Lepidochronological analysis in the Mediterranean seagrass *Posidonia oceanica* : State of the art and future developments

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ABSTRACT

After blade shedding in the seagrass *Posidonia oceanica*, leaf sheaths remain attached to the rhizome and can persist for several decades within a "matte". They show, according to their insertion rank, cyclical variations in parameters such as thickness and tissue structure. These cycles are always annual, and may be influenced by environmental parameters such as light, temperature or water movement. The study of cyclic changes in *Posidonia oceanica* sheaths is thus a technique that can be used to investigate past changes in the near-shore environment. By analogy with dendrochronology, this study is termed lepidochronology. A number of applications of lepidochronological analysis have already been successfully tested : (i) Estimation of the growth rate and annual production of rhizomes; (ii) Modelling of leaf renewal cycles (*e.g.* rhythm of leaf formation and of leaf fall, leaf longevity); (iii) Detection and dating of previous flowering from the remains of flower stalks inserted between the sheaths; (iv) Evaluation of primary production. This new technique is much faster than the classic methods and has the further advantage that primary production for previous years can also be estimated. Twenty-two sites in the Mediterranean sea, between 0.7 and 32.0 m depth have been investigated using this technique.
The possibility that chemical events (*e.g.* heavy metals) may be memorized in *Posidonia oceanica* sheaths and rhizomes is currently being investigated.

RÉSUMÉ

Analyse lépidochronologique de la phanérogame marine *Posidonia oceanica* : Bilan et prospectives

Chez la phanérogame marine *Posidonia oceanica*, après la chute du limbe, le pétiole reste attaché au rhizome et peut persister plusieurs décennies au sein de la matte. En fonction de leur rang d'insertion, ces pétioles montrent des variations cycliques de leur épaisseur et de leur anatomie. Ces cycles sont toujours annuels mais peuvent être influencés par des paramètres du milieu comme la lumière, la température ou l'hydrodynamisme. L'étude de ces variations cycliques apparaît comme une méthode qui peut être utilisée dans l'étude de l'évolution du milieu littoral. Par analogie avec la dendrochronologie, cette technique est appelée lépidochronologie.
Plusieurs applications de la lépidochronologie ont déjà été testées avec succès : (1) Estimation de la vitesse de croissance et de la production annuelle de rhizomes; (2) Modélisation du cycle de renouvellement des feuilles (*e.g.* rythme de formation, rythme de chute et longévité); (3) Mise en évidence et datation

d'anciennes floraisons à partir de restes de pédoncules floraux intercalés entre les pétioles; (4) Evaluation de la production primaire. Cette nouvelle technique, plus rapide que les méthodes traditionnelles, permet également d'évaluer la production primaire des années antérieures. Vingt-deux stations du bassin méditerranéen, situées entre 0,7 et 32,0 m de profondeur, ont ainsi été étudiées. La possibilité que des caractéristiques chimiques du milieu (e.g. concentration en métaux-traces) puissent être mémorisées dans les pétioles et les rhizomes de *Posidonia oceanica* est en cours d'investigation.

INTRODUCTION

Posidonia oceanica (Linnaeus) Delile, a marine phanerogam abundant in the Mediterranean, grows between the surface and 30 to 40 m depth in extensive beds called meadows ("herbiers" *in* Molinier and Picard, 1952).

Posidonia oceanica rhizomes grow in the vertical (orthotropic rhizomes) or horizontal plane (plagiotropic rhizomes); these intertwined living or dead rhizomes, together with the sediment which fills the interstices, constitute a most characteristic structure, "the matte". At the rhizome apex, *Posidonia oceanica* shoots, of distichous phyllotaxy, consist of 4 to 8 leaves.

When the leaves die, only the blade falls away; the sheath remains attached to the rhizome. Sheaths and rhizomes decay little and can persist within the matte for millennia (> 4 600 years, Boudouresque *et al.*, 1980).

Sheath thickness shows cyclic variations according to insertion rank along the rhizome (Crouzet, 1981; Pergent *et al.*, 1989). These cyclical variations have a chronological significance with each cycle corresponding to a one-year period (Pergent, 1990). This phenomenon appears to resemble variations observed in tree ring thickness (Douglass, 1936). By analogy with dendrochronology, the study of these cyclical variations and the main related phenomena has been termed "lepidochronology" (Boudouresque *et al.*, 1983; Pergent, 1990).

The analysis of a large number of rhizomes, sampled over a one year period or at intervals at numerous sites around the Mediterranean, from shallow to deep water, has made it possible (i) to establish a standardized procedures for studying this phenomenon; (ii) to determine its characteristics with accuracy; and (iii) to develop a wide variety of applications.

LEPIDOCHRONOLOGICAL ANALYSIS

All the rhizomes studied (both orthotropic and plagiotropic) show cyclical changes in sheath thickness along the rhizomes. The fact that these chronological cycles are always apparent whatever the locality (Albania, Algeria, Corsica, Egypt, France, Greece, Italy, Sardinia, Spain, Tunisia, Turkey), depth (from the surface to 43 m depth) and sample period attests to their universal character in *Posidonia oceanica* in the Mediterranean Sea (Pergent *et al.*, 1989).

The variations in sheath thickness that are observed along the length of the rhizomes are accompanied by corresponding cyclical changes in the anatomical structure of the sheaths, apart from the epidermis (Pergent *et al.*, 1989). The variations observed in sheath thickness along the rhizome can be explained on the one hand by variations in the number of layers of cells, such as parenchyma and dorsal sclerenchyma, and on the other hand by changes in the shape and appearance of cells with, for example, ventral sclerenchyma cells that are palisadic to a varying degree.

These cyclical variations in sheath thickness and anatomical structure are generated at the apex of the rhizome. In fact, the appearance of a new sheath corresponds to the shedding of the blade of the oldest leaf. The thickness of these newly formed sheaths shows cyclical variations with two annual inversions : a minimum thickness and a maximum thickness (Pergent *et al.*, 1989). Depending on depth and locality, the minimum thickness appears in late winter or spring, and the maximum thickness in the autumn. For a particular site, dates of changes in sheath thickness are consistent from year to year. Consequently, the cycle periodicity observed at the rhizome apex is annual. After the death of leaves, turgescent or emptying phenomena may appear, inducing considerable variation in sheath thickness, although modification of the number of cells or their differentiation cannot occur (dead organ). The synchronization observed between cycles of sheath thickness and anatomical cycles can then extend the chronological significance of the cycles observed at the rhizome apex to the totality of cycles present along the rhizomes. The cycle period, delimited by two thickness minima, is called a "lepidochronological year" (Fig. 1). The dates of the appearance of thickness minima in fact differ from the rhythm of calendar years (1 January-31 December) to a greater or lesser extent, according to the site.

Anatomical variations in sheath structure, which generate the thickness cycles, would appear to be linked to the longevity of the leaves. At Ischia (Pergent and Pergent-Martini, 1991), the sheaths exhibiting maximum or minimum thickness correspond respectively to leaves whose mean longevity is very high (10.2 months) or very low (7.7 months). The annual cycle of sheath thickness variation would appear to reflect the annual cycle of leaf longevity variation. Further investigation is required to confirm or otherwise this hypothesis.

Figure 1

Arrangement of sheaths along an orthotropic rhizome, recorded in 1986, and corresponding cycles of variation in sheath thickness acording to their rank. Chronological significance of cycles ("lepidochronological years") is also indicated (from Pergent et al., 1989).
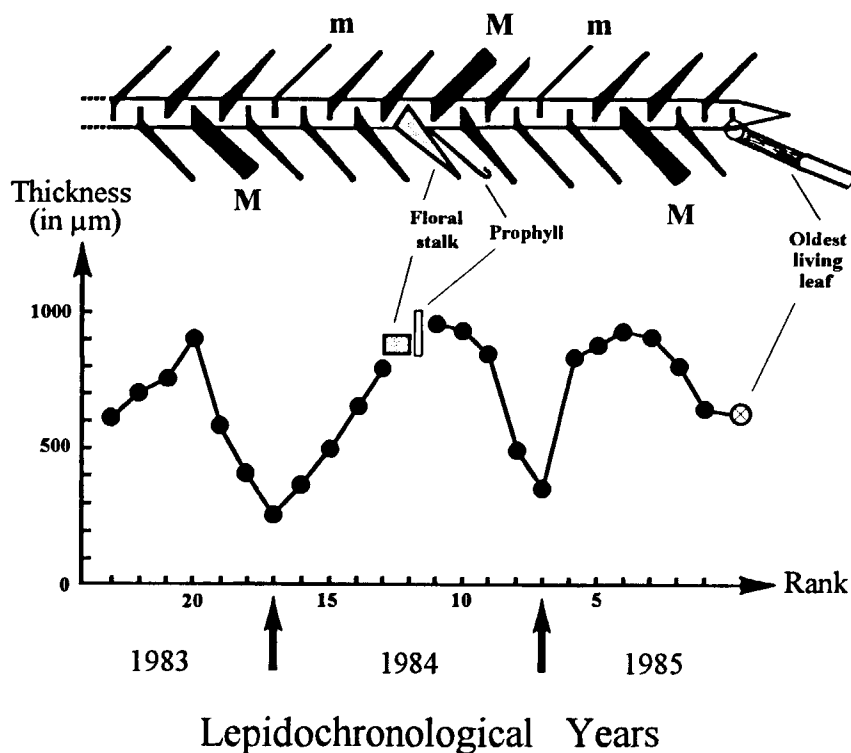
SIGNIFICANCE OF CYCLICAL VARIATIONS

The various parameters which characterize the cycles (range, period, mean thickness) are subject to modulations. These modulations are not random and could indicate the plant's response to internal or external factors.

The mean thickness of sheaths varies between sites according to four factors (Pergent, 1990) :

(i) Light : the mean thickness of sheaths increases with a decrease of light intensity (depth and/or turbidity);

(ii) Water movement : the mean thickness of sheaths is higher in sites where water movement is greater;

(iii) Water temperature : at the same site, significant positive correlation is observed between temperature and sheath thickness;

(iv) Locality : the mean thickness of sheaths varies according to the sample site.

At several sites, exceptional years (unusual sheath thickness) can be observed (Pergent, 1990). These years correspond to cycles showing unusual maximum or minimum thickness for all rhizomes at the site.

The cycle period appears to be mainly affected by internal factors; the number of sheaths may be greater for cycles showing remains of floral stalks, indicative of previous flowering. Nevertheless, at some sites (shallow water or lower limits) variations caused by external factors may be observed. For example, an increase in depth or turbidity induces a reduction in the mean number of sheaths per cycle. Variation of light intensity could explain this phenomenon.

Along rhizomes where a large number of annual cycles are apparent, cycle periodicity > 1 year may appear. Differentiation between cycles is facilitated by the particular structure of sheaths showing minimum thickness (e.g. anatomy). These cycles are rather similar to cycles observed in

the thickness of tree rings (Fritts, 1976). By using spectral analysis (Calmet et al., 1981), which is a statistical procedure often used in dendrochronology (Tessier, 1978), different multi-annual cycles, of 3, 6 and 11 years, appear (Pergent, 1990). The regularity of these cycles and the absence of synchronization between the different rhizomes seems to suggest that they are of endogenous origin.

Besides the now well established chronological significance of these cycles, it would appear that other parameters are recorded within the sheaths of *Posidonia oceanica*. It thus seems that in *Posidonia oceanica*, the sheaths act as a multichannel recording device, with each channel (parameter showing cyclical variation) recording a distinct signal superimposed on a clearly defined chronological signal.

Lepidochronology, which is based on similar principles to dendrochronology and dendroclimatology, can provide a means of exploiting the natural biological archives of virtually all the marine littoral zones of the Mediterranean.

DIRECT APPLICATIONS OF LEPIDOCHRONOLOGY

The discovery of lepidochronology and, in particular, the possibility this valuable tool offers of dating rhizome segments and the sheaths attached to them with considerable accuracy, can provide solutions to a whole range of problems. It can offer both quicker, more practical ways of dealing with questions that have already been studied using classical techniques, and solutions to problems that were hitherto unsolved.

A number of applications have already been tested successfully :

(i) Since the age of a particular section of rhizome can be determined, and segments corresponding to one or several

years can be removed from it, the rate of growth, and the proportion of the production required for rhizome growth can be accurately estimated (Sanchez Lizaso, 1989; Pergent and Pergent-Martini, 1990). The mean growth of orthotropic rhizome ranges from 3.4 to 20.9 mm per year, according to depth and locality. The mean growth rate appears to be very closely related to the sedimentation rate (Boudouresque et al., 1984; Pergent and Pergent-Martini, 1990). The mean annual production of orthotropic rhizomes was found to range from 24 to 120 mg dw per rhizome (4 to 66 g dw per m²).

(ii) The number of leaves formed during an annual cycle corresponds to the number of sheaths per cycle. Significant changes were observed as a function of depth and locality. The mean number ranges from 6.3 to 8.3 leaves per year, but for a given cycle, the number of leaves per cycle ranges from 4 to 11. These changes could be related to external factors (e.g. mean light and temperature from year to year) or internal factors, such as the distance between the orthotropic rhizome apex and its connection to the parent rhizome.

(iii) Modelling of leaf renewal cycles (rhythm of leaf formation and of leaf fall, leaf longevity). By monitoring month-by-month the progression of the most recent sheath thickness cycle, it is possible to determine the rhythm of leaf fall (Pergent and Pergent-Martini, 1990; 1991). The fall of a leaf (oldest adult leaf) corresponds to the appearance of a new sheath in the cycle; maximum and minimum thickness rank for the previous cycle are used as points of reference. The rhythm of leaf formation is calculated from two parameters : variation of leaf number per shoot and rhythm of leaf fall. In fact, the rhythm of leaf formation, for a given period, corresponds to the variation in the number of leaves per shoot added to the number of leaves fallen during the same period (Pergent and Pergent-Martini, 1990; 1991). Leaf longevity is deduced from the formation period of a new leaf within the shoot (rhythm of leaf formation) and the fall date of this new leaf.

Although new leaves appear throughout the year, a decrease or even interruption of leaf formation in winter and at the beginning of spring can be observed (Pergent and Pergent-Martini, 1990). In all sites studied, the leaves fall throughout the year; however, the rate of leaf fall is not constant and an acceleration in summer, related to the ageing of the adult leaves, is observed. Leaf longevity ranges from 7 to 14 months, depending on the period during which the leaf appears on the shoot (Pergent and Pergent-Martini, 1990; 1991).

(iv) Detection and dating of paleo-flowering from the remains of flower stalks inserted between the sheaths (Pergent et al., 1989). The remains of flower stalks and the associated prophyll are inserted between the thickest sheaths (Fig. 1). The appearance of these floral stalks is characteristic, and cannot be confused with sheaths (morphological and anatomical structure). It is possible, using retro-dating from the cycle where the floral stalk remains were found to the rhizome apex, to determine the flowering year corresponding to the floral stalk remains found between the sheaths. Flowering is a rare event and it occurs at a season (autumn) in which field observations are usually scarce. At Banyuls-sur-Mer and in Corsica, numerous flowerings, the oldest dating from 1942, which passed unnoticed at the time, were detected (Pergent et al., 1989).

Analysis of our results and those of several other investigations into Posidonia oceanica flowering (Giraud, 1977; Thelin and Boudouresque, 1985), shows that flowering appears to be synchronous in the Mediterranean basin. Flowering seems to occur more often in shallow sites than in deeper ones. Since the use of lepidochronology can make available a far greater quantity of data than can be derived from field observations, it should lead to a better understanding of the climatic factors that control flowering.

(v) Better understanding of the processes involved in the formation and dynamics of mattes (Boudouresque et al., 1986). By accurate dating of each rhizome section within the matte, these authors elucidated the dynamics of a peculiar type of Posidonia oceanica meadow, the hill-type meadow (Boudouresque et al., 1985). The dynamics of two other types of meadows, namely the tiger-type meadow (Boudouresque, 1986) and the stairs meadow, will be studied in the same way.

(vi) Recording of atmospheric fallout using, for example, caesium 137 (^{137}Cs) analysis (Calmet et al., 1988). The maximum activity of ^{137}Cs in sheaths occurred in groups produced between 1960 and 1964, a period corresponding to a fallout peak. The distribution of ^{137}Cs activity by rhizome age group indicates an apparent time lag, perhaps due to transport of material in the rhizome. As a result, sheaths of Posidonia oceanica appear to be able to memorize a chemical event for at least 30 years, and Posidonia oceanica could thus prove to be a valuable tool for marine pollution surveys.

FIRST EXAMPLE OF INDIRECT APPLICATION : ATTEMPT OF ESTIMATION OF PRIMARY PRODUCTION

Primary production in marine phanerogams is usually measured by one of two methods :

(i) the leaf marking method, based on the growth rate of the biomass (Zieman, 1974; Sand-Jensen, 1975; Nienhuis and De Bree, 1980; Ott, 1980; Bay, 1984; Romero-Martinengo, 1985);

(ii) the oxygen method (Clough and Attiwill, 1980; Ott, 1980) and ^{14}C method (Wetzel, 1964; Mc Roy, 1974; Drew and Jupp, 1976; Capone et al., 1979; Libes, 1986), based on measurement of photosynthesis.

The respective advantages and disadvantages of these methodological approaches and their significance (total production or net production) have been discussed in the literature (Zieman, 1974; Mc Roy and Mc Millan, 1977; Plante-Cuny and Libes, 1984).

In the Mediterranean, measurements of Posidonia oceanica primary production have generally been carried out at very shallow sites, largely because of the technical difficulties involved in traditional measuring techniques. The values obtained, even when they refer to net production (leaf marking method), generally deal only with the blade part of the total production.

The possibility of estimating Posidonia oceanica net primary production by a new technique (lepidochronology) that takes these tissues into account would therefore seem to be of particular interest (Pergent and Pergent-Martini, 1990;
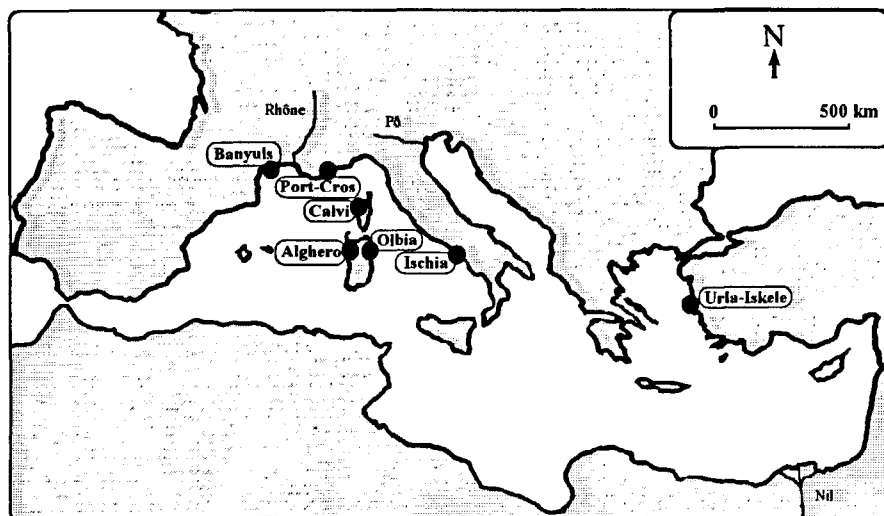
Figure 2

Map of the Mediterranean Sea showing locations of the study sites for assessment of primary production.

1991; Pergent *et al.*, 1992; Pergent *et al.*, 1994). Lepido-chronology has the additional advantage both of being very rapid and of allowing work to be carried out at any depth : from the surface to the lower limit (30 to 40 m depth).

For this investigation, primary production in the *Posidonia oceanica* meadow was measured at 22 sampling sites at various depths throughout the Mediterranean basin (Fig. 2; Tab. I).

Table I

Sample sites and mean annual primary production of orthotropic rhizomes of Posidonia oceanica (leaves and rhizomes); estimated by lepidochronological method. n = mean number of shoots studied monthly; * = occasional samples. Data for Ischia from Pergent and Pergent-Martini (1991) and Pergent et al. (1994).

Sites	Foliar production (mg dw/shoot)	Rhizome production (mg dw/shoot)	Meadow density (Nb shoots/m²)	Leaf production (g dw/m²)	Rhizome production (g dw/m²)	Total production (g dw/m²)
Banyuls/Mer (Fr)						
-0.7 m (n = 21)	340	51.3	1278	434.5	65.6	500.1
-2 m (n = 21)	310	51.3	1163	360.5	59.7	420.2
-12 m (n = 21)	920	57.8	535	492.2	30.9	523.1
-19 m (n = 21)	970	45.8	367	356.0	16.8	372.8
Port Cros (Fr)						
-0.7 m (n = 21)	1 250	45.5	942	1 177.5	42.9	1220.4
-2 m (n = 21)	1 390	59.4	645	896.6	38.3	934.9
-11 m (n = 21)	1 230	80.2	317	389.9	25.4	415.3
-23 m (n = 21)	850	33.0	283	240.6	9.3	249.9
-32 m (n = 21)	1 140	59.7	205	233.7	12.2	245.9
Calvi (Corsica, Fr)						
-10 m (n = 15)	1 530	56.7	480	734.4	27.2	761.6
Ischia (Itl)						
-5 m (n = 20)	1 540	80.0	473	728.4	37.8	766.2
-10 m (n = 20)	1 320	120.0	351	463.3	42.1	505.4
-15 m (n = 20)	950	80.0	253	240.4	20.2	260.6
Alghero (Sard., Itl)						
-5 m (n = 160)*	530	55.3	525	278.3	29.0	307.3
-10 m (n = 260)*	660	72.8	518	341.9	37.7	379.6
-15 m (n = 80)*	830	63.9	395	327.9	25.2	353.1
Olbia (Sardinia, Itl)						
-7 m (n = 10)*	770	24.3	164	126.3	4.0	130.3
-10 m (n = 30)*	920	34.5	240	220.8	8.3	229.1
-20 m (n =10)*	800	25.9	381	304.8	9.9	314.7
Urla (Turkey)						
-0.7 m (n = 20)	1 090	47.3	1129	1 230.6	53.4	1284.0
-2 m (n = 20)	1 000	55.7	510	510.0	28.4	538.4
-5 m (n = 20)	700	52.3	450	315.0	23.5	338.5

Primary leaf production (sheath and blade) was estimated on the basis of three parameters according to Pergent and Pergent-Martini (1991) :

(i) the number of leaves produced annually (rhythm of leaf formation in Pergent and Pergent-Martini, 1990); (ii) the mean length of blades and sheaths for the leaves in rank 1 (growth completed, *in* Giraud, 1977) and (iii) leaf density, which corresponds to leaf weight by unit of length (Sand-Jensen, 1975).

On the basis of these three parameters, primary leaf production (sheath plus blade) per year can be estimated by applying the formula :

PI = N x L x D

N = mean number of leaves formed annually

L = mean length of the oldest adult leaves still in possession of their apex (estimated over a one-year period)

D = leaf density

Estimated primary leaf production per shoot (sheath plus blade) of *Posidonia oceanica* shows wide variation according to depth and site (Tab. 1), and ranges from 310 to 1540 mg dw/shoot/year. Primary leaf production per m^2 generally decreases with depth; this phenomenon is correlated with the decrease in the density of the meadow (number of shoots per m^2) at greater depths (Tab. 1). Primary leaf production estimated per unit area ranges between 126.3 and 1230.6 g dw/m^2/year.

Leaf production was estimated separately for the sheaths and blades, at three sites situated around the island of Ischia (Pergent and Pergent-Martini, 1991; Pergent *et al.*, 1994). Sheath production accounts for between 21 and 23 % of primary leaf production and around 20 % of total primary production (leaves and rhizomes).

Mean rhizome production, estimated by lepidochronology, ranges from 4.0 to 65.6 g dw/m^2 /year (Tab. 1). For the whole set of 22 sampling sites, mean rhizome production accounts on average for 6 % of total primary production. Root production is not taken into account since it is generally very low (less than 2.5 %, *in* Ott, 1980).

Variations in leaf and rhizome production per shoot in the *Posidonia oceanica* meadow are mainly related to the locality (significant difference using a One-Way Analysis of Variance, confidence level = 95 %); depth-related variations are not significant.

Our results can be seen to be perfectly consistent with those obtained by classical methods (*e.g.* leaf marking). It should however be pointed out that primary production values obtained by lepidochronology take into account both the blade and sheath. In addition, with this new method it is also possible to estimate rhizome production, which can amount to 10 % or more of leaf production (Tab. 1).

As a general rule, the highest values were recorded at Ischia, Calvi and Port-Cros. They correspond to dense meadows exhibiting a high degree of vitality (Giraud *et al.*, 1979; Bay, 1984; Mazzella *et al.*, 1986; Pergent and Pergent-Martini, 1988). Conversely, the lowest values were recorded in the meadows on the Sardinian coast (Alghero and Olbia).

The morphometric ratio of sheath length to overall leaf length (Giraud, 1977; Pergent and Pergent-Martini, 1991) can serve to estimate past primary production. On the basis of this ratio, at three sites at Ischia (Pergent *et al.*, 1992), primary production appears to be relatively stable at depth (site I20 = Ischia -20 m) and over a period of five years. Nevertheless, there is greater variation by year for the shallower sites (sites I10 = Ischia -10 m and I05 = Ischia -5 m). These variations appear to be synchronous; primary production for these two sites reached its maximum in 1986 and 1990 and minimum in 1989 (Pergent *et al.*, 1992).

This new technique for estimation of the primary production of *Posidonia oceanica* meadows presents considerable advantages : (i) primary production can be estimated rapidly, and therefore on a large scale in terms both of depth and area covered; (ii) leaf base (sheath) and rhizome production, as well as blade production, can be taken into account; (iii) past primary production can be estimated to provide a chronological series for a given station.

While our findings confirm the high rate of productivity of this phanerogam (130.3 to 1284.0 g dry weight per m^2 per year), it would appear that the data recorded in the literature, which is generally based only on findings from surface stations, greatly overestimates the total production of the *Posidonia oceanica* meadow (extrapolation to the whole bathymetric range).

SECOND EXAMPLE OF INDIRECT APPLICATION : STUDY OF POLLUTANT ACCUMULATION

The marine phanerogams are generally capable of concentrating trace metals, and the pollutant levels recorded in their tissues tend to mirror the concentrations occurring in the environment (Bryan, 1971; 1984). Stenner and Nickless (1975), for example, found concentrations of zinc, copper and lead in *Zostera sp.*, that were equal to or double the normal rate in polluted areas. Similarly, Augier *et al.*, (1978) have shown that *Zostera marina* Linnaeus and *Posidonia oceanica* provide a good indication of mercury content levels in sea water.

We therefore felt it would be of interest :

(i) to investigate whether *Posidonia oceanica*, like other phanerogams such as *Halodule wrightii* Ascherson (Pulich, 1980) and *Posidonia australis* (Ward, 1987), might be biological indicators of the quality of the environment, by studying concentrations of contaminants occurring in the living plants. Several previous studies had in fact suggested that the leaves and rhizomes might concentrate stable pollutants such as mercury (Augier *et al.*, 1978; Maserti *et al.*, 1988), copper, lead and cadmium (Chabert *et al.*, 1983), the organochloride compounds - DDT, lindane and PCB (Chabert *et al.*, 1984) and certain artificial radionucleids (Florou *et al.*, 1985; Calmet *et al.*, 1988).

(ii) to see whether the rhizomes and sheaths memorize the concentrations occurring in the environment during their formation, which would make it possible to reconstitute a chronological sequence over long periods of time (more

than 10 years), as has been demonstrated for [137]Cs (Calmet *et al.*, 1988).

Three replicates of 15 orthotropics rhizomes of *Posidonia oceanica* were collected on each site by scuba-diving at -10 m depth. They were dissected according to the lepido-chronological method. *Posidonia oceanica* leaves were separated by type (adult or intermediate). Sections of rhizome, delimited by two minimum thicknesses (corresponding to one annual cycle), were also selected.

For this study the mineralization of samples (blades, sheaths and rhizomes) was performed in Nalgene FEP Teflon bottles, using the microwave method, in a mixture of nitric acid and oxygenated water. Mercury measurement was achieved using a flameless atomic absorption spectrometer (MAS 50 of Perkin Elmer); the others metals were analyzed by Induction Coupled Plasma Spectrometer.

The first measurements carried out indicate that trace metal concentrations (Cd, Cu, Fe, Hg, Pb, Zn) differ significantly according to the living tissue studied on the basis of Giraud's classification (1977) : blades and sheaths of adult leaves, intermediate leaves (leaves without sheaths, which are not fully grown). These observations are quite consistent with the bibliographical data, which show that in the marine phanerogams, the distribution of pollutants in the tissues appears to be highly diversified, and varies in relation with the growth of the plant and the level of contamination of the sea water (Ward, 1987). In the three sites studied (Marseilles, Calvi, Ischia) concentrations in the intermediate leaves are lower than those in adult leaves for almost all the metals investigated; it would appear therefore that the phenomenon of accumulation might be correlated with the age of the leaf (Fig. 3). Brinx and Lingby (1982) also noted an increase in concentrations of cadmium, lead and zinc in relation with the age of the leaves in *Zostera marina*. In *Posidonia oceanica*, Calmet *et al.* (1991) observed a preferential contamination in adult leaves, after the Chernobyl fallout, probably because they were in contact with soluble radionucleids for a longer period than the intermediate or juvenile leaves. Never-
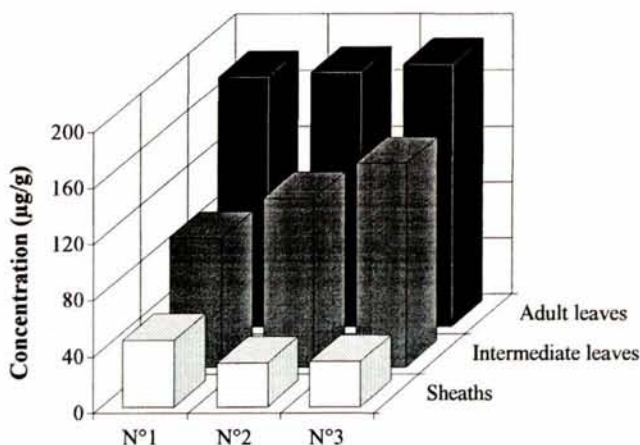
theless, to check whether or not there is any seasonal factor, further measurements, carried out at other periods of the year are required in order to confirm or refute this hypothesis.

New investigations carried out on mercury in the sheaths of *Posidonia oceanica*, show that concentrations are not random. Concentrations are strongly correlated to weight of sheaths (Fig. 4). The explanation for this phenomenon may perhaps be found in the localization and distribution of the sites of mercury fixation in the sheaths of *Posidonia oceanica*. By erasing this correlation it is possible to assess the difference between theoretical concentration (only due to the weight) and observed concentration (Fig. 5). It then appears that this difference reflects seasonal patterns of accumulation. They provide evidence of the occurrence of cycles of mercury concentration, according to sheath insertion rank. These cycles are synchronized with the sheath thickness variation cycles .

If the sheaths are grouped by lepidochronological year, variations in trace metal content are observed from year to year (Fig. 6). These variations are not random (Spearman's rank correlation coefficient : $r_{Pb/Cu} = 0.78$, $r_{Cu/Zn} = 0.90$, $r_{Pb/Zn} = 0.93$, significant level = 99 %) and reflect variations in stable pollutant concentrations in the environment.

Similarly, trace metal concentrations differ, in both the sheaths and rhizomes, according to the site. The first indications suggest that trace metal concentrations in the sheaths (except Cd) recorded in the Bay of Calvi (Corsica)
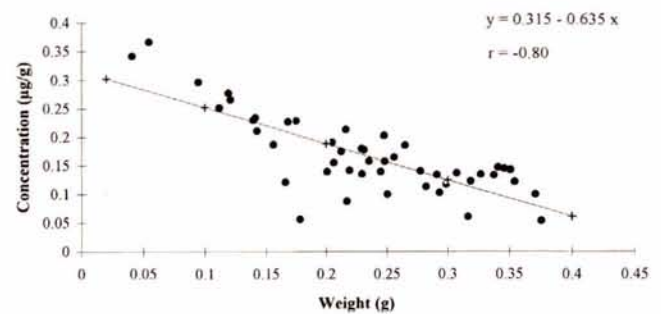
Figure 4

Concentration of mercury, according to dry weight of sheaths (Calvi, January 1993, -10 m).
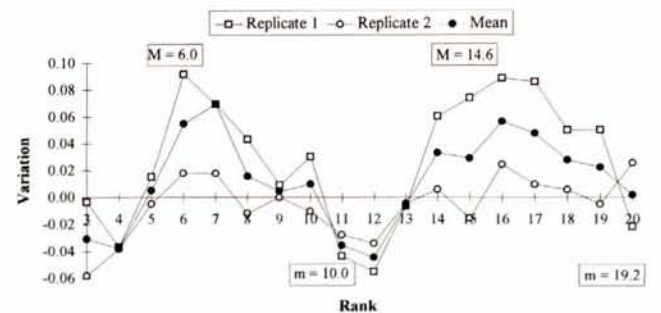
Figure 5

Variation (in µg/g dry weight) between theoretical and observed mercury concentration, in Posidonia oceanica *sheaths, on the basis of the rank, from the more recent (rank 3) to the oldest (rank 20) (Calvi, January 1993, -10 m). Position of maximum (M) and minimum (m) sheath thickness is also noted.*
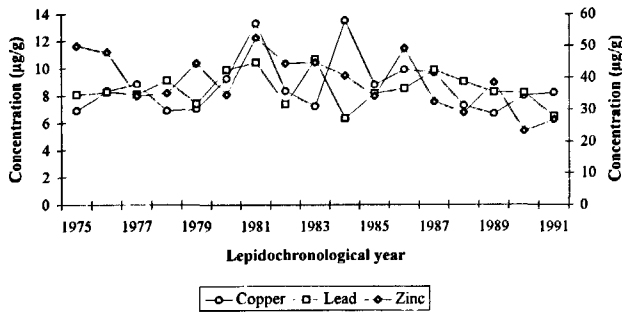
Figure 3

Concentration of zinc (in µg/g) in Posidonia oceanica *leaves (adult and intermediate) and sheaths, for three replicates from Calvi (April 1992; -10 m).*

Figure 6

Mean concentration of copper, lead and zinc (in μg/g dry weight) in sheaths (three replicates) on the basis of lepidochronological years, Calvi (April 1992; -10 m).
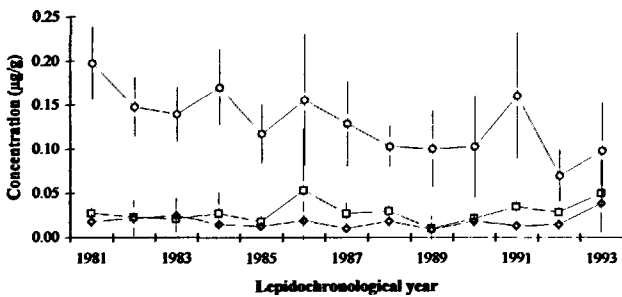

Figure 7

Concentration of mercury (μg/g dry weight) in sheaths on the basis of lepidochronological year and of the sites, for similar depth (-10 m). Standard deviation is also noted (3 replicates). o = Marseilles, □ = Ischia, ◆ = Calvi.

are lower than those recorded in the Gulf of Marseilles in proximity to the urban sewage outlet and in Ischia island (Fig. 7). Similarly, the concentration in iron is highest in samples taken in a *Posidonia oceanica* meadow around the island of Ischia (Italy), where a characteristic feature of the site is the presence of a number of iron-bearing underwater springs.

In the future, we plan to investigate whether the accumulation of trace metals by various organisms shows significant variations according to the season. We shall therefore analyse trace metal concentrations in the various leaf tissues of *Posidonia oceanica* over an annual cycle.

Other experiments will be carried out in vitro in order (i) to determine whether concentrations recorded in the leaf tissues do in fact reflect variations in concentrations in the sea water; and (ii) to attempt to establish a correlation between the concentrations occurring in the water and those actually accumulated by the living leaves. These experiments will be complemented by analyses carried out *in situ* in areas where complete data on the pollutant inputs is available.

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