EARLY WARNING ASSAYS: AN OVERVIEW OF TOXICITY TESTING WITH PHYTOPLANKTON IN THE NORTH AMERICAN GREAT LAKES

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MANAGEMENT PERSPECTIVE

The use of phytoplankton as test organisms in bioassays has recently gained momentum due to their simplicity, availability, sensitivity, rapidity of analysis, and cost-effectiveness. Increasing emphasis is currently being given to field and *in situ* experiments using indigenous populations, particulary ultra-plankton/picoplankton (20-2 μm) which play a key role in the 'microbial loop' and food chain dynamics. Impact evaluation can be determined at the structural, ultrastructural, and functional level. An array of techniques is available for toxicity testing including the use of either algal cultures or natural assemblages in laboratory or *in situ* experiments, the selection of which depends on the objectives, precision required, and project budget of the particular study. An overview is presented of the various procedures using algae in toxicity testing with a focus on the Great Lakes and an emphasis on field techniques. The effective use and application of such sensitive technology has tremendous potential for early warning detection of ecosystem perturbations in concert with a multi-trophic battery of tests.

PERSPECTIVE-GESTION

Depuis quelque temps, le phytoplancton est de plus en plus employé dans les épreuves biologiques, car il est composé d'organismes simples qu'il est facile de se procurer et qui permettent une analyse sensible, rapide et d'un bon rapport coût-efficacité. On s'intéresse maintenant de plus en plus à l'expérimentation sur le terrain, in situ, portant sur des populations indigènes, et plus particulièrement sur l'ultraplancton et le picoplancton (20-2 um), organismes qui jouent un rôle clé dans la "boucle microbienne" et dans la dynamique de la chaîne trophique. On peut évaluer les effets aux points de vue structurel, ultrastructurel et fonctionnel. Il existe tout un ensemble de techniques pour évaluer la toxicité en utilisant notamment des algues en culture ou des assemblages naturels en laboratoire ou in situ, le choix dépendant des buts visés, de la précision recherchée et du budget dont on dispose. On présente ici les différentes méthodes d'évaluation de la toxicité dans lesquelles on emploie des algues, en traitant plus particulièrement du cas des Grands

Lacs et en insistant sur les techniques d'expérimentation sur le terrain. Une technologie d'une telle sensibilité offre des possibilités extrêmement intéressantes au point de vue de la détection rapide des perturbations de l'écosystème, en combinaison avec une batterie d'épreuves multi-trophiques.

INTRODUCTION

There is a growing global concern over the increasing contamination of our aquatic environments from municipal, industrial, urban, and agricultural sources. This problem has been further complicated by atmospheric pollutants crossing international boundaries and spreading as far as the Arctic regions. However, it is encouraging to see that concern for our environment and a desire to seek solutions has been voiced on an international level (World Commission Report on Environment and Development 1987) and on a national level in Canada (Canadian Environmental Protection Act 1988). The traditional pollution control programs thus far have been confined mostly to the reduction of nutrient enrichment, and very little has been accomplished on the decontamination of toxic substances such as organics and metals in the aquatic environments.

It is now well-established that the impact of contaminants cannot be effectively evaluated solely from chemical analysis, because this approach does not provide the vital data concerning their bioavailability. It is also accepted that an ecosystem approach must be adopted to achieve a more holistic perspective of both the environment and its inhabitants. Such an assessment of the impact of contaminants in an ecosystem is best carried out using aquatic organisms from various trophic levels in comparative bioassays (Gachter 1979; Maciorowski et al. 1981; Bringmann & Kuhn

1980). In the Great Lakes, a multi-trophic battery of tests has been standardized for the bioassessment of Great Lakes "Areas of Concern" (Munawar et al. 1989c; I.J.C. 1987) which includes algal toxicity testing. Although the use of algae in bioassays is not new (Allen & Nelson 1910; Schreiber 1927), it is only recently that these organisms have attracted the attention of toxicologists and regulatory agencies (Rai et al. 1981; Munawar & Munawar 1987). This increased awareness of algae is due to their vital role in the "microbial loop", food chain dynamics, and trophic interactions (Sherr & Sherr 1988; Ross & Munawar 1987; Munawar et al. 1989c). Furthermore, the suitability of algae as test organisms is gaining support due to their structural simplicity, ubiquitous abundance in nature, and the ease of obtaining commercially available algal cultures for laboratory testing (Munawar et al. 1988a). Also, algal toxicity tests are rapid, inexpensive, and sensitive, and can be used effectively to assess those toxic substances which are found in concentrations too low for effective detection by higher trophic level organisms (Munawar & Munawar 1987; Wong & Couture 1986). Phytoplankton in their natural environments, unlike other organisms, are affected directly by both nutrients and contaminants due to their uptake kinetics.

This paper resulted from an invitation by the Conference Committee to review the state-of-the-art use of algae in toxicity testing, with an emphasis on field/in situ investigations. Here we provide a general overview of techniques currently being used for the assessment of environmental hazards caused by both metals and

organic contaminants. A more detailed overview is available elsewhere (Munawar et al. 1988a).

The phytoplankton health assessment strategy in this paper has been conveniently divided into the following categories (Figure 1):

1. Structural indicators (community structure)
2. Ultra-structural indicators (cytological)
3. Functional indicators (physiological/biochemical)

In addition, we also describe new techniques having considerable potential for future environmental hazard assessment. Examples from the North American Great Lakes have been focussed on to demonstrate the usefulness of some of the techniques discussed in this review.

Structural Indicators

Some of the common structural indicators are given in Figure 1. It is apparent that the structural aspect is heavily dependent on standard, microscopical, analytical techniques that utilize all size components of phytoplankton such as picoplankton (<2 um), ultraplankton (2-20 um) and microplankton/netplankton (>20 um). It has been demonstrated that picoplankton and ultraplankton are sensitive to contaminants such as metal mixtures (Munawar et al. 1987a). However, the time-consuming, meticulous nature of taxonomic identification, the scarcity of trained phycologists (Munawar et al. 1987b; Munawar & Munawar 1980) and a lack of standardized, data-processing procedures are all limiting factors in generating

long-term and consistent floristic records to evaluate changes in the community structure. Such long-term species data sets are generally limited in the Great Lakes (Vollenweider et al. 1974; Munawar & Munawar 1986; Stoermer 1978) and as a result, the structural response of phytoplankton to contaminants has received very little attention (Rhee 1988). However, it is encouraging that such studies of structural response are being initiated in the Great Lakes "Areas of Concern" (I.J.C. 1987) such as Toronto Harbour (Munawar 1989). Furthermore, the size assemblage shifts (Munawar et. al. 1987b; 1988b) and biomass spectral changes in contaminated (nearshore) versus offshore areas (Sprules & Munawar 1986; Sprules et al. 1988) are emerging. Traditional microscopy is also now combining with microcomputer-assisted devices to measure size structure. Also, knowledge concerning the "microbial loop" and trophic interactions has rapidly increased since the classical papers by Azam et al. (1983), Porter et al. (1985), Stockner (1988), and Sherr & Sherr (1988). In the North American Great Lakes, Munawar et al. (1987c) and Munawar & Weisse (1989) have demonstrated the sensitivity of autotrophic picoplankton to nutrients and contaminants.

Ultra-structural Indicators

Ultrastructural indicators are worthy of discussion because they represent a field of considerable potential for bioassay technology. It has been recognized since the early years of

electron microscopy that the structure of cell components such as organelles, microtubule arrays, membrane systems, fibrils, and various granules can be interpreted in terms of the compartmentalization of cell function (Brinkley & Porter 1977). In some cases, at a resolution approaching 0.001 μm, the structural-functional relationships can be confirmed down to the level of identifiable macromolecules such as enzymes (phosphatases) (Blum et al. 1965; Dodge 1973), individual structural components (cellulose microfibrils) (Preston 1974), and storage materials (starch granules) (Dodge 1973; Gibbs 1971). Thus, an ultrastructural response to an environmental insult can yield more than just a correlation. Because it is a sensitive measure of a physiological change, such a response can be used inferentially as a guide for selecting measures of both physiological and anabolic responses which might otherwise not be considered. Also, it has potential for permitting development of a structural index of health of unicellular and pauci-cellular organisms. In the case of picoplankton, such a research thrust has already begun (Leppard et al. 1987).

Let us examine the basis for the general statements above and the potential opportunities that they present through technology transfer from cell biology. Several cell compartments of relevance to algal productivity have been "dissected" in such a way that an organelle dysfunction can be diagnosed by an examination of ultrastructure. In addition to the large specialized literature on this subject, several decades of general reference works have

been produced which adequately introduce the subject to the non-specialist who has an interest in transferring the technology into the aquatic sciences. Among these are early general works on structure-function relationships in cell membranes (Rothfield 1971), chloroplasts (Gibbs 1971), and mitochondria (Lehninger 1965). The abundant literature of general findings on these major structures (see Journal of Cell Biology, Journal of Cell Science and Protoplasma) is applicable to algal cell membranes, chloroplasts, and mitochondria; some of these findings were based directly on algal experiments. With respect to the normal ontogeny and/or experimental perturbation of algal intracellular compartments there have been revealing studies on: (A) intracellular membranes in relation to the spatial orientation of organelles (Bouck 1965); (B) assembly and disassembly of the microtubular cytoskeleton (Brown & Bouck 1973); (C) extracellular secretion by the Golgi apparatus (Brown et al. 1973); (D) flagellar structure in relation to flagellar motion (Gibbons 1977; Bouck 1971); (E) the regulation of gas vacuole activity (Walsby 1972); (F) structure-chemistry-function associations in cell wall growth (Preston 1974); and (G) analyses of many other compartments and sub-compartments of relevance to algal productivity in nature (Dodge 1973). As Dodge (1974) has shown, one can classify, at least on a crude scale, many unicellular and small algae by an examination of ultra-structure at high resolution.

The literature above from cell biology provides limited information to the ultra-structural biologist in diagnosing

physiological alterations caused by environmental insults. Despite the limitations, the microscopical technology (a combination of optical microscopy, scanning electron microscopy, transmission electron microscopy, cytochemistry, and energy-dispersive spectroscopy) can be used to assist the aquatic biologist in selecting assays to delineate a biochemical, physiological, and ecotoxicological response by algae to contaminants. This technology transfer has been little exploited in the past, despite the fact that the limitations are not severe.

At this point, two notes of caution are necessary. Firstly, the specialized literature created by the cell biologists is based mainly on whichever algal species was most amenable, for a given kind of study, to the analytical techniques of the moment. Consequently, an incomplete effort was made to provide a coverage of algal types based on their importance to natural ecosystems. Technology transfer may require some adaptation before it can be applied to a species that was of little cytological interest in the past. Secondly, many of the most helpful descriptive works were done several decades ago. Thus, a descriptive work up on a species whose cytological literature is incomplete might require a manual expertise which is great and a time frame which is long.

Functional Indicators

Most toxicity testing falls into the functional indicators category since the assays are rapid, sensitive, and cost-effective

and serve as early warning indicators of ecosystem health. A large variety of physiological and anabolic tests are available to choose from depending on the type of problem, precision required, and budget. Figure 2 summarizes bioassays that are commonly done in laboratory and field/in situ situations. The experiments are conducted using algal cultures, natural assemblages, and in situ procedures such as cages, enclosures, etc.

Impact of metals:

Assays conducted with cultures grown in the laboratory are useful in providing basic information on physiological limits for individual species (Braek et al. 1976; Wong et al. 1978), but the data are extremely difficult to extrapolate to a natural situation due to inherent environmental interactions in each ecosystem. Consequently, our laboratory has adopted a field-to-laboratory approach and an effort has been made to use fresh, natural phytoplankton assemblages as test organisms wherever possible. Several laboratory techniques are available in evaluating the impact of toxicants to phytoplankton. These include in vitro batch and continuous cultures, with the latter using turbidostats or chemostats. Details of these techniques are provided by Malek & Fencl (1966), Rhee (1982), Wong et al. (1983) and Munawar et al. (1988a).

Very little information is available on the interactive effects of metal mixtures on algae, although there is abundant literature available concerning the toxicity of individual metals (Gachter 1976; Rai et al. 1981; Lustigman 1986). Synergistic and

antagonistic effects have been reported which shed considerable light on the complexity of metal mixture toxicity (Gachter 1976; Bartlett et al. 1974; Hutchinson & Stokes 1975; Wong et al. 1978; Munawar & Munawar 1982).

The most common assay used with natural assemblages in the Great Lakes was either ^{14}C size-fractionated primary productivity monitoring, or Algal Fractionation Bioassays (AFB). The impact of various toxic materials, such as sediment-bound or waterborne contaminants and synthetic metal mixtures, on phytoplankton size assemblages was assessed (Munawar & Munawar 1982; 1987; Munawar et al. 1983; 1987a). In total phytoplankton community assays, the impact of contaminants on various components of the test community was either missed or masked (Munawar 1982; Lane & Goldman 1984). In contrast, the AFB simultaneously focussed on the effects of toxic substances on various size fractions by isolating a natural assemblage of diverse species with a wide variety of sizes, physiological requirements, and environmental tolerances. Several papers cited above demonstrate the observed differential response of various size assemblages to contaminant stress and indicate the usefulness, convenience and ease of performing this rapid and sensitive procedure. We have successfully used size-fractionated, primary productivity as an overall indicator of ecosystem health and as a biomonitoring tool for initially understanding an ecosystem (Munawar et al. 1989c). A large number of assays with sediment elutriates and filtered versus unfiltered water was done using offshore phytoplankton as a test population (Munawar et al.

1989c).

As a result of an extensive AFB program, techniques concerning differential filtration, isolation, and concentration were developed. These techniques were used in conjunction with epifluorescence microscopy. Johnson & Sieburth (1982) fractionated organisms to sizes less than 2 um (picoplankton) while we isolated and concentrated organisms with a Gelman 1.2 um Acroflux capsule (Munawar et al. 1987c). These 1.2 um organisms were used in ^{14}C picoplankton bioassays with metal mixtures and the resulting primary productivity was found to be extremely inhibited by the addition of these toxicants. Recent work by Munawar & Munawar (1987) and Munawar & Weisse (1989) indicated that autotrophic picoplankton were scarce or lacking in contaminated environments of the Great Lakes such as the Niagara River, Toronto Harbour, and Hamilton Harbour. The autotrophic picoplankton, on the contrary, were abundant in oligotrophic and mesotrophic systems such as Lakes Superior and Ontario (Munawar et al. 1987b).

Impact of Organic Contaminants:

Very little information is available concerning the impact of organic contaminants on phytoplankton although these toxicants pose a serious threat to fisheries. Field data is lacking and even laboratory work is limited (Rhee 1982; Munawar et al. 1988a). There is a paucity of historical records of phytoplankton abundance and it is difficult to isolate the simultaneous impact of other factors such as nutrient enrichment, food chain changes, and the introduction of exotics which may alter the predator-prey

relationship (Rhee 1988). These problems are dealt with in a detailed review of Great Lakes research by Munawar et al. (1988a). Both enhancement and inhibition of primary productivity due to organic contaminants was observed (Rhee 1982; Wright 1978; Lal and Saxena 1982) although the mechanism of these impacts is not clearly understood. Like the bioavailability of metals, the susceptability of algal species to organo-chlorine compounds results in the community structural changes of relatively resistant species. The impact of synergistic, antagonistic, or additive effects is not well-known for the organic contaminants (Rhee 1988) and much needs to be done in this area of research.

The results of the impact of organic contaminants should receive the immediate attention of researchers due to the obvious human health risks. For example, in the Great Lakes, data has been generated over a number of years for thousands of organic contaminants and a disproportionate number of "non-detectable" values were observed (D. Sergeant, Fisheries & Oceans Canada - Personal Communication). The cost and time to perform the analyses were considerable for minimal gain. Furthermore, other factors such as detection limits need resolution for various organics. In essence, the lower the detection limits, the greater the time and cost of analysis. The "non-detectable" data of the field samples speaks for itself and suggests that the issue of chemical analysis, particularly organic substances, needs a review and change of approach. For example, an alternate but scientifically and economically sound approach could be to have the field samples pre-

screened by means of rapid, sensitive, and cost-effective bioassays to identify samples with toxic effects which then could be subjected to intensive organic analysis. This could be followed by testing with chronic assays. Then, the pre-defined, compound-specific screening of organics could be changed since many acutely or chronically toxic compounds might not be detected and may be treated as interference (D. Sergeant & M. Munawar, Fisheries & Oceans Canada, unpublished data).

Field/In situ and New Techniques

The use of natural assemblages is favoured over laboratory-grown cultures because extrapolation of laboratory data to natural conditions is often difficult and misleading. Toxicity tests using natural assemblages compared to laboratory-grown cultures have yielded results showing enhancement of primary productivity in natural phytoplankton, while the same test indicated inhibition in mixed cultures (Munawar & Munawar 1987).

Our laboratory differentiated between "field" and "in situ" experiments. The former tests utilize natural, as opposed to cultured, phytoplankton but the experiments were conducted in incubators aboard ships. The latter, or in situ experiments, were conducted in the original test site, without the benefit of incubators or other simulating devices. In the past, field/in situ work in the Great Lakes (Figure 2) consisted mainly of incubations in polycarbonate bottles under constant light regimes at ambient

lake temperatures (Vollenweider et al. 1974). For some assays, the bottles were exposed in the lake on moorings to assess the impact of contaminants on the phytoplankton assemblages at various depths in the water column (Munawar et al. 1988a). Flow-through bottles with Nitex nets have been used in Ashbridges Bay, Ontario, by the Ministry of the Environment to assess the impact of effluents on phytoplankton biomass and species composition (K. Nicholls and L. Heintsch, personal communication). Other methods have included various types of enclosures, bags, microcosms/mesocosms, and cages (Goldsborough et al. 1986; Jensen et al. 1972; Gachter 1976; Munawar & Munawar 1987; Munawar et al. 1989c).

The field/in situ assessment of contaminants in the Great Lakes utilizing algae as test organisms included the following effluent impact assessment, dredging/disposal impact assessment, and navigational impact assessment. The effluent impact assessment was conducted in Ashbridges Bay (Munawar 1989) using size-fractionated primary productivity and filtered versus unfiltered assays with offshore phytoplankton. The dredging and disposal assessment dealt with monitoring productivity for pre-dredging and post-dredging activities (Munawar et al. 1989b). Navigational impacts were evaluated using a similar approach with bioassays being conducted before and after ships' passage in Toronto Harbour (Munawar et al. 1989b).

The environmental bioassay technology is advancing rapidly as a multi-trophic and multi-disciplinary approach and bioassessment is no longer confined to fish lethality testing. Algal assays,

ignored in the past, are again receiving considerable attention from both academic and industrial researchers. The assays are proving to be sensitive, rapid, and cost-effective. Moreover, the resolution of food chain dynamics along with the microbial loop concept is another valid reason for the study of algae as an effective and early warning indicator of contamination and ecosystem health. Several new techniques (Figure 2) are developing such as video analysis systems, in situ plankton cages, limited sample bioassays, epifluorescent microscopy, and flow cytometry (Munawar et al. 1989a; 1989c; Munawar & Munawar 1987; Weisse 1989a; 1989b; Yentsch et al. 1984; Berglund & Eversman 1988). These new techniques, together with a wide variety of existing procedures and computer-assisted methodologies, provide an excellent array of tests to assist in environmental protection and conservation of endangered aquatic environments.

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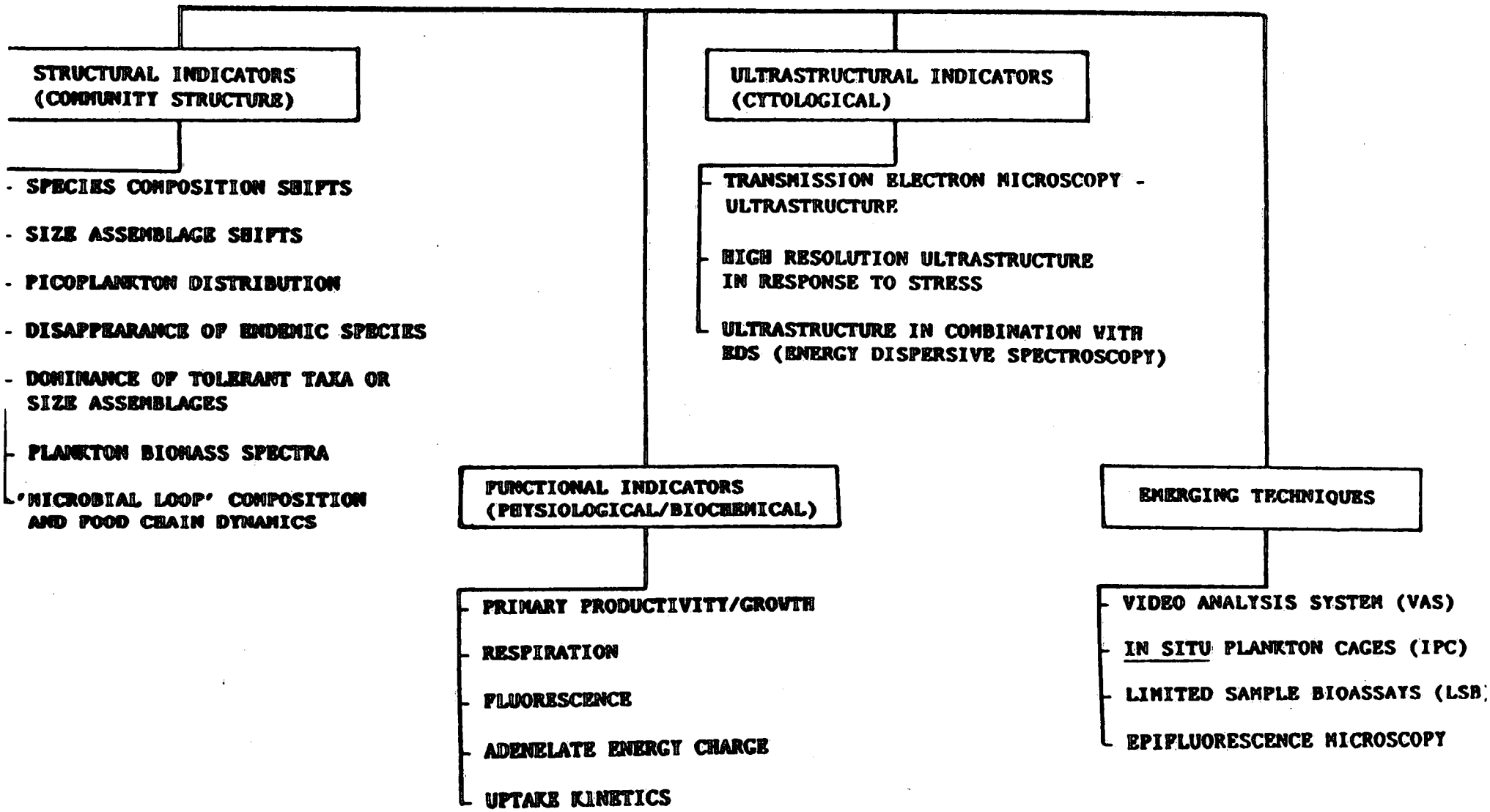
PHYTOPLANKTON HEALTH ASSESSMENT



PHYTOPLANKTON BIOASSAYS

LABORATORY

ALGAL CULTURES

1 Batch cultures

2 Continuous cultures (turbidostat/chemostat)

3 Microplate algal assay

4 Selenastrum capricornutum (carbon-14 uptake)

5 Ankistrodesmus bibrianus (two-chamber device)

6 Video analysis system (fluorescence)

7 Limited sample bioassay (LSB) (with bottom or suspended sediments)

NATURAL ASSEMBLAGES

8,9 Algal fractionation bioassay (AFB)

Carbon-14 uptake
Biomass
Pigments

10 Picoplankton assay (carbon-14 uptake)

11 Picoplankton (epifluorescence)

12 Biosensors

13 Video analysis system (fluorescence)

FIELD/IN SITU

13 Bottles/moorings

14 Flow-through bottles

15 Periphyton enclosures

16 Dialysis bags

17 Microcosms/Mesocosms

11 In situ plankton cages

18 Effluent impact assessment (municipal/industrial)

19 Dredging/disposal impact assessment

11 Navigational impact assessment

NEW TECHNIQUES

11 Video analysis system (VAS)

11 In situ plankton cages

7 Limited sample bioassays (LSB)

20,21 Flow cytometry

Numerals indicate citations relevant to the techniques given in the references.

1 Rhee 1980;
1,2 Malek & Fencl 1966;
3 Blaise et al 1986;
4 Ross et al 1988;
5 Ahtl et al 1989;
6 Mayfield & Munawar 1988;
7 Munawar et al 1989a;
8 Munawar & Munawar 1982;
9 Munawar et al 1987b;
10 Munawar et al 1987a;
11 Munawar & Munawar 1987;
12 P.Hansen, personal communication;
13 Munawar et al 1988a;
14 K.Nicholls, personal communication;
15 Goldsborough et al 1986;
16 Jensen et al 1972;
17 Gachter 1976;
11 Munawar & Munawar 1987;
18 Munawar 1989;
19 Munawar et al 1989b;
11 Munawar & Munawar 1987;
20 Yentsch et al 1984;
21 Berglund & Eversman 1988.

FIG. 2.