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SYMPOSIUM

PROTOZOA AS INDICATORS OF ECOSYSTEMS

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The activities of mankind which introduce excess nutrients and/or pollutants of various kinds into lakes, streams, estuaries, soils and the air, can, and often do, cause significant changes in aquatic and terrestrial environments world-wide. The pollution problem is critical because of the pressures of increasing populations, industrial growth and intensification of agricultural production. Conflicts of interest arise everywhere as challenges to resource use and natural state ('beauty') are turned into public issues. Are all changes harmful?... destructive? How do we measure change? Is there an urgency for us to find out and measure it? Can we reverse deterioration which has already taken place? How do we measure restoration? How do we control and regulate point sources of pollution? How can we tell whether regulated potential pollution sources are complying with abatement procedures? What are the criteria of 'acceptable' risks or damages? These questions, and many more are not simple matters of common-sense, administrative judgement or precise scientific endeavor ... they call for wisdom and an amalgam of talents which is only now being developed in our society.

The holding of this 7th International Congress of Protozoology for the first time in a developing nation seemed reason enough to take stock of what protozoologists were doing to involve themselves in the assessment of ecosystems. The timeliness of this symposium topic was counterpointed by the publication in *Science*, just the day before the Congress opened, of a case on the effects of years of experimental acidification of a small Canadian lake (Schindler *et al.*, 1985). The study which simulated the effects of acid rain was carried out over an 8 year period by adding sulphuric acid. As the researchers found out, without surprise to protozoan or algal ecologists, the first irreversible disturbances to the 'simple organisms' were taking place even before significant pH changes were noted. The mass of the phytoplankton remained relatively constant, but new species appeared, and the numbers of organisms small enough to be eaten by the indigenous zooplankton declined as the food web began to be less well integrated. Filamentous algae eventually bloomed and the normal prey of the lake trout (shrimp, minnows and crayfish) died. The shrimp died from hydrogen ion toxicity; the minnows became less fecund at low pH; and crayfish egg masses were infested with a fungal parasite. The trout starved, became more cannibalistic and researchers predicted that within a decade the lake would become fishless.

Some protozoologists have long recognized the potential use of the organisms they study as ecological indicators. The foraminifera in particular have been especially useful in this regard since their skeletal remains in undisturbed recent sediments can trace distribution patterns for centuries or millenia prior to the introduction of anthroprogenic effects (e.g. Watkins, 1961; Bandy *et al.*, 1965; Roa and Roa, 1979; Schafer, 1970; Setty and Nigam, 1984; Sieglie, 1971). There really is no agreement among protistan ecologists as how best to apply our knowledge to the assessment of ecosystem stress. The speakers for the symposium were chosen to represent different points of view, approaches and applications.

One point of view was presented by Ernst A. Nusch, of the Laboratory of the Ruhr River Association. His approach, which is representative of a large portion of the scientific community, is to select particular organisms which represent sensitive functions in the ecosystems of concern and then perform bioassays under very precise and reproducible conditions. As he pointed out, time has long passed since there have been proponents who believed it was possible to find the 'most sensitive organism' in an ecosystem. Through his efforts a standardized *Paramecium* bioassay has been accepted for inclusion in the batteries of useful test organisms used by the international community for toxicity testing.

Cultures of *Paramecium caudatum* used for testing are grown in an aqueous suspension of dried turnip chips (0.5 g/l). Test organisms are harvested at the end of the exponential phase (approx. 2 weeks) and transferred to standard dilution water used for the bioassays. Knowledge of the well known geotactic behaviour of *Paramecium* is used to separate them from their turnip growth medium. Cultures are placed in a separatory funnel connected to another funnel filled with the artificial test water. When the cocks between the funnels are opened the protozoa swim up into the test medium. This procedure avoids the possible deleterious effects which might be introduced by filtration or centrifugation of the paramecia.

Clear cut results for *Paramecium caudatum* acute bioassay tests are available in 24–48 hr. The test criterion is mortality after 24 hr as compared to a control batch without test substance. The organisms are counted microscopically after lugol-fixation. Three concentration levels are useful as indicators: (1) LC_0—the highest concentration of the test substance without lethal effects; (2) LC_{50}—the concentration of the test substance which kills 50% of the population in 24 hr; (3) LC_{100}—the concentration of the test substance which kills all of the test population in 24 hr. Dr Nusch showed the audience impressive examples of the results of this toxological bioassays and their reproducibility. Some of these results have been published (Nusch, 1982). The discussion that followed raised the question: What does a 24 hr LC_{50} of *Paramecium caudatum* mean for the functioning of the ecosystem as a whole? The suggestion that microcosm bioassays might be more representative of ecosystems fell to the same criticism as single species tests. No matter what views the discussants expressed there was consensus that acute bioassays with single species under constant conditions had the advantage that they could yield accurate and reproducible results.

Dr Wilhelm Foissner, Institut fur Zoologie, University of Salzburg, Austria, showed how the knowledge of soil protozoa is easily applied to evaluation of terrestrial ecosystems. Although terrestrial protozoa are often ignored they can be an important, or even a dominant, part of the standing crop of soil fauna. Like plants, protozoa can be used for soil and site characterization. Experimental field and laboratory studies show that soil testacea and ciliates react very quickly to changes in soil water content and agricultural practices such as soil compaction or aeration, fertilizers, pesticides and acid rain (Figs 1–3). Changes are recognizable in species abundances and diversity. As examples he cited studies of testacea and nematodes from alpine pasture plots in which the top soil was removed. Even 5–7 years after the grading, the abundance and biomass of the testacea and nematodes was only 10% of controls. Diversity was down by 50%. Experimental compaction of soils reduced soil protozoa considerably. Ciliates were more sensitive to compaction stress than the testacea. In studies of fertilized farming plots the earthworms seemed to be unaffected by conventional farming practices, while organic farming produced higher biomass and abundance of testate amoebae and nematodes. Acidification of soils reduces the abundance and diversity of soil protozoa. Restoration after 'acid rain' is rapid after application of lime. Protozoan abundance and species richness doubled within a few months after application. The discussion which followed Dr Foissner's presentation centered on the seemingly relative lack of interest in this aspect of protozoology and its application. One possible reason brought out in the discussion was that there are really no convenient single sources of information on soil protozoa and their identification.

The remaining symposium speakers used colonization methods to assay responses of freshwater communities to environmental stress. Dr James R. Pratt, University Center for Environmental Studies and Department of Biology, Virginia Polytechnic Institute and State University U.S.A., described the conceptual aspects of this approach. He uses an artificial substrate, pieces of polyurethane foam, as the vessels in which protozoan microcosms are allowed to develop and are experimentally manipulated. Those who follow this approach believe that toxicity testing based on target species is insensitive to important interactions and compensatory reactions of many interlocking species which are present in complex natural communities. They also believe that their microcosms contain enough important components to exhibit properties and processes which occur in the ecosystems they attempt to model. In the hands of properly trained practitioners, community composition and diversity seem very sensitive biological indicators of environmental change. Protozoa are easy to harvest from polyurethane foam units (PFU). Gentle squeezing is all that is necessary. Dr Pratt described the research that he, Dr John Cairns Jr, and the rest of their group at Virginia Polytechnic Institute were doing with PFUs. Multivariate analyses comparing community structure to abiotic factor showed that protozoan communities are capable of distinguishing small differences in water quality. He also gave examples of how PFUs were used as actual evidence of ecosystem stress or recovery.

Dr Shen Yunfen, Institute of Hydrobiology, Academia Sinica Wuhan, PRC, described the studies she did with Dr Cairns and his group at Virginia Polytechnic Institute and those following her return to China. Her study site in Virginia was a small stream near Backsburg called Cedar Run. The stream receives storm water run off and discharged effluents from a sewage treatment plant and an electroplating plant (from which the effluent contains heavy metals, cyanide, chloride and sulphides). Testing with PFU communities was carried out in both the field and in the laboratory.

The MacArthur–Wilson equilibrium model was used to explain colonization of PFUs in stations in the presence of stream-borne toxicants. The presence or absence of certain protozoan texa also provided some indication of stress. For example, *Coleps* was absent in samples stressed by heavy metals. Pollution intolerant taxa such as *Mallomonas* and *Cryptomonas ovata* were present only at reference or extreme downstream stations.

Predictions based on the results of laboratory tests of effluents on protozoan communities in PFUs were in good agreement with the results obtained by incubating PFUs at appropriate stations in Cedar Run. In China she applied PFU methodology to a series of five oxidation ponds associated with Ya-Er lake in Hubei Province, 40 km from Wuhan. Since 1962 the lake has been polluted by wastewater from a pesticide plant which produced parathion, malathion, dimethaote and HCH (hexachlorocyclohexane). Operational experience with the serially linked ponds has shown that the system is quite effective in pollution control. Removal efficiencies for the pesticides range from 77 to 98%. The total numbers of bacteria decreased serially in ponds as they became

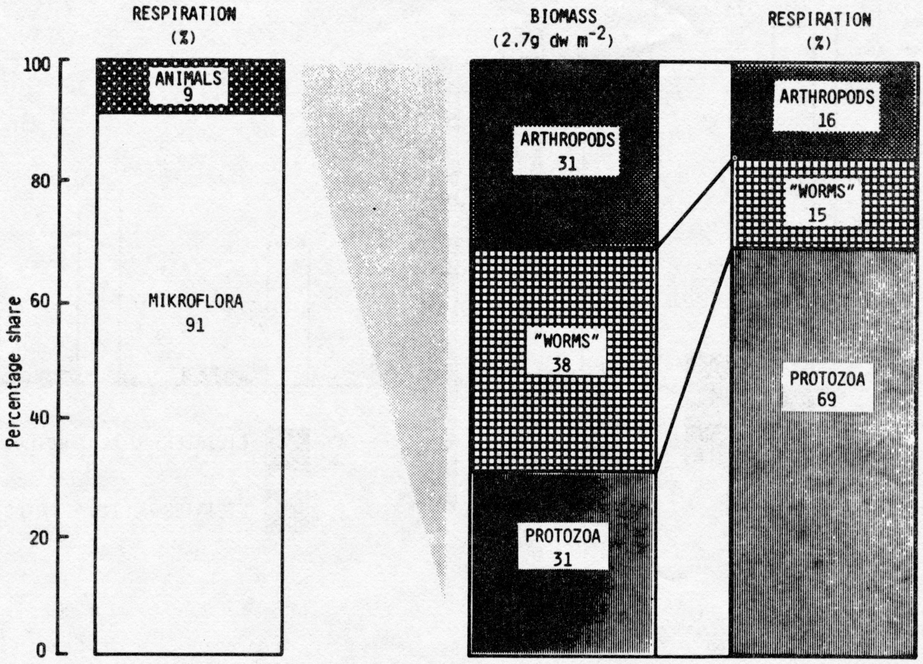

Fig. 1. Contribution of the protozoa to the biomass and respiration of the soil animals. The graphs show the mean of 14 ecosystem studies from various sites of the world. From Foissner (1986).

further removed from the pesticide plant. Total chlorophyll, overall respiration rate of the microbial community and species diversity in the protozoan community increased as ponds became further removed. She studied the cost effectiveness of various sampling protocols and decided that examining 1 or 3 day PFU protozoan communities is good enough for routine biological monitoring. During the discussion which followed her paper she discussed PFU training programmes she conducted in China and how PFU monitors detected various pollution accidents.

Dr M. Cs. Bereczky, Hungarian Danube Research Station of the Hungarian Academy of Sciences, Göd,

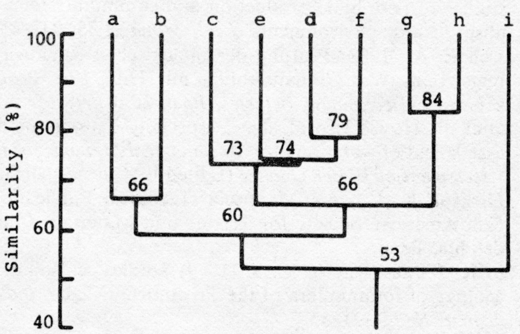

Fig. 2. Habitat discrimination (clustered species similarity index of Sørensen) by soil ciliates. (a) Twelve sites in the Austrian Alps (Glockner area); (b) 11 sites in the Austrian Alps (Gastein area); (c) 2 corn fields (Austria, Tullnerfeld area); (d) 2 xerothermic uncultivated grasslands (Austria, Tullnerfeld area); (e) 2 humid lowland sites (Austria, Tullnerfeld area); (f) 1 beech forest (Austria, Tullnerfeld area); (g) 4 corn fields (Austria, Salzburg area); (h) 6 meadows (Austria, Salzburg area); (i) 2 spruce forests (Germany, Ulm area). From Foissner (1986).

Hungary, described the aufwuchs approach to water quality evaluation. Microscope slides were fastened into plastic frames and then submerged in the Danube at different depths. Samples were removed from the river 2, 4, 8, 16 and 32 days following exposure. The slides were examined microscopically and species diversity (H) and evenness (E) were calculated (Bereczky *et al.*, 1983). The temporal development of the protozoan communities was in agreement with classical island colonization theory (MacArthur and Wilson, 1967). The sessile community reached its most developed state on day 8, after which, more mobile species became more numerous with diatom communities as their focus. Aufwuchs or periphyton (diatometer) monitors have long been used as routine water quality assay tools (e.g. McIntire and Phinney, 1965; Patrick, 1973; Rodgers *et al.*, 1978). Dr Bereczky pointed out that examination of protozoan communities which develop on such slides would add much more information and perhaps finer distinction to this type of routine bioassay. The discussion that followed the presentation pointed out some of the shortfalls of both the aufwuchs and PFU methods. The former seems to 'catch' many more sessile colonizing species and bias observation away from the motile forms. The latter does the opposite. The sessile species are less likely to be discharged when the PFUs are squeezed in the sampling process.

In the general discussion which followed the symposium, the participants focused on the value of protozoa as indicators of water quality. There was general agreement that protozoan species diversity is as useful a monitor, as any other protistan group, in the routine assay of environmental quality. The drawback for protozoa, as is true for every group, is that there has been a general tendency in bio-

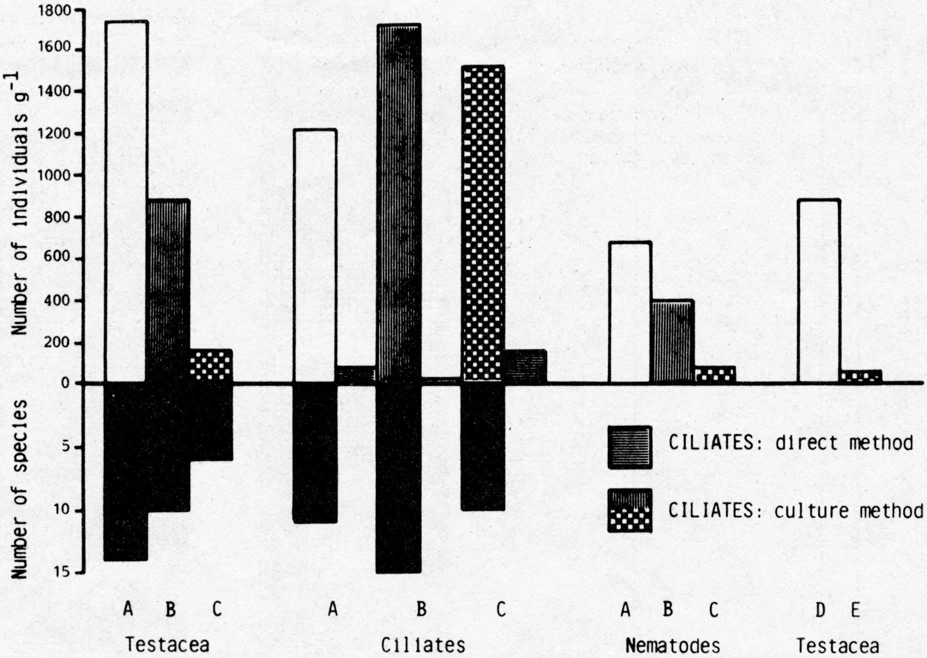

Fig. 3. Abudance and species number of the testacea, ciliates and nematodes in soils of graded ski runs in the Austrian Central Alps. A, B, C—3 grassland sites at the Schlossalm near Bad Hofgastein (Salzburg), about 1950 m NN. Abundance (individuals g^{-1} dry weight of soil) and species number were determined by direct observation in a watered soil suspension. In addition, the culture method of Buitkamp (1979) was used for the ciliates. The graphs show the mean of 3 samples during the vegetation period. The differences between all sites are significant ($P \leqslant 0.05$) for the testacea and the nematodes, but not for the ciliates ($P \geqslant 0.05$). A = undisturbed alpine pasture (control); B = marginal area of the ski trail, about 30 m to the west of site A; C = center of disturbance of the ski trail, about 50 m to the west of site B. Constructed from the data of Foissner *et al.* (1982). D, E—2 forest (*Pinus cembra*) sites at Obergurgl (Tyrol), about 2070 m NN. Abundance (individuals cm^{-3} soil) was determined by direct observation in a watered soil suspension. The graphs show the mean of 4 samples during the vegetation period. D = undisturbed forest (control); E = graded ski run without trees. Constructed from the graphs of Laminger *et al.* (1980). From Foissner (1986).

logical science for new students to shy away from taxonomically oriented or based studies. There was a perceived need to make the relevant taxonomic literature more easily available to neophytes who might consider protozoa as research tools.

REFERENCES

Bandy O. L., Ingle J. C. and Resig J. M. (1965) Modification of forminiferal distribution by the Orange County Outfall, California. *Ocean Sci. Ocean Engng* **1,** 54–76.

Bereczky M. Cs., Oertel N. and Nosek J. N. (1983) Structural investigations of periphytic protozoan communities in 3 layers of the Danube River. In *Periphyton of Fresh Water Ecosystems* (Edited by Wetzel R. G.). (W.) Junk, The Hague.

Buitkamp V. (1979) Vergleichende Untersuchungen zur Temperaturadaption von Bodenciliation aus klimatisch verschieden Regionen. *Pedobiologia* **19,** 221–236.

Foissner W. (1986) Soil protozoa: Basic problems, ecological significance, adaptation, indicators of environmental quality. In *Progress in Protozoology*, Vol. 1. Biopress, Bristol, U.K.

Foissner W., Franz H. and Adam H. (1982) Terrestische Protozoen als Bioindikatoran im Bodon einer planierten Ski-Piste. *Pedobiologia* **24,** 45–56.

Laminger H., Geisler-Moroder K., Siess A., Spiss E. and Spiss B. (1980) Populations dynamik terrestricher Protozoen (Testacea, Rhizopoda) in zentralalpine Lagen Tirols. I. Untersuchungen subalpiner Boden in Raum Obergurgel (Tirol/Osterreich). *Arch. Protistenk.* **123,** 280–323.

MacArthur R. H. and Wilson E. O. (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, N.J.

McIntire C. D. and Phinney H. K. (1965) Laboratory studies of periphytic production and community metabolism in lotic environments. *Ecol. Monogr.* **35,** 237–258.

Nusch E. A. (1982) Prufung der biologischen Schadwirkungen con Wasserinhaltsstoffen mit Hilfe von Protozoentests. *Decheniana-Beihefte, Bonn* **26,** 87–98.

Patrick R. (1973) Use of algae, especially diatoms in the assessment of water quality. In *Biological Methods for the Assessment of Water Quality* (Edited by Cairns J. Jr and Dickson K. L.), ASTM Special Technical Publications 528. American Society for Testing and Materials, Philadelphia, Penn.

Rao K. K. and Rao T. C. S. (1979) Studies on pollution ecology of foraminifera of the Trivandrum Coast. *Indian J. mar. Sci.* **9,** 31–35.

Rodgers J. H. Jr, Clark J. R., Dickson K. L. and Cairns J. Jr (1978) Nontaxonomic analyses of structure and function of augwuchs communities in lotic microcosms. In *Microcosms in Ecological Research* (Edited by Giesy J. P. Jr), U.S. Dept of Energy CONF 781101. National Technical Information Services, Springfield, Va.

Schaffer C. T. (1970) Studies of benthonic foraminifera in the Restigouch estuary: I. faunal distribution patterns near pollution sources. *Maritime Sediments* **6,** 121–134.

Schindler D. W., Mills K. H., Malley D. F., Findlay D. L., Shearer J. A., Davies I. J., Turner M. A., Linsey G. A. and Cruikshank D. R. (1985) Long term ecosystem stress: the effects of years of experimental acidification on a small lake. *Science* **228,** 1395–1401.

Setty M. G. A. P. and Nigam R. (1984) Benthic foraminifera as pollution indices in the marine environment of West Coast of India. *Riv. It. Paleont. Strat.* **89,** 421–436.

Sieglie G. A. (1971) A preliminary note on the relationships between foraminiferas and pollution in two Puerto Rican Bays. *Caribb. J. Sci.* **11,** 93–98.

Watkins J. G. (1961) Foraminiferal ecology around the Orange County, California, ocean sewer outfall. *Micropaleontology* **7,** 199–206.