

# Seed nutrient content and nutritional status of *Posidonia oceanica* seedlings in the northwestern Mediterranean Sea

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**ABSTRACT:** We studied nutritional constraints on growth of *Posidonia oceanica* seedlings, morphometry and elemental content of mature seeds, and seedlings stranded on a beach of the northwestern Mediterranean Sea on 3 dates (May 2004, May 2005 and December 2005). The mean total nitrogen (N) and phosphorus (P) content of *P. oceanica* seeds was high compared with closely related species. Seeds varied considerably in mass, and their nutrient and carbon (C) content increased linearly with mass. This indicates substantial variation in the amount of reserves initially available for seedling growth. Stored reserves in seeds were used by seedlings within 6 to 8 mo after germination: P was retrieved with greater efficiency (86%) than was N (69%) and C (20%). From May to December of the 2nd year (2005), the tissue nutrient concentrations of seedlings declined due to fast utilization and translocation of nutrients from the internal pools to support summer growth. Instead the C concentration in roots and rhizomes increased, indicating an accumulation of C. Seedling tissue N and P concentrations were above the critical levels reported for seagrasses, and were also lower than those of plagiotropic rhizomes of *P. oceanica* collected in the same period. These results suggest that P may play a major role in constraining growth of *P. oceanica* seedlings.

**KEY WORDS:** Seeds · Seedlings · Nutritional constraints · Morphometric analysis · Elemental analysis

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## INTRODUCTION

Seagrasses can reproduce both vegetatively by ramets and sexually by seeds. In some species, however, sexual recruitment is severely constrained by depressed seedling growth and survival (Hootsmans et al. 1987, Duarte & Sand-Jensen 1990, Terrados 1993, Olesen & Sand-Jensen 1994, Balestri et al. 1998a). One of the major factors likely to reduce seedling survival is the incapacity of seedlings to acquire sufficient nutrients to support continued growth in environments where nutrients are scarce or patchily distributed. However, attempts to assess the importance of nutrients in constraining seedling growth for seagrasses have been few (Robertson & Mann 1984, Duarte & Sand-Jensen 1996, Nielsen & Pedersen 2000, Walker

et al. 2004). Duarte & Sand-Jensen (1996) have found that P limitation may be the major cause of failure of seedlings to develop new patches in the Mediterranean seagrass *Cymodocea nodosa* (Ucria) Aschers. On this basis, the authors supposed that severe nutrient deficiency is also the reason for the low reproductive success of *Posidonia oceanica* (L.) Delile, the endemic and dominant seagrass species in the Mediterranean Sea.

This slow growing species forms large meadows in oligotrophic areas subjected to seasonal variation of nutrients, i.e. relatively high concentrations of N and P in late autumn–winter and low concentrations in spring–summer (Ballesteros 1989, Alcoverro et al. 1995, 1997, Vidondo & Duarte 1995). Nutrient limitation in mature stands may be high from May to

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October, and may involve both N and P (Alcoverro et al. 1997). To compensate for seasonal nutrient limitation the plant has evolved a complex strategy of nutrient conservation and recycling (Alcoverro et al. 1995, 1997, 2000, 2001, Invers et al. 2002, Lepoint et al. 2002). Flowering and fruit production is highly variable in space and in time, with intensive synchronized flowering episodes occurring at 8 to 10 yr intervals (Balestri & Cinelli 2003, Balestri 2004, Diaz-Almela et al. 2006). At maturity (early spring) the positively buoyant fruits are transported by currents away from parental shoots, enabling dehisced seeds to colonize sites outside the range of vegetative growth (Buia & Mazzella 1991, Balestri et al. 2006). Recruitment by seeds has been observed only occasionally in the field (Buia & Mazzella 1991, Balestri et al. 1998b, Piazzi et al. 1999, Diaz Almela et al. 2008). Although densities as high as to 480 germinated seeds  $m^{-2}$  have been detected in favorable sites, available data indicate that only a few seedlings can initiate new patches (Balestri et al. 1998b, Alberto et al. 2003, Jover et al. 2003, Migliaccio et al. 2005, Balestri & Lardicci 2008, Diaz Almela et al. 2008). It seems that newly established seedlings rely on seed C-based reserves for most of the 1st year (Balestri et al. 1998b, Belzunce et al. 2005), but subsequently a large proportion of them (ca. 70%) perish possibly due to environmental stresses (Balestri et al. 1998a, Piazzi et al. 1999, Balestri & Bertini 2003, Balestri & Lardicci 2008). Virtually nothing is known, however, on seed nutritional quality and nutritional status of seedlings during early growth.

In nutrient-poor habitats, the ability of seedlings to become established and cope with environmental stressors (i.e. nutrients, defoliation, herbivores and pathogens) depends, in large part, by the amount of reserves originally available to the seedlings (Foster 1986, Mazer 1989, Houssard & Escarré 1991, Seiwa & Kikuzawa 1991, Milberg & Lamont 1997, Bonfil 1998, Tyler & Zohlen 1998, Naegle et al. 2005). A number of studies have demonstrated that seed mass is a major determinant of seed reserves, and substantial seed mass variation exists at many scales (i.e. among populations and plants and within plants) in a range of terrestrial species (Ågren 1989, Matthies 1990, Mehlman 1993, Obeso 1993, Lord 1994, McKee & Richards 1996, Navarro 1996, Krannitz 1997, Vaughton & Ramsey 1998, Simons & Johnston 2000) because of reduced resource availability throughout the growing season, or architectural or physiological constraints operating during seed provisioning (Susko & Lovett-Doust 2000, Vaughton & Ramsey 2001). Differences in seed mass may affect seedling recruitment via effects on seedling growth and survivorship. Generally, under nutrient-limited conditions, seedlings from small seeds have low ability to sustain normal seedling growth, whereas

those from large seeds have energy and nutrients necessary for maximizing initial growth and are able to recover from stress (Vaughton & Ramsey 1998, Susko & Lovett-Doust 2000, Vaughton & Ramsey 2001). Therefore, information on the degree of variation in seed quality may be important when assessing the causes for reducing recruitment success and the capacity of seedlings of *Posidonia oceanica* to cope with nutrient stress.

After the latest important flowering of *Posidonia oceanica* recorded in the Mediterranean Sea (Balestri 2004, Diaz-Almela et al. 2006), a large number of fruits and seedlings detached from a meadow off the coast of northwestern Italy after storms and were deposited on a beach on 3 different dates. This reproductive material offered an opportunity to (1) determine the degree of variation in seed mass and the influence of seed mass on the seed reserve (C, N and P) content and (2) evaluate which putative elements might be limiting to growth in seedlings. To achieve these goals, we examined the changes in C, N and P concentrations (and their atomic ratios) in the different components of seedlings (seed remains, leaves, leaf scars, roots and rhizomes) over the sampling events. The C and nutrient concentrations in the different tissues of seedlings and adult shoots were also compared.

## MATERIALS AND METHODS

**Plant material and processing.** Fruits of *Posidonia oceanica* were collected in May 2004, while seedlings and rhizome fragments were collected in May 2005 and December 2005 (Table 1) on a beach of Tuscany (Italy, Ligurian Sea, northwestern Mediterranean; 43° 37' N, 10° 20' E). On the basis of available data on waves and wind direction (National Wavemetric Net, Istituto Superiore per la protezione e la ricerca ambientale, available at [www.idromare.it](http://www.idromare.it)), the fruits have been assumed to come from a large *P. oceanica* meadow located offshore (Meloria Shoals). The seedlings had sediment particles still attached to roots; they were evidently established in some unknown

Table 1. *Posidonia oceanica*. Type of material examined, sampling date and sample size

| Plant material          | Date        | Size |
|-------------------------|-------------|------|
| Mature fruits           | 7 May 2004  | 30   |
| Live seedlings (1 yr)   | 10 May 2005 | 38   |
| Dead seedlings          | 10 May 2005 | 34   |
| Live seedlings (1–2 yr) | 4 Dec 2005  | 14   |
| Plagiotropic rhizomes   | 4 Dec 2005  | 3    |

area close to the meadow in May to June 2004 and then dislodged from the substrate by storms. Water column nutrient concentrations at the site were low and corresponded to oligotrophic conditions as reported by Sistema Difesa Mare (Si.Di.Mar. 2005). As seeds are likely to germinate within a few days after fruit dehiscence (Balestri et al. 1998b), the seedlings collected in May 2005 were about 12 mo old at the time of collection (1 yr seedlings) and those of December were about 19 mo old (1 to 2 yr seedlings). Many seedlings collected in May did not show standing leaves and were thus categorized as dead seedlings.

In the laboratory, seeds were extracted from dehiscent fruits (Fig. 1A). To examine natural variation in seed mass, mature seeds (i.e. seeds with a developed plumule) were individually weighed after oven-drying at 70°C for 48 h ( $n = 30$ ). Seeds determined to be aborted or damaged were discarded. To examine seedling development, morphological measurements

(number of standing leaves, length of the longest leaf, number of leaf scars, number of shoots, length of rhizome, number of roots and length of longest root) were carried out on a subsample ( $n = 6$ ) of live seedlings in each sampling event. The total number of leaves produced during the shoot's life span of a seedling was estimated as the sum of the number of leaf scars and the number of standing leaves. The number of leaves produced by seedlings collected in May 2005 (i.e. at the end of their 1st year) corresponded to the annual leaf production rate of seedlings. This value was used to estimate the average monthly leaf formation rate of seedlings. The morphological characteristics of dead seedlings were also examined and compared with those of live seedlings collected in the same period ( $n = 10$ ). The number of leaves that had been produced by dead seedlings was derived from the number of leaf scars present along the rhizomes. The remaining seedlings were partitioned into their tissue compo-

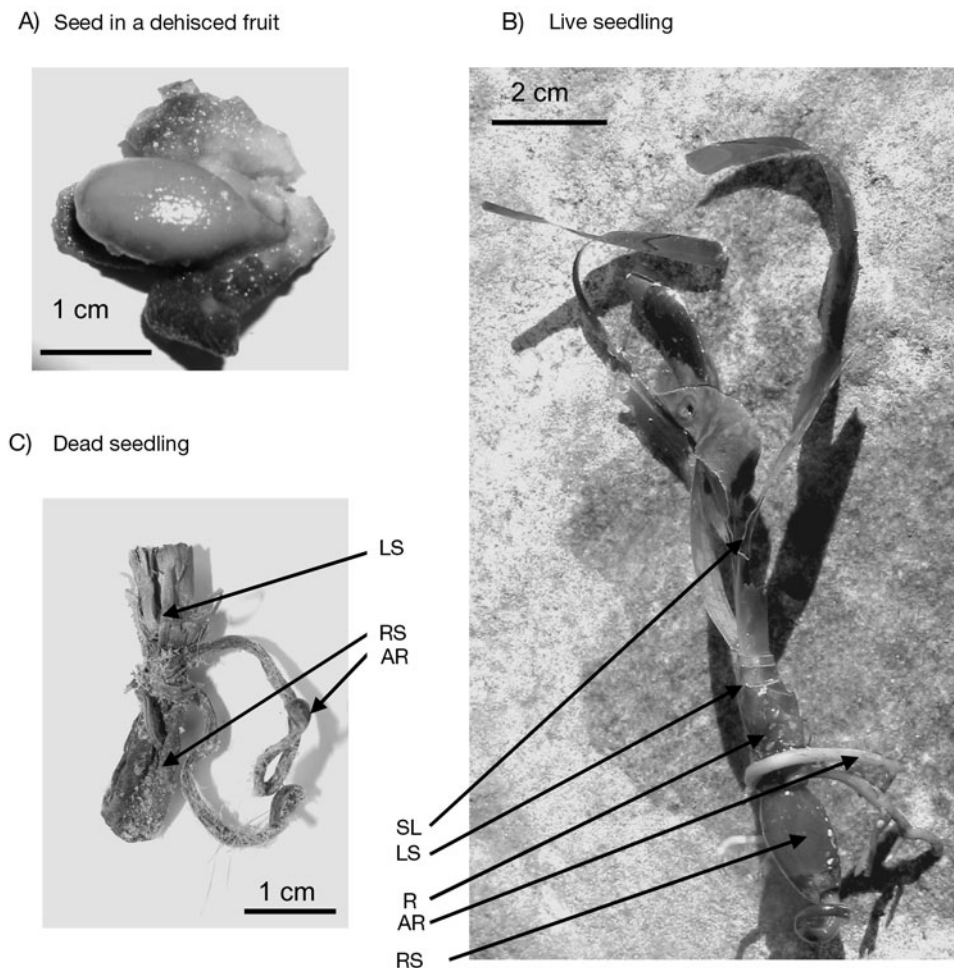


Fig. 1. *Posidonia oceanica*. (A) Mature seed in a dehiscent fruit, (B) live seedling and (C) dead seedling. Arrows shows the tissue components of seedlings used in the elemental analysis. AR: adventitious roots; SL: standing leaves; LS: leaf scars; R: rhizome; RS: remains of seed

nents (leaves, leaf scars, rhizome, root and remains of seed if present) (Fig. 1B,C) to examine the nutritional status and the potential role of each tissue component in the seedling nutrient economy. Seedlings with signs of herbivore damage were discarded. Finally, to compare the nutrient concentrations of seedlings and adult shoots, 3 plagiotropic fragments with the apical shoot and 2 to 3 lateral shoots were selected and then cut into segments that were separated into tissue components.

**Elemental analysis.** Mature seeds, and the seedling and shoot biomass fractions were dried at 70°C and placed under a glass bell with fuming HCl (37% analytical grade, Merck) for 24 h to remove all traces of calcareous epiphytes, and then ground into a homogeneous powder (MM 301 microgrinding device, Retch). Elemental analyses of C and N were performed on dry samples (2 to 6 mg) using a C:N:S analyser (NA 1200, Carlo Erba). Elemental analysis of P was performed using an inductively coupled plasma-mass spectrometer (Elan DCR II) on a 10 mg distilled water (DW) sample after digestion with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in an Ethos D microwave digester. Total reserves of seeds (C, N or P, mg seed<sup>-1</sup>) in fruits were calculated from the mass (mg dry weight [dry wt]) of each seed multiplied by the relative C, N or P concentration (% dry wt). Since a single seedling did not provide adequate tissue material for elemental analysis, the material from the corresponding tissue fractions of 2 or 3 seedlings was combined into a single sample.

**Data analysis.** To examine variation in seed mass the coefficient of variation (CV = 100 × SD/mean) for seed dry mass was calculated. The influence of seed mass on seed C, N or P content (mg seed<sup>-1</sup>) was examined by least-squares linear regression analyses on log-transformed variables. Multivariate analysis of variance (MANOVA) was used to test for differences in the growth characteristics of live seedlings between the sampling events (May versus December). The significance of multivariate main effects was determined from Wilks' A statistics. When MANOVA indicated significant differences between events, univariate ANOVAs were run on each of the response variables examined. The benefit of using multivariate analyses was protection against Type I error resulting from multiple tests of correlated variables. One-way ANOVAs were used to test for differences in the total number of leaves and roots produced by seedlings between dead and live seedlings collected in May.

To examine the dynamics of the pool of reserves initially available for seedling growth, differences in C, N and P concentrations in seeds from different sources (mature fruits versus 1 yr live seedlings versus 1 to 2 yr live seedlings versus dead seedlings) were tested for significance using 1-way ANOVA followed by Tukey's post-hoc comparison tests.

Standard all-pair *t*-test comparisons ( $\alpha = 0.05$ ) were used to determine whether mean C, N or P concentrations in live seedlings differed statistically across the different tissue types (leaves, roots, rhizome and leaf scars). One-way ANOVAs were also performed to test for differences in the mean C, N or P concentrations and atomic N/P and C/N ratios in the seedling tissues between the sampling events. Finally, 1-way ANOVAs were performed to test for statistical differences in the C, N and P concentrations and N/P and C/N ratios in tissues between seedlings and rhizomes collected in December 2005. Variables were appropriately transformed when necessary to normalize data distributions and stabilize error term variances.

## RESULTS

### Seed mass and nutrient content

Seed mass ranged from 0.32 to 0.65 g dry wt (mean ± SD = 0.45 ± 0.05 g dry wt). The coefficient of variation in seed mass was 15.2%. The distribution of seed masses (Fig. 2) tended to be skewed to the right and also exhibited positive kurtosis.

The mean C, N and P concentrations were 36.13 ± 0.16%, 1.49 ± 0.05% and 0.13 ± 0.003%, respectively. The C, N and P content of seeds (Fig. 3) increased linearly with seed mass, as indicated by the slope (*b*) of the regression functions fitted to log-transformed variables (C:  $b = 1.01 \pm 0.03$ , N:  $b = 0.80 \pm 0.21$  and P:  $b = 0.81 \pm 0.26$ ). Most of the variation in the C content of seeds was explained by seed mass (97%). However, seed mass did not explain as well the total variance in the N (32%) and P (24%) content of seeds. The linear relationships between seed mass and seed content

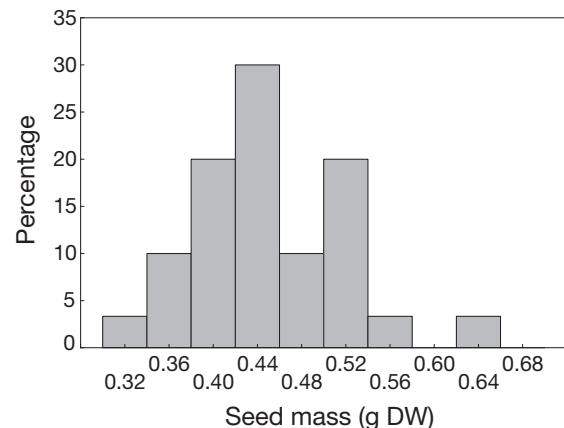


Fig. 2. *Posidonia oceanica*. Frequency distribution of seed mass (g dry wt) (n = 30)



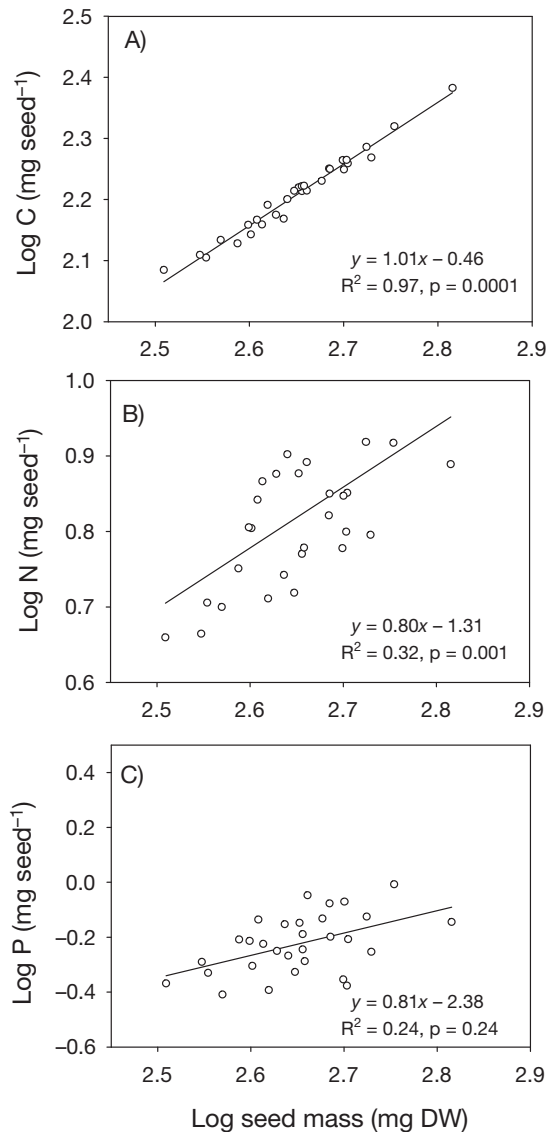


Fig. 3. *Posidonia oceanica*. Relationships between seed mass (mg dry wt) and the (A) C, (B) N and (C) P content (mg dry wt seed<sup>-1</sup>) of seeds. Least-squares linear regression fitted to log<sub>10</sub> transformed variables (solid lines). The coefficient of determination ( $R^2$ ) and the significance of the coefficient ( $p$ ) are provided ( $n = 30$ )

indicated that the increase in seed content per unit of seed mass was relatively uniform across seeds.

#### Seedling growth characteristics and tissue elemental analysis

A seed still attached to the rhizome was present in 92% of the collected seedlings. The dry mass of seeds in the seedlings of May 2005 ( $0.09 \pm 0.03$  g dry wt) was

about  $\frac{1}{4}$  of the initial mass of seeds in fruits. Elemental concentration between seeds in fruits and seeds attached to seedlings are compared in Fig. 4. The average concentrations of C, N and P in seeds declined significantly from mature fruits to 1 yr old seedlings and did not change significantly between May and December 2005 (C:  $F_{2,6} = 15.76$ ,  $p = 0.004$ ; N:  $F_{2,6} = 9.48$ ,  $p = 0.01$ ; P:  $F_{2,6} = 91.62$ ,  $p < 0.0001$ ). It was also true for the atomic N/P ratios ( $F_{2,6} = 6.40$ ,  $p = 0.03$ ; Fig. 4D). No differences were observed between dead seedlings and live 1 yr old seedlings (C:  $F_{1,4} = 1.87$ ,  $p = 0.24$ ; N:  $F_{1,4} = 0.53$ ,  $p = 0.83$ ; P:  $F_{1,4} = 0.76$ ,  $p = 0.43$ ; N/P ratio:  $F_{1,4} = 0.16$ ,  $p = 0.70$ ).

The biometry of seedlings was variable (Table 2). The seedlings consisted of one shoot, a very small rhizome (1 to 3.5 cm in length) with 2 to 5 adventitious roots. In May 2005 the number of standing leaves varied from 2 to 8 leaves (mean  $\pm$  SD =  $5.5 \pm 1.0$  leaves per seedling, CV = 18.1%) and the maximum leaf length was  $13.1 \pm 1.7$  cm (CV = 12.9%). The total seedling biomass was also variable, ranging from 0.19 to 0.73 g dry wt (mean =  $0.45 \pm 0.13$  g dry wt; CV = 28.8%). MANOVA showed significant differences in seedling development between the 2 sampling events ( $F_{7,4} = 11.55$ ,  $p = 0.001$ ). Seedlings from May were collected 12 mo after germination (1 yr old seedlings) and were smaller in size compared with those collected in December, i.e. 19 mo after germination (1 to 2 yr old seedlings), exhibiting a lower number of leaf scars and a shorter rhizome (Table 2). However, the number of standing leaves and roots per seedling was similar, and leaves and roots were longer compared with those of December (Table 2). In total the number of leaves produced by seedlings from May was ca. 16, and this corresponded to the number of leaves produced by seedlings during the 1st year. The seedlings collected in December 2005 had produced ca. 23 leaves in total; thus, ca. 7 new leaves were produced between May and December 2005 of the 2nd year. As the mean rate of annual leaf production was 16 leaves yr<sup>-1</sup>, on average a new leaf was produced by seedlings approximately every 25 d. The seedlings collected in May categorized as dead seedlings did not show standing leaves and their rhizome were only a few millimetres in length: the number of leaf scars ( $10 \pm 0.5$ ) and roots ( $3.5 \pm 0.5$ ) per seedling was comparable with that of live seedlings collected in the same date ( $F_{1,10} = 0.55$ ,  $p = 0.47$ ;  $F_{1,10} = 3.18$ ,  $p = 0.10$ ). Since dead seedlings had produced only 10 leaves in total, leaf production would be ceased several months before collection, possibly 7 to 8 mo after germination.

Elemental C, N and P concentrations and atomic N/P and C/N ratios in seedlings from May and December 2005 are shown in Fig. 5. In terms of mean elemental concentrations in each tissue type, leaves were richer

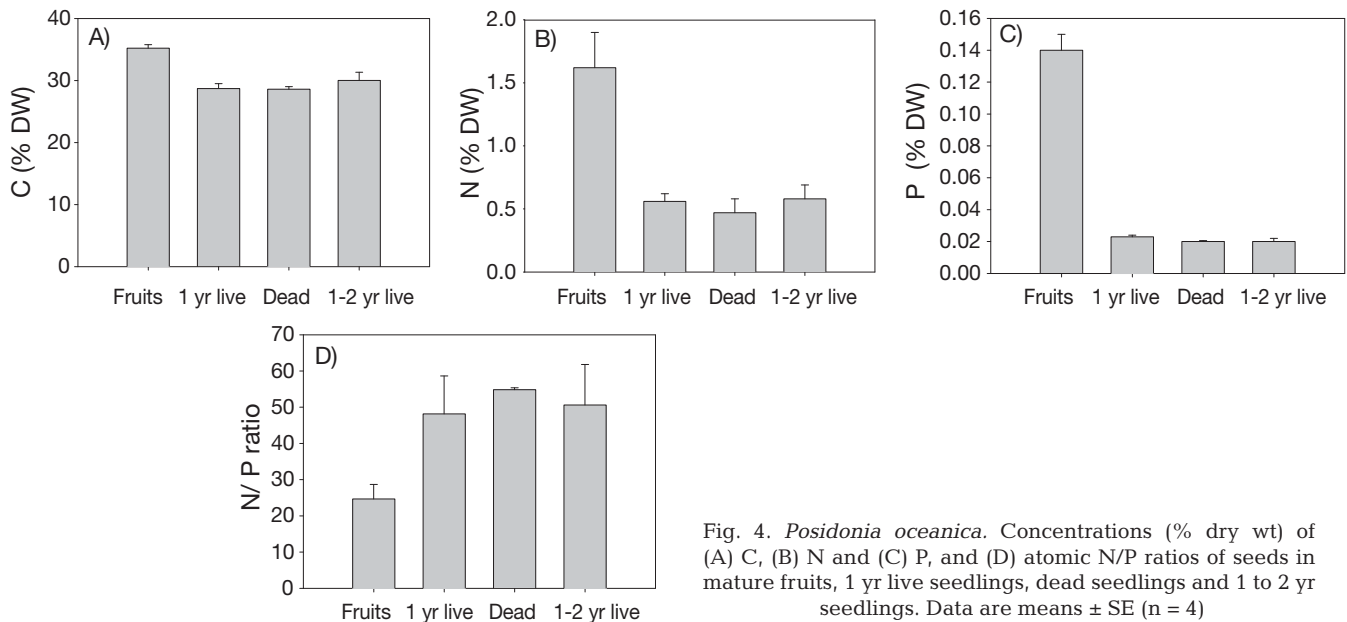


Fig. 4. *Posidonia oceanica*. Concentrations (% dry wt) of (A) C, (B) N and (C) P, and (D) atomic N/P ratios of seeds in mature fruits, 1 yr live seedlings, dead seedlings and 1 to 2 yr seedlings. Data are means  $\pm$  SE (n = 4)

Table 2. *Posidonia oceanica*. Morphological measurements for 1 yr and 1 to 2 yr live seedlings, and results of 1-way ANOVA testing for the effects of sampling event (May versus December 2005) on each morphological variable. Data are mean  $\pm$  SE, n = 6

| Dependent variable        | Mean $\pm$ SE  |                | MS     | F     | p       | Transformation  |
|---------------------------|----------------|----------------|--------|-------|---------|-----------------|
|                           | May            | December       |        |       |         |                 |
| Number of standing leaves | 5.5 $\pm$ 1.0  | 5.6 $\pm$ 0.5  | 0.08   | 0.01  | 0.89    | None            |
| Number of leaf scars      | 10.5 $\pm$ 0.4 | 16.8 $\pm$ 0.9 | 120.23 | 37.21 | <0.0001 | None            |
| Length longest leaf (cm)  | 13.1 $\pm$ 1.7 | 7.8 $\pm$ 1.3  | 85.73  | 5.91  | 0.03    | None            |
| Leaf width (cm)           | 0.50           | 0.51           | 12.26  | 45.54 | <0.001  | Log(x)          |
| Number of roots           | 4.6 $\pm$ 0.3  | 5.5 $\pm$ 1.0  | 0.02   | 0.16  | 0.69    | Square root (x) |
| Length longest root (cm)  | 4.0 $\pm$ 0.4  | 2.3 $\pm$ 0.5  | 9.36   | 6.55  | 0.02    | None            |
| Rhizome length (cm)       | 1.4 $\pm$ 0.1  | 3 $\pm$ 0.4    | 3.49   | 54.94 | <0.001  | Log(x)          |

in C in May, whereas rhizomes and roots were richer in C in December. Leaves and rhizomes were always richer in N, while rhizomes were richer in P (Fig. 5). In May the C concentration in leaves was significantly higher compared with that of December, while the concentrations of the other tissues were significantly lower (Fig. 5A, Table 3). The N and P concentrations in leaves, rhizome and scars in May were significantly higher than those in December, while root concentrations of N were not (Fig. 5B,C, Table 3). There were significant differences in the atomic C/N ratio of all tissues between sampling events (Fig. 5E, Table 3). The atomic N/P ratio of scars was higher in December than in May, while the N/P values of the other tissues were comparable (Fig. 5D, Table 3).

Seedlings tended to have lower leaf C, N and P and N/P leaf ratio concentrations than adult plants (Fig. 6A–D), although the differences were not statistically significant (C:  $F_{1,4} = 0.56$ ,  $p = 0.49$ ; N:  $F_{1,4} = 6.10$ ,

$p = 0.06$ ; P:  $F_{1,4} = 0.26$ ,  $p = 0.63$ ; N/P:  $F_{1,4} = 1.62$ ,  $p = 0.27$ ). Roots and scars of adult plants were impoverished in N ( $F_{1,4} = 16.61$ ,  $p = 0.01$  and  $F_{1,4} = 65.17$ ,  $p = 0.001$ , respectively) compared with seedlings, but had similar P ( $F_{1,4} = 0.97$ ,  $p = 0.37$  and  $F_{1,4} = 0.09$ ,  $p = 0.76$ ) and C values ( $F_{1,4} = 1.64$ ,  $p = 0.06$  and  $F_{1,4} = 0.001$ ,  $p = 0.97$ ). Rhizomes of adult plants were impoverished in C ( $F_{1,4} = 109.26$ ,  $p = 0.0004$ ), but their N ( $F_{1,4} = 1.31$ ,  $p = 0.31$ ) and P ( $F_{1,4} = 0.15$ ,  $p = 0.71$ ) concentration was comparable with those of seedlings (Fig. 6A–D). The C/N ratio of leaves of adult plants was lower ( $F_{1,4} = 8.03$ ,  $p = 0.04$ ) than that of seedlings, while no significant differences were detected for the other tissues (roots:  $F_{1,4} = 3.18$ ,  $p = 0.14$ ; scars:  $F_{1,4} = 1.75$ ,  $p = 0.25$ ; rhizome:  $F_{1,4} = 1.89$ ,  $p = 0.24$ ; Fig. 6E). Finally, no significant differences were detected for tissue C or P concentrations between dead and live seedlings, while the N concentrations were lower in dead seedlings than in live seedlings (Table 4).

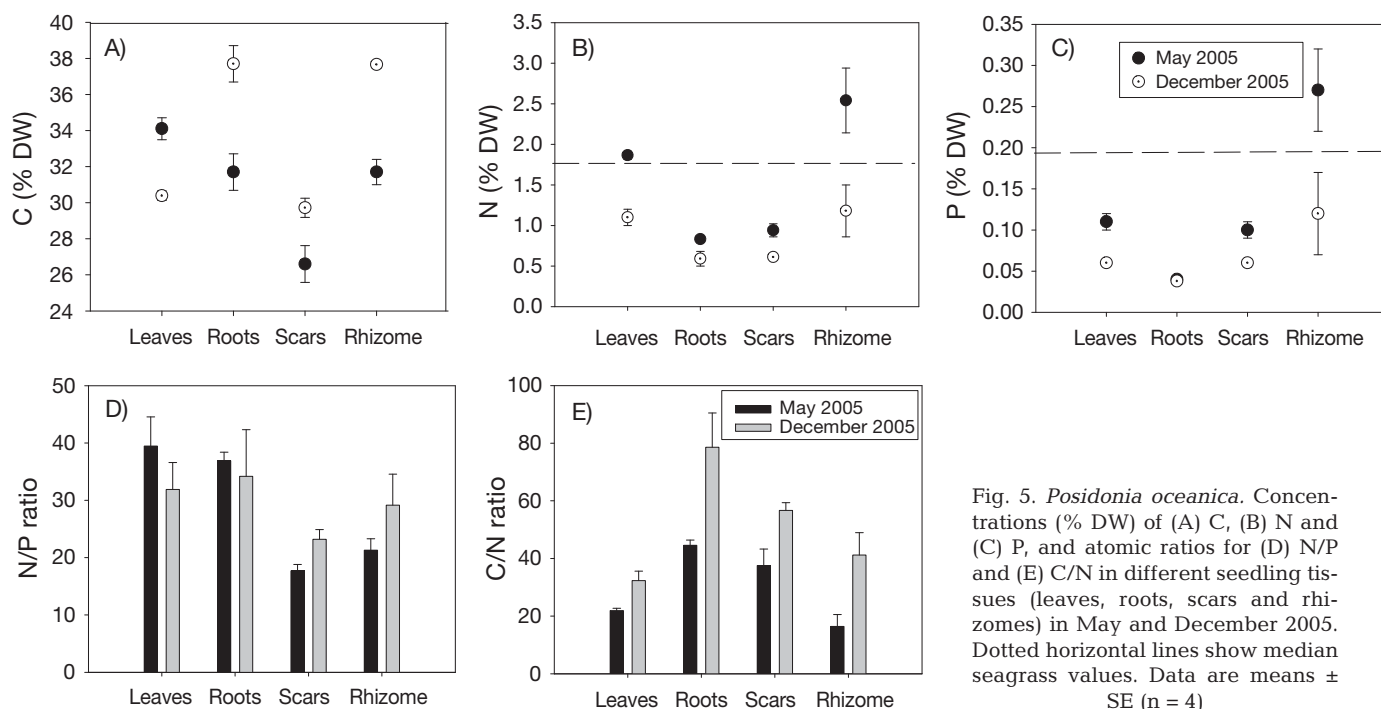


Fig. 5. *Posidonia oceanica*. Concentrations (% DW) of (A) C, (B) N and (C) P, and atomic ratios for (D) N/P and (E) C/N in different seedling tissues (leaves, roots, scars and rhizomes) in May and December 2005. Dotted horizontal lines show median seagrass values. Data are means  $\pm$  SE (n = 4)

Table 3. *Posidonia oceanica*. Results of 1-way ANOVAs testing for the effects of sampling event (May versus December) on the C, N and P concentrations, and C/N and atomic N/P ratios for each tissue type (leaves, leaf scars, rhizome and roots) of live seedlings. Results of Tukey's tests are also reported where values differ significantly. ns: not significant; n = 4

| Dependent variable | Tissue type | MS      | F     | p      | Transformation | Tukey's test   |
|--------------------|-------------|---------|-------|--------|----------------|----------------|
| C                  | Scars       | 34.76   | 34.31 | 0.001  | None           | December > May |
|                    | Rhizome     | 71.21   | 64.51 | 0.0002 | None           | December > May |
|                    | Leaves      | 34.19   | 40.57 | 0.0007 | None           | May > December |
|                    | Roots       | 58.51   | 13.26 | 0.01   | None           | December > May |
| N                  | Scars       | 0.22    | 15.95 | 0.007  | None           | May > December |
|                    | Rhizome     | 3.64    | 8.32  | 0.02   | None           | May > December |
|                    | Leaves      | 1.11    | 38.29 | 0.000  | None           | May > December |
|                    | Roots       | 0.11    | 4.77  | 0.07   | None           |                |
| P                  | Scars       | 0.007   | 13.84 | 0.009  | None           | May > December |
|                    | Rhizome     | 0.057   | 7.13  | 0.039  | None           | May > December |
|                    | Leaves      | 0.004   | 7.17  | 0.03   | None           | May > December |
|                    | Roots       | 0.00018 | 5.55  | 0.056  | None           | ns             |
| C/N ratio          | Scars       | 1196.21 | 52.38 | 0.003  | None           | December > May |
|                    | Rhizome     | 1223.45 | 7.64  | 0.03   | None           | December > May |
|                    | Leaves      | 0.296   | 14.32 | 0.009  | Log(x)         | December > May |
|                    | Roots       | 0.56    | 10.11 | 0.01   | Log(x)         | December > May |
| N/P ratio          | Scars       | 61.89   | 7.46  | 0.03   | None           | December > May |
|                    | Rhizome     | 120.59  | 1.77  | 0.23   | None           | ns             |
|                    | Leaves      | 114.76  | 1.17  | 0.32   | None           | ns             |
|                    | Roots       | 14.76   | 0.107 | 0.75   | None           | ns             |

## DISCUSSION

One of the basic features that contribute to the continued success of a species in a nutrient-poor environment is the capacity on the part of seeds to sequester

and concentrate a considerable proportion of the plant's resource of the limiting nutrients (Pate & Dell 1984). Australian seagrasses belonging to the Posidoniaceae (*Posidonia australis* Hook f., *P. sinuosa* Cambridge and Kuo and *P. coriacea* Kuo and Cambridge) are likely to

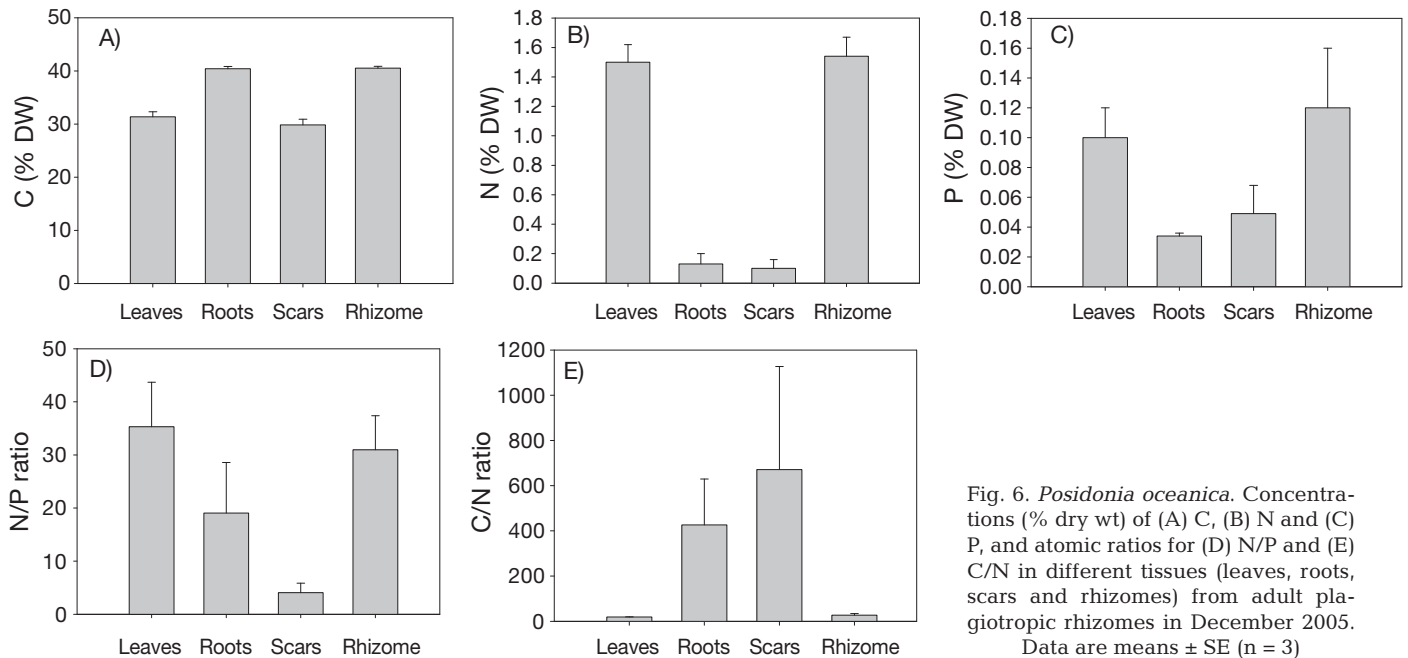


Fig. 6. *Posidonia oceanica*. Concentrations (% dry wt) of (A) C, (B) N and (C) P, and atomic ratios for (D) N/P and (E) C/N in different tissues (leaves, roots, scars and rhizomes) from adult plagiotropic rhizomes in December 2005. Data are means  $\pm$  SE (n = 3)

Table 4. *Posidonia oceanica*. Results of 1-way ANOVAs testing for the effects of seedling status (dead versus live) on the C, N and P concentrations for each tissues type (leaf scars and roots) of seedlings (May 2005). Results of Tukey's tests are also reported where values differ significantly. Values in round brackets indicate mean ( $\pm$ SE) concentrations of the elements in each tissue type. n = 10

| Dependent variable | Tissue type | MS     | F     | p     | Concentration   | Tukey's test |
|--------------------|-------------|--------|-------|-------|---|--------------|
| C                  | Roots       | 0.050  | 0.022 | 0.88  | Dead (32.73 $\pm$ 0.68%)<br>Alive (32.86 $\pm$ 0.59%) |              |
|                    | Scars       | 2.13   | 3.96  | 0.16  | Dead (25.61 $\pm$ 0.45%)<br>Alive (26.50 $\pm$ 0.40%) |              |
| N                  | Roots       | 0.821  | 77.40 | 0.000 | Dead (0.43 $\pm$ 0.04%)<br>Alive (0.84 $\pm$ 0.02%)   | Dead < Live  |
|                    | Scars       | 5.94   | 1.03  | 0.025 | Dead (0.72 $\pm$ 0.15%)<br>Alive (1.16 $\pm$ 0.10%)   | Dead < Live  |
| P                  | Roots       | 0.0002 | 2.46  | 0.13  | Dead (0.04 $\pm$ 0.003%)<br>Alive (0.05 $\pm$ 0.001%) |              |
|                    | Scars       | 0.005  | 2.83  | 0.11  | Dead (0.16 $\pm$ 0.01%)<br>Alive (0.13 $\pm$ 0.005%)  |              |

invest a substantial amount of P and N into the seed reserves to provide newly established seedlings with nutrients for several months after germination (Hocking et al. 1980, Tomlinson 1982, Kuo & Kirkman 1996, Walker et al. 2004). The present study demonstrates that the seeds of *P. oceanica* had, on average, higher N concentration (1.6% versus 0.7 to 1.3%) but lower P concentration (0.14% versus 0.18 to 0.36%) compared with the Australian *Posidonia* species. Instead, compared with other Mediterranean seagrasses such as *Cymodocea nodosa*, the N seed concentration was lower (1.6% versus 2.3%) and P was equivalent (0.14% versus

0.13%, Duarte & Sand-Jensen 1996). In terms of absolute quantity of nutrients, however, the seeds of *P. oceanica* contained ca. 6.78 mg of N and 0.63 mg of P, whereas this amount reached only ca. 2.80 mg of N and 0.47 mg of P in *P. australis* seeds (Hocking et al. 1980) and ca. 0.32 mg of N and 0.02 mg of P in *C. nodosa* (Duarte & Sand-Jensen 1996). The ability of *P. oceanica* to produce such nutrient-rich seeds may be a strategy to allow prolonged seedling development in environments where nutrients are in concentrations low enough to limit the establishment of other species such as *C. nodosa* (Pérez et al. 1991, 1994).



This study also documents substantial variation in the mass of seeds produced by *Posidonia oceanica* within the study population, which is consistent with degree of variation reported for some terrestrial plants (CV = 13.6 to 23.6%, Obeso 1993, Susko & Lovett-Doust 2000). The C, N and P content of seeds increased linearly with seed mass, indicating that the availability of these elements may constrain seed provisioning and impose a cost on producing larger seeds in terms of limited environmental nutrients. Since flowering in *P. oceanica* requires a considerable demand on reserves of useable nutrients, especially N and P (Gobert et al. 2005), the production of seeds of variable masses could be related to within-meadow differences in the resource available to reproductive shoots, or development constraints operating during fruit maturation within individual shoots. Further studies are needed to assess whether seeds of *P. oceanica* below a certain mass have enough reserves to protect seedlings from nutrient stress during the 1st year of establishment.

The analysis of the nutrient and C content of *Posidonia oceanica* seeds in mature fruits and in seeds remained attached to dead seedlings indicates that the N and P reserves were retrieved from the hypocotyl to the seedling axis within 7 to 8 mo after germination, with 69 and 86% efficiency, respectively, assuming no losses due to leaching, while only 20% of the C reserves were used. As the seed nutrient concentrations in live seedlings were similar to those of dead ones and did not change significantly with age, the reserves that remained uncommitted were incorporated in structural tissues of the seed, rather than contributing to seedling growth. Morphological examination of live seedlings revealed that ca. 7 leaves were produced between May and December of the 2nd yr; thus, the nutrient requirements for the production of new biomass would have been met by active uptake of external nutrient supplies and reallocation of existing material.

The uptake of nutrients by leaves and their storage within different plant components (rhizomes, in particular) in autumn through winter and remobilization in spring through summer is an important strategy adopted by *Posidonia oceanica* to obtain independence from seasonal variations in nutrients in the environment (Alcoverro et al. 1997, 2000, Lepoint et al. 2002). Results presented here indicate that the distribution of nutrients within seedling tissues was comparable with the pattern reported for adult plants (Alcoverro et al. 2000, Invers et al. 2002, Lepoint et al. 2002), with high values of P and N in rhizomes and minimum values in roots. The decrease in the nutrient tissue concentrations observed between May and December may indicate that in this period the nutrient demands for active leaf growth exceed uptake, and part of this

demand was met by the reserves accumulated in excess of growth demands during the previous winter. The concomitant increase in the C concentration in rhizomes and roots indicates storage of the carbon taken up in excess in summer, in accordance with that found in adult plants (Alcoverro et al. 2001). It appears, therefore, that the rhizome contributes significantly to the nutrient economy of seedlings during the 1st year, while the contribution of roots appeared to be limited.

Analysis of the leaf nutrient content of seedlings shows that in May the P concentration was below the median values characteristic of nutrient-deficient plants reported for seagrasses (0.2%), while the N concentration was close to the median values for seagrasses (1.8%, Duarte 1990). In December both P and N concentrations were below the median values reported for seagrasses (Duarte 1990), and were also lower compared with apical shoots in plagiotropic rhizomes although the differences were not statistically significant. The leaf N/P ratio decreased from 39 (May) to 31 (December) as a result of decrease in N and little variation in P, and these values were in the range considered to be P-limited (30 to 40, Duarte 1990).

In summary, the results of this study indicate that *Posidonia oceanica* produce large seeds containing more mineral nutrients (N and P) compared with some closely related species. It seems that during the early seedling developmental phase the seeds act to supplement environmental supplies of N and P to ensure rapid growth of shoot and roots. Resource constraints, however, may affect the nutritional quality of seeds with potential important consequences for seedling performance. Seedlings appeared to be primarily limited by P during their second growing season. This lends support to the hypothesis that growth limitation due to nutrient deficiency may be a cause of mortality for seedlings established on poor-nutrient patches. These findings may have important implications for the restoration of *P. oceanica* meadows, as the use of adequate fertilization practices is expected to enhance the success of future seedling transplantation programs. Clearly, determination of the nutrient content in uprooted seedlings cannot be the only criterion for the assessment of the extent of nutrient deficiency experienced by seedlings of *P. oceanica*. Further long-term studies and experimental tests on nutrient addition or the uptake of nutrients and other essential elements in seedlings growing in the field are needed.

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