THE NEWMAN METHOD, MODIFIED FOR THE ESTIMATION OF THE TOTAL THALLUS LENGTH OF FILAMENTOUS ALGAE*

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ABSTRACT. — NEWMAN's (1966) method of total root length estimation is modified and adapted to measure total length of filamentous algae in culture (i. c. *Polysiphonia*). This method makes possible a measurement and an estimation of the growth of the algae in culture without disturbing them. The petri dishes are placed on a 1 x 1 mm grid and the filament/grid intersections are counted. Total filament length (T) is given by $T = \pi N (L \times W)/4 (L \times W)$, with N = the number of intersections, L = length and W = width of area in which the thallus is situated. Total filament length of *Polysiphonia* in culture estimated by the Newman method is compared with camera lucida measurements. The correlation between the results obtained by the 2 techniques is very high ($r = 0.97$). The maximum deviation between the means of the total length obtained by the 2 methods was 7,2 % : these means are not significantly different (Student t-test). The Newman estimation method required less time than direct measurement, and its results are not significantly different.

RÉSUMÉ. — La méthode de NEWMAN (1966) pour estimer la longueur totale des racines est modifiée et utilisée pour mesurer la longueur totale d'algues filamenteuses en culture (notamment *Polysiphonia*). Avec cette méthode il est possible d'estimer la croissance d'algues en culture sans les perturber. Les boîtes de Pétri sont placées sur un grillage à mailles de 1 x 1 mm et les points d'intersection entre les filaments et le grillage sont comptés. La longueur totale des filaments (T) est donnée par $T = \pi N (L \times W)/ 4 (L \times W)$ avec N = le nombre de points d'intersections, L = la longueur et W = la largeur de la surface dans laquelle le filament est situé. La longueur totale des filaments de *Polysiphonia* en culture obtenue par la méthode de Newman a été comparée à la longueur totale mesurée à la chambre claire. La corrélation entre les 2 techniques est très bonne ($r = 0,97$). La plus grande différence entre les moyennes des longueurs totales obtenues par les 2 méthodes est de 7,2 %. Les moyennes des longueurs totales ne sont pas différentes de façon significative (Student t-test). L'estimation de la longueur totale avec le grillage est plus rapide que la mesure directe. Les résultats sont très fiables.

KEY WORDS : filamentous algae, measurements, NEWMAN's method.

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* Communication présentée au Colloque de la Société Phycologique de France à Caen (25-27 avril 1986), en hommage à Madame le Professeur P. GAYRAL.

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INTRODUCTION

Some of the commonly applied techniques for measuring growth and estimating growth rates of algae in culture are : counting cell numbers (BONEY, 1960), determining cell volumes (DRING, 1967), dry weight (BOALCH, 1961), total pigment or protein content (HO et al., 1983), making measurements on xerocopied thalli (PATWAY & VAN DER MEER, 1984), microscopically measuring filament length according to RUSSELL (1963). Most of these are inappropriate for a continuous monitoring of the cultures.

During a revision of *Polysiphonia* section Oligosiphonia (Rhodophyta) a technique was needed for estimating the total thallus length and for surveying the cultures during a long period without disturbing or cutting up the algae into pieces.

NEWMAN (1966) established a formula $R = NA/2H$ for estimating the total root length in an extracted sample. Root length (R) was measured by counting the number of intersections (N) per regular area (A) of the roots with randomly located and oriented lines, of total length (H). The lines were provided by a hair-line in a microscope eye-piece. MARSH (1971) simplified the method by placing a grid under the roots and counting the intersections with the horizontal and vertical lines of the grid. We have slightly modified the method of NEWMAN to measure the total thallus length of filamentous algae in culture. The results obtained by this method are compared with camera lucida measurements.

METHODS

The method described by NEWMAN (1966) requires the image of a line engraved on an eye-piece to be superposed on a uniformly arranged field of roots. By adapting the Newman formula it is possible to use a grid which can be superimposed on any system of filaments to enable a rapid estimation of length in culture and even in the field. This method requires no re-arrangement of the subject unless teasing out concentrations for easy counting.

The further modification of NEWMAN's (1966) method consists of a variable circumscription of the thallus in the grid (depending on the size of the thallus).

In the mentioned formula A and H are to be replaced so that :

$$T = \frac{\pi N (L \times W)}{2 (A + B)} \quad (1)$$

with T = (estimated) total thallus length
N = number of intersections with the grid
L = length of the rectangular area
W = width of the rectangular area
A = total length of the vertical grid lines in the area = W times L
B = total length of the horizontal grid lines in the area = L times W

The formula can be simplified :

$$T = \frac{\pi N (L \times W)}{4 (L \times W)}$$

$$= \frac{\pi}{4} N$$

$$T = 0.7853 N \quad (2)$$

Multiplied with the grid unit, viz by 1/2 for 1/2 x 1/2 mm grid squares, T gives millimetre measurements.

Formula (2) can be used for larger thalli (more than 100 intersections with the grid) because the formula has become independent of the area.

Formula (2) accords very well with the formula proposed by MARSH (1971), tested on roots by TENNANT (1975), and the formula derived by McINTYRE & PIRIS (1981).

The measurements of the cultures of *Polysiphonia* growing in petridishes were executed under a binocular lens, using a 1 x 1 mm grid.

If the thallus consisted of erect filaments, a coverslip was placed on top of it to flatten the thallus.

Counts are made of the filament/grid crossings. One value is accorded to a filament crossing a line, a filament apex touching a line and a curved portion touching a line; two values to curved portions which lay on and along a line and to a filament overlying a grid cross. For a larger thalli, the counting procedure is to be simplified by counting the intersections with the horizontal lines first and then those with the vertical lines.

The rectangular area is defined by the size of the thallus fragment. The limits of the area are the lines nearest to this fragment which are not crossed by any filament.

Thallus species of *Polysiphonia* cf. *urceolata* (Dillw. ex Lightf.) Grev. were measured at the start of a salinity experiment, after 14 days preconditioning by incubation at salinities to be used in the experiment.

RESULTS

We compared the grid method figures with lengths measured on camera lucida drawings of the thalli (Fig. 1).

The total length of the thallus fragments measured from the drawing (A) and that with the 1 x 1 mm grid (B) using the adapted formula (1) showed a highly significant straight line relationship. For all the lengths measured ($n = 50$) at the different salinities we could calculate a very high correlation coefficient ($r = 0.979$) between the lengths obtained by the two methods.

For the length of the pieces per salinity ($n = 10$) the correlation coefficients (r) varied between $r = 0.936$ and $r = 0.985$ (Table 1). The correlation

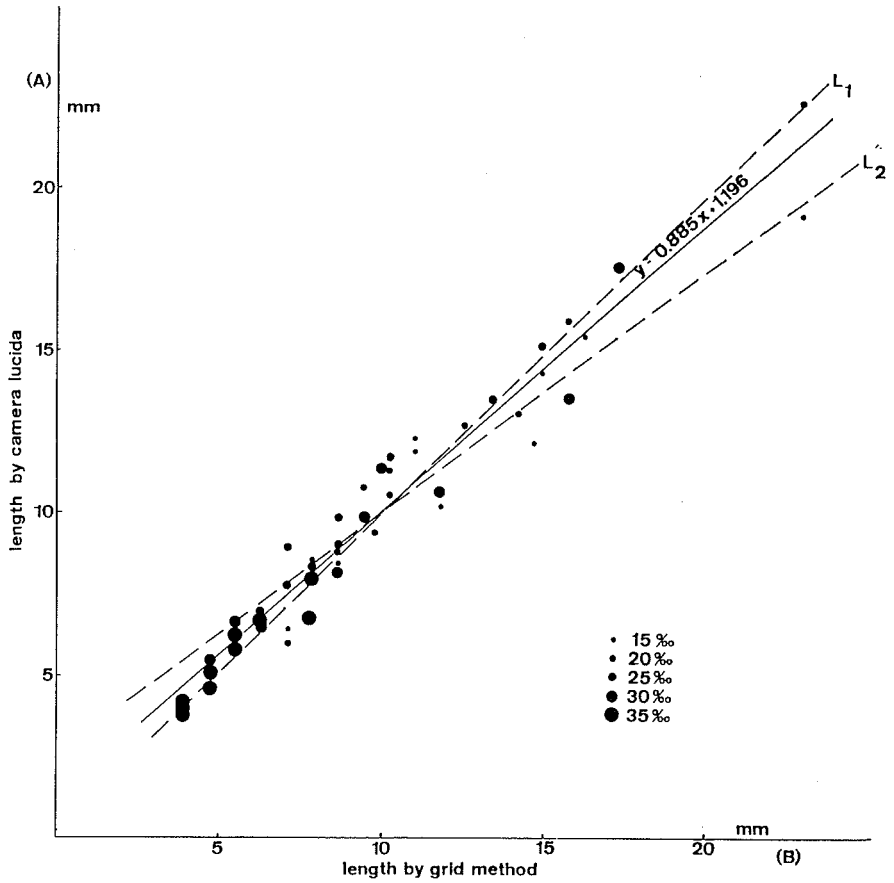

Figure 1 — The relation between the total thallus lenghts measured on drawings made by camera lucida (A) and by the grid method (B) for thallus pieces of *Polysiphonia* cf. *urceolata* (Dillw. ex Lightf.) Grev., incubated for 14 days in different salinities. L_1 ($y = 0.980x + 0.230$) and L_2 ($y = 0.740x + 2.677$) are the 99.9 % confidence limits for the regression line ($y = 0.885x + 1.196$).

between the means is very significant.

We also compared the means of the total thallus length per salinity obtained by the two methods with the Student t-test (Table 1).

We can accept $H_0 : \bar{x}_{(1)} = \bar{x}_{(2)}$ for all the salinities.

The means obtained by the two methods are to be considered as not significantly different.

The maximum deviation (Δ %x) between the means obtained by the two methods is approximately 7.2 % for the means of 10 observations. If the number

Table I – Means (x), standard deviations (s) of total length of *Polysiphonia* cf. *urceolata* (Dillw. ex Lightf.) Grev. thalli and correlation coefficients (r) and t-values (t) between the values obtained by measuring drawings made by camera lucida (A) and by the grid method (B) for different salinities after 14 days incubation at these salinities.

	15°/₀₀		20°/₀₀		25°/₀₀		30°/₀₀		35°/₀₀	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
$\bar{x}$	12.83	13.59	12.13	12.02	10.41	9.66	10.14	10.29	5.60	5.42
s	4.77	5.75	4.51	4.60	2.76	2.99	3.78	4.21	1.41	1.50
Δ % $\bar{x}$ (1)	5.92		-0.91		-7.20		1.48		-3.21	
t	-0.32		0.05		0.52		-0.08		0.28	
r	0.973		0.985		0.978		0.982		0.936	
r^2	0.947		0.970		0.956		0.964		0.876	

$n(1) = 10 \quad n(2) = 10$

critical values t-test $t_{0.05(18)} = 2.101 \quad t_{0.001(18)} = 3.922$

r $t_{0.05(18)} = 0.444 \quad t_{0.01(18)} = 0.561$

of observations is increased, a higher precision can be obtained, even within the 5 % of the true population mean (McINTYRE & PIRIS, 1981).

For measurements on thalli of *Polysiphonia* species, there was no significant difference between the lengths measured with a 0,5 mm x 0,5 mm grid and a 1 mm x 1 mm grid.

The time spent measuring a thallus piece by the grid method was considerably shorter than measuring the drawings made by the camera lucida.

DISCUSSION

The modified line intersection method has been proved practicable and advantageous to measure growth and growth rates of filamentous algae in culture which need continuous monitoring. There is no difficulty in determining the total length of the samples, even without taking the thallus out of the culture dishes. There is no need to cut up the thalli.

The amount of time spent measuring the total length is much shorter than the time spent with other methods (RUSSEL, 1963; DRING, 1967; BOALCH, 1961;..).

The reliability of the method is high, even with a small number of measurements.

For *Polysiphonia* species a 1 x 1 mm grid was very satisfactory, but the smaller filamentous algae (e. g. *Ectocarpus* spp., *Rhizoclonium* spp...) a closer

grid will give better results, and for larger filamentous algae (e. g. *Gracilaria* spp...) a wider grid would be appropriate. Besides estimating total length of filamentous algae, this method can also be used in determining the total outline of foliaceous thalli.

ACKNOWLEDGEMENTS

I wish to thank P. Danneels, B. Demuynck, P. Vervalcke and Q. Vyvey for valuable suggestions about the application of the technique on roots. Dr. Coppejans gave helpful criticism on the manuscript. Special thanks are extended to P. Goetghebeur for correction of the English text.

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