest successes have highlighted the adverse effects of non-point sources on water quality. The run-off from soil and city streets, the effects of fertilizers an pesticides used in agriculture and the movement of nutrients from soils disturbed during timber felling are some of the examples of this type of water pollution. They are generally much more difficult to deal with and may even require a whole new approach to agricultural and forestry operations so that the water quality in our lakes and rivers can be retained or improved.

Although present day standards for potable water can usually be met by using available treatment methods, the presence of small quantitites of chemicals that may cause cancer and other illnesses may be increasing and their detection and removal by chemical or biological means is an area requiring considerable work.

That viruses in water supplies are a hazard to health is agreed, but the techniques for their detection and the means of their elimination are not well understood. A survey by the Standing Biological Working Party showed that no laboratory in New Zealand was in a position to test water for viruses on a routine basis, and it is clear that much remains to be done about this aspect of water quality in New Zealand.

Not all aspects of water quality assessment of importance have been covered in this publication and further reviews will be published from time to time as they become available.

A.P. MULCOCK
Chairman,
Standing Biological Working Party,
Water Resources Council

A survey and assessment of some methods being used for biological assessment of water quality in New Zealand

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This report is a collation and partial synthesis of the author's views, views expressed in the literature, and the views of twenty-two practising freshwater biologists in New Zealand, on various methods for the biological assessment of water quality and the suitability of these methods for New Zealand waters.

Fifteen techniques, which were identified as being of possible use in establishing biological water quality guidelines for classifying and managing New Zealand's inland waters, are considered.

Recommendations for future research include perfection and standardisation of methods for use throughout New Zealand.

CONTENTS

I Introduction

Techniques which have proved useful in testing and monitoring water quality in the United States have used bacteria, seston, chlorophyll, diatoms, phytoplankton, periphyton, primary productivity, macrophytes, benthic invertebrates and aquatic vertebrates (Weber 1973a, b). Detailed descriptions of the methods are available in several handbooks (e.g. APHA 1975; Slack *et al.* 1973; Weber 1973b; Lind 1974; Vollenweider 1969).

This review covers 15 techniques which may be, or prove to be, useful in establishing water quality guidelines for classifying and managing New Zealand's inland waters. In compiling it, the comments of biologists with practical experience of using the methods in New Zealand, were obtained. An attempt is made to indicate the extent of current use of the techniques in New Zealand, some advantages and disadvantages of these and the equipment, personnel and training required. Each technique is evaluated for its potential usefulness in water quality assessment and management in New Zealand and the possibility of establishing guideline limits for water of different trophic or pollution status.

Received 25 October 1976

Standing Biological Working Party of the Water Resources Council 1979: A review of some biological methods for the assessment of water quality with special reference to New Zealand. *Water and Soil Technical Publication 18 :* 5–19

Some techniques are reviewed in more detail than others because they have been used more widely, are more controversial, or appear to show particular promise for use in New Zealand.

Biological techniques which fall into the categories of bacteria, diatoms, periphyton, algal taxonomy and macrophytes are the subjects of separate reviews and are not included here.

The applicability of the techniques reviewed here to receiving waters (lakes, streams, rivers) and effluents is given in Table 1.

Table 1 Applicability of selected techniques to receiving waters and effluents.
x = suitable, (x) = possibly suitable, - = unsuitable.

	Lake	Large slow river, estuary	Stream or swift river	Effluent
Oxygen[1]	x	x	x	(x)
Biological Oxygen Demand (BOD)[1]	-	-	-	x
Secchi disc visibility	x	x	-	-
Phytoplankton productivity	x	x	-	-
Phytoplankton biomass:				
Volume	x	x	-	-
Chlorophyll *a*	x	x	-[2]	-
ATP	x	x	x	(x)
Seston	x	x	x	(x)
Plant bioassay:				
Algal assay — test organisms	x	x	x	x
— natural populations	x	x	-	x[3]
Tissue analysis	x	x	(x)	-
Activity	x	x	(x)	-
Zooplankton:				
Indicator organisms, diversity	x	x	(x)[4]	-
Biomass	x	x	(x)[4]	-
Benthic fauna (lakes):				
Indicator organisms, diversity	x	x	-	-
Biomass	x	x	-	-
Benthic fauna (streams):				
Indicator organisms, diversity	-	x	x	-

1 Not reviewed here
2 Technique useful for attached algae (periphyton) in streams
3 Stimulatory or inhibitory effects of effluents on the natural flora of the receiving waters
4 "Drift" fauna of typically benthic organisms which have become suspended in running waters - not true zooplankton

II Techniques

A. Secchi disc visibility

One criterion of high quality water for many industrial, aesthetic and recreational purposes is the absence of colour and turbidity. The transparency of water to light is influenced by dissolved and particulate matter present in the water. A rough estimate of this transparency may be obtained by the use of a Secchi disc. (The theory and practice of the Secchi disc experiment are discussed by Tyler 1968.) In most lakes, turbidity is caused mainly by algae in suspension (phytoplankton) so that Secchi disc depths give a fair indication of planktonic productivity or biomass. Studies overseas and in New Zealand have shown good correlations between Secchi disc depths and the trophic state of lakes as indicated by other parameters (Shapiro *et al.* 1975; McColl 1972).

METHOD

The method is described in several handbooks e.g. Lind (1974). A weighted circular place, usually 20cm in diameter and usually painted with alternating black and white quadrants, is lowered into water on a calibrated line. The line is attached by a ring to the centre of the disc so that it hangs horizontally. The disc is lowered vertically until it disappears, then lowered a little further and raised until it reappears. Secchi disc transparency is the average of the depth at which it disappears and reappears.

Transparency readings are affected by:

(a) organic particles in suspension (algae, yeasts, bacteria, zooplankton, detritus, etc.);
(b) inorganic particles in suspension (clay, glacial silt, sand, etc.);
(c) coloured compounds in solution (humic compounds, dyes, etc.);
(d) weather conditions (sun, cloud cover);
(e) wave action (roughness of the water surface);
(f) time of day (angle of the sun);
(g) eyesight of the observer;
(h) shadow of the boat or jetty;
(i) reflectance of the surface paint on the disc;

(j) angle of the line suspending the disc;
(k) size of the disc;
(l) markings of the disc;
(m) personal errors.

PRESENT USE IN NEW ZEALAND

Although 20 cm diameter discs are used by most workers, a 30 cm diameter oceanographic disc may be required in deep clear lakes where Secchi depths greater than 20 m may be encountered. Most workers use black-and-white discs but at least two use, and strongly recommend, all-white discs. Appropriate intervals for line marking will vary with water transparency but 0.1 m intervals are the most common. Secchi disc depths are measured, traditionally, in the open water of the main basin(s) of a lake and additional sampling sites in semi-enclosed bays, offshore from urban developments, or in turbid inflows, are recommended.

One measurement at each sampling site is considered sufficient, provided all precautions are taken. In lakes where more than one site is sampled, the mean of the measurements at all sites is used.

To reduce errors associated with water movements, several workers recommend viewing the disc through a glass-bottomed container or water telescope. All measurements are best done near true noon and by the same person; failing this, cross-checks should be carried out between operators. Weather conditions (cloud, rain, wind) should be recorded with the date and time of a measurement.

The frequency of sampling will depend to some extent on the size and accessibility of the lake and whether or not it stratifies thermally in summer. Frequency varies from fortnightly (small lakes) and monthly (large lakes) to as infrequently as once each season. It must be noted, however, that some lakes show pronounced and sudden variations in turbidity so that too few readings may be misleading. Several workers recommend extra measurements during spring and early summer when algal productivity is usually maximal, and during overturn, flooding, and blooms of algae or blue-green bacteria. It is suggested that the minimum Secchi disc depth recorded in the spring/summer period may be the most useful index of water quality.

ASSESSMENT

Early limnological surveys in New Zealand included measurements of Secchi disc depths. As Secchi disc depth is often the only parameter in common between early and recent surveys in New Zealand it is a useful guide to long-term trends in water transparency.

Secchi disc depth is an index of turbidity which can be caused by suspended inorganic material (e.g. silt) and organic matter (e.g. algae, bacteria, etc.). Although Secchi disc depth gives a rough indication of algal biomass in most lakes, the value of the method as an indicator of phytoplankton biomass or productivity is severely limited in lakes which contain significant amounts of clay, silt or humic compounds. For this reason, flood conditions or stormy weather which can bring sediments into suspension should be noted. In the absence of these limitations, Secchi disc transparencies may be useful for comparing productivities of different lakes (e.g. McColl 1972; Mitchell 1971; Green 1975). Within any one lake, however, changes in Secchi disc transparency may, or may not, reflect changes in productivity (Fish 1975; Mitchell 1971).

The method is simple and very little instruction and experience are required to carry it out correctly. However, to interpret the reasons for changes in transparency a limnologist may need to be consulted.

It should be possible to establish guidelines to assess the trophic state of lakes based on Secchi disc depths. The guidelines would almost certainly differ for different regions of New Zealand, and more data are required before guideline limits can be set. However, tentative guidelines for some North Island lakes suggested by three workers are.

Trophic status	*Secchi disc depth (m)*		
	*	**
Oligotrophic	>10	>9	>5
Mesotrophic	5–10	5–9	2–5
Eutrophic	0–5	<4	<2

* Lakes of the volcanic plateau of the North Island, New Zealand. (Ranges based on mean monthly measurements with a water telescope.) It was noted that in waters where Secchi disc depth is ≤2 m the method cannot be used to distinguish degrees of eutrophy.
** Minimum Secchi disc visibility in spring and summer.

RECOMMENDATIONS

(a) It is recommended that the measurement of Secchi disc depth should be included in water quality guidelines for lakes and reservoirs, especially for those in which the influence of inorganic suspended matter and humic compounds is insignificant.

(b) If adopted, the technique should be standardised (e.g. disc marking, use of telescope). The possibility of using simple indices incorporating Secchi disc depth for assessing lake water quality for management purposes should be considered, for example, the index of White (1976).

B. Phytoplankton productivity

Water quality is reflected in the species composition and abundance of plankton, particularly algae (phytoplankton). For many purposes, the factor of greatest interest is the rate at which new organic matter is formed by photosynthesis and accumulates within the system. This rate, termed the primary productivity, can be measured as biomass, dry weight of organic matter, carbon content, oxygen evolved, or assimilation of ^{14}C-labelled carbon dioxide. The most popular methods are the "Oxygen, Light-and-Dark-bottle Method" and the "^{14}C Method".

The principle advantages of the Oxygen Method lie in its independence from restrictions associated with the use of radioisotopes and in the cheaper equipment and materials needed to measure oxygen than ^{14}C. Disadvantages are that the method is unsuitable when productivity is low (see below). Best results are obtained in eutrophic waters in which productivity is between about 3 and 200 mg carbon m^{-3}. $hour^{-1}$ (Slack *et al.* 1973).

The principle advantage of the ^{14}C Method is excellent sensitivity which allows primary productivity to be estimated when this is very low. Low productivity is characteristic of oligotrophic lakes and frequently also of eutrophic lakes at certain times of the year. It can result also from the presence in water of substances inhibitory to photosynthesis. Thus it is a useful method for determining the effects of pollutants and nutrients on an aquatic community.

METHODS

Both methods are described in several handbooks (e.g. Lind 1974; Vollenweider 1969; APHA 1975; Slack *et al.* 1973).

PRESENT USE IN NEW ZEALAND

In New Zealand to date, the Oxygen Method has mainly been restricted to studies in eutrophic lakes and oxidation ponds, and measurement of primary productivity by the ^{14}C Method has been confined to research projects carried out by a few scientists despite the fact that the method was first used here 16 years ago.

Two variations of the method are being used: *in situ* incubation for a 4h midday period (ideally under standard weather conditions) and, laboratory incubation to eliminate weather variations (e.g. Burnet & Wallace 1973). Both methods have shortcomings, some of which can be overcome by strict standardisation of method (Vollenweider 1969).

The number of sampling sites depends on the size and shape of a lake and the objectives of the study. Generally one open water site is adequate for small lakes but more sites are required in large lakes because of horizontal variations in plankton distribution; additional sites in semi-enclosed bays or areas of special interest are sometimes required.

When a lake is stratified thermally, sampling is commonly carried out at seven depths at each site; this number may be reduced when a lake is not stratified. Frequency of sampling varies from fortnightly for a detailed study to bi-monthly for monitoring. Because a series of samples is examined at each site on each sampling day, the need for replication is reduced. Thus, one replicate at each depth is adequate.

ASSESSMENT

The Oxygen Method is adequate for use in oxidation ponds and eutrophic lakes provided all recommended precautions are taken and the method is standardised.

The high sensitivity of the ^{14}C Method suggests that it has potential for detecting eutrophication at its earliest stages before changes in chlorophyll, transparency or chemical indices are detectable (e.g. Mitchell 1975).

Field operation of both methods is technically simple but considerable training is needed to get reliable results. Skill in analytical methods is required; and for the ^{14}C Method laboratory assistance in preparing radiochemicals, measuring radioactivity of samples and calculating results is essential. A central laboratory could provide this service as is done overseas. To interpret the results, a biologist familiar with the ^{14}C Method would be required.

There are continual improvements being made to the ^{14}C Method many of which require increasingly expensive equipment and materials. Two scientists consulted recommend the inclusion of ^{14}C productivity measurements in water quality guidelines and think that guideline limits can be established for defined management objectives. Another did not recommend the method for general use, contending that not only is it difficult at present to obtain technicians and scientists with the requisite skills, but also that productivities measured by the ^{14}C Method correlate well with other parameters (chlorophyll, water transparency, oxygen levels) which are measured more easily and therefore are preferable for wide use, e.g. the methods suggested by Tunzi and Porcella (1974).

RECOMMENDATIONS

If the ^{14}C Method is to be used more widely for establishing water quality guidelines in New Zealand, consideration should be given to:

(a) Setting up a suitably equipped and staffed central laboratory for the routine preparation of radiochemicals, measuring radioactivity of samples and analysis of results.

(b) Standardisation of methods.

C. Phytoplankton biomass

Large crops of phytoplankton are the most obvious manifestation of eutrophication. The sizes of these crops reflect the integration over time of several important parameters of lake condition, viz. nutrient levels, temperature and light. Measurements of algal biomass can be obtained from measurements of algal volume, chlorophyll, adenosine triphosphate (ATP) and dry weight. Some comments on the first three techniques are given below. Other information on collecting, preserving and counting algae is given by Cassie elsewhere in this publication.

Although nuisance-causing algae and bacteria are comparatively large and hence can be collected with a fine net, sedimentation volumes or dry weights together with net sampling are almost valueless as estimates of phytoplankton biomass because buoyant colonial blue-green bacteria and *Botryococcus* spp. are difficult to sediment; also, varying proportions of the cells, if favourably aligned, will slip through even fine netting, and rotifers, ciliates and juvenile crustaceans will be inextricably retained with the algae.

1. Algal volume

Quantitative phytoplankton samples are collected and the volume of representative cells of each algal species are calculated either automatically or from the mean dimensions of cells, assuming that their forms correspond roughly to simple geometrical solids (Findenegg 1969). The sum of the volumes of individual cells in a known volume of water is the algal volume.

METHOD

The method is described in several handbooks (Lind 1974; APHA 1975; Vollenweider 1969; Slack *et al.* 1973; Weber 1973b).

Several methods of subsampling and examination are suitable:

Counting chambers
(e.g. Sedgwick-Rafter, Petroff-Hausser, haemacytometer)

ADVANTAGES – fast sample preparation, 3-D shape of algae retained, conventional microscope suitable.

DISADVANTAGES – time consuming analysis, only suitable at high algal densities.

Inverted microscope method

ADVANTAGES – 3-D shape of algae retained, delicate algae more likely to be preserved intact.

DISADVANTAGES – inverted microscopes are expensive, sedimentation chambers delicate and easily broken; sedimentation for several hours is required before samples can be examined; samples must be stored in liquid form for future reference. The method is time consuming if done sufficiently precisely to meet statistical requirements.

Membrane filter method
(combined with microscopic examination)

ADVANTAGES – faster sample preparation; storage on slides for later reference is less bulky than samples preserved in liquid.

DISADVANTAGES – unless filtration pressure is very low, cell damage to delicate species may result. The method is time consuming.

Electronic particle counter
(with cumulative volume readout)

ADVANTAGES – automation, no eyestrain, very fast; useful for pure cultures (e.g. in bioassays).

DISADVANTAGES – the expensive equipment requires careful maintenance; no taxonomic information is obtained and the method is rarely suitable for natural phytoplankton communities because colonial and filamentous forms cause problems by blocking the aperture tubes; no discrimination between dead and living material or between organic and inorganic particles is possible. Volumes must be calculated for each algal species several times during the year as the mean cell volume of individual species can fluctuate seasonally.

Colonial forms pose problems and eutrophication is often manifested in an increase of colonial blue-green bacteria and diatoms. Because colonies differ widely in size at any one time and with time, either individual cells or all colonies should be counted or a multiplication factor for converting colony counts to cell counts must be obtained by counting the cells in a statistically acceptable sample of colonies on each sampling day.

PRESENT USE IN NEW ZEALAND

This is discussed in a paper by Cassie elsewhere in this publication.

ASSESSMENT

Any of the four methods listed above could be carried out by a trained, careful technician, but good equipment (microscope or particle counter) is essential and the work is fairly time consuming. Supervision would be required initially and a limnologist must be consulted for interpretation of results.

Although taxonomic identification is not essential for calculating algal volume, phytoplankton samples should be monitored taxonomically for changes in species composition and abundance. Ideally, the latter should be quantitative.

Vollenweider (1968) gives the following scale for the maximum plankton density that may develop during a year (expressed as $cm^3.m^{-3}$):

Ultra-oligotrophic	< 1
Mesotrophic	< 3– 5
Eutrophic	5–10
Highly eutrophic	> 10

The trophic status of four lakes in New Zealand, assessed according to Vollenweider's (1968) scale, was found to be consistent with trophic assessments based on other parameters (Burns & Mitchell 1974; Fish 1975; Baars-Kloos 1976). Therefore, both maximum phytoplankton volume (Vollenweider 1968) and mean summer phytoplankton biomass (Burns 1975; Burns & Mitchell 1974) appear to be fairly good indices of the trophic state of lakes.

RECOMMENDATION

It would not seem worthwhile at present to include algal volume as a routine parameter for assessing lake water quality, because algal biomass can be assessed more easily from measurement of chlorophyll or ATP.

2. Chlorophyll *a*

Chlorophyll *a* is normally the most abundant and important pigment in photosynthetic organisms, in which it constitutes approximately 1–2% of the dry weight of organic material. The pigment can be extracted quantitatively and measured either spectrophotometrically or by the more sensitive fluorometric method.

METHODS

These are described in all standard handbooks (e.g. APHA 1975; Lind 1974; Vollenweider 1969; Slack *et al.* 1973; Weber 1973b).

Chlorophyll *a* measurements are affected by:

(a) the type of filter (membrane or glass fibre, Long & Cook 1971);
(b) solvent used for extraction (methanol, acetone);
(c) completeness of extraction (affected by time, species, and whether cells mechanically crushed or not);
(d) degradation pigments (phaeophytin and phaeophorbide, Tett *et al.* 1975);
(e) dominant species of algae (affects chlorophyll extractability);
(f) turbidity (centrifugation required);
(g) wavelengths at which absorbance measured;
(h) equation used to estimate chlorophyll content from absorbance;
(i) method of storing samples prior to analysis (affected by acid conditions and exposure to light);
(j) time elapsed between sample collection and analysis.

PRESENT USE IN NEW ZEALAND

Samples of water can conveniently be collected at the same open water sites used for measurement of transparency and primary productivity. Two methods of sampling are in use in lakes.

(a) An integrated sample from several depths in the photic zone is collected by filling a vertical tube (e.g. 3–4 cm diam., 3–5 m long plastic tube weighted at the bottom).
(b) A non-metal closing bottle is used to obtain samples from discrete depths. Several depths should be sampled, including 1 m below the surface and spread throughout the photic zone. It has been suggested that samples should always include 1 m below surface, 1 m above bottom, and depths just above and below a thermocline.

The method of sampling should be stated. Although several methods of analysis have been suggested. usually only chlorophyll *a* (not *b* and *c*) is measured and a correction is made for phaeophytins.

The water samples are transferred to polythene bottles, kept cool, in the dark, and are filtered within 24 h. Opinions differ on how to keep samples for longer than 24 h if this is unavoidable. Some recommend refrigeration of the liquid samples and others freezing, but for short periods only; several workers have advocated filtering the samples and storing the filters in darkness at O°C.

Most workers measure chlorophyll extracts spectrophotometrically, although the use of the more sensitive fluorometric method (APHA 1975) is increasing in New Zealand.

The frequency of sampling depends on the objectives of the study and the resources available. Monthly sampling is most common in large lakes, but small lakes or lakes under

special study should be sampled every two weeks. At a minimum, sampling should cover spring and summer when lakes in New Zealand generally develop their annual maximum algal crop.

The number of replicate samples collected at each sampling site varies. Generally one or two vertical profiles of bottle samples are collected, or three to four integrated samples.

Several recommended procedures include noting algal and bacterial blooms, water discolouration and weather conditions at the time of sampling. Thorough mixing of the water samples before they are subsampled, low filtration pressure to minimise cell rupture and the use of glass fibre filters (rather than cellulose acetate) and chilled acetone have been advocated as ways of increasing the precision of the method.

ASSESSMENT

Chlorophyll *a* content is probably the best, readily measured, single biological indicator of the state of eutrophication of standing waters. Good correlations between chlorophyll *a* concentrations and nitrate and phosphate levels in winter have been obtained (e.g. Lund 1970; McColl 1972; Dillon & Rigler 1974). Chlorophyll *a* content may also be a useful index of the trophic state of large rivers and of lakes in which inorganic suspended materials or humic compounds decrease the value of Secchi disc measurements. Because large seasonal variations in chlorophyll content may occur, chlorophyll *a* must be monitored routinely if it is to be used to assess the trophic state of a range of lakes. Ideally it should be used in combination with other parameters (e.g. Secchi disc transparency or ash-free dry weight of seston to obtain the Autotrophic Index, p.1030, APHA 1975).

The sensitivity of the techniques is potentially very high, especially if a fluorometer is used, and correction for phaeophytins probably improves the sensitivity. Accurate chlorophyll *a* measurements require considerable skill and inexperienced operators may make large errors so that supervision is essential initially.

Although superficial interpretation of the results requires little background knowledge, deeper and wider interpretations call for limnological training and several years experience in the field. Attempts to relate chlorophyll *a* concentrations to algal numbers or biomass require a knowledge of algal physiology and an appreciation of the precision of the methods used.

It would be worthwhile to include chlorophyll *a* concentrations in water quality guidelines for New Zealand. However, preliminary standardisation of the technique would be essential. It was generally agreed that guideline limits for the chlorophyll *a* content of lakes could be established. However, because the chlorophyll *a* content of lakes varies continuously, limits would have to be set arbitrarily in relation to the purposes for which lakes were to be used or classified.

Although the distinction between oligo-, meso- and eutrophic conditions is somewhat arbitrary and there is considerable overlap between adjacent categories, the following broad guidelines, based on the "maximum chlorophyll *a* concentration" recorded at any depth or at any time in the year, apply to many North Island lakes. Chlorophyll *a* maxima generally occur in early summer.

	$\mu g.l^{-1}$
Oligotrophic	< 6
Mesotrophic	6-30
Eutrophic	> 30

In any one lake there may be marked fluctuations in chlorophyll *a* concentration within a season. The largest fluctuations generally are found in eutrophic waters in summer. Suggested seasonal changes and within-season fluctuations in chlorophyll *a* content of eutrophic waters are:

	$\mu g.l^{-1}$
Winter - all depths	4-10
Summer -epilimnion	15-60
Summer - hypolimnion	0-4

A possible alternative to "maximum chlorophyll *a* concentration" is "annual mean chlorophyll *a* concentration". The latter would require that sampling be done regularly throughout a year, whereas the former would reduce the sampling period to spring and early summer in most lakes. There would seem to be considerable merit in deriving guideline limits from a combination of parameters (e.g. Secchi disc, seston, chlorophyll *a*) rather than from chlorophyll *a* content alone. Concomitant with chlorophyll *a* measurement, the dominant algal taxa in the samples should be recorded.

RECOMMENDATIONS

(a) Consideration should be given to including measurements of chlorophyll *a* in water quality guidelines for lakes, reservoirs and large rivers in New Zealand. The simplified method described by Lind (1974) may have considerable merit for routine use in water quality assessment and monitoring.

(b) Standardisation of a method of measuring chlorophyll *a* is essential.

(c) The possibility of deriving an index or guidelines based on a combination of parameters, including chlorophyll *a*, to characterise water quality in relation to use (e.g. White 1976) should be investigated further.

3. Adenosine triphosphate

Adenosine triphosphate (ATP) is present in all living cells at relatively constant concentrations per unit dry weight. Therefore, the concentration of ATP in water provides a measure of the biomass of living material in the water e.g. bacteria, algae, zooplankton etc (p.1035 APHA 1975). ATP is not associated with non-living material and it is inactivated rapidly upon death of cells. Thus, ATP assays can be used for immediate detection of lethal responses to toxic substances or to environmental stresses, e.g. hot water discharges (Brezonik *et al.* 1975).

ATP assays have been used successfully to:

(a) Determine bacterial biomass in situations where almost no phytoplankton or zooplankton exist (Hamilton *et al.* 1968; Cavari 1976).

(b) Confirm that chlorophyll *a* provides a good measurement of algal biomass in mixed waters (Paerl *et al.* 1976; Brezonik *et al.* 1975).

(c) Monitor zooplankton mortality resulting from entrainment at a power plant (Drew *et al.* 1976).

(d) Determine if nutrients were limiting in natural waters since Cavari (1976) has shown that nutrient deficiencies in algae limit ATP synthesis.

METHOD (See APHA 1975)

ATP is extracted from cells and used to provide the energy source for the light-emitting oxidation of luciferin, under the influence of an enzyme, luciferase, both of which are extracted from firefly lanterns. The light emitted is a function of ATP concentration and is measured quantitatively. There is a range of ATP photometers available commercially but liquid scintillation counters can also be used for bioluminescence assay. Several precautions are required as pointed out by Sutcliffe *et al.* (1976) and strict standardisation of the assay procedure is essential.

PRESENT USE IN NEW ZEALAND

The method is being used at several research institutions in New Zealand.

Frequency and depth of sampling depend on the objectives of the investigation. Duplicate ATP samples are usually taken each time phytoplankton is enumerated. Commonly, 100-500 ml of water are filtered from oligotrophic lakes, 25-100 ml from mesotrophic lakes and <25 ml from eutrophic lakes. Zooplankton and sediments may cause problems. Zooplankton are largely removed from samples by prefiltering the water through netting of approximately 125 μm mesh. The problem of separating large protozoans and rotifers from equally large phytoplankton remains unsolved. Sediment-containing samples will not allow 100% extraction of ATP with tris buffer as ATP is retained on the sediment particles (Paerl pers. comm.). Various methods for extracting ATP from sediments have been devised overseas (Bancroft *et al.* 1976; Patterson *et al.* 1970; Lee *et al.* 1971).

ASSESSMENT

The method is extremely sensitive. For example, it is easy to detect 0.001 μgATP.ml^{-1} corresponding to a microbial biomass of 1.25 μgC.l^{-1} if 1 litre is filtered. Therefore, it is unlikely that the ATP content of any lakewater in New Zealand will be beyond the limits of detection. The analytical procedure is rapid, reproducible and highly sensitive (Holm-Hansen & Booth 1966; Holm-Hansen & Paerl 1972). It is relatively inexpensive and instrumentation is stable and reliable (APHA 1975).

A technician can be trained in 2 h to prepare the samples and enzyme, and to operate an ATP photometer. The older model photometers with larger photomultiplier tubes are more sensitive than newer, compact and more expensive versions (Paerl pers. comm.). Fifty samples can be analysed in 2–3 h. Since the enzyme decays with time a standard series of ATP concentrations should be run every 15–20 samples.

It is unlikely that standard conversion factors for converting ATP content to cellular carbon content for mixed communities of organisms will be found. The C:ATP ratios for algae and zooplankton differ from those of bacteria so that additional steps are necessary before biomass can be apportioned among members of the planktonic community and total biomass calculated (Holm-Hansen & Paerl 1972; Jassby 1975).

The merits of ATP analyses as general water quality indicators are currently being evaluated. Since this assay measures total microbial ATP content it may be of use in rapidly assessing the growth potential of water in any lake or stream and coupled with chlorophyll analyses, especially in vertically mixed systems, it is of potential use for rapidly detecting increases in numbers of micro-organisms. A rough set of standards, in terms of μg ATP.l^{-1} which may be used to typify or categorise lakes on a trophic basis, has been established (Paerl pers. comm.).

D. Seston

The productivity of a lake is often reflected closely by the amount of organic matter in suspension in the water. Suspended particulate matter or seston includes phytoplankton, bacteria, fungi, zooplankton, detritus and inorganic particles. A rough estimate of the organic component of the total particulate matter may be obtained by igniting a weighed sample of seston and subtracting the weight of ash (inorganic residue) that remains. This estimate is termed, more accurately, the ash-free dry weight of seston.

METHOD

Details of the method are described by APHA 1975 (pp 94–8), Slack *et al.* 1973 (pp 54–7) and Lind 1974 (pp 77–80).

PRESENT USE IN NEW ZEALAND

The method is being used in several laboratories.

ASSESSMENT

The method is simple, straightforward and easy to carry out, but standardisation is essential (e.g. pore size of filter used, temperature of muffle furnace). The method only provides a crude estimate of organic matter present, mainly because ignition can also produce decomposition or volatilization of some inorganic salts.

E. Plant bioassay

1 Algal assay

Algal bioassay is based on Liebig's "Law of the Minimum" which states that "growth is limited by the substance that is present in minimal quantity in respect to the needs of the organism". Algal assays (Algal Assay Procedure: Bottle Test 1971, APHA 1975 p. 744) are intended primarily for use in the following situations:

(a) Assessment of a receiving water to determine its nutrient status and sensitivity to change.
(b) Evaluation of materials and products to determine their potential effects on algal growth in receiving waters.
(c) Assessment of effects of changes in waste treatment processes on algal growth in receiving waters.
(d) Assessment of the impact of nutrients in tributary waters on algal growth in lakes and receiving waters.

Algal assay tests (Algal Assay Procedure: Bottle Test 1971) have been used to:

(a) Identify algal growth-limiting nutrients.
(b) Determine biologically the availability of algal growth-limiting nutrients.
(c) Quantify the biological response to changes in concentrations of algal growth-limiting nutrients.

Two kinds of bioassay are commonly used, those which use test organisms and those which use natural populations of micro-organisms.

(a) Bioassay with test organisms

METHOD

Selected algae are added to the water to be tested and algal growth is determined at appropriate intervals. The refined Algal Assay Procedure (AAP) : Bottle Test (1971) recommends three species as test organisims. *Selenastrum capricornutum, Microcystis aeruginosa* and *Anabaena flos-aquae.* These species, under standardised culture conditions, give a range of responses reflecting the nutritional state of the waters being tested. Recently, three diatoms have been added to the list of test organisms for fresh waters (APHA 1975 p. 784).

USE IN NEW ZEALAND

The test organisms are being used for research in at least one laboratory in New Zealand.

ASSESSMENT

Payne (1975) claims that the method can be used to:

(a) Assess the enrichment potential of sewage (or other inputs) in particular lakes. The test algae are grown in lakewater and in lakewater spiked with sewage. The population growth response (abundance) of the test alga is followed over a period of 2-3 weeks.

(b) Determine the nutrient(s) which limit algal growth in particular lakes with a view to decreasing the levels of these nutrients and hence to reducing algal growth in the lakes. The growth of the test algae in lakewater is compared with their growth in defined media and the growth response to spike additions of nutrients (e.g. P, N, Fe, P + N) is used to determine whether levels of the spike nutrients in the lake were limiting algal growth.

The AAP: Bottle Test is now widely used in the U.S.A. to study eutrophication (Golterman 1975). Several precautions are recommended including the use of several test organisms because different species respond differently to a given nutrient limiting condition (APHA 1975, pp 750–5). However, there are still serious limitations and difficulties even with the "refined procedure". Some of these are:

(a) Because native organisms (algae, yeasts and bacteria) in the lakewater compete with the test algae they must be removed from the water to be tested. Three pretreatments are available but all change the chemical characteristics of the lakewater (Filip & Middlebrooks 1975). These pretreatments are:

(i) Filtering through glass fibre and membrane filters to remove indigenous algae and particulate phosphate. This method is used if the aim is to determine the growth response to growth-limiting nutrients which have not been taken up by filterable organisms, or if it is desired to predict the effect of adding nutrients to a test water at a specific time.

(ii) Autoclaving to solubilize the nutrients in the native organisms and release them for use by test organisms. This method may be used if the aim is to determine the amount of algal biomass that can be grown from all nutrients in the water, including those in the plankton. It also raises pH, causes precipitation of some chemical species and loss of CO_2.

(iii) Exposure to U.V. light to cause autolysis of native organisms, and oxidation of organic and inorganic nitrogen.

Weiss (1976) suggested that the growth response of test algae in filtered water gave a good measure of *ambient growth potential* of the water, whereas the growth response in autoclaved water (which had been filtered subsequently to remove particulates) gave a good measure of *total growth potential.*

(b) Growth of one or two test algal species does not necessarily indicate the growth potential of the water for other algal species with different nutrient requirements.

(c) Test algae "adapt" (by natural selection) to the higher nutrient concentrations in cultures. Algae in lakes may form dense populations at nutrient levels below those required by test algae.

(d) The AAP inoculum densities (*Selenastrum* 1.0×10^6 cells.l^{-1}, *Microcystis* 5.0×10^4 cells.l^{-1}, *Anabaena* 5.0×10^7 cells.l^{-1}) are more appropriate for testing sewage effluents than natural lake waters (Golterman 1975).

(e) The element limiting growth in bioassays may not be the element which should be the target for decrease in the lake. For example, if a lake shows an increased algal crop due to increased phosphate input, another element, for example iron, may then become limiting and in bioassays of the lakewater with test algae, iron will be the growth-limiting element and an erroneous recommendation to remove iron from the lake may be made.

(f) Bioassays, like chemical assays, are restricted to the nutrient conditions at one point in time. Effects of continuous input of nutrients and effects of nutrient recycling in a lake may be overlooked.

(g) In a joint evaluation of the AAP: Bottle Test using the same water distributed to each of 8 laboratories and a standardised assay protocol, the interlaboratory precision was low (>30% variation) according to Weiss and Helms (1971).

(h) Guideline Limits. Weiss (1976) used the AAP: Bottle Test, with *Selenastrum capricornutum* as the test organism, to determine limiting nutrients in 44 lakes, reservoirs, impoundments and rivers in North Carolina. Analysis of the results of 345 assays carried out at several seasons of the year revealed that the ratio of soluble inorganic ntrogen to soluble inorganic phosphorus in the water gave a good indication of nutrient limitation:—

when N/P	>13	water P-limited
when N/P	9–12	both nutrients limiting
when N/P	<8	water N-limited

(i) Experimental design, data analysis and evaluation require a knowledge of algal physiology and statistics.

(b) Bioassay using natural populations

Although AAP: Bottle Tests have proved adequate in numerous eutrophic and mesotrophic lakes they are often too insensitive to be applicable to oligotrophic lakes.

METHOD

The general method, described by Goldman (1969) has been modified by workers to meet individual needs (e.g. Allen 1972). Gerhart and Likens (1975) have compared four modifications.

USE IN NEW ZEALAND

In situ algal bioassays with natural flora are being used by several workers in New Zealand to test the response of natural phytoplankton populations to nutrient additions, especially in oligotrophic waters. For example, at Lake Taupo, water is collected from just below the surface in the open water and dispensed into specially cleaned 4 litre polythene bags. Nutrients, followed by ^{14}C-bicarbonate, are added directly to the bags which are then tied securely and returned immediately to the lake where they are incubated for 5 days to 2 weeks depending on the growth response. Records of water temperatures, ambient nutrient concentrations and sunlight hours are kept. At the end of the incubation period subsamples are taken from each bag for analyses of ^{14}C incorporation, chlorophyll *a* and total pigment accumulation, ATP content of the particulate material, and cell numbers.

ASSESSMENT

The main advantage of this technique is that it is applicable to the oligotrophic waters which characterise many New Zealand lakes.

The additional advantage of analysing algal abundance by several methods is that phytoplankton growth in response to nutrient additions measured by one method can be cross-checked with the response measured by other methods. Often the result of one method of analysis may show stimulation in response to a particular nutrient while the results of other methods fail to reveal stimulation. This is especially true when chlorophyll *a* and ^{14}C uptake are compared with ATP estimates and cell counts. It has been shown repeatedly (Paerl pers. comm.) that micronutrients often stimulate chlorophyll *a* production in cells with little or no concomitant biomass increase. If chlorophyll *a* were the only method used for assessing biostimulation, a wrong conclusion might be reached. In oligotrophic waters, where small additions of nutrients will have a greater effect than in eutrophic waters, measurement of the response by several methods offers greater sensitivity than can be gained by the use of a single method.

At present the three useful methods of analysis, in order of decreasing sensitivity, are ^{14}C uptake, ATP assay and chlorophyll *a*. ^{14}C uptake is the most sensitive method of measuring phytoplankton productivity and it is widely applicable.

ATP assay ranks second in sensitivity and is widely applicable to aquatic ecosystems. The main shortcoming lies in the failure of the method to discriminate phytoplankton from bacterial biomass. However, in assessing planktonic response to the addition of nutrients, biomass of the total community is often a more realistic parameter.

Chlorophyll *a* stimulation is a marginally sensitive parameter in oligotrophic lakes because unrealistically high doses of nutrients often have to be administered in order to elicit a response. The reliability of chlorophyll *a* as a sensitive growth indicator will need to be examined for individual situations. A combination of chlorophyll *a* and ATP analyses often helps to distinguish algal from bacterial stimulation. New methods which are being evaluated for use with natural phytoplankton communities include the use of "continuous flow chemostats" (e.g. Gerhart 1975).

RECOMMENDATIONS

(a) Further research in New Zealand on algal bioassays to answer questions relating to the availability to algae of nutrients in various forms (e.g. insoluble, complexed, particulate) and the fertility or toxicity of an input of sewage, industrial or agricultural waste into a lake or river, would seem worthwhile.

(b) Algal assay procedures should be kept under scrutiny with a view to their possible use in New Zealand.

(c) The possibility of establishing N/P ratio guidelines relating to the limitation of these nutrients in lakes in New Zealand warrants further investigation.

Research is being carried out into other plant bioassays which may be useful in assessing water quality. Of these tissue analysis and activity are described briefly below. Both are more suitable for use on aquatic macrophytes than phytoplankton, as they are suitable only with species which form monospecific blooms.

2. Tissue analysis

The relation between the concentration of an essential element in a bloom-forming species of organism (or in specific parts of an aquatic angiosperm of known physiological age) and plant growth is established from laboratory cultures. The critical concentration (concentration of the element in the tissues which is just inadequate for maximum growth) is identified (Fig.1).

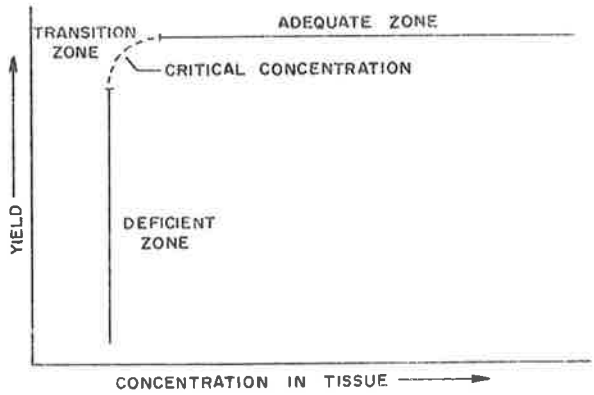

Figure 1 Schematic diagram of the relationship between plant growth and the concentration of an essential element in specific plant parts of a definite physiological age. (From Ulrich 1961, cited in Gerloff 1969).

A comparison between the concentration of the element in samples of algae or bacteria collected from blooms and the critical concentration is made to establish whether growth of the organisms in the lake is being limited by the supply of that element (Gerloff 1969). Critical N and P concentrations for the bloom-forming blue-green bacterium, *Microcystis aeruginosa*, and for several aquatic angiosperms have been established (Gerloff & Skoog 1957).

ASSESSMENT

Tissue analysis provides an integrated expression of the availabilities of an element in the various parts of a lake or stream from which the plant absorbed nutrients during growth. The method promises to be useful but has yet to be developed to the point of general reliability and usefulness (Gerloff 1969).

3. Activity

Plants limited in growth by the supply of available nitrogen will absorb ammonium-nitrogen (NH_4-N) in the dark four to five times more rapidly than will plants with surplus or adequate nitrogen.

Plants limited by the supply of available phosphorus contain little or no extractable orthophosphate (PO_4-P) and have as much as 25 times the alkaline phosphatase activity of plants grown with surplus phosphate.

METHOD

Rate of absorption of NH_4-N in the dark, amount of PO_4-P extracted by boiling water and alkaline phosphatase activity in phosphate-free media are measured to follow changes in nutritional status of nitrogen and phosphorus in algae and in aquatic weeds in relation to changes in supply of these elements (Fitzgerald 1969).

The different species in mixed blooms must be separated for assay because nutritional requirements of nitrogen-fixing and non-fixing species may differ. In aquatic macrophytes the physiological age of the plant and the parts assayed (terminal shoots preferably) must be standardised as in tissue analysis.

F. Zooplankton

1. Indicator organisms and diversity

The concept that the presence of a particular species is indicative of the existence of certain environmental conditions in water is an attractive one. However, for a species to be useful as an indicator of these conditions, it must have a rather narrow range of suitable environmental conditions that are known and of interest to man. Few species satisfy these criteria (Warren 1971; Hart & Fuller 1974).

METHODS

The place, method and frequency of sampling is governed by the biology of the indicator organisms (see below). Organisms larger than Protozoa are collected with pumps, traps and/or nets, the mesh size of which is small enough to catch the juvenile stages. Suggested methods and fixatives for various groups or organisms occur in several handbooks, e.g. Lind 1974; APHA 1975; Edmondson and Winberg 1971; Slack *et al.* 1973.

USE IN NEW ZEALAND

The zooplankton in lakes and ponds throughout New Zealand have been sampled sporadically for more than three decades. The distribution of the major groups of zooplankton reported and methods of collection are given below.

PROTOZOA: Cosmopolitan species occur in New Zealand but so far they have received little study (Stout 1970). Collections are made by taking water samples from various depths.

ROTIFERS: Cosmopolitan species occur in New Zealand and are often abundant. This group is receiving more study but identification to species level is often difficult, requiring patience and experience. Errors in identification could confound the interpretation since species which are similar morphologically can have very different ecological requirements. Although different species and assemblages of species can be found in waters of different trophic status there is much overlap and seasonal variation. Some species have restricted pH and salinity tolerances and may be more sensitive indicators. Littoral rotifer communities, which are among the most diverse in species composition, are potentially sensitive indicators of water quality. At present, too little is known about the ecology, composition, or variation within these communities for them to be used. Collections are made with a 70μm mesh net in open water and among weed beds.

CRUSTACEAN PLANKTON: The species diversity in New Zealand is very low and most species are fairly widespread. Therefore, it is unlikely that any of them will be useful as indicator species (Chapman *et al.* 1975). Collections are made with nets in open water and among weed beds.

ASSESSMENT

(a) In New Zealand most species of zooplankton seem to be found in a wide range of habitats and the variations which occur may not be related to water quality.

(b) Considerable training and experience are required to identify species and to interpret the results.

(c) Changes in dominance of zooplankton through time in some New Zealand lakes have been noted and it has been suggested that these changes may be related to changes in productivity (Stout 1970; Mitchell 1975). However, it is unlikely that zooplankton will be useful as indicator organisms in New Zealand lakes.

RECOMMENDATION

Zooplankton diversity or indicator organisms should not be included in water quality guidelines.

2. Biomass

It has been suggested that the average biomass of plankton over a year might indicate the trophic state of lakes in New Zealand (Stout 1970).

METHOD

Samples of plankton are collected from known volumes of water. The dry weight and ash weight are determined and the weight of organic matter per unit volume of the water sampled is calculated (Slack *et al.* 1973).

USE IN NEW ZEALAND

The technique has not been used widely in New Zealand. Generally, sampling has consisted of paired vertical net hauls collected at fortnightly or monthly intervals from the deepest part of the lake, with additional paired hauls in semi-enclosed bays or other areas of interest. Samples were oven-dried at 50–60°C, avoiding temperatures > 60°C, and ashed at 600°C.

Methods of collection and drying need to be standardised.

ASSESSMENT

The method requires care but is straightforward and little training is needed to carry it out. Considerable experience is needed to interpret the results. In combination with other parameters, biomass may be a good index of water quality. However, there is insufficient information available to assess its potential usefulness.

The plankton biomass found in four South Island lakes (see below) suggests that it may be possible to establish guidelines related to trophic state (Stout 1970).

Lake	Trophic status	Mean dry wt ($mg.l^{-1}$)
Manapouri	Oligotrophic	0.58
Grasmere	Mesotrophic	3.28
Rotorua*	Eutrophic	13.02
Rotoiti*	Eutrophic	12.75

*Kaikoura lakes

RECOMMENDATION

The possible value of plankton biomass in assessing water quality merits further study. Standardisation of methodology is essential (e.g. size of net, mesh size, drying temperature etc.).

G. Benthic fauna (lakes)

1. Indicator organisms and diversity

The concept of benthic indicator species is similar to that of zooplankton indicator organisms (see previous section and Hart & Fuller 1974).

Potentially the most useful groups are likely to be oligochaete worms, especially tubificids (blood worms), and chironomids (midges).

OLIGOCHAETES: Increased organic pollution of waterways is usually accompanied by decreased tubificid diversity (Brinkhurst & Cook 1974). However, Aston (1973) in a review of tubificids and water quality, was able to conclude only that with increasing organic pollution tubificids became more abundant and the proportion of *Limnodrilus hoffmeisteri* increased. He found no foolproof method of using tubificids as an index of pollution.

CHIRONOMIDS: These organisms have been used as indicators of the trophic condition of lakes in the Northern Hemisphere (Brinkhurst 1974).

ASSESSMENT

Very little is known about the benthic fauna of New Zealand lakes, especially of South Island lakes. It appears unlikely that single species of benthic invertebrates will be useful as indicator organisms. The presence or absence of a species or group could be influenced by factors unrelated to the trophic state of a lake.

OLIGOCHAETES: The species appear to be cosmopolitan and species diversity appears to be low in New Zealand, although more taxonomic studies are necessary to confirm this.

CHIRONOMIDS: Low species diversity in New Zealand makes it unlikely that chironomids could be used here.

2. Biomass

The value of benthic fauna in assessing pollution and productivity, or the trophic state of lakes is discussed by Brinkhurst (1974). However, as noted above, knowledge of the species diversity, distribution, abundance and ecology of benthic macroinvertebrates in New Zealand lakes is inadequate, being limited largely to a few lakes in the Rotorua region (Forsyth 1975) and scattered lakes in the South Island (e.g. Graham 1976; Winter 1964: Stout pers. comm.).

METHODS

Most benthic sampling is done with a corer or Ekman grab. The methods are described in many handbooks e.g. Lind 1974, APHA 1975, Slack *et al.* 1973, Edmondson and Winberg 1971, Weber 1973b. The frequency of sampling, number of samples taken and location of sampling stations in a lake depend on the objectives of the study.

Graham (1976) used an Ekman grab and stratified random sampling. Forsyth (pers. comm.) sampled along a transect through the deepest part of the lake and took paired samples at 2.5m and then at 5m depth intervals in lakes up to 30m deep and at 10m intervals in deeper lakes. Sampling was done monthly over one year. Samples were washed through a series of sieves, the mesh of the smallest being 0.5mm, and the animals preserved in 2% formaldehyde.

Forsyth's experience (pers. comm.) in North Island lakes has shown that the abundance of oligochaetes does not change throughout the year, molluscs reach a peak in October-November and insects in August. He has suggested that instead of an extensive sampling programme spread over a year it may be possible to obtain an indication of water quality from a single sample of the number of individuals present at the end of winter (August) before chironomids begin their spring emergence. He suggests that the pre-emergent standing crop provides a measure of the maximum potential of a lake to support benthic fauna. As the bottom waters of a lake contain oxygen at this time of year, any confounding effects of hypolimnetic oxygen depletion which may occur in summer are minimised.

ASSESSMENT

Adequate representative sampling of benthic fauna still poses problems which are not overcome easily. In addition, the collection and sorting of samples is time-consuming. Rough sorting of samples requires little training but species identification and interpretation of results requires specialist training and experience.

In the Rotorua-Taupo region eutrophic lakes can be distinguished from oligotrophic and mesotrophic lakes on the basis of the pre-emergent standing crops of fauna, expressed numerically. Eutrophic lakes have >1000 individuals $.m^{-2}$ whereas oligotrophic and mesotrophic lakes have <1000 individuals $.m^{-2}$ (Forsyth pers. comm.). Total biomass of the pre-emergent standing crops expressed as dry weight may prove more sensitive as a gross indicator of water quality, especially if used in conjunction with other parameters.

If opportunities for sampling a lake are limited, a single measure of the benthic fauna may be of more value in assessing water quality than a single chemical analysis because the former reflects conditions prevailing in the lake over the previous year rather than conditions prevailing temporarily.

A survey of benthic fauna in a wider variety of lakes and reservoirs throughout New Zealand is necessary before the value of benthic macroinvertebrates as indicators of water quality can be assessed.

H. Benthic fauna (streams and rivers)

1. Indicator organisms and diversity

Community composition and diversity is now recognised as being much more reliable than particular indicator organisms for evaluating environmental conditions (Warren 1971). Recently, a number of formal systems for the biological assessment of pollution based on community composition have been suggested. These systems range in complexity from simple ratios of abundance of species groups to complex numerical indices of diversity based on identification to species level. Changes in community diversity resulting from changes in water quality are reflected in changes in the 'diversity index' value. The reduction of complex biological changes to simple numbers is particularly appealing to engineers and other non-biologists involved in water quality management. Consequently, the value of diversity indices in water quality guidelines is receiving considerable scrutiny.

Indices based on numbers and species of benthic animals in rivers must be interpreted with caution as several factors unrelated to water quality affect diversity. These include:

(a) substrate heterogeneity (heterogeneity increases diversity);
(b) competition and predation;
(c) method of sampling (sampler used, depth to which sampled, time spent collecting each sample);
(d) time of year (seasonal changes affect diversity);
(e) size of sample area;
(f) size of mesh through which sample is sieved;
(g) time elapsed since pollution occurred (diversity increases with time after pollution ceases):

(h) historical factors (rivers of naturally low diversity decrease the sensitivity of the method);
(i) catastrophic natural events (floods, droughts);
(j) level of taxonomic identification and discrimination in sorting samples.

Critical reviews of diversity indices and the potential usefulness of those based on stream invertebrates for water quality assessment in New Zealand have been the subject of two recent BSc Honours theses (Lear 1972; Mace 1975). Both authors concluded that most indices had serious shortcomings and/or could be misleading. Simple biological indices, or tabulated raw data in the hands of experienced biologists, are probably more valuable in detecting slight environmental changes than complex formal methods (e.g. diversity indices).

METHODS

These are described in several handbooks e.g. Slack *et al.* 1973, Edmondson & Winberg 1971, Weber 1973b, APHA 1975, Lind 1974.

Hughes (1975) compared 4 sampling methods and concluded that the Box or Surber sampler, although criticized (Kroger 1972), was the most suitable for community studies. Beak *et al.* (1973) recommended the use of artificial substrates to assess water pollution.

USE IN NEW ZEALAND

The location of sampling sites, frequency of sampling and method of sampling vary with the objectives of a study. All workers sample riffles and most sample also in the various other habitats in streams, e.g. pools, flats. Where discharges occur it is essential to sample representative habitats upstream as well as downstream of the discharges.

Surber samplers or Waters and Knapp (1961) samplers of base area 0.04–0.1 m^2 fitted with netting (mesh size 100–500 μm) are used for quantitative work; timed "kick samples" (Mills 1971) and a pond net suffice for qualitative studies. Core samplers are sometimes used on soft bottoms. Depth of penetration of samplers is governed by the design of the sampler and the type of bottom but uniformity within a study is essential.

Preferences in the use of preservatives differ. Live material may be examined but preservatives such as ethanol, isopropanol, formaldehyde and acetone, alone, or in various mixtures, may be used. Flotation, screening, staining, subsampling and the use of electric fields (Fahy 1972) may be used to aid sorting samples.

The frequency of quantitative sampling varies from fortnightly to twice per annum although most workers sample four times per annum. Replication is essential and at least two samples per site should be collected but three samples are preferred.

Most workers measure several physical and chemical parameters at each sampling site e.g. temperature, current velocity, dissolved oxygen, pH, turbidity, total solids, organic carbon in the substrate, various anions and cations including heavy metals, and forms of nitrogen and phosphorus. The extent of these measurements depends on the study objectives.

ASSESSMENT

The equipment is simple and methods are straightforward so that little technical training is needed for routine sampling and coarse sorting of macrofauna. Site selection, detailed sorting, species identification, data analysis and interpretation require an experienced biologist.

Stream biota are more sensitive than chemical tests for long term assessments of the effects of mild or subtle types of pollution. For this reason most workers consider that macroinvertebrates are of potential value in assessing water quality in streams and rivers in New Zealand. There are, however, difficulties when using macroinvertebrates. These are:

(a) Little is known about the fauna of streams and rivers in New Zealand and pollution often occurs in lowland streams where highly modified catchments confound the effects of pollution on the fauna.
(b) More taxonomic work is needed to provide keys for many groups of macroinvertebrates so that they can rapidly be identified to species.
(c) Accurate species identification takes time and expertise.
(d) Several of the popular numerical indices of pollution which have been developed overseas are unsuitable for general use in New Zealand (e.g. Lear 1972; Mace 1975). However, the Shannon-Weaver Index (Shannon & Weaver 1949) needs further evaluation.
(e) Faunal surveys have not so far revealed any species which may be considered an indicator species but more work on oligochaetes is required.
(f) The amount of time needed for sorting and identification of animals imposes practical limitations on the widespread use of any method, index, or formula which for accuracy requires many replicate samples and identification to species.

Recognising these difficulties, several workers are using simple biological indices which relate to water quality in their own region and which may be able to be used more widely in New Zealand. These indices, all of which are based on samples collected quantitatively, are:

DOMINANCE HIERARCHY: In this system, the samples of animals collected can be separated into four classes depending on the numerically dominant groups present (Gibbs pers. comm.).

A. Dominated by mayfly and caddisfly larvae
B. Dominated by chironomid larvae and snails
C. Dominated by annelids
D. Benthic fauna absent

This classification defined water quality in rivers in the Hutt Valley but has not been tested elsewhere.

BIOMASS RATIOS (Weights): In this method the weights of major groups of organisms are compared (Little pers. comm.). For example, the ratio of tubificids to all other invertebrates was found to be satisfactory for indicating gross pollution. The ratio of tubificids to chironomids, and the ratio of these two groups to the remaining invertebrates, indicate the proportion of suitable trout food and are particularly satisfactory for fisheries management purposes (Little pers. comm.).

Adequate samples of fauna from two sites in a flat and two in a riffle were collected at each station four times per annum (16 samples at each station) and preserved. At a standardised time after preservation samples were spun dry in a modified centrifuge tube for a specified time and at a specified speed, and weighed. Empirically derived correction factors were used to convert weights of preserved material to the equivalent live weights.

The ratios obtained correlate with water quality and fish production in one part of the North Island (Little pers. comm.) but they require testing elsewhere.

BIOMASS RATIO (Numerical): In this method the numbers of the mayfly *Deleatidium* spp. in quantitative samples taken in riffles are compared to the numbers of oligochaetes in the same samples (D:O). Within one river, for each eroding substrate, a change in ratio from substan-

tially over 1.0 to substantially less than 1.0 indicates significant organic pollution. The method is very sensitive to mild pollution and becomes less useful with gross organic pollution as the ratio tends to 0 (Hirsch 1958; Dacre & Scott 1973). The ratio works well in parts of Otago and Southland but it requires testing elsewhere.

These three biological indices have the advantage of being simple; minimal taxonomic identification is required which speeds up sample sorting, and statistical requirements of sampling are minimal. To date, these indices have been used only in localised areas of New Zealand. It is not known how sensitive and how widely applicable they are.

It is probably unrealistic to expect that biologists will be able to provide a single index of water quality based on benthic fauna that is capable of use throughout New Zealand and, preferably, is capable of legal interpretation. However, it may be possible to derive simple objective indices with regional relevance to water quality.

RECOMMENDATIONS

(a) More information is needed on distribution, abundance and taxonomy of benthic invertebrates in New Zealand in relation to water quality and pollution.

(b) The potential use of simple biological indices warrants further investigation. In particular, several ratios merit assessment in a wider variety of streams throughout New Zealand. These are:
   (i) the numerical ratio — *Deleatidium* spp : oligochaetes (D:O)
   (ii) the biomass ratios — tubificids:all other invertebrates; tubificids:chironomids: and these two groups:the remaining invertebrates.
   (iii) the dominance hierachy —
      A - dominated by mayfly and caddisfly larvae
      B - dominated by chironomid larvae and snails
      C - dominated by annelids
      D - benthic fauna absent.

If benthic macroinvertebrates are included in water quality guidelines for streams and rivers, consideration must be given to standardising some aspects of sampling (e.g. mesh size, type of sampler).

III Summary of main conclusions

A. Criteria for assessing water quality must be sufficiently flexible and numerous to accommodate the requirements of different water uses.

B. No *single* criterion is likely to be adequate; a combination of biological and chemical criteria is often required.

C. Biological criteria which are used overseas must be tested in New Zealand before they are adopted for use in assessing water quality in this country.

D. Assessment of the potential usefulness of biological criteria in classifying and managing inland waters in New Zealand is hampered by a dearth of information, inadequate taxonomic knowledge and insufficient comparable data.

E. There is a need for more information from a wide variety of lakes, streams and rivers throughout New Zealand before the value of some biological methods can be assessed and before tentative guideline limits can be set for values of other biological measurements.

F. The taxonomy of some groups of freshwater organisms is still incomplete and there are no suitable keys for the identification of others. Work on organisms that could be useful as indicators should be encouraged.

G. Biological methods which are to be used for the assessment and routine monitoring of water quality in New Zealand should be standardised as far as possible to facilitiate comparison of results.

H. Limnologists, or suitably qualified biologists, should be consulted prior to setting up any programme to assess or monitor water quality; they should also be consulted when results are being interpreted in relation to water quality.

I The biological methods and criteria covered in this review which appear most likely to be useful in assessing and monitoring the quality of effluents and receiving waters are:
   1. Effluents: Bioassay, using algal assay proced ures.
   2. Receiving waters:
      a. Lakes — Secchi disc transparency
         Chlorophyll *a* content
         Multiple method bioassays
      b Streams & Rivers — Simple macroinvertebrate indices.

Secchi disc transparency and chlorophyll *a* content are suitable for routine monitoring; multiple method bioassays are more suited to special studies and research.

J. Some of the methods and criteria identified in I immediately above require further testing in New Zealand conditions. They are:
   1. The American Algal Assay Procedures utilising test organisms to test the fertility or toxicity of an input of sewage, industrial or agricultural waste to a lake or river.
   2. Indices and guidelines based on a combination of parameters including Secchi disc transparency and chlorophyll *a*. An early evaluation of the index proposed by White (1976) to assess water quality in lakes and reservoirs for management purposes, is recommended.
   3. Simple indices based on dominance hierarchies or on ratios of biomass and numbers of the major groups of macroinvertebrates to assess water quality in streams and rivers.

K. If the ^{14}C method is to be used more widely for establishing water quality guidelines in New Zealand, consideration should be given to setting up a suitably equipped and staffed central laboratory for the routine preparation of radiochemicals, measurement of radioactivity of samples and analysis of results.

IV Acknowledgements

I am grateful to the biologists who replied to my questionnaire on biological methods for assessing water quality.

A more detailed report, in which comments of 22 biologists who responded to a questionnaire circulated by the author are collated, is available on request from the National Water and Soil Conservation Organisation, Ministry of Works and Development, Wellington.

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Algae in relation to water quality

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The general relationships between algae and water quality are reviewed. Various diversity systems for assessing the degree of pollution of a water body by algae are described and assessed.

The toxicity of algae, and algae as eutrophication indicators, are discussed. A brief review of algal sampling procedures in use overseas is also included.

It is recommended that future research in New Zealand should be directed towards greater knowledge of benthic algal communities, along the lines of similar schemes propounded by workers overseas. The applicability of biological systems of water quality assessment used overseas may then be assessed for New Zealand conditions.

CONTENTS

I Introduction

This review discusses a few relevant papers and books that have a direct contribution to make towards the investigation of water quality from an algal point of view. The importance of algal communities as indicators of the condition of a particular body of water is so great that it should not be ignored. Algae can be used: (a) as a measure to determine the presence or absence of organic and inorganic wastes; or (b) to measure the degree of recovery from pollution by these wastes. Eutrophication, i.e. the natural or man-made addition of plant nutrients to water, may be either beneficial or undesirable. Algae act in a beneficial way when they:

(a) oxygenate water;
(b) remove nutrients from water (as in sewage treatment ponds);
(c) provide material for fertilizers;
(d) yield useful chemicals for medicine and industry;
(e) control sodium chloride concentration and pH;
(f) are used for bioassays;
(g) grow on slow sand filters to improve the final quality of water.

Algae act in a harmful way when they:

(a) accumulate in large masses to form water blooms, especially in calm sunny weather;

Received 21 August 1978, revision received 19 December 1978

Standing Biological Working Party of the Water Resources Council 1979: A review of some biological methods for the assessment of water quality with special reference to New Zealand. *Water and Soil Technical Publication 18* : 21-30

(b) cause gastric, respiratory and skin disturbance in man;
(c) produce substances which are lethal to fish, birds and domestic animals;
(d) generally reduce recreational and amenity uses;
(e) clog up filters in water supplies;
(f) give water an unpleasant taste and odour;
(g) corrode concrete and metal (Palmer 1962; Hart 1974; G. Wood 1975).

An invaluable aid to all methods used in assessing water quality is the Biological Field and Laboratory Methods Manual (Weber (Ed.) 1973). This covers among other topics, standard techniques used in applied research on productivity of plankton and periphyton. It includes a detailed account of methods used in algal assay, and suggests several methods of conducting bioassays given in APHA (1971). The chief difficulty in using algae to assess water quality lies in the lack of trained personnel with sufficient knowledge of taxonomy and ecology in the different groups.

II Algae as pollution indicators

According to Hynes (1964), biological assessment of pollution has many advantages over non-biological, and it may indicate pollution when other methods fail to do so. Species diversity seems to be the most reliable and generally applicable means of assessing the biological effects of pollution (Mitchell 1972). Change in dominance of benthic diatoms has been used by Cholnoky (1968) as a reliable measure.

A Systems of assessment of pollution

1 Patrick's System

Patrick (1949, 1951), using numbers of species in seven different taxonomic groups of plants and animals, classified watercourses as:

1. Healthy — dominants are diatoms and green algae. All recorded species of insects, crustacea and fish are above 50% of the biota.
2. Semi-healthy — an irregular pattern with more individuals of a given species.
3. Polluted — balance upset, favourable conditions for blue-green algae, green algae, and certain rotifers. Reduction in insect and fish species.
4. Very polluted — conditions toxic to plant and animal life.
5. Septic.

Patrick selected the average number of species present in each taxonomic group of organisms occurring in a normal healthy stream as the number equalling 100 per cent. The number of species in each taxonomic group found at nine separate stations was then evaluated, and expressed as a percentage of its occurrence in a normal stream. The general effect of pollution seems to be a reduction in numbers of species. In a healthy stream many species should be present, but none should predominate. In a polluted stream the reverse situation often prevails, with dominance of one or only a few species. In such a stream there is a marked reduction in numbers of species of insects and fish. Patrick (1977) has elaborated on this system in a lengthy discussion on the effects of pollution on diatoms; but she cautions that diatom communities change regardless of pollution effects, and that it is important to recognise changes from sensitive to tolerant species.

ASSESSMENT

Drawbacks to the use of this system are that it is not precise, and there is a significant time lag between the onset of toxic conditions and the laboratory determination of the response of the microbial community (Cairns & Lanza 1972). Also it has been stressed by Fjerdingstad (1971) that different species in one genus may have different pollution ratings.

2. Kolkwitz and Marsson's Saprobic System

The method of Kolkwitz and Marsson (1908, 1909) is based on division of a sewage-loaded stream into zones of pollution:

(a) polysaprobic	gross pollution
(b) alpha-mesosaprobic	increased pollution
(c) beta-mesosaprobic	slight pollution
(d) oligosaprobic	purification

Each zone is characteristised by indicator species (*Leitformen*). The system was fully reviewed by Leibmann (1962), and Schoeman (1973) has detailed its deficiencies – chiefly that natural conditions are not catered for and there is no reduction phase as defined by Kolkwitz and Marsson. Bick (1963) has reviewed the methods used in central Europe for biological estimation of water pollution levels. He reported that three methods have been used:

1. Samples are taken, all animal and plant species listed, and water quality is assessed on the basis of either occurrence and frequency of indicator organisms or composition of the community; i.e. direct ecological methods.
2. Estimation of bio-activity is made by indirect physiological methods and by counts of bacterial numbers.
3. A test organism may be inoculated into the water, and its growth pattern used as an index of water quality.

3. Pantle and Buck's System

According to Bick (1963) the most promising system devised up to that date was that of Pantle and Buck (1955). These authors calculated a Saprobity Index with a high degree of statistical accuracy as the indicator value of each species in Liebmann's (1962) list of indicator organisms. They allotted a value to each species according to its frequency, and its position in the Saprobity system of Kolkwitz and Marsson (1908, 1909).

Saprobic zone rating:

oligosaprobic indicator organism	S = 1
beta-mesosaprobic indicator organism	S = 2
alpha-mesosaprobic indicator organism	S = 3
polysaprobic indicator organism	S = 4

They characterize the species found as follows:

(a) found only by chance	h = 1
(b) occurring frequently	h = 3
(c) occurring in abundance	h = 5

The Saprobity Index may be calculated using the equation

$$S = \frac{\Sigma rh}{\Sigma h}$$

where S = Saprobity Index, r = saprobic zone rating, h = frequency of occurrence of single species.

Saprobity Index (calculated for each locality)	*Degree of pollution*	
1.0–1.5	very slight	(oligosaprobic)
1.5–2.5	moderate	(beta-mesosaprobic)
2.5–3.5	heavy	(alpha-mesosaprobic)
3.5–4.0	very heavy	(polysaprobic)

ASSESSMENT

Although Pantle and Buck's (1955) scheme was applied to flowing waters, Schrader (1959) used it to assess pollution levels in reservoirs. This is the standard method in East Germany.

Other workers have added refinements to the system. The saprobity system has been challenged by Hynes (1960) and others on the grounds that it cannot be applied successfully to waters other than slow and evenly flowing rivers, i.e. it is not readily workable in turbulent or standing waters and those which have been dammed up or made into canals.

4. Fjerdingstad's System

Using different species of micro-organisms and communities common in Denmark, Fjerdingstad (1950, 1964, 1965, 1971) has recognised:

A. Autecological groups

These were further divided according to the species they contained:

	Number of species
1. Saprobiontic (coenobiontic) species — occurring only in the most heavily polluted waters (large numbers of individuals).	67
2. Saprophilous (coenophilous) species — occurring in polluted water, but with a wide range of tolerance.	66
3. Saproxenous (coenoxenous) species — occurring in non-polluted water, but tolerant of pollution.	34
4. Saprophobous species — those unable to exist in polluted water.	17

B. Synecological communities

The communities he considered, distributed in nine zones in order of decreasing pollution, were typified by the following genera and species:*

Zone	*Communities*
1. Coprozoic zone	*Bodo* (Chrysophyta - also classed as Protozoa - Curds 1975) Other genera of bacteria
2. Polysaprobic zone	*Euglena* (Euglenophyta - also classed as Protozoa - Curds 1975) *Chlorobacterium* (phototrophic bacteria) *Rhodopseudomonas* (*Rhodobacterium* - phototrophic bacteria) *Thiobacterium* (sulphur bacteria)
3. Polysaprobic zone	*Beggiatoa* (gliding bacteria) *Thiothrix* (gliding bacteria) *Euglena* (Euglenophyta)
4. Polysaprobic zone	*Oscillatoria* (Cyanophyta - also classed as Cyanobacteria - Stanier *in* Bergey's Manual, 1974) *Sphaerotilus* (Mycophyta)
5. Mesosaprobic zone	*Ulothrix zonata* (Chlorophyta) *Stigeoclonium tenue* (Chlorophyta) *Oscillatoria* (Cyanophyta)
6. Mesosaprobic zone	*Cladophora fracta* (Chlorophyta) *Phormidium* (Cyanophyta)
7. Mesosaprobic zone	*Batrachospermum* (Rhodophyta) *Lemanea* (Rhodophyta) *Cladophora glomerata* (Chlorophyta) *Ulothrix zonata* (Chlorophyta)
8. Oligosaprobic zone	*Draparnaldia* (Chlorophyta) *Vaucheria* (Chlorophyta) *Meridion* (Chrysophyta)
9. Katharobic zone	*Chlorotylium* (Chlorophyta) *Draparnaldia* (Chlorophyta) *Hildenbrandtia* (Rhodophyta - encrusting red algae) *Chamaesiphon* (Cyanophyta) *Calothrix* (Cyanophyta)

* Algal classification after Round (1965)
Bacterial classification as in Bergey's Manual (Buchanan & Gibbons (Eds.) 1974).

ASSESSMENT

Fjerdingstad (1950, 1964, 1965, 1971) did not favour mathematical methods, but found it necessary to make subjective estimations of frequency of species. This system is readily applied in that it relies upon relatively few indicator organisms.

5. Schoeman's System

Schoeman (1973) used diatoms as a measure of water quality in the upper Orange River Catchment of Lesotho, South Africa. Permanent slides were made of benthic diatoms, and up to 400 individuals of each species present were identified and counted on each slide. The percentage frequency of each species in the diatom association was calculated and the pollution tolerance of each species was based on the concentration of nitrogenous compounds in the water. Schoeman (1973) suggested that the nitrogen-heterotrophic species, i.e. species which use organic nitrogenous compounds, are partly responsible for self-purification of polluted waters as they de-aminate amino acids (i.e. convert nitrogenous compounds to ammonia). Algae may break down organic nitrogenous compounds in excess of their needs and are thus able to take part in the purification of the water

ASSESSMENT

Although of great importance, the usefulness of this system is restricted by the general lack of knowledge of the diatom species, and the time involved in counting each slide. Electron microscopy is necessary in many cases for a correct taxonomic diagnosis to be made. Lange-Bertalot and Bonik (1976) have been enabled by this means to distinguish between species of *Navicula* with different tolerances to organic pollution in the River Main near Frankfurt.

6. Palmer's System

To cater for the needs of practical workers involved with algae in the treatment of water supplies, Palmer (1962) in U.S.A. has produced an ecological classification of algae, as follows:

1. Taste and odour algae (e.g. sweet or bitter, flower-like, fishy, earthy) - 39 species.
2. Filter-clogging algae (mainly diatoms) - 43 species.
3. Polluted water algae - 50 species.
4. Clean water algae - 46 species.
5. Algae growing on reservoir walls.
6. Plankton and other surface-water algae.

Later, Palmer (1969) assessed and published works of 165 authors and drew up a list of 50 algae in order of their importance as pollution indicators.

ASSESSMENT

The successful application of this system depends on accurate identification of the species. Palmer makes it obvious that identification to genus alone is not sufficient, since different species within the same genus frequently have different tolerances to pollution. In Lake Rotorua, *Melosira granulata*, the dominant diatom, is 38th on Palmer's list of 50 (Cassie 1974).

7. Lowe's System

In a report on environmental requirements and pollution tolerance of freshwater diatoms, Lowe (1974) has assembled data from 48 published papers, covering 300 diatom species. Their reactions to pH, nutrients, salinity, organic pollution and habitat requirements, are recorded and their seasonal distribution and temperature requirements are tabulated. A concensus of opinions was taken as the standard for each taxon. The scheme is applicable to diatoms from both phytoplankton and periphyton.

ASSESSMENT

As with Schoeman's system, a profound knowledge of the 300 diatom taxa referred to, as well as that of their ecological and physiological parameters, is necessary for its adequate use.

B Toxic algae

A number of algae, mainly blue-green species (regarded by some workers as bacteria), known to be toxic to animals and humans, have been reported from time to time in New Zealand lakes and waterways (Flint 1966; Connor 1977; Lam 1977). These include (Fig.1):

Anabaena circinalis (blue-green)
Anabaena flos-aquae (blue-green)
Microcystis aeruginosa (= Anacystis cyanea) (blue-green)
Aphanizomenon flos-aquae (blue-green)
Coelosphaerium kuetzingianum (blue-green)
Nodularia spumigena (blue-green)
Prototheca wickerhamii (colourless)

When such algae occur in rivers, lakes and ponds in bloom proportions, usually in summer and autumn, a thick scum may form on the surface. Nearly all species possess potent endotoxins with effects ranging from acute discomfort to death if disintegrating cells are ingested in large quantities by animals (Gorham *et al.* 1964; Schwimmer & Schwimmer 1964, 1968).

Anabaena circinalis, widespread in the North Island, may release a toxin after ultrasonic treatment. When injected intraperitoneally into mice, this toxin will kill them in 5–30 minutes (May & McBarron 1973). However, the species is not always harmful to animals when it blooms.

Anabaena flos-aquae, widespread in both islands, has not yet been recorded as a potent producer of toxin in New Zealand. Upon disintegration this organism has been reported to liberate a toxic alkaloid, which may cause the death of animals. It is known to have killed mice in 1–2 minutes (Gentile 1971); however the toxin is not present in every strain.

Microcystis aeruginosa (= Anacystis cyanea), the commonest toxin-forming blue-green alga in the North Island, is reported to have caused the death of lambs and cattle. Both toxic and non-toxic strains possess a cyclic polypeptide (Gorham *et al.* 1964). Lam (1977) has calculated that 544 mg of *Microcystis* would be necessary to kill a man weighing 68 kg, but he would have to drink 2.7 l of water, containing 1×10^7 cells/ml of a toxic strain of *Microcystis*. Thus death of humans from *M. aeruginosa* toxin is very unlikely. However, sheep, cattle and pigs may die if they have ingested a large quantity of the toxic colonies. Diarrhoea may be caused in human beings if they drink water contaminated with large quantities of *Microcystis* sp. (Aziz 1974).

Aphanizomenon flos-aquae, recorded from a few lakes in both islands, has also been reported to possess toxic and non-toxic strains, but the toxic strain is less poisonous than either *Anabaena circinalis* or the toxic strain of *Microcystis aeruginosa* (Connor 1977). Barica (1975) has recently reported fish kills in Canadian prairie ponds due to *A. flos-aquae.*

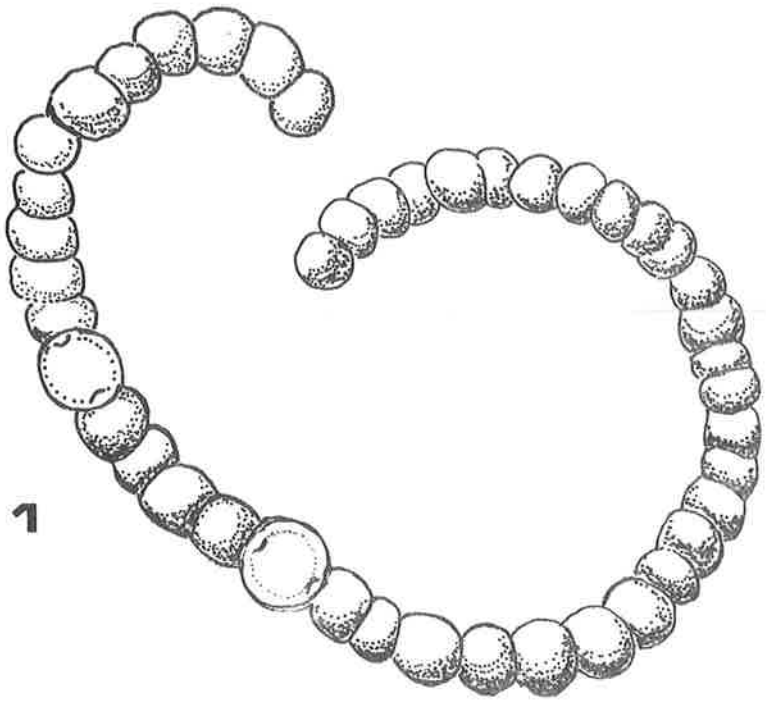
Coelosphaerium kuetzingianum, which sometimes blooms in eutrophic lakes of the North and South Islands, synthesizes a nerve- and muscle-blocking agent (Gentile 1971).

Nodularia spumigena, reported from dune and brackish lakes in both islands, is said to have killed steers, dogs and sheep (Flint 1966; Connor 1977). In many cases bacteria associated with the blue-green algae have been shown to produce toxins which aid in causing slow death of the animals which have ingested the algae (Flint 1966).

Prototheca wickerhamii, a saprophytic colourless unicellular species resembling *Chlorella* and assigned to the algae on account of its metabolism, has been isolated in New Zealand from a human ear, and from a human bloodstream (Joshi *et al.* 1975). This disease is known as Protothecosis and causes skin lesions which are extremely hard to eradicate (Tindall & Fetter 1971).

Figure 1. Toxic algae recorded from New Zealand. (2, 5, 6 after Prescott 1962; 8 after Flint 1975)

1. *Anabaena circinalis* Rabenhorst (× 1500)
2. *Anabaena flos-aquae* (Lyng.) de Brébisson (× 1000)
3,4. *Microcystis aeruginosa* Kuetzing (× 850)
5. *Coelosphaerium kuetzingianum* Naegeli (× 1000)
6. *Aphanizomenon flos-aquae* (L.) Ralfs (× 750)
7. *Prototheca wickerhamii* Tubaki and Soneda (× 1000)
8. *Nodularia spumigena* Mertens (× 1250)



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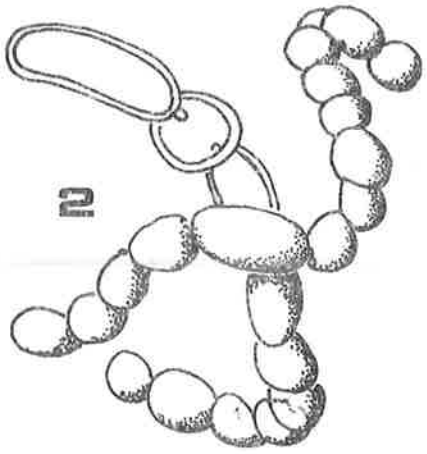

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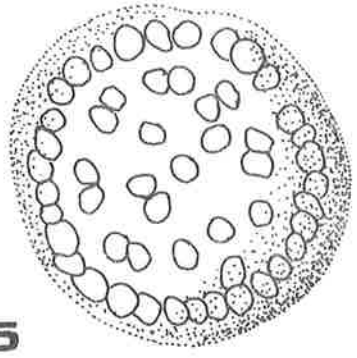

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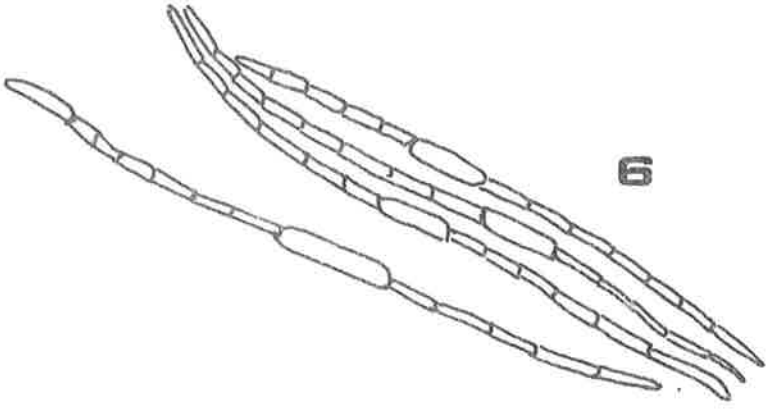

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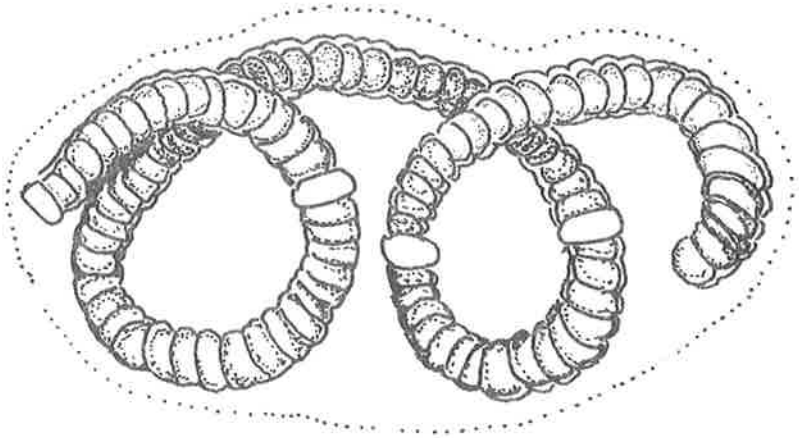


8

III Algae as eutrophication indicators

The standing crop (biomass), or the amount of organisms present at a given instant of time in unit volume or area, is greater in eutrophic than in oligotrophic waters. Algae are a major constituent of standing crop in most waters.

Phytoplankton may be estimated by numbers, volumes and pigments (see paper by Dr Carolyn Burns for greater detail). Counting procedures are involved and time-consuming, and give no relation to biomass according to cell volume. However, much useful information can be gained from cell counts, especially in assessing dominance. In general, the greater the cell numbers per unit volume, the more eutrophic the water (G. Wood 1975).

Incipient eutrophication is indicated by a quantitative increase in biomass of macrophytes and periphytic algae inshore, or in that of planktonic algae (Vollenweider 1968). There is usually a decrease in oligotrophic species, an increase in single species dominance, accompanied by a decline in oxygen in the hypolimnion, a change in water colour and a build-up of the nutrient level in the sediments. According to Vollenweider (1968), abundance of the diatom *Fragilaria crotonensis* marks the onset of eutrophication.

A Phytoplankton communities

It is desirable to define the phytoplankton communities in a given body of water. Hutchinson (1967) has described 13 different types:

	Phytoplankton association	Dominant genera
1.	Oligotrophic desmid plankton	*Staurastrum, Staurodesmus*
2	Oligotrophic diatom plankton	*Cyclotella, Melosira, Fragilaria, Asterionella, Rhizosolenia*
3	Botryococcus plankton	*Botryococcus, Dinobryon, Peridinium*
4.	Chrysophycean plankton	*Dinobryon, Tabellaria*
5.	Oligotrophic chlorococcal plankton	*Oocystis*
6.	Oligotrophic dinoflagellate plankton	*Peridinium, Ceratium*
7.	Mesotrophic or eutrophic dinoflagellate plankton	*Peridinium, Ceratium, Peridiniopsis (= Glenodinium)*
8.	Eutrophic diatom plankton	*Asterionella, Fragilaria, Synedra, Stephanodiscus, Melosira*
9.	Mesotrophic or eutrophic desmid plankton	*Staurastrum, Cosmarium*
10.	Eutrophic chlorococcal plankton	*Pediastrum, Scenedesmus*
11.	Myxophycean plankton	*Microcystis, Aphanizomenon, Anabaena, Oscillatoria, Nodularia, Spirulina*
12.	Euglenophyte plankton (polluted water)	*Euglena*
13.	Bacterial plankton	coloured sulphur bacteria

B Phytoplankton indices of diversity

The most suitable index as a guide to eutrophication appears to be the Compound Index of Nygaard (1949), who defined the Compound Index as the number of species of Chlorococcales, Myxophyceae, centric diatoms and some species of Euglenales divided by the number of species of desmids.

< 1 = oligotrophic
1 = mesotrophic to eutrophic
> 1 = eutrophic
> 5 = very eutrophic to polluted

Bayly and Williams (1973) have calculated indices for Lake Sarah, South Island, New Zealand from the data of Flint (1938); and for Lake Rotorua, North Island, New Zealand, from Cassie (1969). They found the index for Lake Sarah to be 0.91 and that for Lake Rotorua to be 2.23. Cassie (1978) has worked out further indices for several North Island lakes. Patrick (1977) has pointed out that this type of formula can only be applied in habitats where desmids are common, and that dominance is not considered in its application.

Brook (1965) found that while about 60% of the commoner desmids in British lakes occurred in oligotrophic habitats, up to 25% were usually in eutrophic ones. Therefore, reliable results from this method depend on a precise knowledge of the nutritional requirements of each species as well as on accuracy of identification.

Other indices proposed by Nygaard include Myxophyceae/Desmidiacae, Chlorococcales/Desmidiacae, Centrales/Pennales (centric/pennate diatoms), and Euglenineae/Myxophyceae + Chlorococcales.

Cholnoky (1968) used changes in dominant species of diatoms to measure changes in amount of pollution, however it is difficult to distinguish this effect from changes which occur due to natural causes of succession.

Mathematical indices of diversity are not discussed in this review. The reader is referred to Patrick (1977) for a full discussion of this topic with reference to phytoplankton in general; and to Archibald (1972) for a discussion on the various methods as they have been applied to diatom communities.

C Periphyton communities

Sládečková (1962) has described methods for investigation of the periphyton community. A more recent approach is outlined by Weber (1970), who maintains that the general pattern of water quality can be established on the basis of diatom populations alone, without other knowledge of environmental conditions. Density of diatom cells on glass slides was compared with density of diatoms in the natural plankton community. *Gomphonema parvulum* and *Nitzschia palea* were the most abundant diatoms in the vicinity of pollution sources, whereas *Cocconeis placentula* dominated above the outfalls and in the oligosaprobic zone downstream.

Northcote *et al.* (1975) sampled benthic algal communities on floating, anchored wooden substrates in the lower Fraser River, Canada, at four different seasons. Wooden surfaces were selected as permitting more valid comparisons from station to station. Areas were selected with representative growth of attached algae, and samples were collected with a tube 27.8–29mm in diameter. Two algal samples were taken from a single substrate and combined in one sampling bottle to reduce variability in the quantity of biomass. Diatoms have also been used by Schoeman (1976) in South Africa to indicate water quality.

D Phytoplankton Volumes

Willén (1976) gave detailed instructions for determining size and volume of individual phytoplankton species belonging to different taxonomic groups of algae.

IV Sampling methods

Examination of living specimens is desirable if accurate identification of most algae to species level is to be made. In many groups, including the majority of flagellated forms, cell characteristics as seen under the light microscope will be drastically altered by the addition of any preservative. However, if cell counts were required, preservation is necessary in order to prevent change in abundance of organisms due to cell division, bacterial decomposition and grazing by animals. Long-term preservation is necessary for species records. Both fresh and preserved samples should therefore be collected at the same site. The size of each sample will depend on the density of micro-algae.

A Collection

1. Phytoplankton

(a) Net samples

(i) *Horizontal net hauls* Concentrated samples of the larger species of phytoplankton may be obtained by towing through the water a conical-shaped net of fine mesh (no. 20) bolting silk or monofilament nylon. A metal hoop at the wider end is attached to the tow rope at three points by a bridle. A metal, plastic or glass container (the "bucket") closes the narrow end (Boney 1975). The net can be towed by a slowly moving boat, or cast from the shore.

(ii) *Vertical net hauls* A weight can be attached to the rope holding the net to induce it to sink vertically to a known depth. Some nets have a throttling device round a canvas sleeve at the wider end (Boney 1975. Fig. 6-1, a-d).

ASSESSMENT

Net samples cannot be used for quantitative studies, since volumes of water filtered are variable, and only a part of the phytoplankton population is collected. Smaller forms (nanoplankton) will pass through the meshes.

(b) Tube and water bottle samples

(i) *Hose pipe* A portion of garden hose up to 5 m long and weighted at one end may be lowered into the water. When filled, the upper end is closed at the surface and the lower (weighted) end is hauled in by a cord. The water sample, which is of known volume, can then be transferred to a container of similar volume (Boney 1975).

ASSESSMENT

Useful in calm inshore parts of lakes, especially over weed beds.

(ii) *Water bottles* The type of sampler used will depend on the habitat. Kemmerer, Juday and Van Dorn samplers (Weber 1973) all consist of cylinders stoppered at both ends, to allow free passage of water while lowering. A messenger is dropped down the cable to close the cylinder ends at the required depth. Non-metallic samplers are essential for algal assays or primary productivity surveys. Nansen reversing bottles are often used for sampling in deep waters. The volume of each sample will depend on the type of analyses to be made. Each sample should be labelled with date, time of collection, and weather conditions.

2. Periphyton

Sampling techniques for periphyton have been described by a number of workers, including Margalef (1949), Blum (1960), Sládečková (1962), Wetzel and Westlake (1969), Weber (1970), Jones (1974) and Northcote *et al.* (1975).

(a) Scraping and peeling methods (Jones 1974; Northcote *et al.* 1975)

Algae are either scraped from surfaces, or peeled off in a covering synthetic film, or grown artificially on glass microscope slides suspended in the water.

(b) Styrofoam float (Weber 1970)

A styrofoam float (30.5 × 30.5 × 5.1 cm) supports a central plexiglass cradle which holds several 2.5 × 7.6 cm glass microscope slides. Two slides are exposed at each station for two weeks. Slides are then removed from the sampler, placed in a small screw-cap bottle with about 70 ml of 5% formalin and transported to the laboratory.

(c) Nylon brush periphyton sampler (Stockner & Armstrong 1971)

A nylon toothbrush is glued to the end of a 100 ml plastic syringe. This is placed against a rock and the piston of the syringe rotated, thus dislodging epilithic algae. Algae are drawn up into the syringe.

ASSESSMENT

Scraping causes great variability in quantity of sample, there is frequently considerable loss in cell numbers, and therefore populations tend to be under-estimated. If slides are used, the settling flora may well be different from that occurring naturally in the same area. The nylon brush method is good for sampling epilithic algae on individual rocks, but does not cater for variability between rocks. An average of results from three rocks is desirable (Ennis 1975).

B Preservation

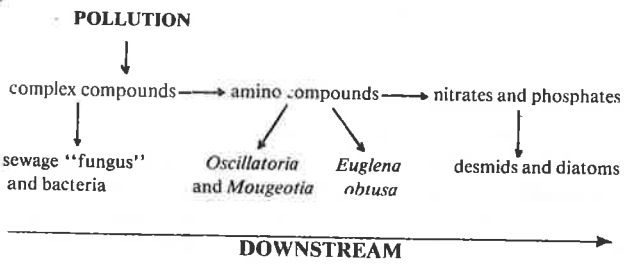
The most suitable preservatives for freshwater algae are formalin (3-5%) with a neutralizing agent for diatoms (R.D. Wood 1975), dilute Lugol's iodine to give the colour of pale tea (Boney 1975), or merthiolate, for staining cell contents and removing gas vesicles in blue-green algae (Weber 1970, 1973). Lugol's iodine has the disadvantage of obscuring the shape and form of chloroplasts. It is also only a short-term preservative, lasting up to about a year. A good long-term preservative is formalin-acetic-alcohol, with added glycerine to preserve filamentous forms. Glycerine is a hindrance, however, in samples containing diatoms which are to be used for scanning electron microscopy. These are best preserved in 5% neutral formalin.

V Discussion and conclusions

Up to the present time phytoplankton algae in New Zealand have received most attention as indicators of water quality chiefly with respect to the degree of eutrophication of lakes (Cassie 1969, 1974, 1978; Flint 1975). Flint (1975) has classified a number of New Zealand lakes into three categories: eutrophic, mesotrophic and oligotrophic, on the basis of their phytoplankton populations from her own identifications. Cassie (1974) has tried to relate dominant species of phytoplankton in Lakes Rotorua and Rotoiti to Palmer's system, and other lakes (Rotoma, Rotoehu, Waikaremoana) to Nygaard's compound index (Cassie 1978). A taxonomic account of algae in Auckland Regional Auth-

ority sewage treatment plant oxidation ponds has been prepared by Haughey (1968, 1969). Lam (1977) has surveyed the blue-green algae in the Waikato River and this is the first study of New Zealand freshwater phytoplankton algae where field observations have been backed up by culture experiments.

By contrast, benthic algae have received little attention to date. One river survey by Cameron (1970) on the effects of organic pollution on the biota of the Heathcote River, Christchurch, has related different algal genera to stages in break-down of organic matter. Results were summarized in the following diagram (after Cameron 1970):



Sewage "fungus" (*Sphaerotilus* sp.) and other bacteria thrived on complex compounds, whereas blue-green algae and flagellates (not identified) flourished on partly broken-down amino compounds, with increasing distance from the source of pollution. Diatoms and desmids (not identified) were shown to be dominant where organic waste was converted into simple nitrates and phosphates.

It is obvious from the work of Schoeman and Archibald (1976) in South Africa and that of Lange-Bertalot and Bonik (1976) in Germany that a knowledge of the taxonomy of centric and pennate diatoms in both light and electron microscopes (scanning and transmission) is imperative for a proper assessment of the components of diatom populations, before these can be accurately related to water quality. In many cases, the differences between species do not show up under the light microscope, despite the fact that each species has its own value as an indicator of water quality. Lowe's (1974) assessment of three hundred diatom species in relation to nine different parameters is a very useful guide to any worker in this field — provided he or she is familiar with the species concerned.

Future research in New Zealand should be directed towards a greater knowledge of benthic algal communities, along the lines of schemes already propounded by workers overseas; but any study should be preceded or accompanied by some training in the challenging discipline of algal taxonomy

VI Glossary

alpha-mesosaprobic describing a moderate-heavily polluted zone

Bacillariophyceae diatoms

benthic attached to a substrate in an aquatic environment

beta-mesosaprobic describing a mild-moderately polluted zone

bioassay quantitative estimation of biologically active substances by the amount of their actions in standardized conditions on living organisms

biomass the total weight of living organisms per unit area of space

blue-green algae (or blue-green bacteria) single cells, colonies or filaments, mainly blue-green in colour, without a nucleus and with a bacteria-type cell organisation (see Myxophyceae)

centric disc-shaped (of diatoms)

Chlorococcales unicellular or colonial microscopic green algae without flagella

Chlorophyceae unicellular, colonial and filamentous green algae with cells possessing one or more nuclei and chloroplasts

Chrysophyceae unicellular or colonial microscopic yellow-brown algae with one flagellum

coenobiontic = saprobiontic

coenophilous = saprophilous

coenoxenous = saproxenous

coprozoic describing the most polluted zone

desmids microscopic green algae with cells in two halves connected by an isthmus

diatoms unicellular yellow-brown algae (Bacillariophyceae) with porous walls of silica in two box-like halves

dinoflagellates unicellular brownish algae with a planktonic and benthic stage, sometimes with a plated shell (theca) and two flagella in grooves at right angles to each other. (Dinophyceae)

Dinophyceae dinoflagellates (q.v.)

Euglenophyceae unicellular, often metabolic, green algae (i.e. changing shape), which ingest food and have reserves of paramylon

eutrophic rich in mineral nutrients and organic matter

eutrophication increase in available mineral nutrients and organic matter

green algae unicellular, colonial, filamentous or parenchymatous primitive aquatic plants with a nucleus and green chloroplasts

heterotrophic requiring organic material from the environment

hypolimnion the lowest stratum in a stratified lake

katharobic describing the cleanest zone

Leitformen indicator species

mesosaprobic describing a partly polluted zone

Myxophyceae blue-green algae (q.v.)

oligosaprobic describing a clean-water zone

oligotrophic poor in mineral nutrients and organic matter

pennate box-shaped, with or without a slit or raphe; of diatoms

periphyton algae living on or close to a substrate which is mostly composed of larger aquatic plants

phytoplankton floating microscopic algae, mostly carried passively by water currents

polysaprobic describing a grossly polluted zone

red algae unicellular, filamentous or parenchymatous aquatic plants in which the major pigments are red and blue-green

Rhodophyceae red algae (q.v.)

rotifers wheel animalcules; tiny metazoan animals which swim and feed with a ciliated band or "wheel"

saprobiontic describing organisms confined to heavily polluted waters

saprobity degree of pollution

saprophilous describing organisms which occur in polluted water, but with a wide range of tolerance

saproxenous describing organisms which prefer non-polluted water but which will tolerate pollution

sessile without a stalk

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Submerged macrophytes as biological indicators of water quality

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The potential and theoretical limitations of using submerged macrophytes as biological indicators of water quality in New Zealand are discussed.

Factors which influence the presence of rooted macrophytes in a particular habitat and factors to be considered when assessing the vigour of submerged macrophytes are suggested.

It is concluded that the response of submerged macrophytes in recognisable water types is of particular moment to water plant management, but should also provide a useful complement to a systems analysis of water quality.

CONTENTS

I Introduction

Submerged plants have two distinct advantages as indicators of water quality. Firstly, they are growth-limited by available light, and are therefore sensitive to water transparency and colour, factors which are of prime importance when considering aesthetic water quality. Secondly, eutrophication results in an increase of available nutrients for plant growth, and the most direct and meaningful measure of eutrophication is in-water plant production.

Macrophytes have two advantages over microphytes as biological indicators:

(a) they are readily identified in the field;
(b) rooted submerged macrophytes have a fixed depth range, hence their compensation depth can be determined directly in the field.

The following discussion attempts to summarize the potential and the theoretical limitations of using submerged macrophytes as biological indicators of water quality in New Zealand.

II Factors influencing the presence and vigour of submerged rooted macrophytes

The factors which influence or limit the presence of rooted macrophytes in a particular submerged habitat include:

(a) shoreline physiography, e.g., cliff face or gentle slope;
(b) substrate, e.g., sand, sandy-silt or silt;
(c) exposure to wave action, e.g., shores exposed to or protected from prevailing winds acting over a significant fetch;

Received 10 May 1977

Standing Biological Working Party of the Water Resources Council 1979: A review of some biological methods for the assessment of water quality with special reference to New Zealand. *Water and Soil Technical Publication 18* : 31–4

(d) velocity of water currents, e.g., adaptation to dislodgement forces;
(e) water level fluctuations;
(f) water transparency and colour;
(g) climate;
(h) water temperature, e.g., seasonal variations and/or thermal inflows;
(i) water depth, e.g., available light, pressure, and the depth of the summer thermocline;
(j) altitude;
(k) nutrient availability from the water and/or sediments;
(l) availability of species capable of colonising the habitat;
(m) inter-life-form competition;
(n) presence of toxic substances;
(o) weed control measures.

The net effects of shoreline physiography, exposure, current velocity and water level fluctuations, will determine the type of substrata present in a given habitat. Submerged rooted plants are generally restricted to those sites where sediment particle size is in the sand to mud classes. This excludes such localities as high current habitats, vertical cliff faces, and exposed gravel beaches. Once an area is colonised by vegetation fine particles may accumulate, which in turn encourage more biological activity. Thus primary colonisers such as *Ranunculus fluitans, Myriophyllum elatinoides,* and *Lilaeopsis lacustris* can pave the way for the establishment of other species which thrive best on sandy-silt or silty substrata.

Aquatic plants vary in their light, nutrient, and climate requirements as do terrestrial species, and, provided physical factors do not exclude submerged macrophytes, the floristics of the submerged vegetation will reflect the growth requirements of the species present.

Submerged macrophytes are an important life form only in oligotrophic to mildly eutrophic waters. Planktonic algae and/or free floating macrophytes are more successful competitors in mildly eutrophic to hyper-eutrophic habitats.

The presence of species able to colonise a particular habitat is of particular moment in New Zealand. There are now some 30 species of introduced submerged macrophytes in this country which have yet to exploit their potential geographic range. It is necessary therefore, not only to score the presence and absence of indicator species, but to examine the vigour of eco-dominants at a particular locality, because a species may persist in a less than optimal environment until it is subjected to competitive pressure from other species which are more suited to that particular environment.

III Factors to be considered when assessing the vigour of submerged macrophytes

There are four factors to be considered when assessing the vigour of a submerged macrophyte. These are:

(a) depth range;
(b) production rate;
(c) growth form and canopy cover;
(d) tissue-nutrient content.

There are two groups of submerged macrophytes in New Zealand with regard to depth ranges. The first group includes widespread species such as *Elodea canadensis* and *Nitella hookeri.* These species have a depth range which is dependent upon the transmission of photosynthetically useful light through the water column. The second group, which includes *Lagarosiphon major* and *Potamogeton ochreatus*, have a constant depth limit in a wide range of oligotrophic-mesotrophic waters. It is only in mildly eutrophic and turbid waters that this depth range is reduced. Species in the first group are indicative of available light in relation to water depth where the same species have reached equilibrium in different habitats.

The most informative data on species vigour come from a comparison of the productivity of a given species, at a given depth and season, in different habitats. Techniques for assessing primary production in aquatic environments have been reviewed in I.B.P. Handbook No. 12 (1974).

Many submerged aquatic plants have a constant growth form which may change depending on environmental factors such as nutrient availability and light, depth or climate. The percentage cover of species such as *Elodea canadensis* is related to fertility levels in the water or sediments.

Luxury uptake of elements such as nitrogen and phosphorus in submerged macrophytes has been proposed as an indication of available nutrient levels in fresh waters (see Gerloff & Krombholz 1966).

IV Discussion

Coffey (1974, 1975) has discussed the performance of submerged macrophytes in relation to water quality in the Waikato River and its associated lakes. The scheme he proposed forms the basis of predictive waterplant modelling studies being conducted by the Ministry of Agriculture and Fisheries for New Zealand lakes at present.

It remains to integrate these data with other criteria of water quality used in New Zealand.

V Conclusions

Water quality is a complex concept and the standards or criteria on which it is assessed should be related to water use requirements. Biological indicators will not be useful in all instances. Chemical/bacteriological assays are, for example, a more direct and meaningful measure of water quality as it relates to treatment required for domestic supplies.

The growth of a particular macrophyte will reflect its total environmental requirements. The absence or poor growth of a test organism can be due to such a wide range of factors that it is only a presence record or a positive response which is significant. A tentative rating of performance of common New Zealand submerged macrophytes in relation to water quality is presented in Tables 1 and 2.

Table 1 Performance of selected submerged macrophytes in relation to water quality

Species	Water quality					
	OLIGOTROPHIC		MESOTROPHIC		EUTROPHIC	
	flowing	static	flowing	static	flowing	static
Callitriche stagnalis Scop	2	1	3	1	1	1
Ceratophyllum demersum L.	–	–	3	2	3	3
Chara corallina Klein ex Willd	1	2	1	3	–	–
Egeria densa Planch	–	–	–	–	3	3
Elodea canadensis Michx	1	2	1	3	1	1
Isoetes alpinus T. Kirk	2	3	–	1	–	–
Lagarosiphon major (Ridl.) Moss	2	3	1	2	1	1
Myriophyllum elatinoides Gaud	2	3	1	2	1	1
Myriophyllum propinquum A. Cunn	1	3	1	1	1	1
Nitella hookeri A. Braun	2	3	2	2	1	1
Potamogeton cheesmanii A. Benn	1	3	1	2	–	1
Potamogeton crispus L.	1	1	2	2	3	3
Potamogeton orchreatus Raoul	2	3	1	1	1	1
Ranunculus fluitans auct N.Z.	3	2	3	3	1	1

Key: 3 optimal performance (see Table 2)
2 less than optimal performance
1 present but not competitive
– not known

Table 2 Optimum performance data for selected submerged macrophytes in New Zealand

Species	*Biomass* g.d.wt.m^2	*Doubling time* days	*Depth range* m	*Maximum height* m	*% cover*
Callitriche stagnalis Scop	x	x	0.0– 1.0b	1.0b	100b
Ceratophyllum demersum L.	1500b	15a	0.5–12.0b	5.0b	100b
Chara corallina Klein ex Willd	x	x	0.1– 4.0b	0.2b	100b
Egeria densa Planch	1200b	8a	0.2– 7.0b	6.0b	100b
Elodea canadensis Michx	1500b	22a	0.3–15.0b	4.0b	100b
Isoetes alpinus T. Kirk	1000b	x	0.0– 6.0b	0.2b	100b
Lagarosiphon major (Ridl.) Moss	1000b	20a	0.5– 6.5b	6.0b	100b
Myriophyllum elatinoides Gaud	100a	x	0.0– 6.5b	3.0b	50b
Myriophyllum propinquum A. Cunn	100a	x	0.0– 4.0b	2.5b	80b
Nitella hookeri A. Braun	400b	x	0.1–35.0b	0.6b	100b
Potamogeton cheesmanii A. Benn	x	x	0.2– 6.0b	3.5b	80b
Potamogeton crispus L.	x	x	0.4– 6.0b	4.0a	100b
Potamogeton ochreatus Raoul	x	x	0.2– 5.0b	3.0b	50b
Ranunculus fluitans auct N.Z.	x	x	0.1– 4.0b	2.5b	100b

Key: x not known a to be confirmed b reliable estimate

When assessing water quality, particularly for general classification purposes, one should take cognisance of a wide range of parameters, and the usefulness of indicator organisms will depend upon stated water use requirements.

The response of submerged macrophytes in recognisable water types is of particular moment to water plant management, but should provide a useful complement to a systems analysis of water quality.

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Some methods for assessing the toxicity of pollutants to aquatic animals

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Laboratory techniques for toxicity tests, suggested methods for laboratory toxicity tests, and on-site techniques for toxicity tests of aquatic organisms are outlined. It is emphasised that because the biological effects of a pollutant are manifested in a variety of ways, the specific technique to be used must be tailored to each specific problem.

Recommendations for future research in New Zealand include identification of sensitive species from various environments and determining the long-term effects of sublethal concentrations of pollutants.

CONTENTS

I Introduction to the biological effects of pollutants

There are no published studies on the effects of pollutants on aquatic animals in New Zealand. Indeed, we know insufficient about the biologies and natural fluctuations in abundance of New Zealand species from freshwater, estuarine and marine environments even to select suitable animals for such studies. Although there is a huge amount of published information on the effects of pollutants on aquatic organisms overseas, it may be unwise to assume that the results are valid for New Zealand, as many pollutants induce a species-specific response. However, we can certainly learn a great deal from the mistakes made, problems encountered and techniques developed by overseas workers, and these aspects are discussed in this article.

Many factors influence the toxicity of pollutants. For example, the quality of the receiving water significantly affects the toxicity of many materials and the sensitivity of organisms to them (Tarzwell 1971). Bryan (1971) listed the following as having considerable effect on the toxicity of heavy metal pollutants on marine and estuarine organisms: (a) the form of the metal in water; (b) the presence of other metals or pollutants in the receiving water; (c) the presence of all those naturally occurring factors which influence the

Received 18 January 1977

Standing Biological Working Party of the Water Resources Council 1979: A review of some biological methods for the assessment of water quality with special reference to New Zealand. *Water and Soil Technical Publication 18* : 35–40

physiology of the organism and the chemical nature of the metal in the water, e.g., salinity, temperature, dissolved oxygen, pH, etc.; (d) the condition of the organism, e.g., its stage in its life-history and its stage in its life-cycle; (e) the size and age of the organism; (f) the activity of the organism; and (g) the previous environment of the organism. All these factors must be taken into account when evaluating safe levels for all pollutants. From this list, it is clear that the same concentration of pollutant will have completely different toxicities in freshwater, estuarine and marine conditions, and in already polluted and non-polluted waters.

Aquatic organisms accumulate pollutants from the water and this again complicates the setting of safe levels (i.e., maximum concentrations of potential toxicants which are not harmful to the aquatic biota with continuous exposure). Concentration factors vary considerably from species to species, but factors of hundreds or thousands are commonly found, and occasionally a factor of a million may be approached, as in the case of some heavy metals (Fukai & Broquet 1965). Many organisms live and feed in sediments and undoubtedly absorb metals and other pollutants from them. Pollutants are also concentrated along the food chains. Thus, it should not be assumed that the concentration of pollutant discharged into an aquatic system is anything comparable to that deposited in the tissues of organisms. Neither should it be assumed that only pollutants derived from solution in the water, either directly or through food chains, are available for aquatic organisms.

Because of the immense amount of information available, I have been very selective in choosing the publications which form the basis of this report. I have concentrated on recent papers which outline practical guidelines for toxicity tests with aquatic organisms, and which are critical of the techniques presently used in such tests. Unless stated otherwise, the information contained here can be applied equally to fish, amphibians and invertebrates from freshwater, estuarine and marine environments. However, I must emphasise that because the biological effects of a pollutant are manifest in a variety of ways, the specific technique to be used must be tailored to each specific problem. I would also like to emphasise that toxicity testing requires a certain amount of skill, and one of the recommendations given by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) was: "To obtain the most meaningful data from a toxicity test with aquatic organisms, the investigator should consult with an aquatic biologist, analytical chemist, biometrician, and aquatic toxicologist before the test".

II Terminology for effects of pollutants

Before describing the tests used to study the effects of pollutants on organisms, it is necessary to define several terms which are currently used to describe their harmful effects. For the most part, the definitions are taken from Water Quality Criteria (1972): (a) **acute** – involves a stimulus severe enough to bring about a lethal response speedily, usually within 4 days; (b) **subacute** – involves a stimulus less severe than an acute stimulus, producing a response in a longer time, may become chronic; (c) **chronic** – involves a lingering or continuous stimulus, often signifying periods of about one-tenth of the life span or more; (d) **lethal** – causes death by direct action; (e) **sublethal** – insufficient to cause death, but may affect migrations, behaviour, incidence of disease, life cycle, physiological processes, genome, feeding and food chains; and (f) **cumulative** – brought about, or increased in strength, by successive additions.

Two broad categories of effect are generally distinguished: acute toxicity, which is usually lethal; and chronic toxicity, which may be lethal or sublethal.

III Methods for assessing toxicity of pollutants

A Introduction to toxicity tests

Information on the effects of pollutants on aquatic organisms comes mainly from bioassays and toxicity tests. These latter terms are not synonymous and the differences between them have been highlighted recently by Brown (1976). A bioassay is a test in which the degree of a known and defined response in a specified living organism is determined in order to quantify the strength of some stimulus (often only one or two levels of intensity being administered). A toxicity test, however, is not concerned with the degree of response but with determining the nature of the responses of any living organism in relation to known concentrations of a stimulus (a wide range of concentrations being given). Thus, in bioassays the concentration of the stimulus is unknown and is indicated by the response of a specified organism. In toxicity tests, the concentration of the stimulus is known and what is determined is the concentration of the stimulus which has no effect, or that which causes a measurable minimal acceptable degree of harm. This "no-effect" level is species- and conditions-specific and therefore cannot be applied generally.

The term "toxicity test" covers a wide range of types of investigations including: (a) tests to compare the relative lethal toxicities to one or more species of aquatic animal of different substances under some fixed, but arbitrary, set of conditions; (b) tests to compare the lethal toxicity to a single species of a given substance under a range of test conditions (e.g., pH, dissolved oxygen, hardness, temperature, salinity) to determine the effects of environmental conditions on toxicity; (c) laboratory tests of the effects of a substance on survival, growth, reproduction etc. on aquatic animals; (d) laboratory and field tests of the effects of a waste (or of a chemical for use in agriculture) on species, on populations and on fisheries; (e) the laboratory use of aquatic animals (usually fish) to monitor for harmful effects of aqueous wastes, and waters being abstracted from rivers for drinking, food processing and irrigation; and (f) the use of fish in cages to monitor harmful effects of river

water or aqueous domestic and industrial wastes (Brown 1976).

Toxicity tests have been used in pollution studies for about 80 years, but unfortunately there has been great diversity in the methods used. Various workers have used different species of test organism, different periods of exposure to the pollutant, waters of different quality, and different ways of reporting the results. The need for standardisation on all aspects of toxicity testing was recognised in 1949 by the formation of a sub-committee of the Research Committee of the Water Pollution Control Federation, U.S.A. (see Tarzwell 1971 for details). The tests and procedures set out by this sub-committee have been revised subsequently. The most recent publication on toxicity testing methods I have read was compiled by the Committee on Methods for Toxicity Tests with Aquatic Organisms (U.S.A. Environmental Protection Agency 1975). This report is essential reading for anyone about to use toxicity tests, and I have cited it extensively in later sections of this paper (see: B—Laboratory techniques for toxicity tests, and C—Suggested standard methods for laboratory toxicity tests).

Toxicity tests may be either short-term (acute toxicity tests) or long-term (chronic toxicity tests), depending on the information required.

1 Short-term (acute) toxicity tests

Acute toxicity tests are generally used to determine the level of a toxic agent that produces an adverse effect on a specified percentage of the test organisms in a short period of time. Because death is a finite response and easily detected (see later criticism by Perkins 1972), the most common short-term measure is the *Acute Mortality Test.* In this test, 50% mortality is the accepted measure of the toxicity of a toxic agent to a group of test organisms, and 96 hours is often a convenient and useful exposure duration. Therefore, the measure of acute toxicity most often used is the 96-hour median lethal concentration (96-h LC_{50}). However, the measure of acute toxicity most often used with daphnids and midge larvae is the 48-hour median effective concentration (48-h EC_{50}) based on immobilisation. [The terms LC_{50} and EC_{50} are consistent with the widely-used terms Median Lethal Dose (LD_{50}) and Median Effective Dose (ED_{50}), respectively, which are also used in the literature. However, it must be noted that "concentration" refers to the concentration of toxicant in the test solution, whereas "dose" refers to the amount of toxicant that enters the test organism.] The median lethal concentration is a convenient reference point for expressing the acute lethal toxicity of a given pollutant to the average or typical animal. It is used to compare the toxic effects of a range of pollutants on one species in order to erect a hierarchy of toxicants; to compare the effects of a single pollutant on a range of different species to select possible "indicator species"; and to compare the effects of pollutants on different stages of the life-cycle of a species to isolate the most sensitive stage in development. However, the median lethal concentration is not a safe concentration, and decisions made after the evaluation of the results from these tests, based on concentrations found to be non-lethal to one half of the test animals within a short period of exposure, are totally inadequate for defining the amounts of a pollutant which can be discharged into a system. Safe levels of pollutants, which permit normal life processes such as reproduction and growth are usually much lower than the LC_{50}. Safe levels are not known for many pollutants, so the trend has been to apply an "application factor" to the recorded LC_{50} of the most sensitive important species in the locality under pollution pressure. Recommended application factors and criteria for safe levels of pollutants are provided in Water Quality Criteria (1972). This publication recommends that for routine assessment and prediction of safe levels, tests for acute lethal toxicity should be carried out and then the lethal concentration be multiplied by the suitable application factor. While some application factors have been derived from chronic or sublethal laboratory experiments and from well documented field studies of polluted situations, others have been somewhat arbitrarily calculated. This is not a satisfactory state of affairs, and the use of application factors must be questioned seriously.

The short-term toxicity test has been strongly criticised by many workers in the field of pollution studies. Perkins (1972) has drawn attention to the fact that acute toxicity tests measure only that mortality which occurs during the period of exposure of the test organisms to the pollutant and not that which affects the surviving animals later. To accommodate the latter, he distinguished three stages of mortality as follows: (a) **concomitant mortality** - deaths that occur during the period of exposure to the toxin; (b) **consequential mortality** - deaths that occur during, and immediately after, the recovery period after exposure to the toxin; and (c) **delayed mortality** - deaths that occur after a period during which the animals have appeared healthy and in which none has died. Gray (1974) criticised the acute toxicity test on the following grounds: (a) it is too short when effluent discharge will be continuous; (b) an organism surviving the test concentrations may not survive for longer periods, may not grow and reproduce at that concentration, or may in nature avoid that concentration; (c) on the assumption that stress increases with temperature, higher summer temperatures are used, yet lower temperatures may induce higher stress; (d) the organisms used are frequently at high trophic levels and are adults, whereas organisms at low trophic levels and larvae or juveniles are often more sensitive; and (e) chemicals are tested singly, whereas in nature mixtures occur. Finally, Brown (1976) has attacked the statistical validity of using the LC_{50} as an accurate expression of the toxic effects of a pollutant. The main criticisms of the LC_{50} are: (a) it is not useful for management purposes as it cannot be applied to the wild population to predict safe levels under conditions of chronic exposure; (b) the precision of the figure is related to sample size (which is not always standardized); and (c) because of unavoidable sampling error, a 50% response in one batch of animals is not always repeated in a second batch from the same population, and can vary between 20% and 80%.

Short-term toxicity tests, however, can be most useful in determining the dilution to be employed in long-term toxicity tests and in comparing sensitivities of various life-stages of the same organism. They are primarily of value in comparing toxicities of a number of pollutants which have similar modes of action.

2 Long-term (chronic) toxicity tests

Chronic toxicity tests at sublethal concentrations of pollutant are essential for determining safe levels of potential toxicants under conditions of continuous exposure. The purpose of these tests is to determine the maximum concentrations of potential toxicants which are not harmful to the aquatic biota with continuous exposure. These longer studies (perhaps lasting for two generations of test organism or for many months) with levels of pollutant which do not kill the test organism are essential for setting water quality standards. At truly sublethal concentrations, a toxic substance may: (a) inhibit or promote growth; (b) influence the reproductive potential by changing the fecundity and brood survival; (c) change behaviour of larvae, juveniles and adults; (d) upset a predator-prey relationship; (e) inhibit or alter the crustacean moult cycle; (f) affect respiration, osmoregulation and ionic regulation; (g) accelerate ageing;

and (h) lower the resistance to disease (Perkins 1972). Long-term tests covering a substantial part of the life-cycle of the organism can be conducted in the laboratory to determine chronic sublethal effects of pollutants. For example, various processes of the organism such as growth rates, respiration, osmoregulation and ionic regulation may be used to evaluate sublethal effects. However, some long-term chronic effects may be more subtle and more difficult to evaluate under laboratory conditions. Examples of these include changes in breeding, changes in migratory behaviour, and the development of a general debility, making the organisms more susceptible to disease, predation or environmental stress. Unfortunately, substantial data on long-term effects and safe levels are available for only a few toxicants. Determination of maximum safe levels of potential toxicants in the aquatic environment is a tremendous job; there are thousands of organisms and thousands of wastes to be considered. Obviously shortcut methods are essential. To reduce the number of organisms to be considered, Tarzwell (1971) has suggested that: (a) only those species of recreational or economic importance and the organisms which serve as their food should be tested; and (b) these economically important species etc. should be further screened to determine the most sensitive species and life-stages to a particular waste or toxicant. The rationale behind (b) is that if the most sensitive species and life-stages are determined and protected, then the whole biota will be protected with a reasonable degree of safety.

While this paper is concerned primarily with the methods used to assess the toxic effects of pollutants on aquatic organisms, it is worth pointing out that a pollutant may also have an effect on the ecosystem not directly related with its effect on an individual species. Ecosystem interactions are difficult to assess in the laboratory and techniques for evaluating them in the field are not completely satisfactory. Thus the final test before applying levels to pollutants, according to Tarzwell (1971), is the use of field studies to determine if the laboratory findings, as to safe concentrations, are adequate to protect the biota under natural conditions, where there is exposure to parasites and disease, and the organisms must compete for food and space and withstand many other stresses.

B Laboratory techniques for toxicity tests

Although toxicity tests with aquatic organisms can be conducted by applying the toxic agent directly to the test organisms, such as by injection or in food, most tests are conducted by exposing the test organisms to test solutions containing various levels of a toxic agent. One or more control treatments are used to provide a measure of the acceptability of the test by giving some indication of the healthiness of the test organisms and the suitability of the dilution water, test conditions, handling procedures, etc. A control treatment is an exposure of the test organisms to dilution water with no toxic agent added. The other treatments are exposures of the test organisms to dilution water with toxic agent added. The toxic agent can be one or more pure chemicals or a complex mixture such as an effluent. Test solutions are usually prepared by dissolving a toxicant in a solvent, preferably water, to form a stable stock solution, and then adding a portion of the stock solution to dilution water. [This may be suitable for freshwater species, but certain salts of heavy metals react with seawater to produce complex mixtures of chemicals and care must be taken to ascertain what chemical salt is added.]

The four main techniques currently used in toxicity tests are the:

Static Technique
Recirculation Technique
Renewal Technique
Flow-through Technique.

1 Static Technique

In the static technique, test solutions containing a toxic agent and the test organisms are placed in test chambers and kept there for the duration of the test. This technique provides the easiest measure of toxicity and is often the only practical means of estimating the influence of variables such as temperature, salinity and existing water quality on the results of toxicity tests. The static technique should not be used for exposures lasting longer than 96 hours.

2 Recirculation Technique

This technique is similar to the static technique except that each test solution is continuously circulated through an apparatus to maintain water quality by such means as filtration, aeration, and sterilization, and then returned to the test chamber.

3 Renewal Technique

The renewal technique is also like the static technique except that the test organisms are periodically exposed to fresh test solution of the same composition, usually once every 24 hours, either by transferring the test organisms from one test chamber to another or by replacing the test solution.

4 Flow-through Technique

In this technique, test solutions flow into and out of the test chambers on a once-through basis for the duration of the test. Two procedures can be used. In the first, large volumes of the test solutions are prepared before the beginning of the test and these flow through the test chambers. In the second, and more common procedure, fresh test solutions are prepared continuously or every few minutes in a toxicant delivery system.

Flow-through tests can obviously last longer than 96 hours as this technique provides for continued addition of test solution to the test chambers, maintains dissolved oxygen, toxicant concentrations and pH at desired levels, and removes degradation and metabolic products. The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) suggests that the flow-through technique be used when other than the static is desired. However, the Committee also recognises the use of the recirculation and renewal techniques in some cases. Obviously, the technique used will depend on the problem being studied.

C Suggested standard methods for laboratory toxicity tests (outline only)

This section is based on the recommendations given in the report — hereafter referred to as "the Report" — of the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Only an outline of the methods is given here to highlight the points which must be considered when using toxicity tests.

1 Equipment

The Committee suggests that: (a) glass, stainless steel and perfluorocarbon plastics be used as construction materials of test chambers, and that rubber, copper, brass or lead should *never* be in contact with the test solution; (b) stainless steel be welded and never soldered; (c) the recommended method for toxicant delivery system for flow-through tests given in the Report be used; and (d) the standard method for cleaning equipment outlined in the Report be used.

2 Dilution water

The Committee states that: (a) the *minimum criterion* for an acceptable dilution water is that healthy test organisms will survive in it for the duration of the test without showing signs of stress; (b) a *more stringent criterion* is that test organisms will survive, grow and reproduce satisfactorily in it; (c) the recommended formulae for reconstituted freshwater and seawater given in the Report should be used to maximize the number of reliable comparisons; (d) when alternative dilution waters are used, they must be uncontaminated, of constant quality and meet the specifications outlined in the Report; and (e) for effluent testing, the dilution water must be a representative sample of the receiving water obtained as close to the point of discharge as possible, but upstream of, or outside, the zone of influence of the effluent.

3 Test organisms

A list of recommended species to be tested in the U.S.A. is given in the Report. If a recommended species is not available, then the Committee suggests that another species of the recommended genus should be used. The Committee strongly urges that identification of any species used be verified. Obviously there is a considerable need to select certain comparable important species for toxicity tests in New Zealand. Gray (1974) suggested the use of sensitive species which are long-lived, have only a slow adaptation capacity and occur in relatively stable environments. This would rule out the use of estuarine species Organisms such as subtidal bivalve molluscs, or polychaetes, may be suitable and can be used for back-up field monitoring, since the organisms remain *in situ* and must tolerate the conditions or perish. Mobile animals, such as fish and crabs, may simply move out of the path of pollutants.

With regard to the test organisms, the Committee recommends that: (a) for effluent tests, the most sensitive important species indigenous to the receiving water in the vicinity of the discharge should be used; (b) for other tests, disease-free fish from hatcheries and laboratory cultures of invertebrates, or "wild" populations from unpolluted waters be used; (c) all organisms must be from the same stock, be healthy, and be as uniform in size and age as possible; (d) immature stages be tested; (e) the methods for collection, care, handling and holding of the organisms outlined in the Report (see also Perkins 1972) be followed; and (f) the times allowed for acclimation listed in the Report be adhered to.

4 Test procedure

The Committee states that test organisms must not be fed while in the test chambers. (This will obviously limit the duration of the experiment and is a recommendation aimed primarily at acute toxicity tests.) The Committee also lists the requirements for: (a) the numbers of animals and numbers of replicates to be used; (b) the limits of the dissolved oxygen concentration in the dilution water; (c) the test temperature (this will depend on the species used, but certain basic limits are outlined in the Report); (d) loading (i.e. the weight of test organism for volume of test solution); (e) the methods of exposure of the toxicant; (f) the method of beginning the test; (g) duration of the test (for acute toxicity tests, the Committee suggests use of 96–h LC_{50}, or 8-day exposure for flow-through tests); (h) the procedure for monitoring the effects of the pollutants on the test organism; (i) the chemical and physical data that need to be monitored during the test; (j) a preliminary, range-finding test; (k) the definitive test; and (l) the calculations with the data (see also Sprague 1969).

5 Reports

The Committee sets out comprehensive details for all the information needed in a report on the results of the tests.

D On-site techniques for toxicity tests

On-site techniques for toxicity tests appear to be in an early stage of development and have so far been used only with fish. Indeed, the whole concept of field toxicity tests, even with fish, has recently been questioned (Brown 1976) on the grounds that the tests offer little or no opportunity for the control of conditions, or for the exploitation of refined techniques, such as is possible in the laboratory. However, the on-site techniques for toxicity testing that I have been able to find are included here for completeness.

1 Static Technique

(i) Berger *et al.* (1967) used large wastebaskets for on-site testing of fish in lakes. (This method could be modified for other aquatic environments, but the main problem would be that the method requires the use of species that could maintain an enclosed reproducing population. It is difficult to get many organisms to reproduce and complete their life-cycles under enclosed conditions.)

(ii) Burress (1975) has tested the suitability of seven types of potential on-site test vessels for toxicity studies using fish, viz. polythene wastebaskets, fibreglass containers, metal lard cans, aluminium pails, stainless steel pails, large glass jars and polythene bags. No single container was superior to the others, but polythene bags were considered to be the most useful, primarily because they are readily portable and are available in many sizes. However, care must be taken when selecting the test site, as rocks, roots and other objects might puncture the bags. Bags can be protected by making a simple enclosure of netting material supported by 4 steel fence posts. (This method was designed initially for 96 h tests and may not be suitable for tests of longer duration. This technique is useful only in lakes, ponds and streams with a slow or moderate current.)

2 Flow-through Technique

Brungs (1973) has described flow-through techniques in which test organisms are kept in a tank on the discharge site and the test solution (or a sample of diluted or undiluted effluent) is allowed to flow through the tank continuously. I have not been able to get a copy of this paper, however, the following appraisal of the method is taken from Hart (1974): "Test organisms are not necessarily species that are present in the receiving water body; rather, they are usually species whose precise tolerance to particular pollutants of concern is known. One of the main shortcomings of such methods is that the tolerance range of species in the receiv-

ing waters may well differ from that of the test organism. In addition, often stream conditions, and the opportunity for bioaccumulation of certain toxic materials through the ecosystem in a stream, are sufficient to damage organisms in-stream, even though the material alone under tank conditions did not damage the organism".

My final comments refer to the use of fish as an alarm system to test the toxicity of waters being taken from rivers for drinking purposes. While the idea is much in vogue, Brown (1976) has itemised the major drawbacks which effectively eliminate the technique as a practical proposition. However, Brown (1976) does recommend the use of fish as part of a wide monitoring programme in pollution studies as follows: "As a component of a water monitoring system the application of fish in a tank is obviously not useless and indeed is to be recommended, even if it only shows from time to time that something is possibly wrong with a water. But an adjunct is all that it should be, and, as such, it need not consist of anything more than a tank through which water is passed and in which fish can be detected as being either "normal" or "abnormal" in attitude and behaviour. Such tests do not seem to be subtle enough for any other purpose and a diversity of test systems and test organisms is to be preferred to a complicated (alarm) system using only fish".

IV Recommendations for research in New Zealand

A Important sensitive species must be identified from freshwater, estuarine (if possible) and marine environments, and their biologies must be fully studied so that effects of pollutants can be properly assessed.

B Workers about to start using toxicity testing techniques are strongly recommended to follow the methods outlined by Perkins (1972) and by the Committee for Methods for Toxicity Tests with Aquatic Organisms (1975).

C Information on the long-term effects of sublethal concentrations of pollutants on the species identified under **A** are required as a prerequisite for setting water quality standards in New Zealand.

V Acknowledgments

I am very grateful to the late Dr C.R. Boyden (Department of Zoology, University of Otago) and Dr M.C. Crawley (Department of Zoology, University of Canterbury) for their constructive criticisms of the manuscript.

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Bacteria and water and waste water quality

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Health and aesthetic problems caused by bacteria in water are reviewed, together with the rationale of water quality monitoring methods that use bacteria as indicators. Methods currently used for the detection of faecal bacteria, bacterial biomass and detection of nuisance bacteria in water are discussed.

It is considered that further research is required in two areas. Firstly, on the methods using bacteria as indicators of water quality, and secondly on the routine detection of viruses in water and waste water.

CONTENTS

I Introduction

The numbers and types of bacteria in bodies of water largely depend on the organic substances present. Inorganic substances which are required by bacteria in small amounts are of lesser importance except where they suppress growth of bacteria or stimulate particular groups of chemolithotrophs. Water is used for domestic, industrial and recreational purposes and can be a vector of disease-causing organisms thus it is necessary to establish if such water is safe for use. In addition, continued input of organic matter stimulates growth of bacteria which deplete the oxygen. This leads to the growth of anaerobic bacteria which produce unpleasant smelling products during metabolism. The reasons therefore, for monitoring water quality are twofold: (a) to protect public health and (b) to ensure the preservation of bodies of water in a pleasant attractive condition.

Not only do we need to detect bacteria in water, but we can use them as indicators of water quality. As indicators they have several advantages over other biological parameters. Firstly, because bacteria reproduce rapidly (under optimum conditions every 20-30 min), tests can be evolved that allow assessment of water quality more rapidly than by using other biological indicators. Secondly, changes in bacterial numbers occur rapidly in response to addition of organic matter and so detection of these changes allows early assessment of a problem.

Waste water quality can also be assessed using bacteria.

Much has been published on the subject of bacteria as indicators of water and waste water quality, but differences in terminology have led to confusion. Modification of tests in contradiction to the principles on which they are based has added to this confusion, which has been further compounded by different laboratories using different methods, making comparison of water quality data from place to place almost impossible.

The literature on bacteria as indicators of water and waste water quality is vast and only selected references are quoted here.

Received 16 November 1976, revision received 17 November 1978

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A Public health and water quality

1. Disease transmission by water

Disease-producing organisms may enter water from the faeces of infected animals (including man), as well as from urine, saliva and mucous, from skin and from carcasses (Holden 1970). While we do not suffer in this country from epidemics of water-borne diseases such as cholera, we do have a high incidence of hepatitis and not infrequent outbreaks of typhoid fever (*Salmonella typhi*) and other salmonella infections. Enterovirus infections are also prevalent and, although they do not cause serious illnesses, result in many man-hours lost per year.

Of the water-borne diseases, those transmitted from infected faeces are the most prevalent. Some come from faeces of animals other than man, and may enter water from freezing works, piggeries, milking sheds and poultry batteries. This contaminated water may then be used for domestic supplies. In many cases, but not all, drinking and domestic water supplies are monitored and treated and so disease transmission is prevented.

Disease is not always contracted from contaminated drinking water. Pollution of water by faecal matter may lead to contamination of foods such as shellfish. These are filter feeders, and disease-causing bacteria and viruses may be concentrated in them, causing unpleasant consequences when consumed.

If water used in food processing is not subject to quality control, transmission of disease may result. Even if disease-causing organisms are not present, evidence of faecal pollution may lead to the rejection of our exported food products. Quality control of water then, is not only a public health matter, but an economic necessity for our food export industry.

Although drinking water and that used for food processing when treated with chlorine may be considered bacteriologically safe, little is known of the fate of viruses in chlorinated water supplies. As viruses can withstand high concentrations of chlorine (Holden 1970) they may survive and lead to disease in man. Faecal contamination of any water to be used for drinking, domestic purposes or food preparation, even if it is to be treated, should therefore be avoided.

Even if one escapes disease by imbibing treated water and by eating carefully prepared food it is still possible to contract a disease by swimming in faecally contaminated water. It is sometimes stated that it is safe to swim in water contaminated by faecal matter as swimmers are unlikely to swallow sufficient organisims to allow a disease to establish. While this may be true for certain disease-causing organisms, it is not for others (US Department of Health Education and Welfare Report 1974). Monitoring of recreational waters is therefore necessary because in New Zealand raw sewage is discharged into many waters used for recreation. Another important factor is that rapid air travel could facilitate the introduction of previously rare faecally-borne diseases.

2. Drug resistance factors and water quality

In the last few years, it has become apparent that there is a need to monitor water quality for public health reasons other than that of transmission of disease. The discovery that drug resistance can be transferred from one bacterium to another (Watanabe & Fukasawa 1961) has added a new dimension to water quality monitoring. Following widespread use of antibiotics, the numbers of bacteria carrying drug resistance factors (R factors) has increased in the gastrointestinal tract of man and animals in some countries (Richmond 1975). Feeding antibiotics to animals to improve growth is not approved of in New Zealand, but widespread administration of antibiotics to the human and animal population has led to a rise in the proportion of gut bacteria carrying drug resistance in certain individuals, as is evidenced by the rise in the proportion of bacteria carrying drug resistance (R) factors found in sewage (D.F. Bacon, pers. comm.; M. Loutit, unpublished results). Bacteria with R factors may be passed between individuals in several ways; through drinking water and by contamination of water used for swimming. R factors in bacteria in this water can then be transmitted to the gut bacteria. Smith (1970, 1971) has shown that R factors in *Escherichia coli* are present in river and coastal bathing waters in Britain in sufficiently high numbers to cause anxiety. While little work has been done on the incidence of R factor containing bacteria in waters in this country, drug resistance factors have been shown to occur in bacteria isolated from water. Cooke (1976a and b) has shown that mussels collected from New Zealand lakes remote from known pollution sources carried coliform bacteria containing R factors. She found, however, the levels to be lower than in shellfish collected from near a sewage outfall; in addition the bacteria from the shellfish near the outfall carried R factors conferring resistance to a greater range of antibiotics. Bacteria carrying R factors conferring resistance to a few antibiotics will occur in most populations in nature, due to the presence of antibiotics produced by micro-organisms in these habitats (Cooke 1976b). If the proportion of bacteria carrying R factors to a larger range of antibiotics increases, a public health problem results, particularly if these bacteria are concentrated by filter-feeding shellfish. For example, if there was an outbreak of shigellosis caused by an antibiotic resistant strain of the pathogen, effective treatment for the disease would not be possible.

3. Metals, bacteria and water quality

Another matter of public health concern relates to the transmission and biomagnification of metals through food chains. The transmission of metals from fish to humans with disastrous consequences was brought to public attention by the discovery of mercury poisoning in a section of the population in Japan. Mercury from industrial processing plants had entered a bay and contaminated fish which were the main source of food for people in that area. While disasters of this magnitude are unlikely to occur in New Zealand because of the smaller industrial development and the varied diet of the population, certain aspects of movement of metals through food chains in relation to public health need to be considered. It has been shown in New Zealand that small amounts of metals in effluents entering waterways can be concentrated by bacteria and passed through food chains to fish (Loutit *et al.* 1973). While the possibility exists that certain metals are implicated in animal (Shaw & Brown 1971) and human disease (Voors 1971) it is desirable that levels of metals in effluents be monitored and toxic compounds removed before discharge. Effluents requiring particular attention are those containing in addition to metals, organic materials likely to cause stimulation of bacterial growth leading to the concentration of the metal at the effluent entry point and preventing its dispersal or dilution.

Shellfish in New Zealand have been reported to contain high concentrations of metals (Brooks & Rumsby 1965) and, as they are filter feeders, the metals have most likely been extracted from water low in metals. There has been a suggestion that bacteria in the gut of the oyster concentrate metal ions (Sayler *et al.* 1975).

B. Aesthetic reasons for water quality control

Bodies of water, fresh and marine, have their own indigenous biota. In unpolluted water the bacteria present are usually Gram negative species of which a large proportion are pigmented. The numbers and types, however, depend predominantly on the nutrients available.

Following rain, many soil bacteria will be washed into the water, but these disappear within a short time. Bacteria and viruses may also enter water in organic wastes and, depending on the quantity of the material, may persist for varying periods within the protection of organic particles.

The addition of organic nutrients to bodies of water usually causes an increase in numbers of bacteria able to use the particular substances added. Should the input of nutrients continue, these bacteria become the dominant population. If there is a high and continued input of organic matter, of either plant or animal origin, the bacterial population places a high demand on the available oxygen leading to a depletion in oxygen: in some cases the body of water may become anoxic. Anaerobic bacteria then proliferate and by their activities cause the water to become smelly and unattractive.

Sometimes the organic matter is in the form of large algal and blue-green bacterial blooms, which after death sink to the bottom of the body of water. Their degradation can lead to the bottom layers becoming anaerobic. Such conditions affect the fauna of the water. Large growths of algae and blue-green bacteria should therefore be controlled, i.e. levels of soluble nitrogen and/or phosphorus should not be allowed to rise to levels at which eutrophication can occur.

II Rationale of water quality monitoring methods using bacteria as indicators

A. Public health monitoring

1. Diseases of enteric origin

If diseases are transmitted by water it would seem logical to attempt to detect disease-causing organisms in suspect water. Unfortunately, except for a few organisms this is difficult because:

(a) excretion or shedding of the disease-causing organisms may be intermittent and the dilution factor may make detection difficult;

(b) culture of the disease-causing organisms may require complex media and methods unsuited to routine investigations.

Because of the difficulties at present associated with detection of most enteric disease-causing organisms it has become accepted practice to use the presence of certain common gut bacteria as an indication that disease-causing organisms may also be present.

The indicator organisms used are non-fastidious inhabitants of the gastrointestinal tract; *Escherichia coli* or *Streptococcus faecalis* are usually used, with *Clostridium perfringens* occasionally the indicator of choice.

While detection of these organisms can only indicate faecal pollution and certainly does not prove that disease-causing organisms are present, the philosophy is, that if faecal pollution has occurred, then there is the possibility that pathogens may be present. Since strains of *E. coli* are becoming increasingly recognised as causes of disease, their presence may indeed indicate a potential for disease.

Further, it is generally assumed that by the time indicator organisms are no longer detectable, potential disease-causing organisms will have died off also. This may not always be true for some pathogenic bacteria, particularly if the quantity of organic matter present is large, and certainly may not be true for viruses.

The use of *E. coli* as an indicator organism is more universally accepted than use of *S. faecalis*. *E. coli* occurs in greater numbers in the faeces of man than *S. faecalis* and *C. perfringens* (Holden 1970). Since, in early studies on monitoring of water quality, only the disease aspect was considered and only diseases spread by water between humans were considered of consequence, *E. coli* was thought to be the most sensitive indicator for this purpose. It is now accepted that animals other than humans carry diseases that can be transmitted to humans, e.g. infections caused by *Salmonella* spp. As *E. coli* also occurs in the gut of animals it is generally considered a suitable indicator organism to detect faecal pollution from animal sources. In animals, however, the numbers of *E. coli* may be fewer per gram of faeces when compared with *S. faecalis*. This does not necessarily detract from the use of *E. coli* as an indicator organism although some authorities use the proportion of 'faecal coliforms' to 'faecal streptococci' as an indication of whether the pollution is of human or animal origin (Geldreich 1972). *E. coli* is preferred as the indicator organism by many authorities because it is said not to multiply outside the gut (Holden 1970). The same claim is made for *S. faecalis* (Geldreich 1972), but under some circumstances both appear to be able to multiply outside the gut. Although *S. faecalis* appears more resistant to destruction than *E. coli* and coliforms (Grabow 1970) it is not as widely used as an indicator.

The tests designed on the basis of using *E. coli* as an indicator organism have been in existence for over 70 years. Recently, because of changes in classification and nomenclature of organisms, modifications to the tests, and increased knowledge, problems have arisen over the interpretation of the results from these tests. In an attempt to overcome these problems the New Zealand Microbiological Society (Report of Recommendations of the Microbiological Society's Committee on Coliform Bacteria 1976) recommended the use of the multiple tube test, but acknowledged that the membrane filter test can be used under some circumstances, and defined terms used in the tests.

The first part of the test for detection of *E. coli* is the presumptive coliform test. This is an indirect counting technique, in which the most probable number of bacteria is estimated following the scoring of the number of tubes of broth that show that the bacteria in the test sample have fermented lactose under set conditions and in a certain time. The number of bacteria occurring is read from mathematical tables based on the probability of the event occurring. It should be remembered that this test has wide confidence limits and the number arrived at is only an estimation that a certain number of lactose-fermenting bacteria are probably present. The bacteria giving a positive reaction in this test are species like *E. coli,* i.e. 'coliform', and are referred to as presumptive coliforms.

The definition of 'coliform' has been and still is a matter of dispute since it is used differently by different workers (Holden 1970). Originally it was assumed that coli-

form bacteria were found only in the gastrointestinal tract of man. We now know that many coliforms exist naturally in soil and water and the gut of other animals and that an increase in the number of presumptive coliforms does not necessarily indicate faecal pollution by humans. All that a positive presumptive test indicates is that some source of organic matter, either of plant or animal origin, has entered the water and this has led to a rapid growth of lactose-fermenting bacteria belonging to the genera *Citrobacter, Enterobacter, Escherichia,* and *Klebsiella.*

Some selection of organisms of faecal origin can be achieved by adding substances such as surface active dyes and bile salts to the culture medium used in the presumptive coliform test. These substances are tolerated better by the gut organisms than by organisms of other than enteric origin but the presumptive coliform test is at best a useful indicator of organic pollution, whether these substances are present or not.

To show that organisms of faecal origin are present it has become common to carry out the lactose fermentation test at an elevated temperature; this test is referred to as the Eijkman test. The method is the basis of the APHA (1975) 'faecal coliform' test. The test was based on the belief that *E. coli* was the only organism capable of fermenting lactose at 44.5°C. It is now known that some *Enterobacter aerogenes* strains, which normally live in water and soil, can also carry out such a fermentation at 44.5°C (Hendricks 1970) as can some *Klebsiella* stains (M. Loutit, unpublished results). The elevated temperature lactose fermentation test therefore is not specific for *E. coli*. Such a fact may be important in monitoring effluents from oxidation ponds where *E. aerogenes* and *Klebsiella* strains occur (M. Loutit, unpublished results).

If *E. coli* is to be used as the indicator of faecal pollution, it is necessary to prove its presence and additional tests are required to do this. These tests are set out in certain publications (WHO 1971; N.Z. Microbiological Society's Coliform Committee Report 1976).

2. Non-enteric diseases

A number of diseases of non-enteric origin, mostly from the skin, mucous and urine of infected individuals and domestic animals, can enter water and subsequently be transmitted to humans.

In swimming pools the mucous and fatty acids from the bathers may protect the organisms they shed from the action of chlorine. Thus species of *Staphylococcus, Streptococcus* and *Pseudomonas* can exist in chlorinated pools in surface films (Cruickshank *et al.* 1975) and serve as foci for infection.

It is usual to detect these organisms by sampling the surface film with the aid of absorbant pads which are subsequently dissolved and used in attempts to isolate the disease-causing organisms (Cruickshank *et al.* 1975).

B. Detection of organic pollution

If organic matter is added to water the numbers of bacteria increase, predominantly the Gram negative chemoorganotrophic aerobes. Organic pollution can therefore be detected by assessing: (a) increase in biomass, (b) increase in viable bacteria, (c) increase in coliforms, and (d) uptake of oxygen.

III Bacteriological methods for monitoring water and waste water

Whatever method is used, care has to be taken to collect samples under aseptic conditions. Multiple samples should be taken over a period of time as results from one sample are insufficient to make a judgement as to whether water is free from organic pollution and/or disease-causing organisms. Each sample must be thoroughly mixed before testing, and if chlorinated water is being tested, chemicals must be added to nullify its effect (APHA 1975).

A. Coliform enumeration and confirmation

The preferred definition of coliform organisms and methods for their detection are given in the New Zealand Microbiological Society's Coliform Report (1976). Further methods are given in the Report on Public Health and Medical Subjects No. 71 (1970), WHO (1971) and by the APHA (1975).

Because different media encourage growth of different genera of bacteria, a 'total' coliform count can never be achieved, although the term total coliform count is used (APHA 1975). Since it is in fact impossible to achieve, it has been recommended that this term not be used (N.Z. Microbiological Society's Coliform Report 1976).

B. Estimation of *E. coli*

It is sometimes necessary to estimate the number of *E. coli* in water, e.g. to assess the numbers of *E. coli* entering rivers by run-off from heavily stocked pasture. In the methods recommended by WHO (1971) a most probable number of *E. coli* can be obtained by the multiple tube method followed by confirmation of the presence of *E. coli* by elevated temperature lactose fermentation and indole production at 44.5°C. However, as previously mentioned, this latter test is not specific for *E. coli* and so many prove inadequate. Streaking on well buffered eosin methylene blue agar followed by the IMVIC series of tests and microscopic examination (APHA 1975), although a more prolonged confirmation procedure, is more reliable.

C. Estimation of 'faecal coliforms'

Tests to estimate 'faecal coliforms' are widely used and involve a multiple tube most probable number technique or a membrane filtration method (APHA 1975), followed by transfer of material from all positive tubes or from colonies, to a selective medium containing lactose. The cultures are then incubated at 44.5°C for 24 hours. It is assumed that only organisms from the gut will ferment lactose at this elevated temperature. However, certain strains of *Enterobacter aerogenes*, found in soil and water and not of faecal origin will also ferment lactose at 44.5°C (Hendricks 1970) so the test is not specific for gut bacteria and it must not be assumed that the term 'faecal coliform' is synonymous with *E. coli*.

D. Detection of 'faecal streptococci', *Streptococcus faecalis* and anaerobic spore-formers (*Clostridium sporogenes*)

WHO (1971) and APHA (1975) give multi-tube methods for estimating the most probable numbers of these organisms. APHA methods (1975) also include a membrane filtration technique and a plate count method for 'faecal streptococci'. Confirmation techniques are given in

both manuals. The range of organisms included as 'faecal streptococci' is discussed in APHA (1975) and it is pointed out that the organisms which could grow in the test media are not only found in faeces so that care must be exercised in interpreting this test. Some of the so-called 'faecal streptococci' are normal inhabitants of soil and vegetation.

E. Detection of water-borne bacterial pathogens

It is possible to detect disease-causing organisms such as *Salmonella* spp. and *Pseudomonas aeruginosa* in water if they are present in sufficient numbers, e.g., during an outbreak of disease, or if the volume of water to be tested is relatively small in relation to the degree of pollution, e.g., a swimming pool. Methods for detection of strains of *Salmonella, Shigella, Pseudomonas, Streptococcus, Staphylococcus, Leptospira* and *Vibrio* are given in the APHA publication (1975). Most of these methods either require selective enrichment techniques or concentration of bacteria in the sample, usually by filtration, or use of other specific procedures (Cruickshank 1975).

F. Viable counts

The numbers of viable chemoorganotrophic bacteria in a water sample give some indication of the degree of organic pollution. Pristine waters contain few bacteria but waters contaminated by organic matter of plant or animal origin support many. These bacteria may be enumerated by thoroughly mixing the sample, making suitable dilutions and plating. The medium used affects the types of bacteria that will grow. For pristine water a non-selective medium low in nutrients is necessary to ensure growth of the indigenous biota. If the water has some organic matter present, media of higher nutrient status can be used. Nevertheless, no one medium will support the growth of all organisms and the estimate of numbers obtained must be considered in this light. A total viable count is impossible for this reason although the term is frequently used. A method that saves considerably in terms of consumables is the drop count method (Sharpe and Kilsby 1971) and if large numbers of samples have to be handled this method has considerable advantages. Until competence in the technique has been achieved however, parallel runs with the conventional dilution plate count method should be carried out.

G. Biomass

An assessment of bacterial biomass can be made using ATP (adenosine triphosphate). This method is discussed in the section "Some biological techniques for water quality assessment" by C. Burns in this publication.

Since ATP is found in many organisms as well as bacteria, estimation of ATP is not specific for bacterial biomass.

H. Biochemical oxygen demand (BOD)

The bacteria in water use up oxygen during metabolism of organic substances. The numbers present will depend on the amount of organic matter available for metabolism. By measuring the amount of oxygen that the bacteria in a sample use, some indication can be obtained of the degree of pollution of the water sample. BOD is a bioassay and is particularly useful in assessing the oxygen requirements of waste waters, effluents and polluted waters. It is useful in measuring waste loading of treatment plants and evaluating the efficiency of such treatment systems.

The technique is described in APHA (1975). It is of doubtful use in measuring the oxygen demand of surface waters particularly in relatively unpolluted streams and lakes, since laboratory conditions may not simulate the natural environment where light, temperature and movement of the water affect the dissolved oxygen.

I. Detection of nuisance bacteria in water

1. Due to inorganic substances

Some organisms can use inorganic substances in water and form coloured compounds, and may at times grow sufficiently to block pipes, e.g., some species of *Gallionella* and *Hyphomicrobium* which respectively deposit iron and manganese around their cells. Enumeration of such bacteria is difficult although isolation is possible (Tyler & Marshall 1967).

Species of *Thiobacillus* that produce sulphuric acid from sulphur and certain compounds of sulphur are the cause of acid mine waters which may cause problems where mine wastes drain into other water ways. The acidity is usually of greater significance than the bacterial growth. Methods for detection of some of these organisms is given in APHA (1975).

2. Detection of organic substances

Some species of bacteria, such as actinomycetes, may give water a musty smell or earthy taste without apparantly causing harm. Detection of these organisms is best achieved by the dilution plate count technique (APHA 1975).

IV Deficiencies of the available methods

No one method is satisfactory for examination of all water and waste water samples, thus for example the BOD test is suitable for load assessment of sewage and other effluents but not for assessing whether pathogens are likely to be present. Further, most methods require considerable manipulative work, and the time lapse before obtaining results is considerable. Many of the techniques have other difficulties, thus the limitations of the test may not be appreciated by the person carrying it out, or the results may be used incorrectly. For example in the estimation of number of coliforms made by the multiple tube most probable number method, the result is not a definitive count but is only what the name implies, a most probable number estimate; the confidence limits of the results are wide and should be appreciated as such.

Difficulties also arise if modifications to the method are introduced without an appreciation of the basis of the test. In some laboratories the number of tubes in the multitube test have been reduced, yet unbelievably, the same statistical tables were used to read the results. While this is not a deficiency of the test, the fact that the basis for the test can be so easily misinterpreted suggests that the method is far from ideal for routine testing.

Similarly it is very easy for the membrane filter technique to be misused, the operator failing to appreciate that different media and filters will give different results. The fact that membranes having more than 200 colonies on them should not be used in estimating numbers because of the interference effects, is often overlooked. The most commonly voiced criticism of the currently used tests is that the techniques are too cumbersome for the large numbers of samples that need to be tested.

V New techniques and the need for further research

In the last few years several new methods for testing water and waste water quality have been suggested. Most are aimed at detecting *E. coli* more rapidly, e.g., the radiometric methods for detecting coliforms (Bachrach & Bachrach 1974) and the use of fluorescent antibodies to detect *E. coli* (Abshire & Guthrie 1972). Others are based on detection of sterols peculiar to faecal matter (Dutka *et al.* 1974). Most of these methods require expensive equipment, but if they are proved more sensitive and reliable than present methods they may be used in central laboratories, where large numbers of samples could then be handled.

Even finding a sensitive, rapid method for detecting bacteria may not be the final solution. There is a need to establish a method for detecting viruses in water, since methods designed for detection of bacteria may not be sensitive enough to indicate the presence of viruses. The literature on virus detection is large: many of the problems are discussed in the publication edited by Malina & Sagik (1974). So far no satisfactory methods exist for routine detection of viruses in water or waste water although tentative methods are described in APHA (1975).

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