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Vegetative Reproduction in *Posidonia oceanica*

I. Effects of Rhizome Length and Transplantation Season in Orthotropic Shoots

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With 6 figures and 2 tables

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Abstract. An investigation was conducted on transplantation in the Mediterranean seagrass, *Posidonia oceanica*. The effects of rhizome length and season of transplantation were investigated over the first year of growth in relation to survival and development in orthotropic shoots. Twelve batches, each composed of 36 transplants bearing one leaf bundle, were detached and planted at the same site and depth. They were fixed horizontally to mesh supports. These twelve batches, comprising three classes of rhizome length (10, 15, and 20 cm), were transplanted at four times of year. Mortality was highest for transplants made in early summer, when temperatures exceeded 20 °C, and lowest for those made in autumn. Although initial rhizome length had no discernible effect on subsequent mortality, it was positively related to the length of the necrosed portion one year later. The most successful transplants, made in autumn with 10 to 15 cm long rhizomes, gave survival rates of 92 to 97 %. These results should help to develop transplantation techniques for restoring damaged sites.

Problem

In *Posidonia oceanica* (L.) DELILE, the principal marine phanerogam in the Mediterranean, vegetative reproduction remains poorly understood. The species reproduce vegetatively essentially by two methods. In the first method the rhizome grows horizontally, but separates from the parent stock by subsequent necrosis of the proximal part. The second method also involves horizontal rhizome growth, yet separation from the parent stock occurs through tearing away of the distal part by water movement (MOLINIER & PICARD, 1952; BOUDOURESQUE & MEINESZ, 1982). While the second method may promote dispersion, the first strategy allows local colonization by horizontal growth at a maximum rate of $3\,cm \cdot a^{-1}$ (MEINESZ & LEFEVRE, 1984). Although the horizontal growth rate of plagiotropic rhizomes has been frequently reported (CAYE,

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1989), little is known about how transplants survive and adapt. Based on studies using maps made 40 years previously, MEINESZ & LEFEVRE (1984) estimated that up to 3 natural transplants $\cdot$ a^{-1} $\cdot$ ha^{-1} could establish on favourable substrates such as "mattes" of dead rhizomes in depths from 6 to 14 m.

In a recent annotated bibliography on worldwide transplantation and culture of seagrasses, MEINESZ *et al.* (1990), have analysed 1000 publications, including 23 reviews, in which the culture or the transplantation, or both, has been described for 37 species. Most of the transplantation experiments (70 %) have been conducted since 1975 on the east coast of the United States, on four species: *Thalassia testudinum* BANKS *ex* KÖNIG, *Zostera marina* L., *Halodule wrightii* ASCHERSON, and *Syringodium filiforme* KUTZING. Since the biology of *P. oceanica* is quite different from that of these four species, its survival and development rates have had to be determined experimentally. Previous work on the transplantation of *P. oceanica* in the Mediterranean (MAGGI, 1972, 1973; CINELLI, 1980; GIACCONE & CALVO, 1980; COOPER, 1982) used heterogeneous material. Furthermore, transplant morphology, numbers of apices per transplant, depth from which transplants were obtained, and other important parameters bearing on transplant survival were not systematically given. OTT (1979) and MEINESZ *et al.* (1991) found that mortality in *P. oceanica* under aquarium conditions is in excess of 50 % during the first year in culture, but falls markedly the following year for surviving transplants. The results of further work – on the

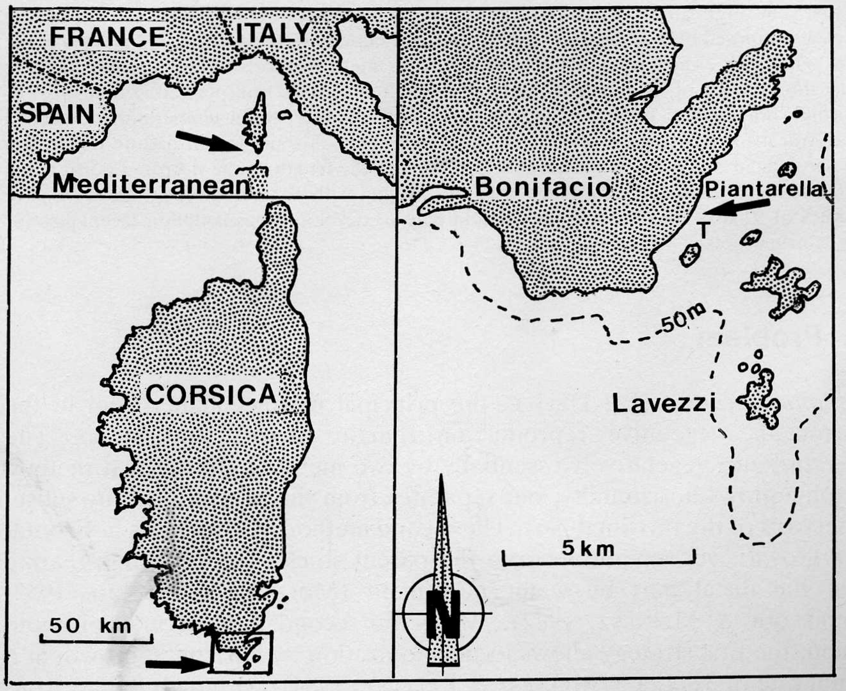

Fig. 1. Study area. T: transplantation site.

Australian species *Posidonia australis* Hook – in which Larkum (1976) followed 80 individuals for between 8 and 12 months and West *et al.* (1990) followed 900 transplants for 3 months, proved rather inconclusive.

Posidonia oceanica presents a very distinct axis dimorphism. The orthotropic axis develops vertically, growing only 0.5 to 1 cm · a^{-1} with little development of ramifications and roots. The plagiotropic axis, by contrast, develops horizontally and grows 3 to 10 cm · a^{-1}, with frequent development of ramifications and roots (Caye, 1980). The change in growth mode from orthotropic to plagiotropic takes place within a year under aquarium conditions (Meinesz *et al.*, 1991). In order to minimize experimental variance, we used material as homogeneous as possible (orthotropic axes with one leaf bundle).

As a step towards development of efficient transplant methods for *Posidonia oceanica in situ,* we investigated the effects of both rhizome length and season of transplanting on survival and adaptation in transplants during the most critical period for survival – the first year after transplantation.

Material and Methods

The work was carried out between June 1988 and June 1990 in the Parc Naturel "Réserve naturelle" of Lavezzi (41°20′ N, 9°15′ E), Corsica, at a site 50 m from the coast, 200 m north of Piantarella at a depth of 5.3 m (Fig. 1). This site is occupied by an extensive *P. oceanica* meadow with two zones: a mosaic of tufts, bearing leaf bundles at high density (≈ 920 · m^{-2}); and a zone, similar in total area to the first, consisting of dead, sandy "matte" and bearing only ten to fifty living leaf bundles · m^{-2}. The sediment is coarse, shelly sand, well sorted by the strong water currents but fixed by interlaced thalli of *Udotea petiolata* (Turra) Boergesen; the alga *Codium bursa* (L.) J. Agardh is present at a density of at least one plant every 5 m^2. The most frequently observed member of the macroscopic epibenthic fauna is the sea urchin *Paracentrotus lividus* (Lamarck), at an approximate density of 5 indiv. · m^{-2}, while the rough pen shell, *Pinna nobilis* L., occurs at a density of about one every 200 m^2.

Three classes of apparent rhizome lengths were used: 10, 15, and 20 cm. We define apparent rhizome length from the point at which the rhizome was sectioned to the extremity of the most distal dead foliar ensheathing the rhizome (Fig. 2). These apparent lengths correspond to real lengths of 7.3, 12.3, and 17.3 cm, respectively. These real lengths were estimated by substracting – from the apparent length – the mean distance between the terminal meristem and the extremity of the most distal, dead, exterior ligula which remained attached to the rhizome. This distance was determined in 50 rhizomes (mean = 2.7 cm, sd = 0.5 cm).

Plants of each rhizome length class were transplanted every three months as follows: 4–6 June 1988; 5–10 September 1988; 5–10 December 1988; 6–10 March 1989.

By means of SCUBA diving, 36 replicates of three length classes were planted at four times of year (432 transplants in all). The selected transplants had vertical rhizomes and a single bundle of regularly formed leaves. In each batch, 36 similar transplants were attached horizontally, 18 per support, 2 to 3 cm apart, to rectangular supports of plastic meshing (50 cm long, 35 cm wide, and of 1 cm mesh size; Fig. 3). The horizontal position of attachment reflects the position adopted by the majority of natural transplants lying on the bottom before fixation.

In order to ensure introduction to a favourable substrate, the supports bearing the transplants were fixed in meadow areas previously cleared by hand. The mesh supports were each anchored to the sea bed by six vertical metallic pegs curved at their ends. The supports were fixed 10 cm from each other in a grid pattern (Fig. 4).

All transplants were examined every three months for mortality, ramifications, and rooting. Ramification was recorded when juvenile leaves became separated by larger leaves. After a year, each support was removed and its transplants examined for: mortality, ramification, presence of roots, and apparent rhizome length. After longitudinal sectioning, the real length of the whole rhizome as well as of the necrosed part was determined.

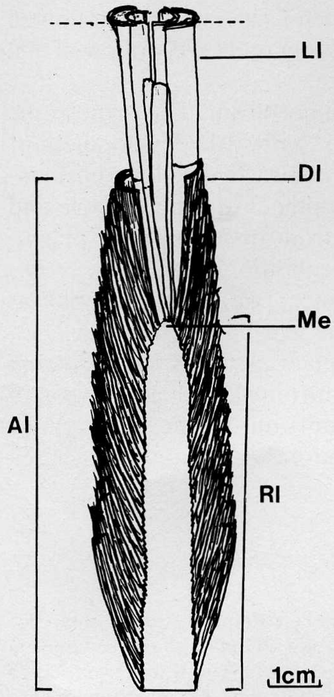

Fig. 2. Longitudinal section of an orthotropic rhizome, showing the different parameters measured. Ll: living leaf; Dl: dead leaf; Me: meristem; Rl: real length of rhizome; Al: apparent length of rhizome.

The surviving transplants from September, December, and March were dissected. Of the June batch, only 12 of the 20 cm batch and 7 of the 15 cm batch were examined. The 10 cm batch was lost. The remaining June 1988 plants were each planted individually and fixed in position by a stainless steel peg. They were harvested in June 1990, and growth in the living portion was measured using lepidochronology (PERGENT *et al.*, 1989), a method based on the positions of the leaf scales.

Differences in survival rate between batches have been judged significant at a probability level >95%.

One-way analysis of variance (ANOVA) (season × initial length) was carried out on the length of the living parts one year later. Significance in difference between batches was evaluated at the 95% level.

Two-way ANOVA was carried out to test the effects of transplantation month and initial rhizome length on both mortality and percent necrosed 12 months after transplantation.

Three-way ANOVA was carried out to test the effects on mortality of: 3-month growth period; transplantation month; initial rhizome length.

The significance of the differences between the survival rates were evaluated with the comparisons of the confidence limits established at a significance level of 95%.

Results

The fixation system using mesh supports was successful, with only one batch of transplants being lost.

Mortality after 12 months varied among batches from 3% to 47% (Table 1). Two-way analysis of variance showed no significant effect of initial rhizome length. The effect of transplantation month, however, was highly significant

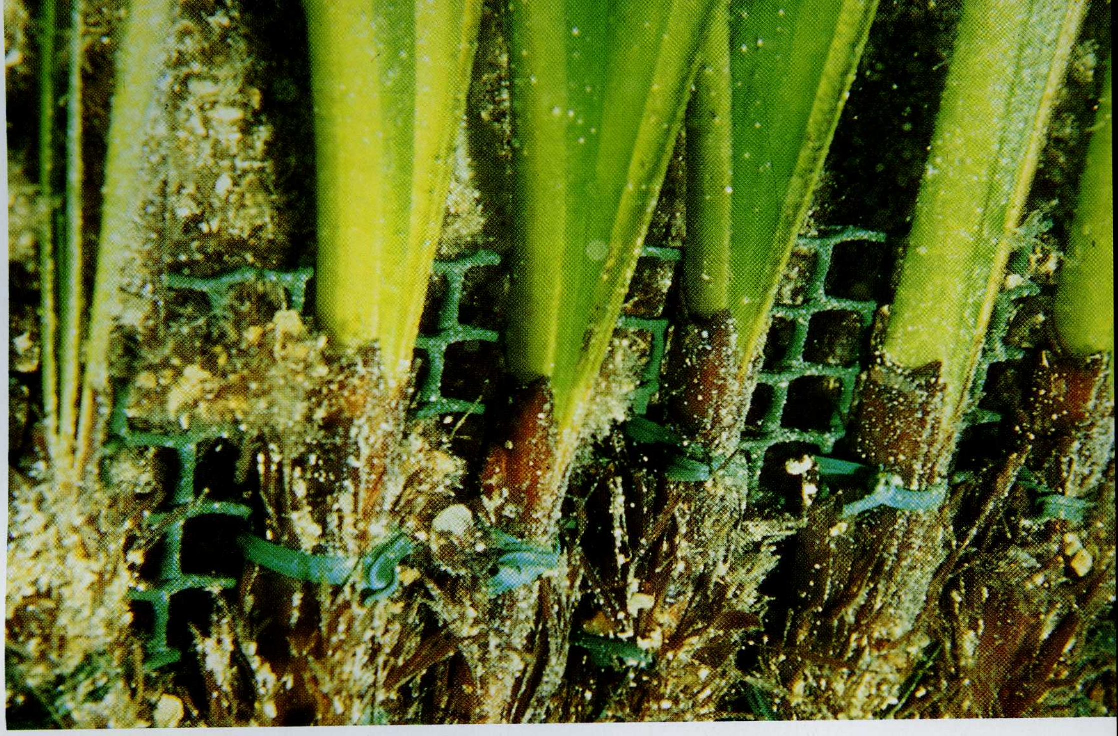

Fig. 3. Photograph showing transplants attached to mesh support.

Fig. 4. Overall view of the transplantation site.

Table 1. Survival, development of rooting, and ramification rates 12 months after transplantation (n = 36 for each batch).

initial apparent length	transplantation month	survival rate (%)	confidence limits	development of survival with roots (%)	shoots with ramifications (%)
10 cm	Jun 1988	supports lost			
	Sep 1988	97	91–100	29	6
	Dec 1988	83	71–95	50	3
	Mar 1989	53	37–69	42	16
15 cm	Jun 1988	67	52–82	38	25
	Sep 1988	92	83–100	30	9
	Dec 1988	75	61–89	41	22
	Mar 1989	81	68–94	69	28
20 cm	Jun 1988	86	75–97	3	19
	Sep 1988	97	91–100	29	6
	Dec 1988	56	40–72	15	5
	Mar 1989	67	52–82	42	17

($P < 10^{-7}$, n = 396 transplants): for rhizomes transplanted in September, mortality after 12 months was only 5 %, compared with 23 %, 29 %, and 33 % for those transplanted in June, December, and March, respectively.

Mortality rates during 3-month periods between observations varied among batches from zero to 39 % (Fig. 5). Three-way ANOVA showed that overall mortality was affected most significantly by growth season ($P < 0.001$, n = 44 batches), being lowest from March to June (0.27 %) and highest from June to September (12 %). The transplantation month affected overall mortality over 3-month periods ($P < 0.05$) similarly to that over 12-month periods.

High variation occurred in the distance from the cut end to the point of cicatrization. This distance was only a few mm in some rhizomes, while other rhizomes survived and developed with only 1–2 cm of living tissue isolated by the scar tissue, the remainder being necrosed. Over the experimental period, most rhizomes showed a net reduction in apparent length (Table 2), apparently due to reduction in the length of the necrosed portions. This is also supported by our observation that the necrosed portions of some rhizomes were in a state of disaggregation. Since shortening in the necrosed portion could not be systematically quantified, the amount of lengthening in the living portion, if any, is impossible to estimate precisely from the measurements in Table 2. For the transplants planted out in June 1988 and sampled in June 1990, lepidochronology revealed the following results on growth. For the two size classes, 15 and 20 cm, mean growth in the living portion reached 1.57 cm (s. d. = 0.56 cm) after the first year. This growth is markedly higher than during the four years prior to transplantation ($0.73 \text{ cm} \cdot a^{-1}$; s. d. = 0.15 cm).

For the different length classes, Table 2 shows the mean lengths of the living portions after a year *in situ*. Two-way ANOVA was carried out to determine whether – one year after transplantation – the length of rhizome occupied by

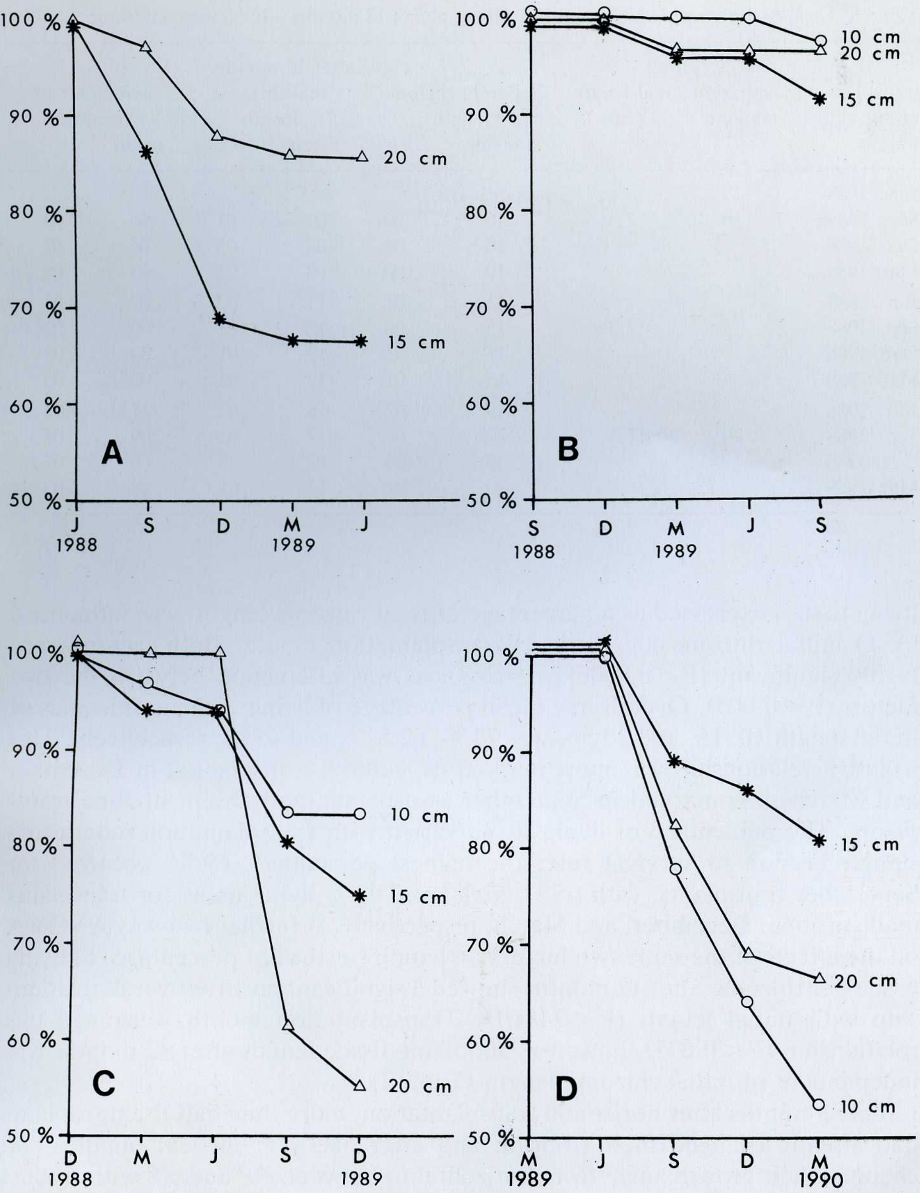

Fig. 5. Survival rates measured every three months for transplants in the three size classes. Transplants made in: A – June 1988; B – September 1988; C – December 1988; D – March 1989.

Table 2. Lengths (in cm) of rhizomes in surviving plants 12 months after transplantation.

transplantation time	initial apparent length	initial real length (cm)	after 12 months: apparent rhizome length mean	s. d.	real rhizome length mean	s. d.	living part of rhizome mean	s. d.
Jun 1988			supports lost					
Sep 1988	10	7.3	10	00	07	01	06	02
Dec 1988			10	00	08	03	06	02
Mar 1989			10	00	08	03	05	02
Jun 1988			15	01	11	02	07	03
Sep 1988	15	12.3	15	00	12	01	09	03
Dec 1988			15	01	12	01	09	03
Mar 1989			15	01	12	01	05	03
Jun 1988			19	02	16	02	11	04
Sep 1988	20	17.3	20	00	17	01	09	05
Dec 1988			20	00	17	01	06	05
Mar 1989			20	01	17	01	05	03

living tissue, expressed as a percentage of total rhizome length, was influenced by 1) initial rhizome length or 2) transplantation month. Both factors were highly significant ($P < 0.00005$, $n = 268$), as was interaction between the two factors ($P < 0.005$). Overall, the mean percentage of living tissue in rhizomes of initial length 10, 15, and 20 cm, was 74 %, 62.5 %, and 45 %, respectively. This negative relationship was most marked in material transplanted in December and March, less marked in September transplants, and absent in June transplants. The percentage of living tissue varied with transplantation month in a similar fashion to survival rate; the highest percentage, 69 %, occurred for September transplants, with 65 %, 62 %, and 43 % living tissue for transplants made in June, December, and March, respectively. A further two-way ANOVA on the effects of the same two factors on length (instead of percentage) of living tissue per rhizome after 12 months showed a significant positive overall relationship with initial length ($P < 0.0001$). Transplantation month weakened this relationship ($P < 0.005$), however, and living tissue length after 12 months was independent of initial rhizome length (Table 2).

Three months after horizontal transplantation, more than half the transplants had already changed their tropism, and after one year all leaf bundles had changed their growth angle from horizontal to between 30° and 60° with respect to the vertical.

Root development was variable according to the individual, and was recorded almost exclusively in summer. Apart from the June transplants, 30 % to 69 % of the surviving plants had roots after 12 months (Table 1). In the surviving June transplants, however, only 3 % to 38 % of the plants developed roots, and these roots had appeared in the summer, within 3 months of planting.

Ramification was also variable. From 3 % to 28 % of the transplants per batch presented apex division after 12 months (Table 1). The September transplants showed least ramification, 6 % to 28 %; the highest percentage, 19 % to 25 %, was shown by the June transplants.

Discussion

Twelve months after transplantation, survival rates ranged from 53 % to 97 %. This is higher than the rate obtained on *P. oceanica* under laboratory conditions: 50 % after 22 months (OTT, 1979), and 41 % after 12 months (MEINESZ *et al.*, 1990).

During the cold season (autumn to end of spring) when the temperature is below 20 °C (Fig. 6), transplant survival remains good. Furthermore, transplants made in September at the beginning of the cold season have had more time to recover from transplantation than the other time-of-year classes, and they survive the summer better. Autumn is the best season for transplantation. Our observations indicate that the high mortality is due to poor cicatrization in rhizomes following artificial transplantation. Cicatrization appears to be less successful at temperatures above about 20 °C. WITTMANN & OTT (1982) also found high mortality, 40 % to 69 %, between May and September (water temperatures 17 to 27 °C) in *Posidonia oceanica* leaf bundles with experimentally sectioned leaves.

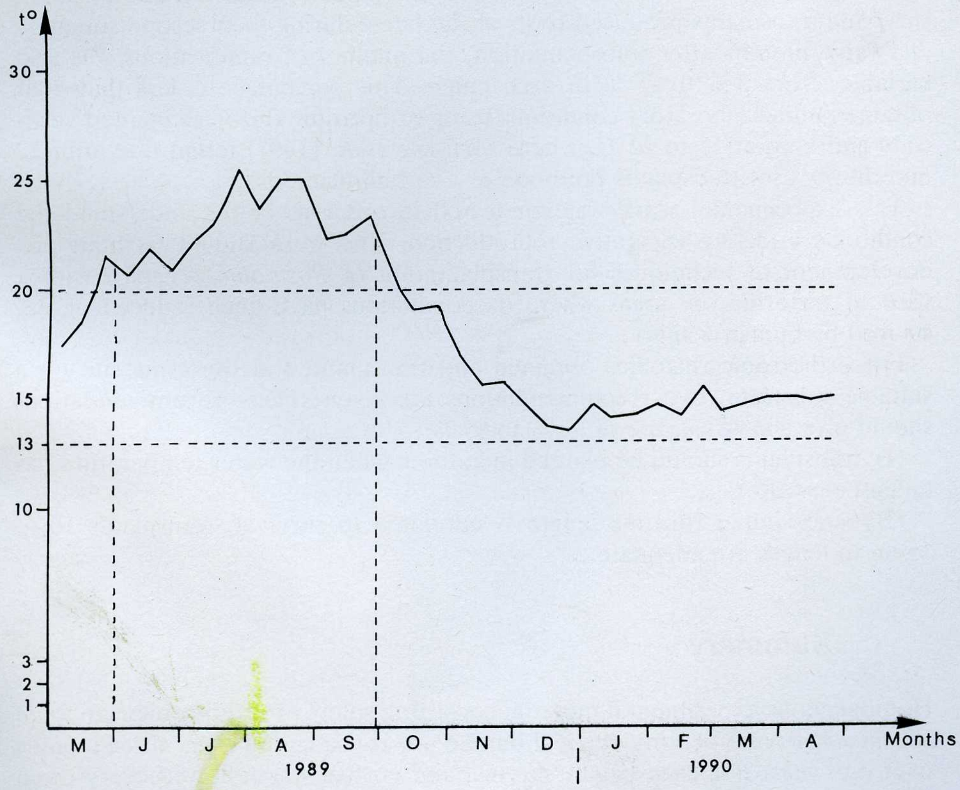

Fig. 6. Water temperature measured at 2 m depth in the Bay of Santa-Manza, 5 km north of the transplantation site. Each point represents the mean over ten days of temperature measured each day.

Although initially longer rhizomes gave relatively higher percentages of necrosed tissue after 12 months, initial rhizome length did not affect survival rate. Necrosis progresses in from the cut surface, suggesting that the apex is somehow protected against attack. CALMET *et al.* (1988) showed that the apical 4.8 cm of *Posidonia oceanica* rhizomes contain more mineral salts, nitrogen, and ^{40}K (reflecting the degree of K fixation) than the more proximal parts. In the present study, irrespective of transplantation month and initial rhizome length, the mean length of the apical portion (isolated by the scar of lignified cells) ranged from 4.5 to 11 cm. Scar formation is thus strongly influenced by distance from the apex and may be controlled by concentration gradients in mineral and organic substances. From *P. oceanica* rhizomes, BERNARD & PESANDO (1989) isolated antibacterial and antifungal substances, which may also be more concentrated in the apical region.

Roots developed only in summer. CAYE (1989), in a description of the biological cycle *in situ,* and MEINESZ *et al.* (1991), in a study of development under laboratory conditions, also found root development confined to the summer. The number of transplants bearing roots after 12 months was very variable, from 3 % to 69 % within batches. Similarly, in the study by MEINESZ *et al.* (1991), of material transplanted under laboratory conditions under May, between 33 % and 50 % of transplants had developed roots after 12 months. All surviving transplants produced roots at the latest during their second summer.

Twelve months after transplantation, the number of ramifications was also variable, from 3 % to 28 % in each batch. This percentage is less than that obtained under laboratory conditions using orthotropic rhizomes planted vertically and kept at 18 to 22 °C, where MEINESZ *et al.* (1991) found that after 12 months 50 % of transplants bore one or two ramifications.

This experimental work was conducted in order to better understand the conditions affecting vegetative reproduction. The work should facilitate the development of techniques for transplantation of *Posidonia oceanica* with a view to restoring the areas where its populations have been reduced or destroyed by human activity.

For orthotropic rhizomes obtained and transplanted at the same site on a suitable substrate, two recommendations are given. These recommendations should give a survival rate of 92 % to 97 %:

(I) transplants should be planted in autumn when the water temperature has fallen below 20 °C;

(II) since initial rhizome length is unrelated to survival, transplants 10 to 15 cm in length are adequate.

Summary

Homogeneous experimental material consisting solely of orthotropic *Posidonia oceanica* rhizomes bearing one leaf bundle was transplanted every three months over one year. For each batch, survival and growth was followed every three months after transplantation. The principal findings are as follows:

1. Material transplanted in December, March, and June showed high mortality in the summer, mean 12-month survival rates for each batch ranging from

53% to 86%. Batches transplanted in September, however, had time to become cicatrized before the summer and showed higher 12-month survival rates, from 92 to 97%.

2. The three initial lengths of rhizome tested, 10, 15, and 20 cm, did not significantly influence survival.

3. The longer the transplanted rhizome is, the longer the necrosed portion after 12 months.

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