Estimating leaf age of the seagrass *Posidonia oceanica* (L.) Delile using the plastochrone interval index

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Accepted 26 April 1994

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**Abstract**

We describe a method to estimate the leaf age of *Posidonia oceanica* (L.) Delile, which yields continuous estimates compared with the discrete estimates of leaf age obtained using the traditional approach. Application of the new method offers considerable advantages to examine aspects of seagrass ecology dependent on leaf age. Regression of age-dependent properties of seagrasses, such as the leaf surface lost to herbivores or epiphyte load, against the continuous estimates of leaf age estimated with the method proposed allows calculation of important rates for seagrass ecology, such as herbivory rate or epiphyte accrual. The interpolation method to estimate leaf age described provides, therefore, an important tool to assess the ecological implications of seagrass leaf age.

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1. **Introduction**

Many important processes for seagrass ecology depend on leaf age, such as photosynthesis (Mazzella and Alberte, 1986), leaf growth (Sand-Jensen, 1975), chemical composition (Thayer et al., 1984; Zieman et al., 1984), epiphyte cover (Borum, 1987; Casola et al., 1987), and grazing loss (Sand-Jensen et al., 1994). Hence there is often the need to measure leaf age as a control variable in quantitative studies of seagrass ecology.

Seagrass leaves are produced continuously by apical meristems according to an alternate pattern, such that older leaves are located in the exterior of the leaf cluster, which allows estimation of leaf age on individual shoots as leaf rank order (Caye and Rossignol, 1983; Casola et al., 1987; Sand-Jensen et al., 1994). The

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leaf age estimates delivered from rank orders are, however, unprecise for the following reasons: (1) the discrete nature of leaf ranks, the number of possible ranks ranging from 2 to 8 (Den Hartog, 1970; Duarte, 1991); (2) the assumption that all leaves with the same rank order, which may differ in age by twice the mean leaf plastochrone interval (PI) (1.1–47.2 days; Duarte, 1991), have the same age. Leaf rank orders can be translated into age as absolute time (days) using knowledge of the PI (e.g. Patrquin, 1973; Ott, 1980; Romero, 1985), but this procedure does not improve the precision of the estimates obtained, which are still discrete and assume the same age for all leaves with the same rank order.

The interpolation method needed to estimate leaf age more precisely, avoiding the discrete scaling of leaf age associated with the use of leaf rank order, has long been available (Erickson and Michellini, 1957; Lamoreaux et al., 1978). This method is based on the leaf PI concept (Askenasy, 1880) and has not yet been applied to estimate seagrass leaf age, although the concept of PI has been used extensively in seagrass ecology (e.g. Brouns, 1985; Duarte et al., 1994).

We demonstrate here, using Posidonia oceanica (L.) Delile as test material, the use of the interpolation method to yield reliable, continuous estimates of seagrass leaf age, and its potential to quantify processes dependent on leaf age, such as herbivory rates and epiphyte accrual.

2. Methods

2.1. The Erickson and Michellini method to estimate leaf age

Erickson and Michellini (1957) expanded the original concept of leaf PI as the time span between the appearance of consecutive leaves (Askenasy, 1880), to refer to the time period for two consecutive leaves to reach the same developmental stage. From this definition, leaf age in PI units can be calculated as the ranked position of the leaf in the shoot, from youngest to oldest, minus 1 plus a fraction corresponding to the age of the youngest leaf, which is, by definition, less than one PI. They then derived an interpolation method, based on the determination of the fractional age of the youngest leaf, to obtain continuous estimates of leaf age, rather than the traditional discrete estimates. To achieve this they assumed, based on previous observations, a constant exponential growth rate \( r \) for the youngest two leaves on the shoot (leaves 1 and 2), the length \( L \) of which is then given by

\[
\ln L_1 = \ln (\text{reference length}) + r \times t_1
\]

\[
\ln L_2 = \ln (\text{reference length}) + r \times t_2
\]

where \( t_1 \) and \( t_2 \) are the ages of the first and second leaves, respectively. The assumption of equal growth rate for the two youngest leaves in the Erickson–Michellini method is supported for P. oceanica (cf. Ott, 1980; Romero, 1985).

Since a new leaf is only detected as it emerges above the sheath of the oldest
leaf, this length is adopted as the reference length \((L_r)\). Because \(t_2 - t_1\) equals a leaf PI, the quotient \(\frac{t_1}{t_2 - t_1}\) represents the age (in fractions of a leaf PI) of the first leaf (Erickson and Michellini, 1957). Hence, rearranging Eqs. (1) and (2), the age of the first leaf is given by

\[
\frac{t_1}{t_2 - t_1} = \frac{\ln L_1 - \ln L_r}{\ln L_2 - \ln L_1}
\]

Once this is known, the age of any leaf on a shoot can be calculated as its rank number minus 1 plus \(\frac{t_1}{t_2 - t_1}\). These age estimates, in leaf PI units, can be transformed to days by multiplying them by the leaf PI equivalence in days.

2.2. Leaf age of Posidonia oceanica

We sampled a \(P.\ oceanica\) meadow (4 m deep, Cala Junquet, NE Spain) at intervals of 4–7 weeks between January 1992 and June 1993. At each visit, we marked, using a modified leaf marking technique (Pérez et al., 1991), the leaves of 15 shoots to estimate the number of new leaves produced per shoot per sampling interval. From 60 shoots collected on each sampling date, we registered the ranked position of the leaves, the lengths of the two youngest leaves and the sheath length of the oldest leaf on each shoot to estimate leaf age in PI units using Eq. (3), which was converted into days by multiplying by the corresponding leaf PI equivalence in days.

The leaf surface consumed by herbivores and the epiphyte biomass were measured on June 1993. The leaf surface lost to herbivores was estimated, for all leaves in the sample, from the area of grazing scars (Ogden, 1990; Sand-Jensen et al., 1994). The leaf surface lost was transformed to biomass (dry weight) from measurements of the specific weight of leaf samples. Epiphytes were recovered by scraping them off apical sections (5 cm long) of each sampled leaf, and weighed, following desiccation at 80°C for 24 h, to estimate biomass. The time available for the development of the epiphyte community equals leaf age.

3. Results and discussion

The rate of appearance of new leaves on \(P.\ oceanica\) shoots varied seasonally, leaves appearing fastest in the fall and slowest in the spring (Fig. 1). We calculated, using the Erickson–Michellini method, the maximum leaf longevity of \(P.\ oceanica\) to be about 1 year (346 days), with the average leaf age ranging from 108 ± 7 to 162 ± 8 days in October and July, respectively. The continuous leaf age estimates obtained using the Erickson–Michellini (E&M) technique were closely correlated \((r=0.90)\) to the discrete estimates derived from the ranked position and leaf PI (Fig. 2), as reflected in the regression equation
Leaf age (days—E&M)

\[ = -2.1(\pm11.7) + 0.97(\pm0.08) \]

Leaf age (days—marking)

\[ R^2 = 0.82, \ N = 32, \ F = 143, \ P < 0.0001 \]

whose slope and intercept are not significantly different \((P > 0.1)\) from 1 and 0,

Fig. 1. Seasonal variation in the leaf appearance rate of *P. oceanica* in Cala Junquet.

Fig. 2. The relationship between leaf age estimated using the E&M method and the discrete estimates obtained from leaf marking techniques. Symbols correspond to estimates derived in spring (○), autumn (□), and summer and winter (●).
respectively. Hence, the E&M method yielded reliable, unbiased estimates of leaf age, with the advantage of having a continuous distribution.

The annual average leaf PI can be estimated, for this one and other species, from a single sampling visit using lepidochronological or reconstruction techniques (Pergent, 1990; Duarte et al., 1994). The annual average leaf PI (±SE) calculated from leaf marking was 49.5 ± 10.8 days, remarkably similar to that obtained in a single visit using reconstructing techniques (47.5 ± 1.1 days, Duarte et al., 1994). The estimates of leaf age calculated with the E&M method using the annual mean leaf PI were also significantly correlated (r = 0.81, P < 0.0001) with the discrete estimates of leaf age. This indicates that it is also possible to estimate the leaf age of *P. oceanica*, and that of other species with limited PI seasonality, from a single sample, albeit at the expense of reduced precision.

The convenience of the use of leaf age in seagrass ecology depends on the cumulative nature of many relevant processes, such as leaf loss due to herbivory (Sand-Jensen et al., 1994) or net epiphyte accrual (Borum, 1987). Herbivory can be calculated as the slope of the regression equation between leaf material lost to herbivores and leaf age (Fig. 3(a)), and the production lost to herbivores corresponds to the total biomass lost over the leaf life span (i.e. at maximal leaf age, Sand-Jensen et al., 1994). This procedure allowed estimation of herbivory on the *P. oceanica* meadow sampled to proceed at a rate of 70 ± 9 µg dry weight (DW) per leaf day⁻¹, and to remove 5.2 mg DW over the leaf life span (Fig. 3(a)). Similarly, the average rate of net epiphyte accrual, an important determinant of seagrass productivity and survival (McRoy and Goering, 1974; Silberstein et al., 1986), can also be calculated as the slope of the regression equation relating epiphyte biomass to leaf age (Fig. 3(b)). We calculated, in this way, epiphytes to accumulate at a net rate of 10 µg DW cm⁻² day⁻¹ in the leaves of

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**Fig. 3.** The relationship between the leaf biomass lost to herbivores (a) and the epiphyte biomass (b) and *P. oceanica* leaf age calculated from the E&M method, and the associated estimates of herbivory rate and epiphyte accretion rate. Values represent means ± SE for leaves grouped in 30-day age classes.
the *P. oceanica* meadow studied. The estimates of these processes obtained with the E&M method are superior to those obtained using discrete estimates of leaf age, because they provide a continuous distribution of data points along the independent variable (leaf age), needed to obtain reliable regression estimates (Sokal and Rohlf, 1981).

In conclusion, our results demonstrate the reliability of the continuous estimates of leaf age derived using the E&M method for *P. oceanica*. Use of this method represents an improvement relative to the traditional discrete method used in seagrass ecology because it avoids the assumption that all leaves with the same rank order have the same age, and the imprecision associated to this assumption. The continuous estimates of leaf age obtained with the E&M method allow a more robust quantification of processes dependent on leaf age, such as herbivory and epiphyte accretion. Hence, this method developed three decades ago by Erickson and Michellini (1957), and firmly established as an important tool in plant science (Lamoreaux et al., 1978), may also open an important avenue for progress in the study of leaf-age dependent aspects of seagrass ecology.

**Acknowledgements**

This research was partially funded by project CI1*-CT91-0952 from the Commission of the European Communities, Project AMB93-0118 of the Spanish Commission of Science and Technology (CICYT) and a CIRIT scholarship to J.C. We thank Carmelito Soneyer for hospitality.

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