A Study of Reproduction and Other Life Cycle Phenomena in Planktonic Protists Using an Acridine Orange Fluorescence Technique*

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Abstract

The percentage of dividing individuals and temporal reproductive patterns were determined for natural populations of several planktonic protists including five species of tintinnids, a dinoflagellate, and a diatom. To obtain these data, a method was used in which the nuclei of planktonic ciliates and phytoplankters can be fluorescently stained with acridine orange at the time of collection and fixation. The technique is simple and can be used routinely in studies of reproduction or other life cycle phenomena of natural protistan populations. For the tintinnids, often more than half of the individuals were in some recognizable stage of fission; periodicity in the division process was only observed once and apparently followed a pulse of conjugation in the population. With the diatom *Ditylum brightwellii* the fluorescent staining technique yielded data on the extent and timing of division which were consistent with, but more complete than, previous enumerations of paired cells.

Introduction

One of the major problems resisting solution in the study of pelagic food chains is that of measuring the growth rate or secondary production of zooplankton. Traditional efforts to estimate the growth of natural zooplankton populations have required extrapolation from laboratory measurements and/or monitoring of single populations for relatively extended periods of time (Mullin, 1969). Both methods are limited. The relevance of laboratory measurements to the natural situation is almost always suspect, and it is generally exceedingly difficult to sample repeatedly a single, well defined, zooplanktonic population for a sufficient period of time to derive the needed demographic statistics. A compromise between the two approaches, the use of large volume containers (e.g. Mullin and Evans, 1974; Beers *et al.*, 1977), has recently shown promise as a partial solution to the problem but is not applicable to the full range of environments and populations of interest.

Edmondson (1960) introduced another technique in which the number of eggs being carried by the females of a population and the length of time required for the eggs to hatch are used to calculate the birth rate of the natural population from a single preserved sample. The Edmondson “egg ratio” technique, and variants thereof, have only occasionally been applied to problems concerning zooplankton growth or production (Edmondson *et al.*, 1962; Hall, 1964; Checkley, 1980). A slight modification of the technique, however, has been successfully applied in defining species-specific growth rates and temporal division patterns in natural phytoplankton populations (Swift and Durbin, 1972; Weiler and Chisholm, 1976; Weiler and Eppley, 1979; Weiler, 1980). In these studies actively dividing and/or recently divided cells were identified to obtain an index of the population’s reproductive (“birth”) rate.

Edmondson (1971) suggested that such a technique could be used with populations of protozoa if a suitable means could be found to identify dividing individuals. In this study we examined reproduction in natural populations of several planktonic protists. Tintinnids, a loricated suborder of the Ciliophora, received particular attention, since they are ubiquitous in marine and estuarine waters, are conveniently sampled, and have been shown to play a significant role in the dynamics of the planktonic food web (Heinbokel and Beers, 1979). In addition we anticipate that the assemblage of tintinnids and other ciliates, with their potential for rapid growth (Heinbokel, 1978) and response to changing conditions, will provide a very valuable model for investigating general features of zooplankton dynamics such as their response to patchy phytoplankton (food) distributions or to pollutants. An

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important part of this work was to develop a technique which could be easily and routinely carried out and which would allow enumeration of dividing organisms with little or no additional effort over that presently required for routine microscopic examination of micro-zooplankton assemblages. While the major emphasis in this paper is on the reproduction of tintinnids, we wish to stress that the technique employed here can be applied easily and simultaneously to other ciliate taxa and to the great majority of phytoplankton species which co-occur in these samples, and that asexual reproduction is not the only life cycle phenomenon that can be profitably studied with this technique.

Materials and Methods

The frequencies of division for five tintinnids, one dinoflagellate, and one diatom from captured planktonic populations were investigated during several experiments undertaken between April 1979 and September 1980. Experiments were conducted for 24- to 36-h periods at various locations within the Chesapeake Bay (USA) and its subestuaries. The specific organism(s) observed, location and starting time for each experiment are as follows: Experiment I, Tintinnopsis acuminata (Daday) (see Kofoid and Campbell, 1929; Gold and Morales, 1976), York River (37°14’N lat.; 76°29’W long.), 1 700 hrs April 24, 1979; Experiment II, Eutintinnuspectinis (Kofoid) and Ditylum brightwellii (West), central Chesapeake Bay (38°23’N lat.; 76°20’W long.), 1 700 hrs August 24, 1979; Experiment III, Tintinnopsis levisgata (Kofoid and Campbell), York River (37°17’N lat.; 76°34’W long.), 1 000 hrs March 18, 1980; Experiment IV, Eutintinnuspectinis (Kofoid), central Chesapeake Bay (38°34’N lat.; 76°27’W long.), 1 000 hrs July 23, 1980; Experiment V, Ceratium furca (Ehrenberg), central Chesapeake Bay (38°04’N lat.; 76°12’W long.), 1 930 hrs August 18, 1980; Experiment VI, Stylicaudapatensis (Cunha and Fonseca) (see Cosper, 1972) and Amphorellopsisacuta (Schmidt), central Chesapeake Bay (37°24’N lat.; 76°05’W long.), 1 300 hrs September 24, 1980.

For all experiments, a 200-l Nalgene cylindrical tank equipped with a stirring paddle and aeration line was filled with surface water. The tank was maintained at ambient surface water temperatures by continuously bathing the outside of the container with bay water. A two-liter sample was taken from the tank each hour, the organisms concentrated on 20-μm Nitex netting, stained with acidulated calcium carbonate buffered formaldehyde containing 5% (v/v) acetic acid, was also diluted 1:19 with sample.

Observations on the nuclear events during the asexual reproductive cycle of Eutintinnuspectinis were made from organisms in an anagnotobiotic culture established September 27, 1979 and maintained for one week at 23°C. The culture was started in a glass carboy containing one-liter cultures each of the microflagellates Isochrysis galbana, Monochrysis lutheri, and Dunaliella tertiolecta and 171 of 20 μm Nitex filtered Chesapeake Bay water (38°45’N lat.; 76°25’W long.). To this mixture approximately two thousand E.pectinis individually isolated from net tows taken at the same location were added. Once the tintinnids reached sufficient numbers (> 1 ml⁻¹), subsamples of the culture were taken periodically and preserved in either the modified Bouin’s fixative or in acridine orange-formaldehyde.

Samples preserved in acridine orange-formaldehyde were concentrated onto 20 μm Nitex netting, washed 2–5 min with tap water, transferred to a Zeiss settling chamber, and examined with a Leitz Divert inverted microscope equipped with a 100 W mercury lamp for epifluorescence (exciting wavelength of 455–490 nm) and a 6 V 15 W tungsten lamp for transmitted brightfield/phase contrast illumination. Bouin’s fixed samples were washed onto 20 μm Nitex netting, stained with acidulated alum hematoxylin (Galigher and Kozloff, 1971), dehydrated through 100% ethanol, and transferred to a type HA Millipore filter. This filter was placed face down and affixed onto a coverslip by exposure to boiling acetone vapors, cleared in xylene, and mounted. Hematoxylin stained specimens were examined with a Zeiss WL brightfield/phase microscope. An Olympus OM-2N was used for photography.

Results and Discussion

Classical cytological staining techniques for nuclei (e.g. Feulgen and hematoxylin stains) have been used to investigate protistan growth dynamics but are much too tedious to be employed in the routine examination of plankton samples. Even the relatively simple aceticarminem stain used by Weiler (1980) requires extensive manipulation of the sample. Acridine orange-formaldehyde, however, has the significant advantage of functioning as a combined fixative, preservative, and fluorescent stain in which samples can be stored (with no special precautions) for a year or longer without deterioration in stain quality. Subsequent handling is minimal, since specimens need only be washed and examined. Furthermore, if screening of appropriate mesh or pore size is used for concentrating and/or washing samples, then quantitative cell counts and classifications of nuclear morphologies of many plankters can be obtained simultaneously.

We generally employ a working solution of 200 μg of acridine orange per milliliter of full strength formaldehyde. This solution is quite stable and after several