Restoration of seagrass meadows: means and limitations

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Abstract

A transplantation experiment of the seagrass *P oceanica* was launched in September 2001. Woven bamboo grids were used as a substrate for transplantation. Biometric parameters were recorded underwater and on samples collected on the transplanted grids. In the best case, the rate of shoot multiplication is 1.4 after 27 months, which could allow to reach the natural shoot density observed in the healthy meadow after 5 years of transplantation. Growth direction shifts from a vertical growth to an horizontal growth during the transplantation. Roots biomass and length increases

spectacularly, but leaf length and weight remain clearly inferior to those of reference plant. This implies that root development is of high priority for the plant and that the transplants do not have a sufficient amount of nutrient to ensure adequate leaf growth. It appears that, after two years, transplantation has very small effect (or very time-limited effect) on sediment parameters. This reflects, in one hand, the fact that sand patches are highly dynamics sedimentary environments and, in other hand, the fact that the establishment of sedimentary characteristic of *P. oceanica* meadow is a long term process. As a consequence of these observations, *P. oceanica* cuttings, even if they develop a high biomass of roots, continue to be affected by sand patch geo-chemistry a long time after their transplantation on the un-vegetated substrate. This is probably a major cause of transplantation experiment failure. It appears that *P. oceanica* transplants display generally lower N and P concentrations in their leaves and rhizomes than reference shoot. Storage function of these organs in winter seem to be not realised which probably diminishes the capacity of the plants to ensure an optimal growth during spring and to resist to nutrient shortage periods (summer and fall). The reason of the low elemental concentrations in transplant is more likely linked to difficulties to incorporate nutrient (i.e. lack of roots) as interstitial data shown that the availability of N and P is higher on the transplanted sand patch than on the reference meadow. The protection of P. Oceanica meadows is essential because its restoration effort is heavy. The transplantation procedure needs divers with underwater experience, needs time for installation and needs one and half year of survey of the grids.

<u>Introduction</u>

Endemic to the Mediterranean Sea, the seagrass *Posidonia oceanica* (L.) Delile forms widespread meadows which exert crucial roles in the coastal waters (see Gobert et al., in press). These meadows cover a surface area of 25,000 to 50,000km^2 representing 1 to 2% of the Mediterranean Sea surface (Pasqualini et al. 1998). Despite their ecological and economical importance, since the sixties, an increasing number of reports documents their ongoing loss or regression in all countries (e.g.Boudouresque et Meinesz 1982). The decline in seagrass meadows and associated communities of plants and animals coverage is generally attributed to anthropic activities (Cambridge et al. 1986, Short and Wyllie-Echeverria 1996). In the damaged areas, healthy meadows have been replaced with deserts of dead matte, sand or mud.

The natural recolonisation of altered sites is rare but does occur (i. e. Pergent-Martini et Pasqualini 2000). The recolonizing ability is poor (i.e. Seddon, 2004) and the process is slow because *Posidonia* are long living species with a low grow and a low occurrence of flowering and success (Boudouresque et al. 1984, Marbà and Duarte 1998, Marbà and Walker 1999). Some mechanical *Posidonia* spp transplantations have been conducted with some success and diverse techniques of *P. oceanica* transplantation have been described (Meinesz et al. 1992, Molenaar et Meinesz 1992, Paling et al. 2001). The success of the transplantation varies greatly (Fonseca et al. 1998) and the processes which influence the performance of the used methodology are generally not discussed. A clear and consistent planning and implementation approach is lacking.

In this paper, we present the results of a two years experimental transplantation project conducted to put in evidence the factors influencing the survival of the

transplanted *P. oceanica* cuttings. We have used woven bamboo grids as support. Biometry, survivorship and multiplication rate of shoots, nutrients dynamics, and impact on sediment parameters have been monitored. We also take stock of necessary means (man and money) to conduct the *P. oceanica* meadow restoration.

Material and method

General

Experimental work was done in the Revellata Bay in front of the oceanographic station STARESO (Calvi, Western Corsica, France) from September 2001 to December 2003 on a sandy patch at 17m depth surrounded by a *P. oceanica* meadow. The transplantation experiment was performed using naturally uprooted shoots (cutting) in order to minimise the impact of transplant collection. These cuttings were collected between 5 and 15 m depth. Shoots with orthotropic rhizomes (maximum 10 cm) were attached with natural links (sisal) on woven bamboo grids ($1m^2$). Two grids were transplanted with 50 orthotropic shoots and three grids with 100 orthotropic shoots. One of these grids was used for a periodical sampling.

Survivorship, multiplication and biometry

In November 2001, on each cutting, initial maximum leaf length, number of leaves per shoot, rhizome length, length and number of roots were measured before the transplantation. These parameters and the multiplication rates of transplants were also measured, *in situ*, in March, June, September 2002 and in March, June, December 2003. Leaf growth was measured using a modified Zieman technique (Zieman et al. 1974). Furthermore, five shoots were sampled on a reference site in the healthy meadow; biometric parameters were recorded. Leaves, scales, roots and rhizomes were lyophilised for 48 h and weighed to obtain biomass data.

Sediment

Sediment level changes were measured, underwater, in March 2002. Zero level was set to the grid it-self. Measurements were done every 30 cm, beginning 25 cm outside of the grids. In November 2001 and December 2003, sediment cores (L: 20 cm, Ø 5cm) were sampled before the settlement of the transplanted grids and after 27 months of transplantation. Reference cores were collected simultaneously in the sand patch near the grids and in the healthy meadow. Each core was cut every 5 cm from the top. Each fraction was dried during 48h at 60°C, weighed before and after drying. Granulometric curves were established using sieves of 8, 4, 2, 1, 0.5, 0.25 and 0.125 mm. Interstitial pore water was repeatedly sampled in sediment of each grid, on the un-colonised sand patch and in the healthy meadow. Nutrient concentrations (i.e. ammonium, nitrates and phosphates) were measured using an analytical automated chain (Skalar, Netherlands). Results are expressed in μM.

Nutrient dynamics in tissues

Nitrogen concentration measurements were performed with an Optima mass spectrometer (Micromass, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy). Phosphorus concentrations were measured with an ICP after micro-wave digestion. Results are expressed as relative concentrations in $mg.g_{dw}^{-1}$.

Results

Biometry

After 27 months, the transplants which had originally a vertical growth have in their majority an horizontal growth. The number of original transplant was initially set to 50 or 100 transplant.m^{-2}. After 27 months, this number decreases (Fig.1) to about 20 transplants for the grids with the lowest density (i.e. 35% of the initial number). The grids with the initial density of 100 transplants show a greater success with about 75 remaining transplants after 27 months. The loss rate of transplants seems generally more important during fall and winter.In September 2001, the number of transplant was equal the number of leaf bundle according to our transplantation design. But, after 6 month of transplantation, the leaf bundle number increases, although between 10 and 15% of initial transplants were already lost. This increase is a consequence of the production of a second (or a third) leaf bundle by transplants. This phenomenon is particularly important in December 2003, when some transplants exhibited till 4 leaf bundles. On the 100 transplants grid, the number of leaf bundles increases till 140 in the final sampling (i.e. 1.4x the initial number of transplanted leaf bundles).

Fig. 1: Temporal evolution of the transplant number (left) and of the leaf number of leaf bundles (right) during the *P. oceanic*a transplantation experiment using an initial density of 50 transplant.m^{-2} (black and open circles) and 100 transplant.m^{-2} (black and open square).

The leaf weight per leaf bundle (Fig.2, left) of transplanted shoots is minimal in December 2003 and maximal in September 2001. It is always lower than that of reference shoots. Both the weights of reference and transplanted shoots show a seasonal variation with a weight increasing in spring and a weight decreasing in fall.

The leaf growth per leaf bundle (Fig.2, right) is minimal in September 2002 for the transplants and the reference shoots. Leaf growth is maximal in June 2003 for the references, but does not show a clear maximum for transplants. Transplant growth is generally lower than that of reference shoots.
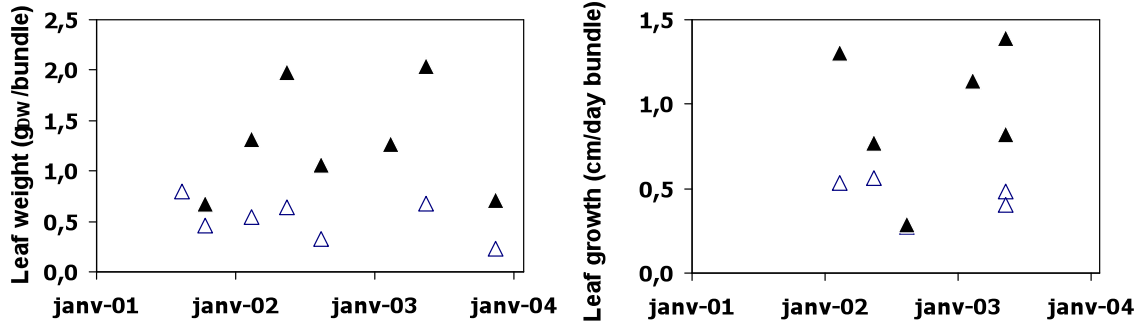

Fig. 2: Temporal evolution of the leaf weight per leaf bundle (left) and of the leaf growth per leaf bundle (right) during the *P. oceanica* transplantation experiment (black triangle = reference site; open triangle: transplanted shoots).

The leaf biomass of transplant represents only 10 to 20 % of the total biomass (Fig. 3) of the transplants. The relative contribution of roots to the total transplant weight increases during this study (from 5% in September 2001 to more than 20% in December 2003). On the contrary, the relative contributions of rhizomes and leaves tend to decrease.
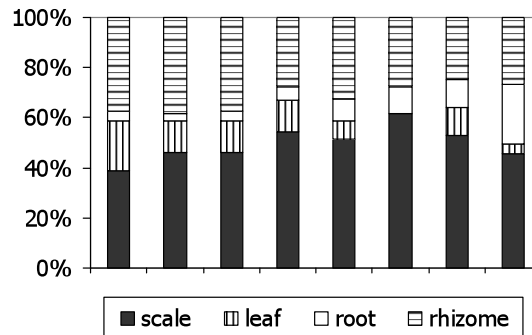

Fig. 3: Temporal evolution (September 2001 to December 2003) of the relative contribution of leaf, root, scale and rhizome to the total transplant weight during a *P. oceanica* transplantation experiment.

Sediment

The transplantation grids were settled directly on the sediment and, consequently, were not covered by sand. After 8 months in March 2002, sediment level measurements indicate that sediment is accumulated on the grid transplanted with 100 cuttings (upon average plus 1.8 cm) but not on the grid transplanted with 50 cuttings (upon average, sediment level decreases by 0.5cm) (Fig. 4). The sediment level outside the grids has changed in a variable way.

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              0   -0.5  -3   -5
          ┌─────────────────────────┐
        0 │ 0                     0  │
      3.5 │     6   2.5   1    1     │  -2
      1.5 │     4    3    3    0     │  -1
      3.5 │     1    3   2.5   2     │  -0.5
      0.5 │   -0.5   0   0.5  -1     │   2
        0 │ 0                     0  │
          └─────────────────────────┘
              1  -2.5   -2   2.5


              0   -1    -2   -3
          ┌─────────────────────────┐
        0 │ 0                     0  │
     -2.5 │   -1.5  -3  -2.5  -4     │   0
       -3 │    0.5   2    2   -0.5   │  -2.5
       -4 │    -1    0   1.5  -0.5   │   0.5
     -3.5 │     0  -0.5   0    0     │   0.5
        0 │ 0                     0  │
          └─────────────────────────┘
             -1   -2    -2   -1
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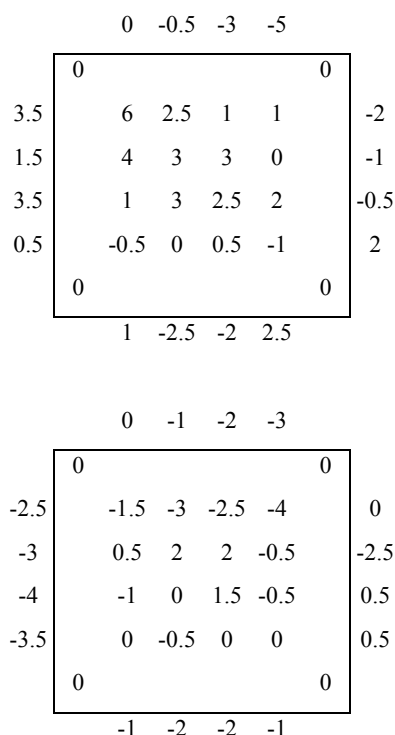
Fig. 4: Sediment level (cm) measured underwater in March 2002 on bamboo grids supporting 100 (upper) and 50 (lower) *P. oceanica* transplants, respectively. The grids are symbolised by the black squares.

In the fractions 1 (0-5 cm) of the sediment cores sampled in the meadow in September 2001, more than 80% of the sediment is constituted by grains smaller than 0.1 mm (i.e. very fine sediment) (Fig. 5, left). This proportion is 50 and 70 % for the fractions of the cores sampled on the sand patch and on the transplantation grids. In November 2003, the fractions 1 of the four cores do not show clear differences. Fractions 2 present the same trends and, globally the same grain size repartition than in September 2001 (Fig. 5, right).

Ammonium concentrations in interstitial pore water ranged from 2 to 50μM and were particularly high both on the transplantation grids and the reference sandy patch. The interstitial pore water sampled in the healthy meadow had lower ammonium concentrations than on the sandy patch. Phosphates concentrations ranged from 0.2 to 2μM and were not different in the 4 investigated sites (Fig. 6). Nitrates concentrations varied from 0 to 0.8μM (Fig. 6). They were generally lower on the transplantation grids than in the healthy meadow or in the reference site on the sandy patch.
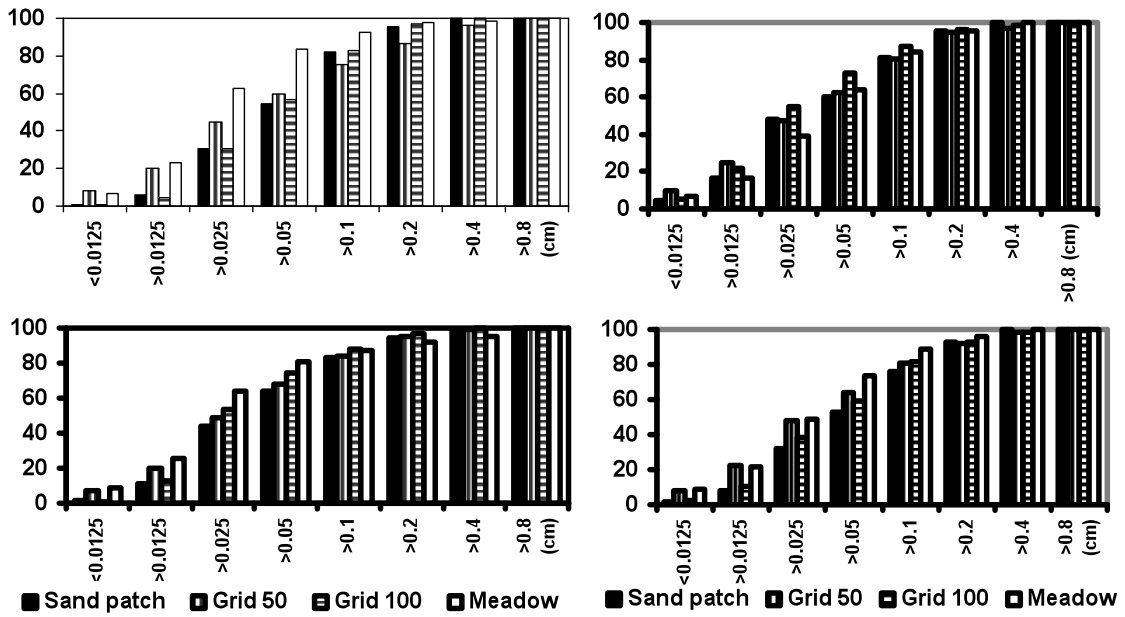
Fig 5: Granulometric curves (%) for fraction 1 (0-5cm) and fraction 2 (5-10 cm) of sediment core sampled in September 2001 (left) and in November 2003 (right).
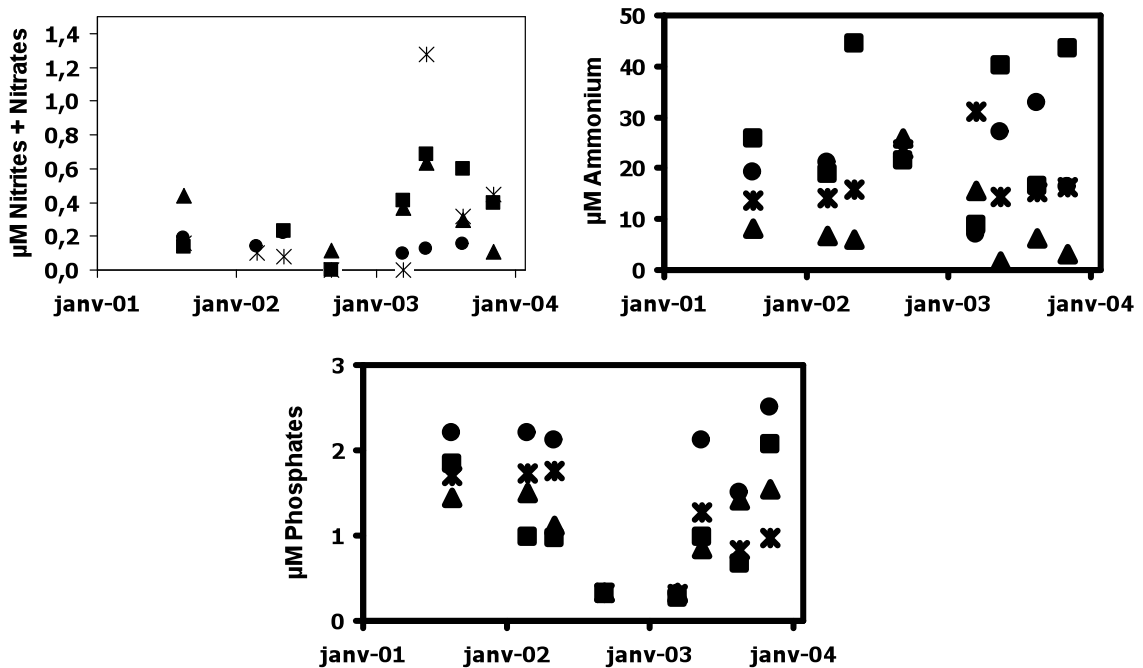

Fig 6: Ammonium, phosphates and nitrates concentrations (µM) in the interstitial pore water sampled on 100 (asterisk) and 50 (black circle) transplantation grids, in the healthy meadow (black triangle) and in a reference point on the sandy patch (black

square). Each point corresponds to five measurements (Standard deviations are not shown for the clarity of the figures).

Nutrient dynamics

Nitrogen concentrations of reference and transplant leaves show a strong seasonal variation with maximal values in early spring and minimal values in fall (Fig.7). N concentrations of transplant leaves are lower than those of reference leaves. Nitrogen concentrations in scales are lower than those of other plant organs. They do not show temporal variations. Roots present also very low N concentrations. N concentrations in rhizomes are of same order than those of transplant leaves, but do not show clear seasonal pattern.

P relative concentrations in transplant organs are generally lower than those of reference shoots, particularly for leaves and rhizomes (Fig 7). Concentrations are quite variable and show a seasonal pattern in the case of transplant and reference leaves with a minimum in fall. Scales of reference and transplant have the lowest N concentrations.
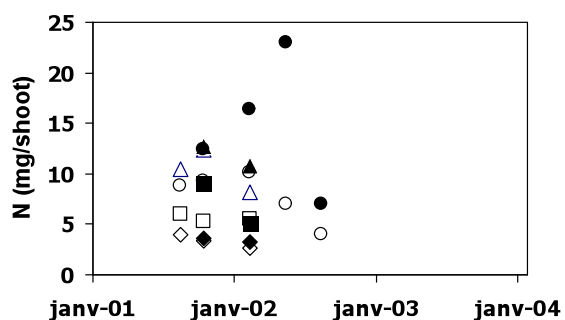

Fig 7: Temporal evolution of the N and P concentrations in organs of *P. oceanica* (circle: leaf; triangle: rhizome; rhomb: scale; square: root) transplants (open characters) and of reference shoots (black circles).

Discussion

Biometry

The transplanted shoots show strong morphological changes after few months. The growth direction changes from a vertical growth to an horizontal growth. This growth direction is a characteristic of colonising shoots. This change is accompanied by a multiplication of the number of leaf bundle. On the grids supporting an initial density of 100 transplants, the number of leaf bundle is multiplied by 1.4 in 27 months, although 20% of the initial transplant died during this period. Although these encouraging results, it must be noted that the natural density of the *Posidonia* meadow at 17m depth is about 300 shoot.m^{-2}. Considering a multiplication rate of 1.4y^{-1} (i.e. a rate which is reached the second year of this experiment), this means that the density measured in the natural meadow could be reached after 4-5 years on these grids. This hypothesis should only be correct if the transplant loss decreases with time due to the development of roots and anchoring of shoots.

The initial number of transplant decreases regularly and slowly on the 100 transplant grids. We have identified three major causes for this decrease: firstly a transplant death due to an initial bad health status of natural *Posidonia* cuttings. This death occurs during the first months of transplantation. Secondly, the links used to attach the shoots on the grids got rotten after about one year. Although the increasing of shoot biomass, many shoots do not have sufficient roots to anchor themselves in the sediment. Thirdly, because of the lack of roots, the shoots are not able to meet their nutrient requirement which could be also a major cause of death for the transplanted shoots (see Fig.7).

The leaf weight and the leaf growth per leaf bundle of transplants are always lower than those of reference shoots. This is partly due to a change in growth orientation and the morphology of transplants, *i.e.* the progressive transition from a vertical (orthotropic) to an horizontal orientation (plagiotropic) which is the colonising form of *P. oceanica* (Meinesz et al. 1993). Plagiotropic shoots generally have a lower leaf weight than orthotropic shoots (Caye, 1980). But this indicates also that the growth conditions are not optimal for the transplants. Leaf growth is minimal in late summer, fall and winter. Late summer and fall are characterised by low nutrient concentrations in the surrounding environment which is limiting for leaf growth. This is also a period of active nutrient resorption from senescing tissues and leaf abscission (Lepoint et al. 2002a). Winter and early spring are periods of low growth but are also periods of nutrient storage occur at very high rate (Lepoint et al. 2002a, b). Late spring is a period of high growth rate when nutrient reserves stored in the leaves and rhizomes are used to produce new leaf biomass.

The relative contributions of *P.oceanica* organs (i.e. leaves, scales roots and rhizomes) to the total transplant weight vary according to the sampling period. Firstly, we observe an increase of the root contribution which is particularly important in the last sampling. In this study, the roots development is quite spectacular for a species qualified of slow growing species. Roots of *P. oceanica* have two functions: to anchor the shoot in the sediment and to incorporate in the shoot the nutrients from the sediment pore water. It appears that the establishment of these two functions is of high priority for the transplant. Rhizomes weight is quite stable. This does not mean that rhizomes do not grew during the transplantation. The transplants have also produced rhizomes during the transplantation. But, in the same time, old rhizome parts often died and got rotten. In some shoots, this was a cause of initial death of the transplant. In summary, the death (and resorption?) of the old part of rhizome is partly compensate by the growth of the new rhizome part. The weight of scales increase during the transplantation, at least at the beginning of the experiment. The scales are the remaining part of leaves after leaf abscission. They are not easily recycled and, therefore, remain attached to the rhizome during many years.

Sediment

Sediment accumulates on grids in various ways, depending of the transplant densities and probably, of the local hydro-dynamism. However, this accumulation is not permanent as, in March 2004, underwater observations show excavation of grid with 100 transplants. Our measurements show that transplant grids act as a time-limited sediment trap in such environment, even at densities of 100 shoot (i.e. a third of the

natural density at this depth). Pore water in sand patch and transplant grid sediment is clearly enriched in ammonium compared to the healthy meadow. On the contrary, nitrates and phosphates concentrations are not different in the two environment types. This means that sediment bio-geochemistry (i.e. organic matter content, bacterial dynamics, …) of the two sites is clearly different (Gobert et al. 2003). Moreover, it appears that the transplantation grids (and the development of *Posidonia* roots) do not affect the measured parameters. This implies that changes of sediment characteristic (and bio-geochemistry) from un-vegetated to vegetated substrate are long term processes. This also implies that transplants could be affected by anoxic sediment process a long time after the transplantation, even if they have developed a relatively high biomass of roots as in this experiment. We observe no clear differences between the granulometric curves done on cores from the sand patch, the transplantation grids or the healthy meadow. It is therefore impossible to know if the transplantation has an impact on sediment granulometry.

Nutrient dynamics

Our data show that transplants display lower nitrogen and phosphorus concentrations than reference plants. Nitrogen, phosphorus could be limiting nutrients for natural stands of *P. oceanica* (Alcoverro et al. 1995) although this species has strategies to uptake or recycle nutrients (e.g. Lepoint et al. 2002b) and display strong physiological integration between connected ramets (Marba et al. 2002). In natural stands, limitation by nitrogen and phosphorus could occur mainly in summer when the concentrations of these elements are relatively low in the environment (Alcoverro et al. 1995). The low N and P concentrations displayed by the transplants indicate that they could be unable to meet their nutrient requirement. In winter and early spring, transplants do not replenish their N reserves which normally occurs in natural stands of *P. oceanica.*. This winter period is very important for *P. oceanica* N budget because it is a period of high nutrient occurrence in the water column and a period of N (and probably P) incorporation (Alcoverro et al. 2000; Lepoint et al. 2002b). The incorporated nitrogen is not immediately integrated to biomass but is stored as free amino acid in leaves and rhizomes. This nitrogen is re-mobilised in spring when the temperature and the light availability increase, making the environmental conditions optimal for *P. oceanica* growth. The transplanted shoots are unable to store nutrient during the winter and spring. The reason of this could be that the N and P availability in the environment of transplant is lower than in the healthy meadow. However, nutrient concentrations in the water column are the same and pore water of sediment on the sand patch is enriched in N and P compared to the healthy meadow (Fig. 6). Therefore, nutrient availability is not the reason of the low N and P concentrations observed in this study. A second hypothesis is that the transplants are not able to meet their nutrient needs because they have the capacity to uptake these nutrients. Indeed, the N budget of *P. oceanica* is very complex (Lepoint & al., 2002b). In natural stand of *P. oceanica*, leaves and roots participate to nutrient uptake. Leaves incorporate nutrients of the canopy water but also exploit the nutrient outflux from sediment (Gobert et al. 2002) Therefore, *P. oceanica* uses both the water column and the sediment as nutrient sources. But, the transplants were only rooted in the second year of the experiment. Therefore, in one hand, the transplants, in absence of roots, had only access to the water column as a nutrient source and, in another hand, the development of new roots have increased the

nutrient needs of the plant. This explains the observed low concentration of N and P in our samples and, probably, it is one of the possible cause of death (or poor health status) of transplants.

Conclusions

The establishment from an un-vegetated environment of the sedimentary characteristics of *P. oceanica* meadow needs a very long time, even if a high root biomass is developed by *P. oceanica*. This implies that bio-geochemistry of transplant is primarily (and for a long time) determined by initial sedimentary characteristic of the transplantation site. A consequence of this observation is that it is very important to establish the initial sedimentary characteristics of a potential transplantation site to estimate the long term success of *P. oceanica* transplantation. The biometric measurements done on the transplanted shoots indicated that transplants had lower biomass and leaf growth than reference plant. The elemental measurements indicate also that transplants display lower N and P concentration than those of reference shoots. This compromises the long term survival ship of *P. oceanica* transplants. Therefore, in future studies, the problem of nutrient incorporation capabilities should be addressed in priority. The simple transplantation design used allows the recovery of a natural *P. oceanica* density in about 5 years. Nevertheless, this method implies the presence of extensive donor meadows at proximity of transplantation site. In addition, this recovery does not mean that the natural complexity and the high bio-diversity associated to *Posidonia* ecosystem is recovered in the same way. At least, the man-power effort induced by a large transplantation operation is time consuming and very expensive (Table 1).

Table 1: time used (one people) for the grid installation and maintenance in the case of *P. oceanica* transplantation on natural sandy patch (UW: underwater work, LW: work in laboratory).

Task	Time used	
Collect of cuttings	1h/4m²	UW
Selection of cuttings	1h/4m²	LW
Installation of grids *in situ*	1h/4m²	UW
Installation of cuttings on the grids	2h/4m²	UW
Survey of grids	1h/4m	UW

For these reasons, projects for protection and conservation of existing *Posidonia* beds should be of high priority and are preferable to transplantation projects.

Acknowledgements

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