COUNTING AND MEASURING LIVING PROTOZOOPLANKTON USING A VIDEO ENHANCED MICROSCOPE AND A NEW MICROPLANKTON CONCENTRATION DEVICE

Tarcisio A. CORDEIRO *
Gotram UHLIG**
Frederico P. BRANDINI*

INTRODUCTION

One of the main logistical problems in the study of planktonic protozoans is the fragility of their body structure, which does not allow staining without changing the cell shape (Choi & Stoecker, 1989) and sometimes disrupting the cell wall. Foissner (1991) and Montagnes & Lynn (1987) proposed staining methods to bring out taxonomic details while allowing for the retention of shape and volume. These methods involve time-demanding procedures, handling of hazardous chemicals and require a large number of individuals per samples. Such conditions are almost impossible to achieve during expeditions on oceanographic vessels, where stations are visited at intervals of a few hours and samples containing high concentrations of organisms tend to be a rare event.

The observation of protozoans in vivo is recommended by many authors (Foissner, 1991; Dale & Burkill, 1982; Uhlig & Heimberg, 1981; Sorokin, 1981), but in these cases, ecological and taxonomical approaches are not compatible. If the objective is morphological description, few individuals are observed in details. Usually the cells are slightly pressed using by the oil immersion objective, causing some deformation in the cell shape. This prevents swimming allowing observation of taxonomic details. Otherwise, if the approach involves cell counting and measuring for biomass estima-

* Centro de Estudos do Mar - UFPR, Av. Beira Mar s/n., 83255-000, Pontal do Paraná, PR, Brazil.
** Zu den Brücken 3, 59427 Unna - Billmerich, Germany

tions, it is almost impossible to obtain morphological details necessary for species identification because the cells have a very short life-span under the microscope. Even with a temperature-controlled observation chamber, the counts must be performed as fast as possible, before the cells collapse due to oxygen depletion or excess of light radiation.

Yet another possibility is the combination of live observations, microphotography and staining techniques as per Maeda & Carey (1981) and Maeda (1986). The combination of two or more methods tends to give the best results. However, it is time demanding, involves higher costs per sample and requires more people.

We present in this paper a simple technique for assessing plankton concentration, cell counts, biomass estimation and species identification, which avoids the use of expensive and frequently dangerous chemicals.

MATERIAL AND METHODS

We used an inverted microscope (Zeiss), a SVHS video camera (Panasonic F15hs), a SVHS video recorder (Panasonic FS88), a video monitor (Sanyo) and a personal computer (Macintosh Performa). The technique was tested on eutrophic shore waters and on oligotrophic open sea waters. The water samples were collected with 10 and 30 liters Niskin type bottle, respectively. Other common plankton laboratory tools will be cited when appropriate.

Concentrated water sample is usually obtained through reverse filtration (Dodson & Thomas, 1978). We developed a simple device to perform a tangential filtration (TFD hereafter; Figs 1 and 2), inspired from the work of Barthel *et al.* (1989). It is made of stable PVC and equipped with a 10 μm Nytal mesh and concentrates up to 20 l of water sample to an average of 60 ml of concentrate. The TFD creates a perpendicular water flow at the mesh surface, allowing organisms to roll under the mesh, instead of being squeezed through the mesh holes.

A graduated transparent bucket allows the discarding of part of the sample, when the mesh becomes suddenly clogged due to unexpected high concentration of suspended material. Therefore, it is important to notice the initial and final amount of water in the bucket. A tap at the bottom of the bucket permits control and interruption of the flow when the mesh becomes clogged.

The TFD is placed in a basin, with the mesh size up, to retain the filtered sample water. When enough water is passed through the TFD, the bucket is disconnected and the TFD is turned on its edge on the basin bottom and with the water entry spout upwards. The mesh is then gently washed with a wash bottle containing filtered sample water, from top to bottom, while removing the device from the basin. When the concentrate reaches about one finger depth, the TFD is placed horizontally and the

remaining mesh surface washed. With the PVC ring and mesh removed, the concentrate is transferred from the TFD to a graduated cylinder to measure the final volume.

Part of the concentrate is then placed in an observation chamber as per, for example, the Uhlig & Heimberg (1981) or the Utermöhl (1958) methods. It is very important to know the chamber dimensions - these values are necessary to estimate the concentration of organisms in the sample. A caliper is usually accurate enough for measuring diameter and height. The chamber height may not be higher than the focus range of the smallest microscope objective, since some organisms (e.g., *Noctiluca*) swim at the top of the chamber, just touching the under side of the cover glass.

At this point it is possible to add anesthetics to slow down the movement of cells. We tested MS222, magnesium chloride, urethan, EDTA and menthol. The best results were obtained with the latter. However, this must be done with care difficult, since different species show different reactions. While some species continue swimming as fast as before, others suffer wall disruption.

RECORDING TRANSECTS

Before starting to record images along transects, it is necessary to calibrate the optical equipment. A micrometer slide is placed in the microscope stage and its distances are measured with the help of a compass in front of the monitor, for each objective to be used. Recording the micrometer slide is also useful to indicate the beginning of a new sample on the video tape. For later calculations, it is also necessary to know the transect width.

Another way of measuring the organisms would be by using a computer and some software for video image analysis. Such software is normally provided with different rulers and in some cases allows to calculate cell volumes. However, they work properly only when the cells have cylindrical to spherical shapes, because they do not recognize a third dimension. The software we tested assumed depth to be equal to diameter.

To facilitate the calculations, it is always preferable to start recording with the largest transect, where the length is equal to the chamber diameter. It is a matter of choice which objective is to be used for the transects. Low magnifications give a wider transect but a poor image definition and leads to the lost of smaller organisms, especially when they are not moving. We used the smallest objective (x5.3), which gave a transect of 750 μm width.

The question of "how many transects should be recorded?" is a matter of experience. Lund *et al.* (1958) and Hasle (1978) discuss in details cell counts with the inverted microscope. Unfortunately, sometimes is difficult to achieve some of their recommendations, like, counting at least a certain number of individuals of the most common species to keep estimates error under 10-20%. The researcher needs to find the best compromise between a minimal sampling error (especially if parametric

statistics are to be used) and hours of recorded images. In shore waters, we needed about 30 minutes to record four transects; in open sea waters, only half this time.

ANALYZING IMAGES

Recorded images can be stored indefinitely. This is very practical, since it is often uncomfortable to measure cell dimensions from a monitor on board a ship. From our experience, it is better to analyze the images on land. Another advantage of using video recording is the possibility of observing all the faces of a swimming cell, which allows a more accurate three-dimensional cell shape simulation.

While the transects are being recorded, it is very common to see cells darting across the monitor; many encounters with protozoans happen very quickly. The video recorder therefore plays a very important role in image analysis. For these cases we recommend to use at least SVHS video recorders, since they have the capability of displaying video images frame by frame, without losing sharpness as do VHS recorders. At times, we could obtain only one frame focused enough for measuring cells axes and structures.

Otherwise, when the cells are not swimming, it is possible to get the best images by using other magnifications and, when convenient, phase-contrast or other light techniques. One may also take advantage of the improvement in microscope resolution inherent to the video equipment. Sometimes it is even possible to identify food particles in the cells. Further discussion about the possibilities of using video enhanced microscopes is given by Weiss *et al.* (1989) and references within, and Taniguchi & Takeda (1988).

To estimate cell concentrations one first needs to know the relationship between the transect volume and the unconcentrated sample. For example, for given transect sizes: length = 26 mm, width = 0.7 mm, depth = 4 mm, the resulting volume is 0,073 ml. With 4 liters of water sample (**ws**) passed through the concentration device and 60 ml of concentrated sample (**cs**), we calculated the equivalent water sample per transect (**wt**, in ml):

$$(ws \bullet 1000) \div cs \bullet 0{,}073 = wt$$

$$(4 \bullet 1000) \div 60 \bullet 0{,}073 = 4{,}82 \text{ ml}$$

This means that all the cells encountered in this transect were living in 4,82 ml of sea water. If 10 individuals of one species were found in this transect, we have:

$$10 \bullet 1000 \div 4{,}82 = 2{,}075 \text{ ind./liter}$$

It is therefore possible to calculate the length of the other transects for a given chamber diameter. It is also possible to draw a model of the chamber to a larger scale and measure the other transects lengths with a good ruler.

FINAL COMMENTS

Even with the better image definition of the microscope inherent to the video equipment, it may sometimes be impossible to identify cells without staining. This can also occur because the image of live specimens sometimes does not match with the illustrations of stained material, and also due to the need to see structures like nucleus and silver-lines. At any rate, if time and material are available, it would be recommended to perform staining techniques when the samples show high concentrations of organisms. Those interested in the study of protozooplankton are encouraged to make experiments, and compare other techniques for cell counting with those described above.

ACKNOWLEDGMENTS

This work was supported by the DAAD (Germany), CNPq and FUNPAR (UFPR). Thanks are due to the staff of the Biologische Anstalt Helgoland (BAH - Germany) and Centro de Estudos do Mar (CEM - UFPR) for providing laboratory facilities. Dr. Peter Martens invited the first author to work at the BAH, and Dr. Paulo Lana made valuable suggestions to improve the text.

ABSTRACT

The microscopic and fragile nature of the protozoans is the major barrier to their study. The most traditional taxonomic literature on protozoans is illustrated with stained specimens, which sometimes do not resemble the living cells. These artifacts are satisfactory for taxonomy purposes, but not for ecological analysis. Staining methods cause the cells to shrink and also cell wall disruption. This work describes a combination of the Utermöhl method and a new concentration device plus video microscopy for analysing living protozoans. The procedure seems to be an accurate and reliable way to assess protozooplankton biomass.

Key-words: protists, protozooplankton, counting, measuring, biomass assessment.

RESUMO

Dimensões microscópicas e fragilidade são sérios obstáculos para o estudo dos protozoários. A maior parte da literatura taxonômica apresenta ilustrações de organismos fixados e corados, que em muitos casos pouco lembra o espécime vivo. Se por um lado estes artefatos têm se mostrado suficientes na taxonomia dos protozoários, isto já não ocorre em trabalhos ecológicos. A fixação e realce com produtos químicos provoca a perda de volume, forma e, freqüentemente, o rompimento da parede celular. Por este motivo, outros autores recomendam a contagem e medição de células vivas. O presente artigo descreve uma nova metodologia para trabalho com organismos vivos, que combina técnicas tradicionais de sedimentação para contagem celular (Utermöhl), um novo procedimento para a concentração de plâncton e a videomicroscopia. O método produz resultados mais acurados e confiáveis nas avaliações de biomassa.

Palavras-chave: protozooplâncton, técnicas de análise, vídeo.

REFERENCES

BARTHEL, U.- G.; SCHANEIDER, G.; GRADINGER, R. & LENZ, J. 1989. Concentration of live pico- and nanoplankton by means of tangential flow filtration. *J. Plankton Res.*, 11: 1213-1221.

CHOI, J.W. & STOECKER, D.K., 1989 Effects of fixation on cell volume of marine planktonic protozoa. *Appl. Environ. Microbiol.*, 55 (7): 1761-1765.

DALE, T. & BURKILL, P.H. 1982. Live counting - a quick and simple technique for enumerating pelagic ciliates. In: Ecology of Marine Planktonic Protozoa, Villefranche-sur-Mer (France), May 1981. *Ann. Inst. océanogr. Paris N. S.*, 58 (suppl): 267-276.

DODSON, A. N. & THOMAS, W. H. 1978. Reverse filtration. In: Sournia, A. (Ed.). *Phytoplankton manual.* UNESCO, U.K, p.104-107.

FOISSNER, W. 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Europ. J. Protistol.*, 27 : 313 - 330.

HASLE, G.R. 1978. Using the inverted microscope. In: Sournia, A. (Ed.) *Phytoplankton Manual*, UNESCO, U.K., p.191-196.

LUND, J.W.G.; KIPLING, C.; LE CREN, E.D. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, 11: 143-170.

MAEDA, M. 1986. An illustrated guide to the species of the families Halteriidae and Strobilidiidae (Oligotrichida, Ciliophora), free swimming Protozoa common in the aquatic enviroment. *Bull. Ocean. Res. Inst. Tokyo*, 21: 1-67.

MAEDA, M. & CAREY, P. G. 1981. An illustrated guide to the species of the family Strombidae (Oligotrichida, Ciliophora), free swimming Protozoa common in the aquatic enviroment. *Bull. Ocean. Res. Inst. Tokyo*, 19: 1-65.

MONTAGNES, D. J. S. & LYNN, D. H. 1987. A quantitative Protargol stain (QPS) for ciliates: method description and test of its quantitative nature. *Mar. Microb. Food Webs*, 2: 83-93.

SOROKIN, Yu. I. 1981. Microheterotrophic organisms in marine ecosystems. In: Longhurst, A. R. (ed.). *Analysis of Marine Ecosystems.* Academic Press. London, UK., p.293- 342.

TANIGUCHI, A. & TAKEDA, Y. 1988. Feeding rate and behavior of the tintinnid ciliate *Favella taraikaensis* observed with a high speed VTR system. *Mar. Microb. Food Webs,* 3 (1): 21-34.

UHLIG, G. & Heimberg, H. H. 1981. A new versatile compression chamber for examination of living microorganisms. *Helgoländer Meeresunters*, 34: 251-256.

UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. int. Ver. theor. angew. Limnol.*, 9: 1-38.

WEISS, D. G.; MAILE, W. & WICK, R. A. 1989. Video microscopy. In: Lacey, Alan J. (Ed.). *Light Microscopy in Biology.* A Pratical Approach. IRL Press, Oxford, p. 221-278.