Measuring size and choosing category size for a transition matrix study of the seaweed *Ascophyllum nodosum*

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ABSTRACT: A nondestructive method for measuring the size of the brown alga *Ascophyllum nodosum* (L.) Le Jol. has been modified in order to estimate the dry weight of tagged individuals for a size-based demographic study. There is a very strong positive correlation between the dry weight (dw) of an individual and a crude measurement of its volume. A total of 786 individuals were sampled on 3 occasions in 2 populations and the maximum length (l) and circumference (c) (in cm), and dry weight (in g) were measured. A model II regression was used to obtain the relationship between dry weight and lc^2, and since there was a significant difference between spring and autumn measurements, 2 predictive equations were needed; for spring $\log_e(dw) = 0.7900 \log_e(lc^2) -2.8547$ (r = 0.99), and for autumn $\log_e(dw) = 0.8605 \log_e(lc^2) -3.3769$ (r = 0.99). It is also possible to use this method on other species of seaweeds. Two methods for choosing the width of size classes were tested. One allowed the division of a population into subpopulations and the use of more than 2 census dates, and was found to be the most appropriate for this study. The first size-class was biologically distinct and was set to 0–5 g, which includes almost all nonfertile individuals. Four more were found by the algorithm and their lower limits are 5, 15, 54, and 190 g and these are used in the size-based demographic study.

INTRODUCTION

The demographic behaviour of an organism is the basis of many ecological and evolutionary studies. One general method to describe the population dynamics of an organism is the Leslie Matrix model (Lewis 1942, Leslie 1945, 1948), which has been used on many different organisms. The model is based on age-specific survival and reproduction, but for many organisms there is a poor correlation between an individual's age and its probability to survive and reproduce (Sauer & Slade 1987 and references therein). For such organisms the fate of an individual is better predicted by its size. For organisms with indeterminate growth through their lives, where individuals might shrink or fragment and still continue to grow, the use of size is preferred to age. This applies to almost all macroalgae and thus size should be used as the categorical variable in almost all demographic studies of macroalgae. The Leslie Matrix model has been extended for stage categories by Lefkovitch (1965, 1967) and a general matrix model for size structured populations is given in Hughes (1984). Ang (1987) used it in a study of brown seaweeds

(*Sargassum* spp.) and recent studies also show that size is a better categorical variable than age even for higher organisms such as vertebrates (Sauer & Slade 1987).

Theoretically it is easy to gather the data for a stage transition matrix by measuring the size of each individual at 2 points in time, but for many macroalgae this is not always easy. The method must be nondestructive and have a high resolution. Parameters such as length, area and volume of the thallus often have a strong correlation to dry weight, although the preferred parameter depends on the species. However, the problem for macroalgae is how to measure these parameters in an easy and precise way. Photogrammetric techniques (Littler & Littler 1985) to estimate the area or length of an individual may be useful for some species, if it is possible to distinguish individuals and the problem of layering is minimal. For some species, where the length of the thallus is highly correlated to dry weight, it is possible to use a standard measuring tape. For *Ascophyllum nodosum* it is difficult to use photogrammetric techniques in dense populations, and length does not correlate well enough with dry weight. Thus some sort of volume measurement must be used. Pielou

(pers. comm. in Cousens 1984) found a high correlation between *A. nodosum* plant dry weight and a crude measure of plant volume. This paper presents a modification of the method described in Cousens (1984), which allows for its use in a stage transition matrix study.

A more general problem with stage-transition matrix studies is choosing the appropriate width of the size classes, because changes in the category size may have a significant effect on the transition probabilities. Vandermeer (1978) described a method to estimate 2 types of errors connected to category size: distribution error (DE) which is large if the chosen category size is too large, and sample error (SE) which is large if the chosen category size is too small. When these errors are estimated, it is possible to choose a category size which is an 'optimal' compromise between the 2 errors. This method has been referred to in a few papers (Bullock 1980, McGraw & Antonovics 1983, Crouse et al. 1987, Huenneke & Marks 1987, Manders 1987) but used in only 2 (Bierzychudek 1982, Fiedler 1987). Moloney (1986) extended Vandermeer's method to include divisions of a population into subpopulations and to use more than 2 census dates. This model has thus far only been used by Moloney (1988). In this paper both methods are tested on 2 populations of *Ascophyllum nodosum* and other considerations concerning category sizes are also discussed.

MATERIALS AND METHODS

This paper is part of a population dynamics study of *Ascophyllum nodosum* (L.) Le Jol. carried out on populations at some small islands 5 km west of Göteborg (57°38' N, 11°45' E) and 10 km west of Tjärnö Marine Biological Laboratory (58°52' N, 11°00' E) on the Swedish west coast. These sites are hereafter referred to as Göteborg and Tjärnö. In the autumn of 1985, 1986 and 1987 all *A. nodosum* individuals in randomly chosen 0.25 × 0.25 m quadrants were tagged around their holdfasts with numbered plastic-cable ties. The size of the individuals (maximum length and circumference as described below) was measured twice each year: in spring just before gamete release, and in autumn. The individuals were followed from the tagging date to their death or to the autumn of 1988, which was the last census date. The sex ratio was 1:1 at both sites, and genets (Kays & Harper 1974) were distinguished according to the method described by Åberg (1989).

Size measurements. A crude estimate of thallus volume (Pielou pers. comm. in Cousens 1984) is achieved by measuring the maximum length (l) and the maximum circumference (c) of each individual (Fig. 1) and lc^2 will be proportional to volume. The length measure-
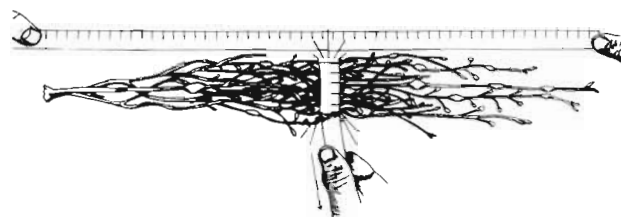

Fig. 1. Schematic drawing indicating how maximum length and maximum circumference are measured

ment is straightforward but the circumference is more crucial. All fronds from one individual are laid parallel, and the circumference of the bundle of fronds is measured in several places until the maximum is found. It is also important to tighten the measuring tape in the same way every time. To obtain the relationship between lc^2 and dry weight (dw), the length and the circumference are measured on a number of individuals outside the tagged areas. These are then harvested, dried and weighed, and the relationship is obtained by regressing dry weight on volume (lc^2). In order to obtain variations between years, seasons and sites, a total of 786 individuals of all sizes was sampled at Tjärnö and Göteborg (outside but close to the area of tagged individuals) in the spring of 1985 and 1986 and in the autumn of 1988. A plastic measuring tape was used to measure maximum length and circumference and the readings were done with 0.5 cm precision. For very small individuals with a circumference less than 1.5 cm it was not possible to use the measuring tape, and their circumference was set to a value of 0.75 cm. To minimize variation in the measurements due to different tightening of the measuring tape all measurements were done by one person. The algae were dried at 60 °C until constant weight (approximately 48 h) and weighed on a precision balance to a precision of 0.01 g. To achieve a normal distribution, both dw and lc^2 were logarithmically transformed. Both dw and lc^2 are subject to error and thus a model II regression was used (Larsen & Marx 1986). A statistical test (Lehmann 1986), which compares the regression coefficients of 2 regression lines, was used to detect differences between sampling sites and dates in order to pool some of the data. The equation of the regression line is used to estimate the dry weight of an individual (in g) by inserting its length and circumference (in cm) from the field measurement.

Category size. The basic data used in Vandermeer's (1978) and Moloney's (1986) models are transition probabilities and here they were taken from the study of the tagged populations. The 2 negatively correlated errors that both models are trying to minimize are somewhat arbitrary. The DE relates to how individuals

are distributed within the size category. If the chosen size category is too large it is likely that only large individuals have the possibility for entering the next size class in one time interval and DE will be high. If the upper limit of the size class is set to a much lower value, the difference between small and large individuals within the class will decrease, and it is possible for small individuals to enter the next size class and DE will be lower. Theoretically, for an infinitely small category size, DE will be zero.

A transition matrix study has a finite number of individuals and the SE depends on how many individuals there are in a size category. If the size category is large it will contain many individuals and SE will be low but if the upper limit of the size class is set to a much lower value it will contain fewer individuals and SE will be higher.

The algorithms for the 2 models are rather different, but they are used in a similar way (for details see the original papers) and the following paragraph summarizes the procedure for choosing category size.

Choose the lower limit of the first size category (containing the smallest individuals) and set the upper limit to a value much greater than the expected maximum size of the category and compute DE and SE. Shorten the category size by setting the upper limit to a lower value and compute DE and SE for this category size. Continue with this until the number of individuals within the category size is less than some predetermined value or until the width of the category size is zero. Make a linear plot of DE and SE against the upper limit of the category size and the intersection point (Vandermeer's model) or the point where DE+SE is at a minimum (Fig. 2) (Moloney's model) this gives the 'optimal' category size. This procedure is repeated with the 'optimal' upper limit as a new lower limit, until all size categories are defined.

Size categories with a biological meaning should generally be preferred to those generated by a model and thus the first category size was set to 0 to 5 g dw, which includes most non-fertile individuals (own unpubl. data).

For the Vandermeer model, which only allows 2 census dates, category sizes were determined for each population at each time interval, with the restriction that each category should include at least 25 individuals at the start of the time interval, which gives a total of 12 category size estimations. With Moloney's model which allows population division in time and space, category sizes were determined for one population (pooled data for the 2 sites) with 7 census dates and for one population with 2 subpopulations (each site seen as a subpopulation) and 7 census dates, with the restriction that each category should at least include 50 individuals at the start of the time interval.
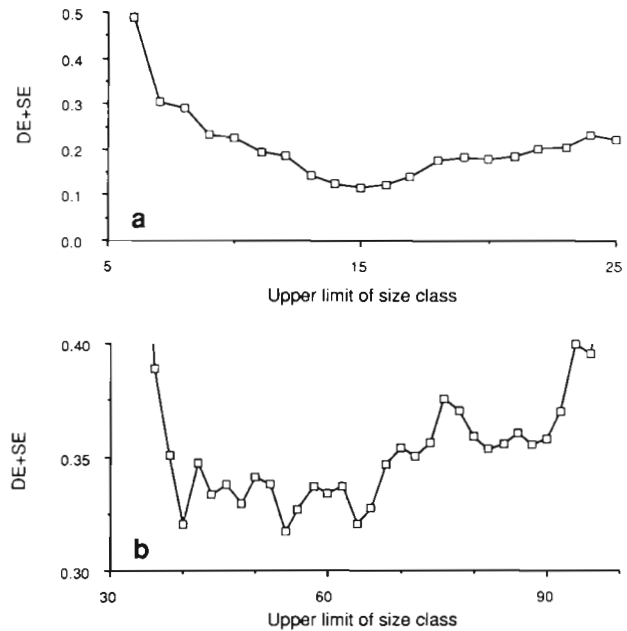

Fig. 2. Two examples of how the upper limit of a size class is chosen with Moloney's (1986) method. The upper limit is chosen where the combined distribution and sample error (DE+SE) has its lowest value. (a) The lower limit is set to 5 (g dry weight) and the upper limit is calculated to 15 (g dry weight). (b) The lower limit is set to 15 (g dry weight) and the upper limit is calculated to 54 (g dry weight)

RESULTS AND DISCUSSION

Size measurements

The scatter plot of all data, for dw against lc^2 (Fig. 3) clearly shows the relationship between the 2 variables. The data were divided into sampling sites and dates and a model II regression was performed. The results are shown in Table 1 and there is a very strong positive
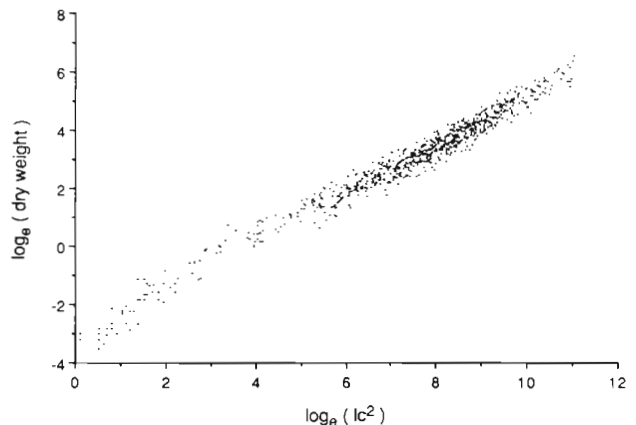

Fig. 3. Relationship between individual $\log_e$(dry weight) and $\log_e(lc^2)$ (l = maximum length, c = maximum circumference of an individual) for all data

Table 1. Regression lines, $\log_e$ (dw) = b[$\log_e$(lc^2)] + a for the sites Göteborg (G) and Tjärnö (T) in spring (s) or autumn (a). dw: dry weight; l: max. length; c: max. circumference; cc: correlation coefficient; N: no. tested

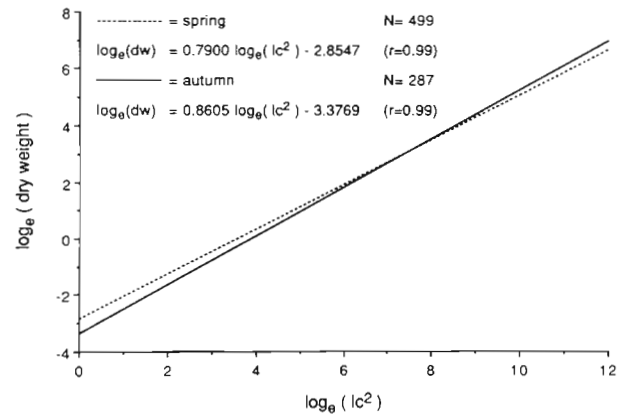| Site	b	a	cc	N
G85s	0.7674	−2.8832	0.9899	107
G86s	0.7854	−2.7625	0.9610	129
G88a	0.8491	−3.3718	0.9704	151
T85s	0.7938	−2.9666	0.9930	132
T86s	0.7507	−2.3785	0.9787	131
T88a	0.8679	−3.3389	0.9931	136

Fig. 4. Regression lines for the relationship between $\log_e$(dry weight) and $\log_e$(lc^2) (l = max. length, c = max. circumference). Individual points are omitted for clarity

correlation between dw and lc^2, with correlation coefficients ranging from 0.96 to 0.99. The results from the model II regression may not be that different from those of a model I regression, because the correlation coefficients are high (Laws & Archie 1981). However, according to Laws & Archie (1981) model II regression should be used in almost all field studies and thus it is the most appropriate for this study. Table 2 shows the results from the tests of the regression coefficients. The trend that can be seen from this is that, regardless of years and sites the regression coefficients are significantly different if the data were sampled in different seasons, but not significantly different if sampled in the same season. Thus the data sampled in the spring was

Table 2. Comparisons of regression coefficients (b1 and b2 from Table 1) for the sites Göteborg (G) and Tjärnö (T) in spring (s) or autumn (a). N: no. tested; ns: p > 0.05; • p < 0.05; •• p < 0.01; ••• p < 0.001

Site	b1	b2	N	p
Between sites, within a year				
G85s – T85s	0.7674	0.7938	235	ns
G86s – T86s	0.7854	0.7507	256	ns
G88a – T88a	0.8491	0.8679	283	ns
Between sites, between years, within a season				
G85s – T86s	0.7674	0.7507	234	ns
G86s – T85s	0.7854	0.7938	257	ns
Between years, within a site, within a season				
G85s – G86s	0.7674	0.7854	232	ns
T85s – T86s	0.7938	0.7507	259	•
Between seasons, between years, within a site				
G85s – G88a	0.7674	0.8491	254	•••
G86s – G88a	0.7854	0.8491	276	•
T85s – T88a	0.7938	0.8679	264	•••
T86s – T88a	0.7507	0.8679	263	•••
Between seasons, between years, between sites				
G85s – T88a	0.7674	0.8679	239	•••
G86s – T88a	0.7854	0.8679	261	•••
G88a – T85s	0.8491	0.7938	279	••
G88a – T86s	0.8491	0.7507	278	•••

pooled as well as the data sampled in the autumn. The regression lines are shown in Fig. 4 and the predictive equation for spring measurements is:

$$\log_e(dw) = 0.7900 \log_e(lc^2) - 2.8547 \qquad (1)$$
$$(r = 0.99, N = 499)$$

and for autumn measurements is:

$$\log_e(dw) = 0.8605 \log_e(lc^2) - 3.3769 \qquad (2)$$
$$(r = 0.99, N = 287)$$

The regression coefficients for Eqs. (1) and (2) are significantly different (p < 0.001). To estimate the dry weight of the tagged individuals of *Ascophyllum nodosum* Eq. (1) was used for spring measurements and Eq. (2) for autumn measurements.

The main difference between this study and that of Cousens' (1984) is that here a logarithmic transformation on both dw and lc^2, and a model II regression has been used, while Cousens used a square root transformation on lc^2 and probably a model I regression. Cousens' method was also tested on the pooled data presented here, which gave the predictive equation:

$$dw = 1.52\sqrt{(lc^2)} - 37.0 \quad (r = 0.90, N = 786) \qquad (3)$$

which is rather similar to:

$$dw = 1.39\sqrt{(lc^2)} - 44.8 \quad (r = 0.94) \qquad (4)$$

found by Cousen. Even though a straight line can be fitted and the correlation coefficients are high, some sort of curve seems to be more accurate (Fig. 5). Both Eqs. (3) and (4) have a negative y-intercept which means that the equations will estimate negative dry weights for individuals with a real dry weight of approximately < 15 g. For a crude measurement of the total biomass in an area this may not be so important, but for demographic studies it is essential that no individuals have negative dry weights. The double

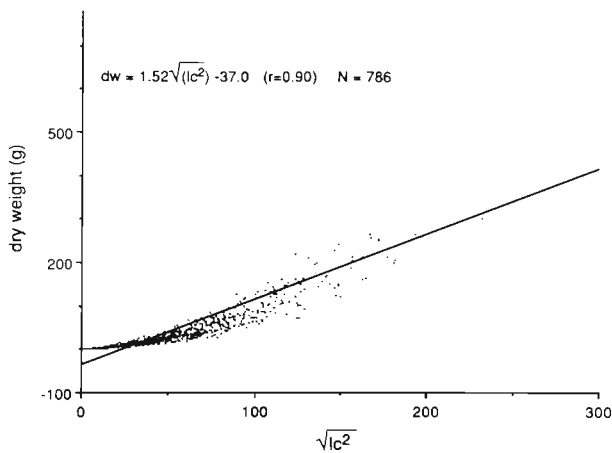$$dw = 1.52\sqrt{(lc^2)} - 37.0 \quad (r=0.90) \quad N = 786$$

Fig. 5. Regression line found using Cousens' (1984) method on all data

logarithmic transformation, which was done to give a normal distribution of the data, also has the advantage that the regression equation rewritten in a non logarithmic way will go through origin. Thus no estimated dry weights will be negative. Eqs. (1) and (2), rewritten become:

for Eq. (1)

$$dw = 0.057 \ (lc^2)^{0.7900} \tag{5}$$

for Eq. (2)

$$dw = 0.034 \ (lc^2)^{0.8605} \tag{6}$$

These relations are shown in Fig. 6 together with that found by Cousens (1984) and the differences for the small individuals are clearly shown (Fig. 6b).

Cousens (1984) did not find significant differences in the relationship between dw and lc^2 for various sampling dates in spring and summer or between different sites, but in this study the comparison of regression coefficients showed a pronounced difference between seasons, and that 2 regression lines, one for spring and one for autumn, were needed. The difference between Eqs. (5) and (6) is most pronounced for fertile individuals with dw > 30 g (Fig. 6b) where individuals with equal lc^2 have higher estimated dry weights in the autumn, and it is probably due to differences in tissue density. Receptables are large in the spring which may affect the density of the individual, or when tightening the bundle of fronds, more water may be included between the fronds. Other variations, within the same season, in dw between individuals with the same lc^2 may be due to different shape e.g. some have a shape like a cylinder while others look like 2 cones. The relationship between lc^2 and dw would be almost perfect if all individuals had the same shape.

One other possible source of error is the measurement of length (l) and circumference (c). The length
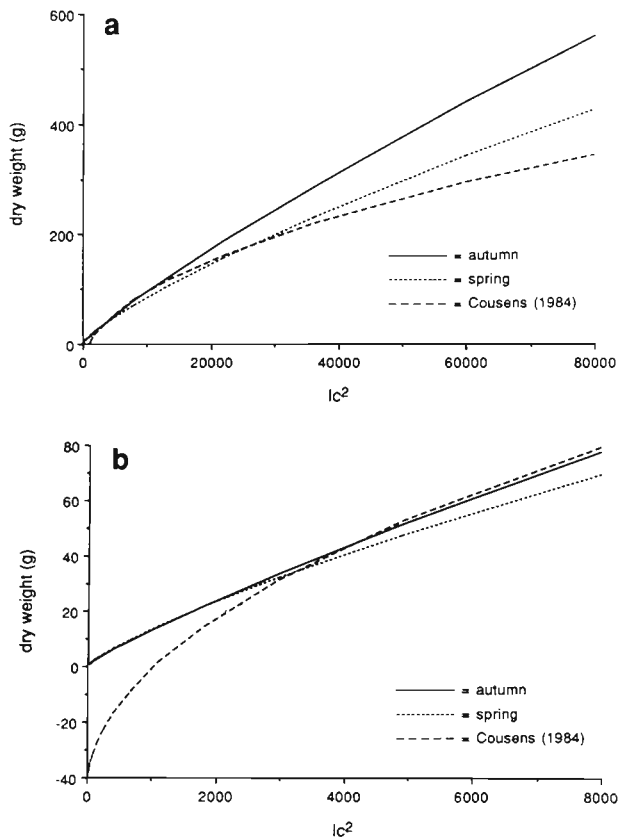

Fig. 6. A comparison of the regression lines for autumn (Eq. 2) and spring (Eq. 1) with that found by Cousens (1984) (Eq. 4) shown on a non-logarithmic graph, where (b) is a close-up of (a). Individual points are omitted for clarity

measurement can easily be done with high precision and the estimated dry weight is not as sensitive to changes in length as it is to circumference, which is squared in the calculation of volume. It is also more difficult to measure the circumference with high precision, because the measuring tape must be tightened in the same way every time. Training is recommended before measuring in the field, especially if several people are involved in the measurements. The circumference may also be overestimated due to epiphytes. There is a large variation in the amount of epiphytes between individuals, with a maximum of 20 % of the total weight and a mean of 3 ± 0.02 % SE (own obs.). Large epiphytes such as the brown alga *Pilayella littoralis* and the red alga *Ceramium rubrum* are often situated in the upper parts of the 'host' plants but the maximum circumference is almost always below this region. Thus epiphytes are not a major source of error when measuring the circumference of *Ascophyllum nodosum* individuals.

This modified method, with increased resolution, makes it possible to follow changes in size on tagged *Ascophyllum nodosum* individuals. Thus it is now pos-

sible to gather the basic data for a size-based transition matrix study. The problem of nondestructive size measurements on seaweeds is general and this method is also applicable to other species of seaweeds. The method is relevant for species with a morphology similar to *A. nodosum*, e.g. the brown algae *Cystoseira* spp. and *Sargassum* spp., the red alga *Cystoclonium purpureum* and many other seaweeds, but it is also applicable to filamentous red and green algae.

Category size

The 12 sets of category sizes found by using Vandermeer's method and those 2 found by using Moloney's method are shown in Table 3 with an example of how the size class limits are chosen (Fig. 2). On the average, fewer size categories were found with Vandermeer's

Table 3. The lower limit of each size class (g dry weight) found by Vandermeer's (1978) and Moloney's (1986) method. Site notation as in Table 1

Site or subpopulation	No. stage categories	Estimated lower limits for categories				
Vandermeer's method						
G85a – 86s	5	0	5	26	65	190
T85a – 86s	4	0	5	30	150	
G86s – 86a	5	0	5	13	30	180
T86s – 86a	5	0	5	13	40	110
G86a – 87s	5	0	5	12	58	150
T86a – 87s	5	0	5	12	30	74
G87s – 87a	4	0	5	24	200	
T87s – 87a	3	0	5	40		
G87a – 88s	5	0	5	18	40	125
T87a – 88s	6	0	5	15	29	85 360
G88s – 88a	4	0	5	23	65	
T88s – 88a	5	0	5	16	55	150
Moloney's method						
Subpopulation 1	5	0	5	15	54	190
Subpopulation 2	5	0	5	17	64	210

algorithm and this is probably due to the fact that fewer transitions were used in each calculation. The 2 sets of category sizes generated with Moloney's model are very similar, with only small differences in base values. The results from Vandermeer's model are more variable, but the geometric means for the third, fourth and fifth base values are 19, 56 and 127 g, in which the third and fourth are rather close to those found by Moloney's model. The fifth is lower, which also indicates the disadvantage of Vandermeer's model, in which fewer transitions are used in each calculation.

In the demographic study of *Ascophyllum nodosum*, different years and populations are compared and thus only one set of category sizes can be used. For this purpose Moloney's model seems to be the most appropriate, although Vandermeer's model generated similar results. To choose between the 2 sets generated with Moloney's model is difficult because the results are very similar, but the magnitude of SE and DE increases with increased number of subpopulations (Moloney 1986) and therefore the case with one population will be used (Table 3).

Studies of size based demography have become more frequent during the last years and the need for choosing appropriate category sizes is thus large. There are 4 possible solutions for choosing category size which all have been used: (1) biologically distinct stages e.g. Crouse et al. 1987; (2) using an algorithm e.g. Bierzychudek 1982; (3) equally large classes, in a linear or logarithmic scale e.g. McGraw & Antonovics 1983; and (4) arbitrary size classes e.g. Hughes 1984. The methods can also be combined (e.g. this study, Method 1 and 2). Classes with a biological meaning are to be preferred, because it is easier to interpret the transition matrix. However, for many organisms it is not possible to find distinct biological classes and thus some other method must be used. Using an algorithm for choosing category size is attractive, but it is desirable to have much detailed information on the species in question so that biologically irrelevant classes, that for some reason may be generated by the model, can be discovered. For *Ascophyllum nodosum* only the first class is biologically distinct and thus the 2 models, where Moloney's showed to be the most relevant, were used. Biologically relevant classes for a perennial seaweed such as *A. nodosum* should be approximately equally large on a logarithmic scale. This is because the very large range of sizes and the fact that larger individuals have the ability to grow much more than smaller ones. The classes found with Moloney's model are approximately equally large on a logarithmic scale and therefore are appropriate to use.

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