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ON THE REMOVAL AND QUANTIFICATION OF ALGAL AUFWUCHS FROM MACROPHYTE HOSTS

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Abstract

Accurate population density measurements of algal aufwuchs associated with macrophyte hosts appears to be fraught with uncertainties. This account details a procedure involving aufwuchs removal by agitation and acid hydrolysis with subsequent quantification by a special Sedgwick-Rafter cell counting technique. The aufwuchs removal efficiency from five hosts of widely differing morphology was calculated to be 98.0% (95% confidence limits: 96.3%, 99.7%) after five 45 second agitation cycles.

Introduction

The removal and quantification (by counting) of algal aufwuchs from macrophyte hosts has received the attention of a number of investigators, and various techniques have been developed. Methods of removal include agitation (Foerster & Schlichting 1965, Knudson 1957), grinding (Douglas 1958), using jets of water and brushing (Hickman 1971), and scraping (Sladeckova 1962, Young 1945). Subsequent quantification by counting has involved the use of inverted microscope techniques (Knudson 1957), the use of membrane filters (Foerster & Schlichting 1965), the use of transects on glass slides (Hickman 1971, Sladeckova 1962), or the use of Sedgwick-Rafter or similar counting cells (Douglas 1958, Slack *et al.* 1973, Sladeckova 1962, Young 1945), and in all of the above investigations results have been expressed in terms of number of organisms per unit of host surface area.

Little objective information, however, appears to be available on the precision and reliability of the various removal techniques, and in cases where such data are presented (e.g., Foerster & Schlichting 1965), a potentially high degree of variability is indicated, thus necessitating the use of 'correction factors'. Moreover, quantification procedures, especially those involving the use of Sedgwick-Rafter cells, apparently assume that similar results (in terms of accuracy) emerge, regardless of the counting regime employed. Woelkerling *et al.* (1975), however, have shown that such assumptions cannot be made. Finally, some authors (e.g. Hickman 1971) note that difficulties may occur in attempting to determine host surface area (see Sladeckova 1962, p. 298 for methods), and this may, in turn, affect results that are expressed in terms of numbers of organisms per unit of host surface area.

These limitations have contributed to the widespread adoption of indirect measurements with the use of artificial substrates (see APHA 1971, Sladeckova 1962 for methods). Artificial substrates, however, not only suffer from the drawback of permitting only indirect measurement, but they also may permit a particular type of selective colonization (Herbst & McNelly 1973, Hickman 1971, Tippet 1970). This, of course, could produce misleading results. Moreover, any natural selectivity resulting from differences in macrophyte host biology (e.g., surface texture, chemical secretion) cannot be detected satisfactorily with the use of artificial surfaces. Thus accurate quantitative (i.e., population density) measurements of algal aufwuchs associated with macrophyte hosts still appears to be fraught with uncertainties.

Preliminary observations on the role of desmids (Desmidiales, Chlorophyta) in Wisconsin lake communities supports the contentions of Griffiths (1928), Hutchinson (1967), Krieger (1933), and others that desmids can occur in substantial numbers in aufwuchs associated

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with macrophyte hosts and that the desmid aufwuchs may be of greater ecological importance than the desmid plankton in a given lake. Consequently, quantitative measurement of aufwuchs appears essential to an understanding of desmid ecology in lakes, and the present investigation has been undertaken in hopes of developing a procedure which would 1) permit direct measurement of aufwuchs on natural substrates, 2) allow consistently for virtually complete removal of aufwuchs from a wide variety of macrophyte host types, and 3) result in a quantification procedure which involves a reliable counting regime, yields population density data, and avoids expressing results in terms of numbers per unit of host surface area.

The procedure

Since aufwuchs attachment to the host surface commonly appears to be effected by mucilage-like polysaccharides (O'Colla 1962), it was hypothesized that aufwuchs removal could be accomplished by a combination of agitation and acid hydrolysis of the polysaccharides with FAA (10:7:2:1::95% ethanol: water: formalin: glacial acetic acid). Initial experiments were conducted on the aufwuchs associated with *Myriophyllum spicatum* L., but the work was eventually extended to include five macrophytes of widely varing morphology (Table 1).

The procedure for the removal and the subsequent quantification which resulted from these experiments may be summarized as follows:

1. Place macrophyte material in a wide mouth jar, add enough FAA to just cover plants, cap jar, and agitate by shaking vigorously (ca. 2 shakes per second) for 45 seconds.
2. Decant through cheesecloth into another container; return macrophyte fragments retained by cheesecloth to original sample. (Removal of material from cheesecloth can be facilitated, where necessary, by rinsing with jets of distilled water from a squirt bottle.)
3. Repeat steps 1-2 until five agitation cycles have been completed.
4. Dry macrophyte material at 70° C for 24 hrs; weigh on an analytical balance.
5. Compute the *total* population of aufwuchs organisms in the accumulated decantation using Sedgwick-Rafter cell counts (Woelkerling *et al.*, 1975; express results in terms of number of organisms per unit dry wt. of macrophyte host.

To insure more accurate results, several factors should be taken into account in applying the above procedure. Care must be exercised in collecting to avoid dislodging very loosely attached forms. The authors generally collected enough 5-15 cm portions of host material to fill a 250 ml jar; subsequent agitation was carried out in a 1000 ml container.

TABLE I

Salient morphological features of the macrophytes studied

Macrophyte	Salient morphological features
Myriophyllum spicatum L.	Numerous finely divided leaves.
Utricularia sp.	Finely divided leaves; carnivorous bladders.
Potamogeton pectinatus L.	Coarse, thread-like leaves from multiple stems.
Sphagnum sp.	Numerous, small, closely appressed leaves.
Nuphar sp. (petioles)	Cylindrical, relatively smooth petioles.

Another aspect concerns the Sedgwick-Rafter cell counting (see Woelkerling *et al.* (1975) for details of the procedure). In the present study the authors employed a regime of 2 counts on each of 12 S-R cells. The volume of the decanted material was adjusted, where necessary, to insure individual Whipple grid tallies of 5-25 at a total magnification of 200x (20x obj.; 10x ocular). This density range appeared to avoid overcrowding and excessive clumping of organisms on the one hand and yet permitted tallies of taxa present in relatively low numbers on the other hand. Dilution was effected with the addition of FAA. Concentration was accomplished with a plankton centrifuge (Kahl Scientific Instrument Corporation, El Cajon, California, Model 020WA106) set at 14,000 rpm and a flow rate of 100 ml/min. The efficiency of the instrument was previously determined (unpublished data) to be 96-99% from comparisons of the residue and effluent counts with the membrane filter technique of McNabb (1960).

The total population (T) of organisms was computed from the equation

$$T = dv \quad (1)$$

where d is the density of organisms per ml of the sample and v is the total volume of the sample's liquid in ml. The procedure for determining d for Sedgwick-Rafter cell counts is outlined in APHA (1971).

Calculation of aufwuchs removal efficiencies

To determine the efficiency of aufwuchs removal, the washing liquid from each 45 second agitation-washing cycle was collected and analyzed separately for each macrophyte. Curves resulting from plots of total counts of aufwuchs present in each fraction vs. washing number for the five macrophytes, displayed an orderly pattern which suggested an exponential decrease (Fig. 1a). Exponential regressions performed with a Hewlett-Packard 1900 B programmable calculator determined approximate functional relationships between total count and washing number for each macrophyte. The equations obtained were of the form:

$$Y_x = ae^{bx} \quad (2)$$

where Y_x is proportional to the number of organisms removed in the X-th washing, e = 2.718, the base of

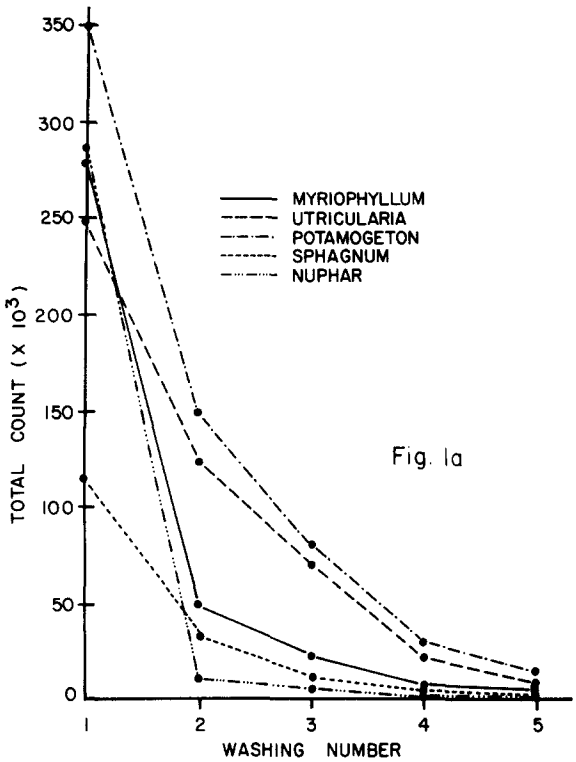

Fig. 1a. Total count vs. washing number for the five macrophytes.

natural logarithms, and a an b are constants determined by regression. The correlation coefficient between Y_x (estimated) and Y_x (observed) ranged from .93 to .98, indicating good curve fits in all cases. Before performing the regressions, the observed Y_x values were transformed to a common scale by assigning a value of 1.000 to the highest count (washing 1) of each macrophyte and computing all other values as fractions of this maximum value.

For a given macrophyte, the total number of organisms removed after n washings is proportional to

$$T_n = \sum_{x=1}^{n} Y_x = ar(1-r^n)/(1-r) \quad (3)$$

and the total number of organisms present is proportional to

$$T = \sum_{x=1}^{\infty} Y_x = ar/(1-r) \quad (4)$$

where Y_x is estimated using equation (2) and $r = e^b$. Efficiencies, E_n, expressed as percent removal of aufwuchs can be calculated by

TABLE II

Exponential equations (regressed) for each macrophyte, efficiencies predicted by each equation and mean efficiencies and confidence intervals.

Macrophyte	Equation	Washing number 1	2	3	4	5	6	7	8	9
		Efficiency (%)								
Myriophyllum spicatum L.	$Y = 1.363\ e^{-.846X}$	57.1	81.6	92.1	96.6	98.6	99.4	99.7	99.9	(100)*
Utricularia sp.	$Y = 2.256\ e^{-.738X}$	52.2	77.2	89.1	94.8	97.5	98.8	99.4	99.7	99.9
Potamogeton pectinatus L.	$Y = 1.877\ e^{-.688X}$	49.7	74.7	87.3	93.6	96.8	98.4	99.2	99.6	99.9
Sphagnum sp.	$Y = 1.319\ e^{-.694X}$	50.0	75.0	87.5	93.7	96.9	98.4	99.2	99.6	99.8
Nuphar sp.	$Y = 3.383\ e^{-1.920X}$	85.3	97.9	99.7	100.0	100.0	100.0	100.0	100.0	100.0
95% confidence interval	lower limit	40.3	69.3	85.7	92.4	96.3	98.1	99.1	99.5	99.7
	upper limit	77.5	93.3	97.5	99.0	99.7	99.9	99.9	100.0	100.0

(5) $$E_n = 100\, T_n / T_x$$

For each macrophyte, efficiencies were calculated for several washing numbers and, as a measure of the variability between macrophytes, 95% confidence intervals were established for each washing number (see Table II). In Fig. 1b, the confidence intervals are plotted against washing numbers. Variability is seen to be the greatest after the first washing (95% confidence limits: 40.3%, 77.5%) and to decline steadily with successive washings until after the ninth washing, the removal efficiency is over 99% (95% confidence limits: 99.7%, 100.0%). If one assumes that the macrophytes studied here represent a range in host morphology likely to be encountered in the field, then the removal efficiency can be expected to fall between 96.3% and 99.7% after five washings.

Discussion

Expressing total population densities in terms of dry weights of macrophyte hosts appears to offer some advantages over results expressed in terms of unit surface area of hosts. Dry weights can be easily and accurately determined whereas estimating surface areas can be laborious and prone to errors. The morphology of many macrophyte species is known to vary from site to site (e.g., *Potamogeton pectinatus* L.). Moreover some species, and even single plants, can vary substantially in morphology at different water depths (Fassett 1957). In addition, most surface area values are based on an average size and shape, a procedure which can introduce errors in the final results. Finally, the morphology of many macrophytes is of such a complex nature (e.g. *Myriophyllum* leaves) that determination of average surface areas by the usual procedure of mathematical approximations produces only rough estimates, at best.

Results expressed in terms of dry weights must be interpreted with caution, however, if comparisons between different types of macrophytes are to be made since surface area to weight ratios may vary. In this study, for example, *Nuphar* petioles probably have a much different surface area to weight ratio than the finely divided leaves of *Myriophyllum*, thus making direct comparisons between the aufwuchs population densities of potentially limited value. Regardless of how the results

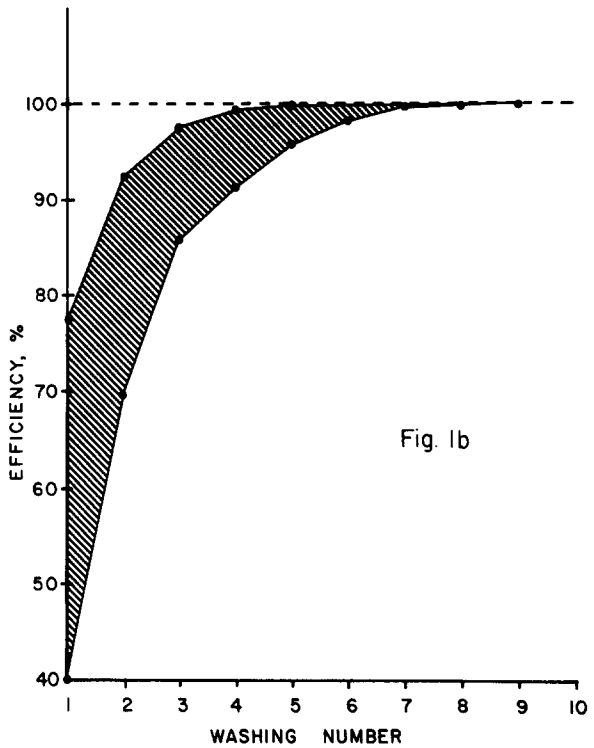

Fig. 1b. 95% confidence belt for removal efficiency based on the five macrophytes.

are to be expressed, however, the aufwuchs removal procedure is applicable.

The proposed procedure assumes that all aufwuchs will be removed after an infinite number of washings; i.e., there are no forms which are entirely resistant to removal. Microscopic examination of the macrophyte material after five washings revealed very few remaining organisms, thereby supporting this assumption. Finally, the force applied in the agitation cycle may not be completely uniform and a machine (e.g., a modified paint shaker) would probably have to be used to standardize the force. However, since removal of the aufwuchs by the procedure described here is nearly 100% after five washings for all of the hosts studied, the use of manual agitation appears justified.

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