A Method for Observation and Enumeration of Epilithic Algae Directly on the Surface of Stones

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Summary. A procedure is described for observation and enumeration of microscopic epilithic algae, directly on stone surfaces. The method involves the use of incident light fluorescence microscopy and provides rapid estimates of total numbers as well as information on the distribution of the organisms. Comparison with conventional procedures, which involve scraping the stone surface, suggest that these latter methods may produce serious underestimates of the algal population. The within-stone and between-stone errors of estimates of the diatom *Cocconeis placentula* are discussed in relation to a) a suitable transformation of the data before use of parametric statistical tests and b) the number of sampling units required to achieve given 95% confidence limits.

Introduction

There have been several studies of periphytic algae (Douglas, 1958; Pieczynska, 1968; Madsen, 1972) and periphytic bacteria (Bott and Brock, 1970). The methodology for quantitative studies of periphyton has been reviewed by Blum (1960), Sladeckova (1962), and Wetzel and Westlake (1969). In most cases samples have been scraped from surfaces (e.g. stones) and then counted in chambers, biomass estimates made, or activity measured. In the case of algae this could involve chlorophyll a extraction or measurement of productivity by the light and dark bottle technique. An alternative to scraping has been to cover the stone surface with a synthetic film which removes the algae when it is peeled away (Margalef, 1949). Other methods have included the use of artificial substrates, usually glass microscope slides, suspended in water bodies, followed by observation of the surface colonization. The objection to any technique based on removal of surface material by scraping, has been the fact that it is very difficult to assess the efficiency of the scraping and recovery procedure. There is ample evidence to indicate that the flora which develops on artificial substrates can differ considerably from that on the natural substrate. Although fluorescence microscopy has been used for examination of algae and bacteria on sand grains (Munro and Brock, 1968) there do not appear to have been any published methods for direct observation of epilithic algae, with the exception of the occasional use of stereomicroscopes for observation of larger forms.
This paper describes the application of incident light fluorescence (epifluorescence) microscopy to direct observation and enumeration of periphyton. The application of the term "periphyton" in this paper is confined to epilithic algae unless otherwise stated.

Materials and Methods

Stones were taken from a local stream (Wilfin Beck) which runs into Windermere, South Basin. A detailed description of the stream has been published by Elliott (1971a). The bed consists of rock of Silurian age which breaks into flat angular stones, and also some depositions of boulder clay and stones.

Stones were sampled by placing them singly into polythene bags which were then filled with water from the stream and sealed tightly in an attempt to protect the stone surface and prevent scratching. The size of stone chosen was usually less than 10 cm (length and width) by 3 cm (depth) but larger stones could be examined, either by modification of the microscope stage, or by breakage of the stone into smaller pieces. The stone can be examined under water with a suitable immersion objective or mounted in a bed of pliable material (e.g. plasticine) and examined through a conventional objective, with or without a coverslip.

There are several possible combinations of filters for excitation and to allow observation of the primary fluorescence of chlorophyll $a$, which is the basis of this method. The excitation wavelength should correspond to blue light (c. 435 nm) and a red fluorescence is observed. The method described below is that which was found to be satisfactory in this laboratory.

Counts were performed on a Leitz Orthoplan microscope fitted with a 100 w Xenon lamp. Excitation was through two heat filters (which absorbed wavelengths > c. 700 nm), a stop filter K 380 nm, and an S 470 interference filter. The settings on the Ploem illuminator were 3 and 3 which was the equivalent of K 495 nm dichroic combination of beam splitting mirror and suppression filter. The image was viewed through a K 530 nm barrier filter. Changes in the combinations of filters used can be made to accommodate differences in fluorescence intensity obtained with different objectives. Although the excitation wavelength used (c. 470 nm) was not the peak for excitation of chlorophyll there is sufficient energy for production of a bright red fluorescence.

The magnification used for counting depended on the size of the alga and the nature of the stone surface. Standard procedure in this laboratory was to use a magnification of 160 × (12.5 × objective) which is close to that used by Douglas (1958). The energy of the Xenon lamp is sufficient to allow counts to be performed at 50 × magnification (4 × objective) when the stone surface is particularly uneven, and magnifications greater than 500 × can also be achieved, particularly on flat surfaces. Known areas of stone surface were counted with the aid of an eye-piece graticule. 30–100 fields per stone were counted, depending on the density of the population.

Results and Discussion

A preliminary survey was conducted on stones from the Wilfin Beck where Cocconeis placentula (Ehr.) is the dominant diatom for much of the year. To determine the distribution of this alga on the stone surface, samples were taken at random, and the relationship between the variance