

## EXECUTIVE SUMMARY

Protozoans are single cells that have evolved into some 30 000 species. They are of major ecological importance in that they consume bacteria and also in that many can encyst or excyst according to the conditions in which they live. Protozoans live in both marine and freshwater conditions and there are four major groups: the amoebae, the flagellates, the ciliates and the sporozoans. The sporozoans are parasitic and, although of immense importance, form a separate field of study – in medical and veterinarian research. They are not dealt with here.

Few studies have been done on protozoans in South Africa, the major work having been done in Europe, the United Kingdom and the United States.

The first component of this report is a literature review that establishes the potential uses of biomonitoring and the systems that are currently in use in South Africa. The South African Scoring System version 4 (SASS4) is currently being used to identify the water quality conditions in a river using the presence or absence of macroinvertebrate taxa. In some parts of Europe and in the UK a similar system, the Biological Monitoring Working Party scoring system is used. Other biomonitoring systems that have been proposed for South African rivers include the Fish Assemblage Integrity Index and the Riparian Vegetation Index. All of these systems require the presence of water in the system in order to produce useful results. SASS4 cannot be used in seasonally dry rivers, ground water and temporary waters, so a system is needed where the permanent presence of water is not crucial.

It would clearly be useful to have additional biomonitoring systems such as

- a backup system to run in parallel with SASS4 in rivers
- means of identifying particular types of pollution (SASS4 merely identifies a general impairment in water quality)
- systems equivalent to SAS4 for wetlands, non-perennial rivers, sediments and ground water.

The potential of protozoans as useful biomonitoring tools was explored by investigating the protozoan assemblages of a number of sites down the length of a small urban river, as well as a variety of wetlands and some borehole waters. The suitability of direct collection, artificial substrates and laboratory cultures for examining protozoan assemblages was investigated.

**The first aim of this project was to investigate and identify those protozoans that could be used as biomonitoring tools and water quality indicators especially for seasonal/ephemeral rivers.**

- Initially we undertook a literature review to establish the purposes of biomonitoring and look at the systems which are used in South Africa.
- Currently the South African Scoring System version 4 is being used to identify the state/condition of the river using the presence and or absence macroinvertebrate taxa to calculate a score which assesses pollution levels.
- In some parts of Europe and in the UK a similar system, the Biological Monitoring Working Party scoring system is used.
- Other biomonitoring systems have been proposed for South African rivers viz. the Fish Assemblage Integrity Index (biological), the Riverine Vegetation Index (biological), the Index of Habitat Integrity (non-biological), the Invertebrate Habitat Assessment System (non-biological) and the Geomorphology Index (non-biological).
- All these systems require water in able to be used. Seasonally dry rivers, borehole/subterranean sources and other temporary water sources cannot be tested with SASS. A system was needed where the presence of water was not crucial.
- The Protozoa are single cells which have evolved into some 30 000 species. They are of major importance in that they consume bacteria and that many can encyst or excyst according to certain conditions.
- Protozoa have many features of single cells (they are eukaryotic and have a system of differentiated areas defined by membranes) but they also live as complete individual organisms, moving, feeding, excreting, reproducing and respiring (Curds, 1992).
- There have been limited studies on Protozoans in South Africa. The major work has, and is, being done in Europe, the United Kingdom and the United States.
- The Protozoa live in both marine and freshwater conditions and there are four major groups. The amoebae, Rhizopoda; the flagellates, Mastigophora; the Ciliates and the Sporozoa. The latter group is parasitic and, although of immense importance, they form a separate field of study – in medical and veterinarian research.
- Protozoa have an outer cell membrane within which is the protoplasm which contains the nucleus. The nucleus is commonly species specific and in the Ciliates there are two types of nucleus in the protoplasm.

- Reproduction is generally asexual, but sexual reproduction does occur. Asexual reproduction is by cell division; longitudinal in the amoebae and flagellates and transverse in the ciliates. There are a number of variations to these systems.

**The second aim of the project was to establish whether certain groups within the protozoans e.g. ciliates are particularly suitable for water quality assessment.**

- In order to make biomonitoring methods accessible to non-biologists it was important to establish whether certain groups, species or populations were more relevant in biomonitoring.
- We decided to carry out four types of sampling which would, we hoped, indicate by their results whether they represented a viable biomonitoring method.
- Lotic sampling along the Liesbeek River, using sites close to those which are regularly sampled for water quality monitoring by the Cape Metropolitan Council, Scientific Services Branch.
- Lentic sampling at various disparate sites in the Cape Peninsula, from pristine to polluted.
- Soil sampling, where soil samples, varying from dry to waterlogged were collected then rehydrated and examined for protozoans present.
- The Liesbeek River is perennial, but water is abstracted from it throughout the year.

**The third aim of the project was to establish whether local taxa are cosmopolitan or at least whether or not they respond to water quality variables in the same way that northern hemisphere taxa do or are specifically endemic.**

- Our findings were that the major species, used for establishing the saprobic index, are indeed cosmopolitan.
- Using this finding on the species found at the sampling sites on the Liesbeek River we were able to ascertain that the river is mildly polluted from Site 2, the Kirstenbosch site. The pollution level increasing to strong pollution at Site 7, the Valkenberg site.

**The fourth aim of the project was to establish preliminary methods for collecting protozoans for the biomonitoring of groundwaters.**

- Protozoans were collected by means of direct sampling with a basting tube in the lotic sites, using Plastic Foam Units (PFU's) in the lentic sites, surface soil and litter collection for soil sample examination and baler samples direct from boreholes for borehole sampling.
- Lotic samples were examined directly but then stored for a minimum of one week in petri dishes in the laboratory. Lentic samples were squeezed out, examined and then stored in the laboratory in sterile sample jars for a minimum of one week. Dry soil and litter samples were held in sterile sample jars for six months before rehydration and examination. Waterlogged soil samples were examined straight after collection, but held in Petri dishes for a minimum of one week. Baled water samples were held for one week after sieving on arrival at the laboratory. All the samples retained their integrity (did not degrade) when held at ambient room temperature.
- Petri dishes and sample jars were initially examined using a dissecting microscope. Thereafter, individual species were removed with a Pasteur Pipette and examined using a concave slide on the compound microscope.
- Various methods, as suggested in the literature, were used to either impede movement and/or stain the species for further identification.
- In this project we rarely identified specimens which were  $> 50 \mu\text{m}$  in length. The exceptions to this had very specialised movement patterns and were identifiable because of this. We were unable to make use of Phase Contrast or Light and Dark Field microscopy.
- However, where water is lentic, underground or present only as a film on subsurface sediments, the use of protozoans as a biomonitoring tool, in tandem with the Saprobic System, may become of major importance in water-poor countries such as Africa.
- Simple but regular biomonitoring methods, such as SASS, which is already carried out by CMC's Scientific Services, should be able to track changes in the condition of the river.
- In the lentic study the effect of site-specific conditions on protozoan communities in wetlands was impossible to separate from the effect of the water quality itself.
- This study was conducted on a very small scale, but it did point to problems in the implementation of any kind of lentic biomonitoring system using protozoans. A new worker would have to become familiar with the identification of organisms and learn the special method of sampling.

- Soil and sediment sampling has a potential as a biomonitoring tool and a key to the identification of species would enable non-biologically trained personnel to undertake biomonitoring.

## **Conclusions**

This study was conducted on a small scale but did point to problems in the implementation of any kind of lentic biomonitoring system using protozoans. Identification of protozoans is difficult and so it would be time-consuming to train biomonitoring technicians: quality assurance of identifications might be a problem.

Protozoans might be useful biomonitoring agents for ephemeral systems, although since many species are able to encyst under unsuitable conditions, results would have to be carefully interpreted.

## **Recommendations**

### **Identification of protozoans**

This project has developed considerable expertise in the identification of freshwater protozoans. In particular, numerous photographs have been taken and video recordings have been made of several taxa.

Independently of this project, Heeg (in press) has produced a brief guide to the identification of freshwater protozoans as part of the WRC-funded project to publish guides to the identification of all freshwater invertebrates. In order to make the best use of the protozoan information in both projects, it would be valuable to collate species lists, keys, photographs and video recordings into a single package for use by future workers on the group.

### **Use of protozoans in biomonitoring**

The rather preliminary results of this project have indicated that protozoans do not offer an easy alternative to the existing SASS biomonitoring system, which uses macroinvertebrates for estimating impairment of water quality in rivers. Developing a similar system using macroinvertebrates for perennial wetlands is likely to be difficult because of the intrinsic differences in water chemistry and other environmental features

between wetlands: it is likely, from the work reported in this project, that this would be true of the use of protozoan assemblages too.

Nevertheless, the real possibility exists of using protozoans in the biomonitoring of various aspects of non-perennial systems and of ground water. The fact that protozoan cysts can persist for some time in a desiccated state offers the possibility that they can provide information on antecedent conditions in dry rivers and wetlands. Further their very rapid responses to inundation means that protozoans should be useful for estimating water quality conditions over relatively short periods of time in ephemeral systems. This aspect should be followed-up.

### **Protozoans in ground water**

The fact that we were unable to find a method for collecting protozoans from ground water should not preclude attempts using a variety of techniques, including artificial substrata, which we did not use in our very brief study of borehole waters.

The National River Act of 1998 requires that a Reserve be calculated for such water resources, though, and we need to continue to investigate protozoans in this regard.

### **Method recommended for further work on protozoans**

Based on the investigations detailed in the report, we offer the following tips for future work on protozoans in biomonitoring studies.

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