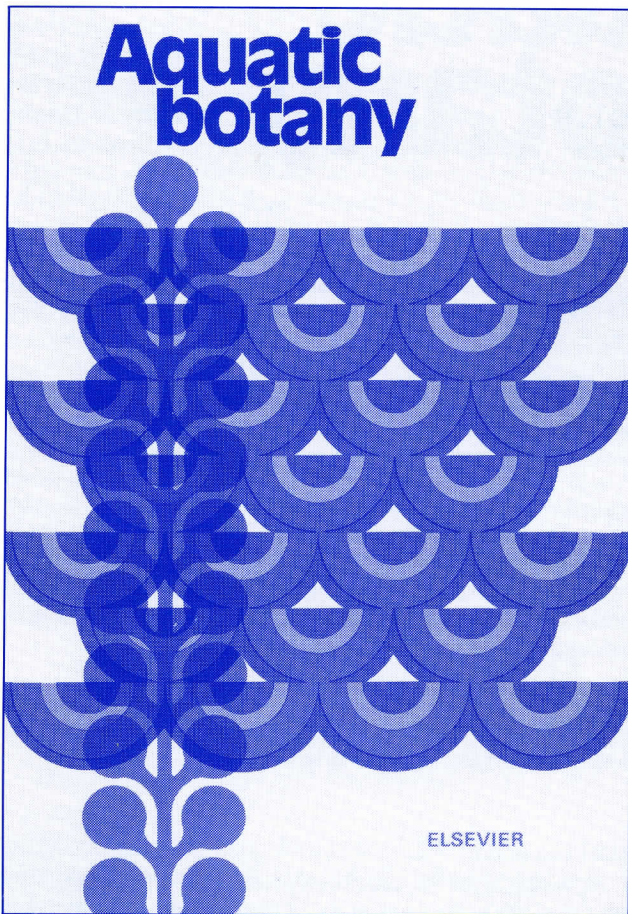


**MEMORIZATION OF NUCLEAR ATMOSPHERIC TESTS BY
RHIZOMES AND SCALES OF THE MEDITERRANEAN
SEAGRASS *POSIDONIA OCEANICA* (LINNAEUS) DELILE**

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REPRINTED FROM:



ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM

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(Accepted for publication 14 December 1987)

ABSTRACT

Calmet, D., Boudouresque, C.F. and Meinesz, A., 1988. Memorization of nuclear atmospheric tests by rhizomes and scales of the Mediterranean seagrass *Posidonia oceanica*. *Aquat. Bot.*, 30: 279-294.

Determination of the age of rhizome sections of *Posidonia oceanica* (Linnaeus) Delile (Potamogetonaceae) by the examination of their dead leaf scales (lepidochronology), makes it possible to cut out the rhizomes in precisely dated 1- or 5-year sections. With increasing age of the section, the annual section length and the C:N ratio increase, while the dry weight per unit length and the ratio of dry weight:ash weight decrease. At a site where the only significant ¹³⁷Cs contamination can be ascribed to global nuclear fallout from bombs, the activity of ¹³⁷Cs was measured in 5-year age groups of rhizomes and scales separately. The maximum activity of ¹³⁷Cs in scales occurred in groups produced between 1960 and 1964, a period during which a peak of activity occurred in fallout. The distribution of ¹³⁷Cs activity in rhizome age groups indicates an apparent lag, perhaps owing to transport of material in the rhizome. In situ dead rhizome scales of *P. oceanica* thus constitute a memory, at least 30-years long, of an environmental chemical pulse. *Posidonia oceanica* could prove a valuable tool for marine-pollution surveys.

INTRODUCTION

The plant

The seagrass *Posidonia oceanica* (Linnaeus) Delile occurs in extensive beds along open shores and in bays, all around the Mediterranean; it grows from

depths of 0 m down to 30–40 m (den Hartog, 1970). Two types of rhizomes are present (Caye, 1980): horizontal, creeping rhizomes (plagiotropous), as in many other seagrasses, and erect ones (orthotropous) with unlimited growth; plagiotropous and orthotropous rhizomes are not irreversibly differentiated, so that orthotropous rhizomes can become plagiotropous, and vice versa. Five to ten leaves occur in each leaf bundle (=shoot) (Panayotidis and Giraud, 1981). Dead leaf sheaths persist on rhizomes after the leaf blades (limbs) have been shed, and are then called “scales”. Calcareous remains of organisms living on the leaves, together with particles suspended in the water, are trapped by the dense leaf canopy and fall down around the shoots, raising the ground level (Molinier and Picard, 1952). The plant may counteract its sinking into the sediment by growing orthotropous rhizomes at a rate of 3–77 mm year⁻¹ (Boudouresque et al., 1984). This process results in the building of a very characteristic type of bottom, called “matte”, a network of living or dead rhizomes in which interstices are filled with sediment. Inside the matte, both rhizomes and scales decay little, and can persist for a long time: Boudouresque et al. (1980) give a ¹⁴C date of 4590 BP for rhizomes collected near Calvi (Corsica). As a result, the matte can be up to 8-m thick (Molinier and Picard, 1952).

Crouzet (1981) has reported cyclic changes in scale thickness along orthotropous rhizomes (100–1300 μm). Such cycles are characteristic of plagiotropous rhizomes as well (Mossé, 1984), and these cycles in scale thickness are a general feature of *P. oceanica* rhizomes all around the Mediterranean, irrespective of locality, depth or season (Crouzet et al., 1983). The space between two consecutive minima of scale thickness is defined here as one cycle of scale thickness. Several anatomical and morphological characteristics of the scales also change cyclically, in association with changes in scale thickness. They include: the number of layers of parenchyma cells; the number of layers of sclerenchyma cells; the tannin cell frequency; scale length; scale width (Pergent et al., 1983); and the rhizome diameter (Pirc, 1983). The chronological meaning of scale-thickness cycles on orthotropous and plagiotropous rhizomes was investigated by Pergent et al. (1983), Crouzet et al. (1983) and Mossé (1984): cycles are annual, with a minimum in scale thickness in February or March, and a maximum in September or October. The study of scale cycles along *P. oceanica* rhizomes was termed “lepidochronologie” (Boudouresque et al., 1983), which we translate into English as “lepidochronology”.

The problem

The possibility of dating sections of rhizome by lepidochronology, the long-term preservation of rhizomes inside the matte, and the distribution of the plant all around the Mediterranean, make *P. oceanica* a useful organism for environmental studies. Moreover, although *P. oceanica* is generally absent around sewage outfalls (Pérès and Picard, 1975), indicating sensitivity to acute pollution, in less polluted environments, *P. oceanica* leaves and rhizomes can

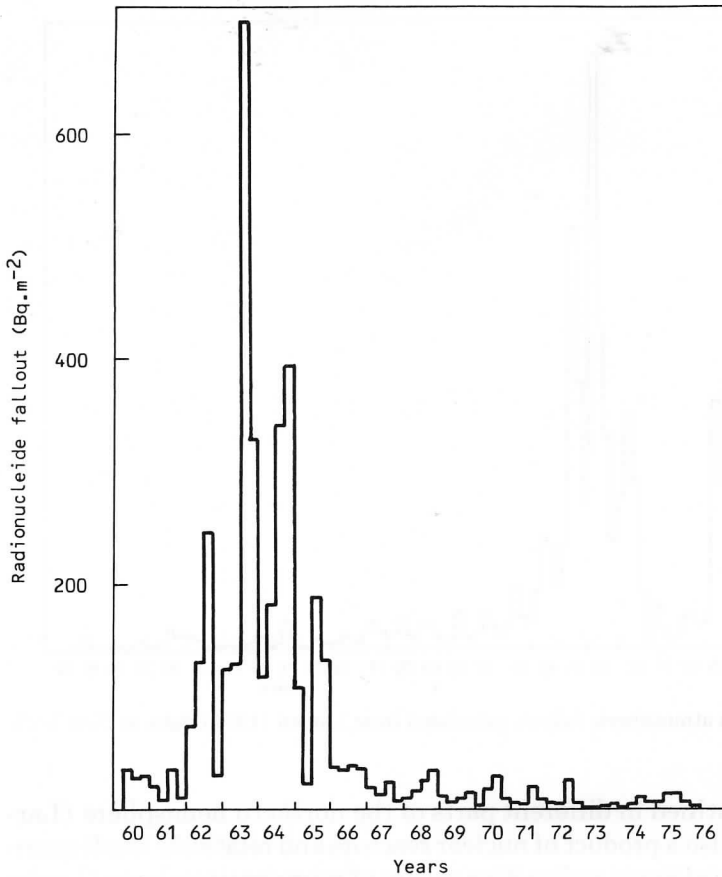


Fig. 1. Evolution of ^{137}Cs atmospheric fallout, calculated from Larsen (1984) data, at Florence, Italy.

concentrate various pollutants, such as heavy metals and organochlorinated compounds: mercury (Augier et al., 1978, 1979, 1984), copper, lead and cadmium (Vicente and Chabert, 1981a; Chabert et al., 1983), DDT, lindane and PCB (Vicente and Chabert, 1981b; Chabert et al., 1984). We formulate the hypothesis that past pollution events are recorded in sections of preserved rhizomes which were developing at the time. Should this hypothesis be correct, *P. oceanica* might be a valuable tool for pollution studies, allowing the determination of time-series of levels of certain chemicals and isotopes in the environment.

To test this hypothesis, we investigated the distribution of ^{137}Cs , an artificial radionuclide, liberated in large quantities among the fission products of atmospheric nuclear weapon explosions (Whicker and Schultz, 1982). Comparison of Figs. 1 and 2 shows that the same sharp peaks in a time-series of ^{137}Cs

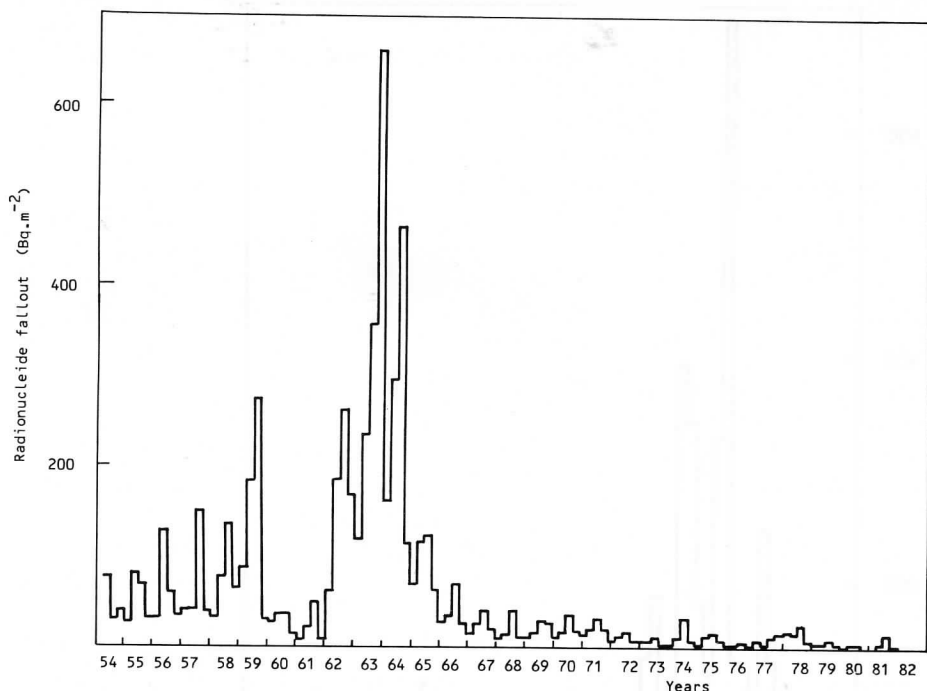


Fig. 2. Evolution of ^{137}Cs atmospheric fallout, calculated from Larsen (1984) data, at New York, USA.

activity may be identified in different parts of the northern hemisphere (Larsen, 1984). ^{137}Cs is also a product of nuclear reactors and relatively small quantities occur in low-level liquid radioactive wastes of reprocessing plants (Luykx and Fraser, 1983). We therefore chose a site protected from the industrial impacts of ^{137}Cs . Significant sources of artificial radionuclides on the French Mediterranean coasts are limited to the area of the Rhône estuary, and in the Natural Regional Park of Corsica anthropogenic radioactivity is overwhelmingly dominated by that caused by past atmospheric weapon testing.

Nuclear testing

The first nuclear device was detonated in the atmosphere at the Trinity site, near Alamogordo (New Mexico) on 16 July 1945, and the last was in China on 16 October 1980. Between these two dates, 423 atmospheric tests were performed, most of them before 1963 (Reiter, 1974b), when the countries considered as major nuclear powers at that time (U.S.A., U.K., U.S.S.R.) signed the Limited Test Ban Treaty forbidding nuclear testing in the atmosphere.

During a nuclear atmospheric experiment, aerosols and debris are produced. The smallest particles, of diameters smaller than $0.35 \mu\text{m}$, stay in suspension, especially if they are injected into the stratosphere, and are widely dispersed over the earth before settling, to constitute the "worldwide fallout". The intensity of atmospheric dispersion depends on the distribution of radioactive debris between the troposphere and the stratosphere, a distribution which is itself determined by the power and the height of the explosion and the altitude of the tropopause. The majority of worldwide fallout produced has been stratospheric, but if the tropopause forms a boundary between troposphere and stratosphere for temperate latitudes, jet streams are associated with breaks in the tropopause and air exchanges take place through the tropopause (Goldsmith and Brown, 1961; U.N.S.C.E.A.R., 1982).

The medium residence time in the stratosphere can vary from 0.3 to 2 years, a function of both the altitude and the latitude of the explosion (Reiter, 1974a). When the radioactive debris penetrates into the troposphere, the Hadley cell transport is then responsible for vertical diffusion of radioactive debris. Sinking of radioactive particles from the troposphere is determined by gravity, air currents and precipitation on the ground or on the sea surface (Mauchline and Templeton, 1964). A fraction of the deposition on land is then washed by surface runoff into littoral waters (Fukai et al., 1981). Then the radionuclides associated with coastal and oceanic surface waters are mixed with deeper water masses. This triple dispersion, atmospheric, oceanic and terrestrial, smooths out the originally very sharp signal of the nuclear weapon test.

Cambray et al. (1978) found that the average activity ratio of $^{137}\text{Cs}/^{90}\text{Sr}$, measured at many sites over a long time, has been fairly constant at about 1.6. From this ratio and from the half-lives of ^{137}Cs and ^{90}Sr , respectively, 30.2 and 28.6 years, the total atmospheric fallout of ^{137}Cs can be estimated from Larsen's (1984) data for that of ^{90}Sr (Figs. 1 and 2).

In sea water, cesium is found as a soluble hydrated cationic form easily available to marine organisms, and less than 1% of its total concentration in open ocean waters seems to be linked with particles (Robertson, 1971).

Chemically, cesium is close to potassium, an element of primary biological importance. Cesium does not seem to be necessary to life, nor is it selectively absorbed, although its physiological behaviour is rather similar to that of potassium in marine invertebrates (Bryan, 1961, 1963).

In the marine environment, ^{40}K , a natural radioactive isotope of stable potassium, is in equilibrium with the latter owing to its very long radioactive half life of 1.3 billion years.

MATERIALS AND METHODS

The study site

The study site was located in Galeria Bay (Mediterranean, Corsica: $8^{\circ}39' \text{E}$, $42^{\circ}25' \text{N}$), at 15 m depth. The *P. oceanica* meadow belongs to Giraud's (1977)

Type II, i.e. shoot-density ranges between 400 and 700 m⁻². Rhizomes were steadily orthotropous down to 75 cm, under the matte surface; below 75 cm, old parts of rhizomes were either dead and cankered, resulting in a breaking point when rhizomes were pulled away and collected, or squaring together with a plagiotropous rhizome.

Orthotropous rhizomes (n=250), 45–75 cm long, were haphazardly collected by scuba diving in August 1984.

Annual lengthening of rhizomes

A sub-sample of 50 rhizomes (from the above sample) was analyzed in order to estimate the annual lengthening of rhizomes. Along each rhizome, scales were removed one by one, according to their rank of distichous insertion, from the oldest, on the lowest part of the rhizome, to the most recent on the top part of the rhizome, ending with living leaves. Changes in scale thickness, which are always conspicuous, were judged by touch; when the narrowest scale (corresponding to the beginning of a new cycle) was met, we cut the rhizome perpendicular to its axis, just below the point of scale insertion. Every rhizome was therefore divided into several tens of sections each corresponding to one year (Figs. 3 and 4). Every rhizome section was given a date, in reverse order to scale removal, i.e. from the top to the lowest part of the rhizome; we are dealing with lepidochronological years and not calendar years: (1) they run from February–March in one year to February–March in the next; (2) they do not fit with the period of leaf life, but with the period of leaf shedding. The rhizome section located between the oldest living leaves and the first minimum of scale thickness corresponds to year 1984; this section (year) is incomplete; the rhizome section located between the first and the second (from the top part of the rhizome) scale-thickness minimum corresponds to year 1983, and so on. The length of annual rhizome sections was measured to within ± 0.5 mm (Table I); annual rhizome section lengths were then pooled into 7 five-year periods (from 1984–1980 to 1954–1950).

C, H, N values

Three to four batches of 5 annual rhizome sections, randomly taken from the 50 available sections for a given year (see above), and corresponding batches of scales, were separately crushed, homogenized and analyzed for C, H and N content. The following years were considered: 1950, 1955, 1960, 1965, 1970, 1975, 1980 and 1984. The analysis was performed by means of a Heraeus CHN-O-rapid, calibrated with acetanilide.

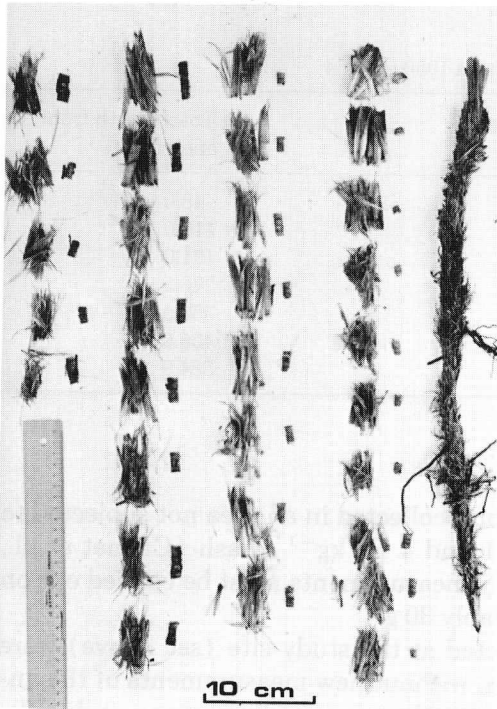


Fig. 3. An orthotropic rhizome before dissection (right); a rhizome divided into 31 annual sections, with corresponding scales on their left side (left).

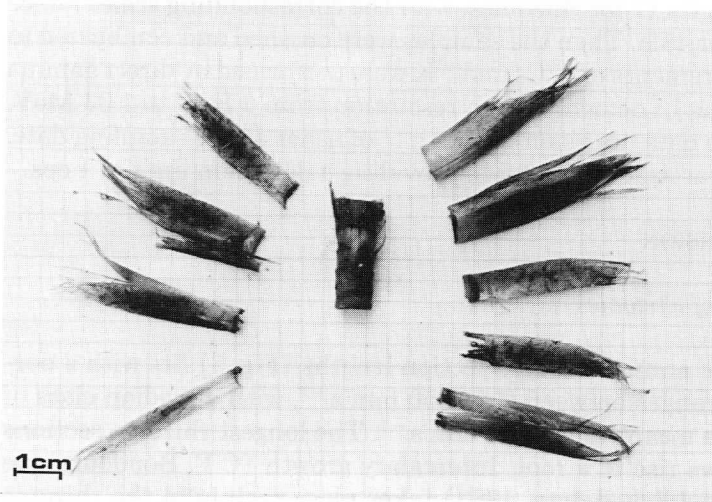


Fig. 4. An annual rhizome section (center) with the nine corresponding scales (left and right).

TABLE I

Length of rhizome sections for 5-year periods, from 1950 to 1984

Five-year periods	Mean length (mm)	Total length from the apex (mm)
1984-1980	48.0	48.0
1979-1975	67.0	115.0
1974-1970	76.0	191.0
1969-1965	85.5	276.5
1964-1960	83.0	359.5
1959-1955	95.5	455.0
1954-1950	129.0	584.0

Radioactivity measurements

The ^{137}Cs levels of *P. oceanica* samples collected in an area not subjected to industrial impacts are low, between 1 and 4 Bq kg⁻¹ of ash (Calmet et al., 1985). The direct gamma radioactivity measurements must be carried out on large quantities of optimal ash, preferably 30 g.

The 200 remaining rhizomes collected at the study site (see above) were directly cut into 7 parts from the apex, without new measurements of the annual length by lepidochronology, following the previous dimensions study (Table I), to establish section length for successive periods of 5 years initiated in 1950. For each part, the corresponding scales were separated from the rhizomes. Annual rhizome sections and the corresponding scales, resulting from the previous study of rhizome lengthening, were added to the corresponding 5-year batches.

The different samples (7 for rhizomes, 7 for the corresponding scales) were first dried at 80°C for 48 h. Then the samples were crushed and combusted to ash at 550°C. Measurements of ^{137}Cs and ^{40}K were performed by direct gamma spectrometry on a Ge-Li detector (high resolution from 2 KeV to 1.33 MeV, efficiency 40%). The data are given in Bq kg⁻¹ of ashes at the sampling date. The counting time was around 1200 mn, providing a detection limit of 1 cps.

RESULTS AND DISCUSSION

Annual lengthening of rhizomes

The distribution of annual rhizome section lengths (Fig. 5) fits with a normal distribution; it ranges between 2 and 50 mm a⁻¹, with a median class of 14-16 mm a⁻¹, and a mean value of 15 mm a⁻¹. The longest rhizome sections (> 40 mm) often give rise to a root. Intercalary growth (C.F. Boudouresque and A. Meinesz, unpublished data, 1983) takes place mainly in the rhizome

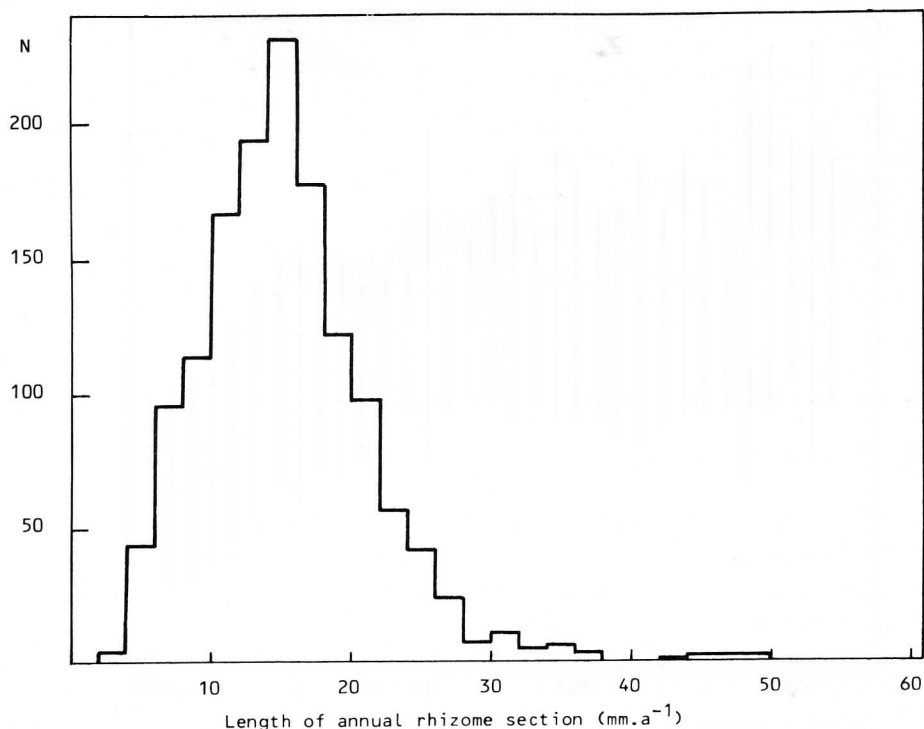


Fig. 5. Distribution of annual rhizome section lengths. Class interval: 2 mm. N = frequency.

section of the current year (i.e. 1984) and to a small extent in that of the previous year (i.e. 1983); accordingly, the year 1984 was not taken into consideration (Fig. 4); removal of 1983 data did not change the histogram shape much.

The mean length of annual rhizome sections (Fig. 6) did not conspicuously change from 1955 to 1969, then they regularly decreased up to 1984. Such a decrease has been previously mentioned from Elbu Bay, Corsica (Boudouresque and Jeudy de Grissac, 1983; Boudouresque et al., 1984). The authors consider it as related to a parallel decrease of the annual sediment input; such a phenomenon can account for a progressive decrease in annual rhizome lengthening when dealing with a "hill type" meadow (Boudouresque et al., 1985), with hills elevated above the sea bottom resulting in local current acceleration, and per descensum flowing by gravity of sediment down the hill slope. In a "plain type" meadow, like the one at the study site, a decrease with time in sediment input does not seem to be a consistent hypothesis; continued observations are required to assess whether or not the decrease of annual rhizome lengthening with time (more exactly with orthotropic rhizome length) is a general phenomenon, and to elucidate mechanisms which could lead to this decrease. For a given year, the annual rhizome lengthening is in any case

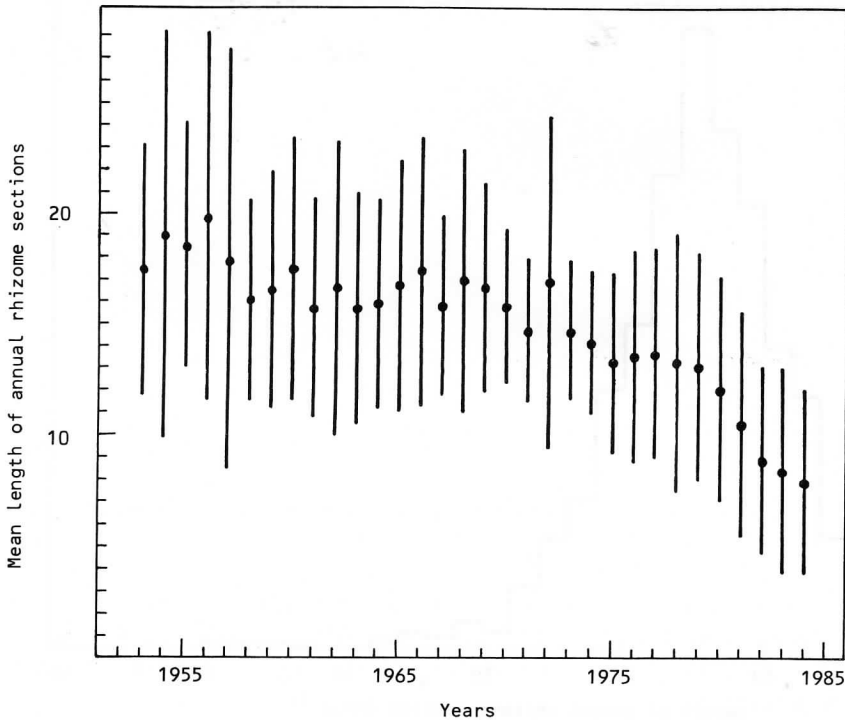


Fig. 6. Mean length (mm) of annual rhizome sections, as a function of age. Vertical bars = 2 s.d.

rather variable from one rhizome to another (Fig. 6). Boudouresque et al. (1984) regard this variability as related to the moving of small sand-waves within the *P. oceanica* meadow.

Rhizome section weight and C, N and K content

The dry weight per unit length of annual rhizome sections (Fig. 7) was nearly constant (around 0.021 g mm^{-1}) from 1949 (proximal and oldest parts of the rhizomes) to 1979, then increased (up to 0.095 g mm^{-1} in 1984). The dry weight:ash weight ratio of annual rhizome sections continuously increased from 1950 to 1984 (Table II); 5.3 to 12.8. The C and H content of rhizome sections did not vary as a function of their age (Table III); on the contrary, the N content significantly increased from 0.8% (1950) to 3.0% (1984); the C:N ratio declined correspondingly. The observed N content was always much higher than published values (0.51%; season not quoted) by Augier and Santimone (1979), but these authors may have analyzed rhizomes together with scales.

The rhizome sections, along which these changes occur, are considered to be alive, according to criteria proposed by Francour (1984): living rhizomes are

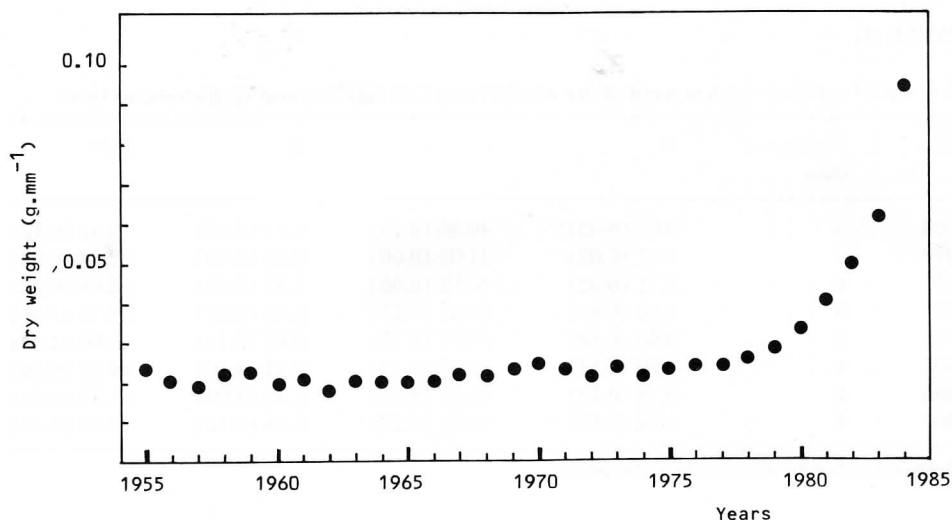


Fig. 7. Dry weight per unit length of annual rhizome sections, as a function of their age. Years earlier than 1955, with less than 10 data per year, are omitted.

pink-edged when broken, with a color and texture gradient from younger (salmon-pink, firm fleshed) to older parts (grey-pink, soft and fibrous). Francour (1984) arrived at these criteria after vital staining of cytoplasm and microscopic observation.

The changes which affect the aspect, together with chemical modifications of rhizomes as a function of rhizome-section age, clearly reflect biological and/

TABLE II

Dry weight, ash weight, dry weight:ash weight ratio and ¹³⁷Cesium content of rhizome sections and corresponding scales

Five-year periods	Dry weight (g)		Ash weight (g)		Dry weight:Ash weight		¹³⁷ Cs (Bq kg ⁻¹ ash)		⁴⁰ K (g kg ⁻¹ ash)	
	RH ^a	SC ^b	RH	SC	RH	SC	RH	SC	RH	SC
1984-1980	71.8	316.9	5.6	54.6	12.8	5.8	LD ^c	LD	171	38
1979-1975	117.7	146.3	10.5	33.2	11.2	4.4	LD	LD	152	24
1974-1970	121.8	140.1	12.7	17.6	9.6	7.9	4.07	3.70	134	28
1969-1965	194.7	315.8	24.6	45.1	7.9	7.0	0.74	4.44	140	31
1964-1960	163.7	286.6	24.6	46.3	6.6	6.2	LD	5.10	133	30
1959-1955	120.1	203.2	21.8	30.1	5.5	6.7	LD	2.22	115	27
1954-1950	25.9	52.6	4.9	10.8	5.3	4.9	LD	LD	100	26

^aRH = rhizome.

^bSC = scales.

^cLD = values equal or below the limit of detection (1 cps).

TABLE III

N, C and H contents (as a percent of dry weight) and C:N ratio for annual rhizome sections

Year	Number of data	N	C	H	C:N
1984	3	3.03 (0.03) ^a	40.96 (0.19)	5.91 (0.03)	13.52 (0.12)
1980	3	1.27 (0.02)	41.09 (0.06)	6.19 (0.03)	32.44 (0.53)
1975	4	1.11 (0.02)	40.52 (0.60)	5.87 (0.05)	36.68 (0.98)
1970	3	1.06 (0.04)	38.91 (0.15)	5.45 (0.07)	36.75 (1.46)
1965	3	0.95 (0.10)	38.74 (0.18)	5.67 (0.17)	40.96 (4.43)
1960	4	0.80 (0.11)	38.72 (0.12)	5.51 (0.10)	48.97 (0.10)
1955	4	0.78 (0.12)	39.16 (0.34)	5.46 (0.09)	51.36 (0.09)
1950	4	0.84 (0.08)	39.01 (0.27)	5.38 (0.14)	46.68 (5.08)

^aBetween parentheses: standard deviation.

or biochemical phenomena. The fact that the rhizomes are regarded as alive does not mean that all tissues are actually working, or even alive, in older parts. During the first 5 years of its ageing, a rhizome section loses a large part of its constituents (organic as well as mineral elements). Later on, it loses principally organic material, especially nitrogen compounds, resulting in an increase of the C:N ratio and in an increase in relative ash content (Tables II and III). A slight decrease in dry weight per length unit should occur although it is not clearly evidenced (may be owing to the small amount of data) (Fig. 4).

As far as ⁴⁰K is concerned, rhizome contents steadily decline, from 171 mg g⁻¹ ash (1984) to 100 mg g⁻¹ ash (1950), whereas they are stable (around 30 mg g⁻¹ ash) in scales (Table II). The decline, from recent to older sections of rhizomes, of ⁴⁰K content, which constitutes a good reflection of total potassium content, an element involved in cytoplasmic processes (e.g. osmotic regulation), could be related to the regression of living tissues with time. For total K content of *P. oceanica* rhizomes, Maubert and Le Goff (1984) give a value of 14 ± 1 mg g⁻¹ ash which is not consistent with our results.

Cesium levels

The composition of scales, particularly in ⁴⁰K (Table II), changes little with time. The activity owing to ¹³⁷Cs in ash from scales was around 2.2 Bq kg⁻¹ for the 1955–1959 period, increasing to 5.1 Bq kg⁻¹ for the 1960–1964 period (Table II, Fig. 8A). That the timing of this increase, corresponds with that of a more marked increase in ¹³⁷Cs activity in fallout (Figs. 1 and 2) is consistent with little post-death gain or loss in material by the scales. From 1965 to 1974, the ¹³⁷Cs activity in ash from scales decreased relatively much less than that

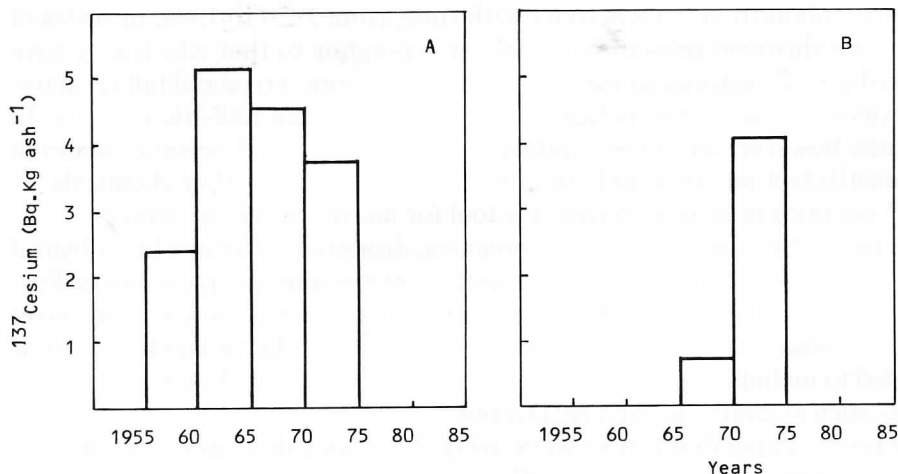


Fig. 8. ^{137}Cs content (Bq kg ash $^{-1}$) of *P. oceanica* scales (A) and rhizomes (B), as a function of their age.

in fallout, from the same period, indicating that decrease in ^{137}Cs activity in the sea could be relatively slower than that in fallout, as suggested above in the section Nuclear testing. Contrary to rhizomes, scales are without doubt dead organs, with no or low exchanges with the living parts of the plant, so that radionuclides included and stored in the scale during the leaf life could not then be removed. The leaf longevity ranges from 22 to 56 weeks, according to Thélin and Boudouresque (1983) whereas rhizome longevity may reach 42 years (Boudouresque, 1986).

In rhizomes, ^{137}Cs was not detectable before 1965 nor after 1974; the greater activity of the two 5-year periods with measurable ^{137}Cs occurred in 1970–1974 (Fig. 8B). Two possible mechanisms may be invoked to explain the apparent lag in the distribution of ^{137}Cs with age in rhizomes relative both to its distribution with age in scales and to its temporal distribution in fallout. Firstly, net transport of ^{137}Cs may occur from older to younger sections of the rhizome by means of the vascular system. Secondly, ^{137}Cs may be at least partially lost from older parts of the rhizome into the sea, perhaps in association with secondary chemical mobilisation (Table II).

CONCLUSIONS

The lepidochronological study of orthotropic *P. oceanica* rhizomes makes it possible to divide them into annual or 5-year sections, and to assign an accurate age to every section. From recent to older sections, the annual section length and the C:N ratio increase, whereas their dry weight per unit length and dry weigh:ash weight ratio decrease. The latter phenomena may result from ageing of rhizome sections.

The distribution of ^{137}Cs activity with time, from 1950 to 1984, in scales of *P. oceanica* rhizomes presents a signal corresponding to that which may have occurred in ^{137}Cs activity in the sea, as estimated from terrestrial fallout activity, residence time in the pelagic environment and the half-life of ^{137}Cs . In rhizomes, however, the corresponding signal shows a lag. *P. oceanica* scales in situ constitute a record of pollution by ^{137}Cs and perhaps other chemicals, so that *P. oceanica* may prove a valuable tool for marine pollution surveys.

The present results show that *P. oceanica*, dissected by lepidochronological procedures, may offer a time-series of certain environmental parameters. Further work should aim to calibrate signals in the time-series, as well as to detect and if necessary, allow for any time shifts in them. The research should be extended to include other chemicals, both inorganic such as heavy metals, and organic such as pesticides and detergents.

The results imply that former work using *P. oceanica* rhizome contamination as an indicator of environmental pollution may have been imprecise in certain ways. Scales have been pooled with rhizomes in chemical analyses, and the age of rhizomes analysed has not been taken into account. We have shown that the chemical content of scales can differ from that of the rhizomes to which they are attached, and that the content of both can vary with age. Some of the variability in data given in the literature may arise from the use of such heterogeneous material.

ACKNOWLEDGEMENTS

The project has been supported by a French Ministère de l'Environnement grant. The authors appreciate the field assistance, advice and companionship of Jean-Robert Lefèvre (CIPALM, Nice) and Charles-Henri Bianconi (Parc Naturel Régional de Corse), and the technical help of Serge Heusner (analyses), Fabrice Di-Santo (drawings) and Dr. I.R. Jenkinson (English translation).

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