Is the apical growth of *Cymodocea nodosa* dependent on clonal integration?

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**ABSTRACT:** The importance of clonal integration for the production of biomass by the apical meristem of *Cymodocea nodosa* (Ucria) Ascherson was tested in situ by experimental manipulation. The production of new biomass by the apical meristem of a horizontal rhizome, as well as the leaf growth of the remaining shoots, was greatly reduced when the horizontal rhizome was severed, even when up to 11 shoots were left connected to the apical meristem of the rhizome. In contrast, the elimination of up to 8 shoots after the 3 apical shoots on a horizontal rhizome did not affect the production of biomass by the apical meristem. These results show that growth at the apical meristem of a *C. nodosa* rhizome depends on resources translocated along the rhizome from shoots situated further than 50 cm from the rhizome apex and that all the individual shoots (ramets) in a *C. nodosa* clone should be considered as one unit. Clonal integration does not depend on the presence of living shoots along the translocation route but is dependent on the integrity of the horizontal rhizome.

**KEY WORDS:** Clonal integration - Growth - Seagrasses - *Cymodocea nodosa*

**INTRODUCTION**

Because of the nature of seagrasses (Tomlinson 1974, Marbá 1995) the integration between shoots (i.e. ramets) may play an important role in nutrient acquisition, growth and reproduction of the clone, as has been extensively shown in terrestrial clonal plants (Hartnett & Bazzaz 1983, Pitelka & Ashmun 1985, Alpert & Mooney 1986, Slade & Hutchings 1987a, b, c, Alpert 1991, 1996). Clonal integration refers to the interdependence between the physiological processes, growth or reproductive output of connected ramets (Hartnett & Bazzaz 1983, Pitelka & Ashmun 1985) and implies the existence of translocation of resources (water, gases, nutrients, or other plant metabolites) between connected ramets.

Although clonal integration has been demonstrated to occur in seagrasses (Harrison 1978, Libes & Boudouresque 1987, Tomasko & Dawes 1989), the evaluation of its importance for the growth and spread of the clone relies on a thin empirical basis. Translocation of photosynthetic products towards organs with high metabolic activity (growing leaves, apical shoots in division, flowering shoots) has been observed in *Posidonia oceanica* (Libes & Boudouresque 1987) and *Zostera americana* (Harrison 1978), and suggested for *Thalassia testudinum* (Tomasko & Dawes 1989). Clonal integration in seagrasses depends on the translocation of resources along the rhizome and requires, therefore, rhizome integrity.

In addition to the examination of translocation of resources, clonal integration may also be inferred by comparing the growth responses of ramets to manipulations of their connections to other ramets (Hartnett & Bazzaz 1983, Pitelka & Ashmun 1985). Leaf growth rates of light-limited shoots of *Thalassia testudinum* were not different from unshaded shoots when the light-limited shoots were connected with the adjacent shoot on the rhizome, but decreased when the rhizome was severed (Tomasko & Dawes 1989). The survival and number of new shoots produced by rhizome fragments of *T. testudinum* increased with the number of shoots initially present in the rhizome fragment (Tomasko et al. 1991) which suggests a direct relation-
ship between the growth of the rhizome and the number of shoots supporting rhizome growth.

The horizontal rhizomes of the Mediterranean seagrass *Cymodocea nodosa* (Ucria) Ascherson remain alive for at least 2 to 3 yr (Duarte & Sand-Jensen 1990a, Terrados & Ros 1992, Pérez & Romero 1994) and connect shoots that may be meters apart (Caye & Meinesz 1985, Marbà & Duarte 1995). These features render *C. nodosa* an appropriate model organism to study clonal integration in seagrasses.

The main goal of this study is to provide, by manipulating the integrity of *Cymodocea nodosa* rhizomes, a test of the hypothesis that clonal integration is an important process for the growth of seagrasses. The vegetative development and proliferation of seagrasses is greatly dependent on the activity of apical meristems (Tomlinson 1974). Vegetative development of *C. nodosa* is the result of the activity of an apical meristem that produces a horizontal rhizome with long internodes and a lateral meristem at each node (Bornet 1864, Tomlinson 1974, Caye & Meinesz 1985). The growth of the apical meristem of *C. nodosa* is hypothesized to depend on resources translocated from other shoots connected along the rhizome (Duarte & Sand-Jensen 1996). This hypothesis could be falsified if growth of the apical meristem of plants having their horizontal rhizome severed to prevent the translocation of resources was similar to that of plants with an intact horizontal rhizome. Moreover, if the rhizome is severed at increasing distances from the apex then an increasing number of shoots will be left connected to the apex. We hypothesized that if clonal integration is important in this species then the growth of the apical meristem will increase as more shoots are left connected (and therefore able to export resources) to the apex (Expt 1). Resources translocated towards the apex may also be used by the shoots located along the horizontal rhizome, so we hypothesized that the leaf growth rate of these shoots will also decrease if the horizontal rhizome is severed (Expt 2). Clonal integration between connected shoots may also be influenced by the distance separating them, and it can, therefore, also be hypothesized that the growth of the apical meristem of *C. nodosa* will decrease as the distance to the nearest connected shoot increases (Expt 3).

**MATERIALS AND METHODS**

The experiments were performed at 2 sites: a shallow (<0.5 m depth) sandy platform occupied by a patchy meadow of *Cymodocea nodosa* (see Duarte & Sand-Jensen 1990b) on the bay side of the sand spit that separates Alfacs Bay from the Mediterranean in NE Spain (41°40' N, 2°47' E) (Expts 2 and 3). All the experiments were performed using horizontal rhizomes of *C. nodosa* that grew centrifugally at the meadow edge ('runners') to standardize the age of the plants used and facilitate the application of the experimental treatments.

**Expt 1.** The goal of Expt 1 was to test the effect of severing the horizontal rhizome at increasing distances from the rhizome apex on the growth of the apical meristem. Between 20 and 22 June 1995, 120 runners of *Cymodocea nodosa* were haphazardly selected at the edges of 5 different meadow patches 20 to 40 m apart from each other (site about 32 to 64 m²) at Alfacs Bay. Each patch was designated as an experimental block and the experimental treatments were also haphazardly assigned and independently applied to each experimental unit (i.e. runner) within the blocks. The experimental treatments (n = 5) consisted of severing an internode of the horizontal rhizome situated at increasing distances from the rhizome apex so that an increasing number of shoots (3, 5, 7, 9 and 11) were left connected to the apical meristem (Fig. 1a). A fluores-
cent-painted plastic cable tie label was placed on the second or third internode (counting back from the rhizome apex) of each horizontal rhizome (Fig. 1a), and the distance between the third shoot and the rhizome apex was measured. The plants were not disturbed in any other way. Control runners were tagged and measured in the same way, but the horizontal rhizome was left intact. Twenty runners (replicates) were assigned to each treatment and the control, representing a total of 120 runners distributed in 5 blocks of 24 runners each.

After 56 to 58 d (17 August 1995), the runners were carefully harvested by excavating the entire rhizome fragment with shoots. Not all of the 120 runners were retrieved at the time of harvest setting the effective replication at n = 18 (control runners), n = 9 (3 shoots'), n = 8 (5 shoots'), n = 10 (7 shoots'), n = 9 (9 shoots'), and n = 13 (11 shoots'). The individual runners were analyzed for the number of new horizontal rhizome internodes produced, rhizome internode lengths, the number of new shoots, and the number of standing leaves per shoot. The width of the second youngest leaf of the apical shoot was also measured as an indicator of shoot size. The new horizontal internodes produced by each runner were dried at 65°C for 24 h to estimate their dry weight.

Differences among treatments in the morphometric traits of the runners were tested using 1-way ANOVA and post-hoc Tukey's HSD tests (Sokal & Rohlf 1981). Prior to the analysis the data were tested for normality and homoscedasticity, and if necessary transformed, to fulfill the assumptions of ANOVA. Whenever transformation did not meet the parametric assumptions a Kruskal-Wallis non-parametric ANOVA was used (Sokal & Rohlf 1981).

Expt 2. The goal of Expt 2 was to test the effects of severing the horizontal rhizome on the leaf growth of the shoots. On 24 August 1995, 24 runners of Cymodocea nodosa were haphazardly selected in 3 sections (blocks) 10 to 20 m apart from each other along the edge of the large meadow in the Bay of Blanes. The leaves of the first 10 shoots on each runner (counting back from the rhizome apex) were marked by punching a hole just below the top of the leaf sheath of the oldest leaf in the shoot to estimate leaf growth (Terrados & Ros 1992). The experiment compared the growth of control plants (intact runners) with treatment plants (runners whose horizontal rhizome was severed at the 10th internode). The treatments were haphazardly assigned and independently applied to each experimental unit (i.e. runner). Twelve runners (replicates) were assigned to both the treatment and the control, representing a total of 24 runners distributed in 3 blocks of 8 runners each.

After 17 d (10 September 1995) the runners were harvested. The leaf biomass of each shoot was divided into 3 fractions: (1) 'new' biomass produced since marking, (2) 'old' biomass, and (3) sheaths. Each of these fractions was dried at 65°C for 24 h and weighed. Leaf growth was estimated as the new leaf biomass produced. The number of new horizontal rhizome internodes produced by each runner, their individual length, and the number of new shoots produced were also recorded. The biomass of the new portion of the horizontal rhizome produced by each runner was recorded after drying at 65°C for 24 h.

The effect of severing the horizontal rhizome on leaf growth was analyzed separately for the shoots present at the start of the experiment ('old shoots') and those produced during the experiment ('new shoots'). Differences between treatments and controls in the leaf growth rate, the number of leaves per shoot, and the number of new leaves produced per shoot were tested using 2-way ANOVA (Sokal & Rohlf 1981) with 'Treatment' (control and treatment plants) and 'Position on the rhizome' (i.e. nodes from the rhizome apex: 1 to 10 in the old shoots, 1 to 3 in the new shoots) as the main effects. Prior to the analysis the data were tested for normality and homoscedasticity and transformed, if necessary, to fulfill the assumptions of ANOVA. Differences in the growth of the horizontal rhizome between treatment and control plants were tested using either the Student's t-test or the non-parametric U-test of Mann-Whitney (Sokal & Rohlf 1981).

Expt 3. The goal of Expt 3 was to test the effect of clipping an increasing number of shoots on the growth of the apical meristem. On 18 June 1996, 60 runners of Cymodocea nodosa were haphazardly selected in 3 sections (blocks) 20 m apart from each other along the edge of the large meadow in the Bay of Blanes. The experiment involved clipping 2, 4 or 8 shoots after the first 3 shoots from the rhizome apex. As a consequence of the clipping, these first 3 shoots were left at increasing distances (about 10, 20 and 40 cm) from other shoots on the rhizome (Fig. 1b). A plastic cable tie label was placed around the 3rd horizontal internode (counting back from the rhizome apex) of each runner. The plants were not disturbed in any other way. Control runners were tagged similarly but had no shoots clipped. Fifteen runners (replicates) were assigned to each treatment and the control, representing a total of 60 runners distributed in 3 blocks of 20 runners each.

After 113 d (9 October 1996), the runners were harvested by excavating the entire rhizome fragment with the attached shoots. Not all of the 60 runners were retrieved at the time of harvest, setting the effective replication at n = 5 (control), n = 5 (2 shoots clipped), n = 6 (4 shoots clipped), and n = 3 (8 shoots clipped). The number of new horizontal rhizome internodes
produced, their individual length and the number of new shoots were recorded for each runner. If rhizome branches were present, their position (node, relative to the first 3 shoots at the setting of the experiment) on the rhizome, the number of horizontal internodes, shoots and length (cm) were also recorded. The new horizontal internodes produced by each runner on the main rhizome and the branches (if present) were dried at 65°C for 24 h and weighed.

Differences among treatments in the total growth of the runners (main horizontal rhizome plus rhizome branches, if present) were tested using 1-way ANOVA (Sokal & Rohlf 1981). Differences among treatments in the growth of the main horizontal rhizome and the rhizome branches were tested using 2-way ANOVA with “Treatment” (control, 2, 4 and 8 shoots clipped) and “Type” (main rhizome and rhizome branches) as the main effects. Prior to the analysis the data were tested for normality and homoscedasticity.

RESULTS

Expt 1

Cutting the horizontal rhizome significantly decreased (p < 0.0001) the number of new horizontal rhizome internodes, the number of new shoots produced, and the growth of the rhizome of Cymodocea nodosa runners (Fig. 2). Not only was the production of new internodes and shoots greatly reduced when the horizontal rhizome was severed, but also their size decreased. The mean length and mass of the horizontal internodes, the number of standing leaves per shoot, and the width of the second, youngest leaf of the apical shoot were all significantly smaller in treatments than in controls (p < 0.0001; Fig. 2). Except for the treatment where 5 shoots were left connected, the specific weight of the horizontal rhizome (mg DW cm⁻¹) also decreased when the horizontal rhizome was severed (p = 0.0013; Fig. 2). Only the control plants produced new branches during the experiment (in 4 out of the 18 control plants retrieved). The production of branches generated, on average, 7.7 ± 5.1 (SE) additional shoots per plant.

Expt 2

Cutting the horizontal rhizome significantly reduced the leaf growth rate of the ‘old shoots’ (Fig. 3a, Table 1).

The leaf growth rates of the shoots present at the start of the experiment decreased towards the apex of the rhizome in both the treatment and the control plants (Fig. 3a, Table 1). The leaf growth rates of the new shoots produced during the experiment tended to increase towards the rhizome apex (Fig. 3a), but the differences between treatments and position on the rhizome (only new positions 1 to 3 were analyzed) were not significant (Table 1).

The number of leaves per shoot was also reduced when the horizontal rhizome was severed and tended to decrease towards the rhizome apex (Fig. 3b, Table 1). The significant effects of treatment and
position were driven mainly by the reduction in the number of leaves of the shoots situated in positions 1 and 2 at the start of the experiment (Fig. 3b; 2-way ANOVA: treatment, $F = 5.524$, $p = 0.0236$; position, $F = 0.739$, $p = 0.3948$). The number of leaves in the new shoots increased towards the rhizome apex in control plants and decreased when the rhizome was severed (Fig. 3b).

The number of new leaves produced by the shoots present at the beginning of the experiment was also reduced when the horizontal rhizome was severed (Fig. 3c, Table 1) and showed a tendency to increase, although not significantly, towards the rhizome apex. The effect of the treatment was driven by the increase in the number of leaves produced by the shoots situated in the positions 1 to 3 at the start of the experiment (Fig. 3c; 2-way ANOVA: treatment, $F = 8.202$, $p = 0.0055$; position, $F = 0.912$, $p = 0.9253$). The number of leaves produced by the new shoots increased towards the rhizome apex in control plants and tended to be smaller in treatment plants (Fig. 3c).

The growth of the horizontal rhizome was also higher in control than in treatment plants (Table 2). The number of rhizome internodes and shoots produced, the growth in length of the rhizome and the rhizome mass produced were reduced when the horizontal rhizome was severed. The specific weight of the rhizome showed no differences between control and treatment plants (Table 2).

The average new biomass produced by a treatment or a control plant ($P_{\text{plant}}$) can be calculated by adding the average new biomass produced by the horizontal rhizome ($P_{\text{hr}}$) and the old shoots ($P_{\text{os}}$), and the average biomass of a new shoot ($P_{\text{ns}}$) times the average number of new shoots produced per plant ($N_{\text{ns}}$) using the equation,

$$P_{\text{plant}} = P_{\text{hr}} + (P_{\text{os}} + 10) + (P_{\text{ns}} \times N_{\text{ns}})$$

Table 1. Results of the ANOVA analysis (F-ratios and p-values) testing the effect of severing the horizontal rhizome on the leaf growth of the shoots (Expt 2). 'Old shoots' are the shoots present on the rhizome at the start of the experiment; 'new shoots' are those produced during the experiment. *Data were log-transformed.
Table 2. Comparison of the growth of the horizontal rhizome of control and treatment (the horizontal rhizome was severed, Expt 2) plants. Differences between the response variables were tested using Student's t-tests or the non-parametric Mann-Whitney U-test (Sokal & Rohlf 1981).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control plants (mean ± SE)</th>
<th>Treatment plants (mean ± SE)</th>
<th>U</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth in length (cm plant⁻¹ d⁻¹)</td>
<td>0.52 ± 0.13</td>
<td>0.10 ± 0.02</td>
<td>25.5</td>
<td>0.0127</td>
</tr>
<tr>
<td>Growth in mass (mg DW plant⁻¹ d⁻¹)</td>
<td>4.5 ± 1.2</td>
<td>0.6 ± 0.1</td>
<td>26.0</td>
<td>0.0138</td>
</tr>
<tr>
<td>Specific rhizome weight (mg DW cm⁻¹)</td>
<td>7.9 ± 0.3</td>
<td>6.7 ± 0.6</td>
<td>1.58</td>
<td>0.1292</td>
</tr>
<tr>
<td>Production of internodes (internodes plant⁻¹ d⁻¹)</td>
<td>0.14 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>3.21</td>
<td>0.0042</td>
</tr>
<tr>
<td>Production of shoots (shoots plant⁻¹ d⁻¹)</td>
<td>0.14 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>3.21</td>
<td>0.0042</td>
</tr>
</tbody>
</table>

These calculations yield a production of 0.271 ± 0.038 (SE) g DW plant⁻¹ and 0.135 ± 0.013 (SE) g DW plant⁻¹ for control and treatment plants, respectively. These results show that there was a 50% reduction in the total biomass produced by the plants when the horizontal rhizome was severed.

DISCUSSION

Our results demonstrate, by showing that the growth of Cymodocea nodosa is dependent on the integrity of the horizontal rhizome, that clonal integration is an important factor controlling growth of the apical meristem of C. nodosa. When the horizontal rhizome is severed the production of new biomass by the apical meristem of a horizontal rhizome is reduced even when 11 shoots are left connected to the apical meristem (Fig. 2). This result indicates that local resources (e.g. photosynthetic activity of the remaining shoots, carbohydrates stored in the rhizome, nutrients) are not sufficient to maintain normal growth rates. Optimal growth of the apical plant parts (i.e. the runners) must, therefore, depend on resources translocated from older shoots in the patch along the horizontal rhizome.

The growth of the plant was not reduced when up to 8 shoots after the 3 apical shoots on the horizontal rhizome were clipped (Fig. 4), which indicates that the

Expt 3

The clipping of shoots did not affect the growth of the apical meristem of Cymodocea nodosa runners. The number of horizontal internodes and shoots produced, the growth in length of the rhizome, the new rhizome mass, and the number of branches produced by the plants were similar in all the treatments (1-way ANOVA, p > 0.05; Fig. 4). The data showed, however, a tendency to a slightly higher growth when the 2 shoots adjacent to the 3 apical shoots on a runner were clipped (Fig. 4).

The relative growth of the main horizontal rhizome versus the branches was, however, affected by the clipping of shoots (Fig. 5). The lengthwise growth of the branches situated on runners that had shoots clipped was higher than those of control plants, as evidenced by a significant 'Treatment × Type' effect (Table 3). Although not statistically significant, the rhizome mass and the number of horizontal internodes and shoots produced by the branches tended to be higher on runners from which shoots had been clipped (Fig. 5).
production of biomass by the apical meristem depends on resources provided by shoots located at distances >50 cm from the rhizome apex. Hence, the integration between connected shoots of seagrasses occurs not only between 2 adjacent shoots (Tomasko & Dawes 1989) but also between shoots separated further on the horizontal rhizome. Furthermore, our results indicate that clonal integration between distantly connected shoots does not depend on the presence of shoots situated along the horizontal rhizome, at least at the spatial scale considered in our experiments. The apical portion of a Cymodocea nodosa horizontal rhizome appears to be a strong sink for resources that are translocated along the horizontal rhizome from other plant parts.

Severing the horizontal rhizome not only has a negative effect on the growth of the apical rhizome meristem, but also reduces the length growth of the remaining shoots (Fig. 3, Table 1). These shoots are, therefore, not able to produce enough resources themselves to maintain optimal growth of the rhizome apex, as evidenced by the fact that the detrimental effect of severing the horizontal rhizome was not alleviated even if 11 shoots were left connected to the apical meristem (Fig. 2). The new biomass (leaves and horizontal rhizome) produced by the first 10 shoots on a Cymodocea nodosa runner decreased by 50% when the horizontal rhizome was severed, indicating that about half of the growth of these apical parts depends on resources translocated from other parts of the plant. This result supports the hypothesis that the first apical shoots on a C. nodosa horizontal rhizome depend on resources (e.g. nutrients) translocated from older shoots (Duarte & Sand-Jensen 1996).

Removal of the apical meristem of a Cymodocea nodosa horizontal rhizome promotes an increase in the branching rate of the rhizome and in the elongation of the rhizome branches (Terrados et al. 1997). The elimination of subapical shoots (Expt 3) had the unexpected effect of promoting the growth of the horizontal rhizome branches, as evidenced by an increase in the growth in length of the branches and a tendency to increase the number of horizontal internodes and shoots produced on those branches (Fig. 5, Table 3). These results show that the control of branch elongation in C. nodosa is not exerted only by the apical meristem of a horizontal rhizome (Terrados et al. 1997), but also results from the joint control of all the shoots present in a certain portion of the horizontal rhizome. Hence, the suppression of branch growth resulting from apical dominance in C. nodosa (Terrados et al. 1997) involves the vertical shoots adjacent to the apical meristem. The promotion of the growth of the horizontal rhizome branches when some shoots are eliminated may be of adaptive value in overcoming the effects of disturbance and the creation of gaps through an increase in the production of new shoots (ramets) that are associated with increased branch growth. In summary, our results demonstrate that clonal integration is a fundamental factor for the clonal growth of C. nodosa, and suggest that a substantial fraction (~50%) of the resources required for apical growth are provided by long-range (>50 cm) transport. These results suggest that future studies of seagrass growth and production must consider the clone rather than the individual shoot (ramet) as the unit of analysis.

Table 3. Summary results of a 2-way ANOVA analysis (Sokal & Rohlf 1981) of the effect of clipping shoots on the growth of the main horizontal rhizome and the branches (Expt 3). Treatment: 0 (control), 2, 4, or 8 shoots clipped. Type: main horizontal rhizome or branches.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment F-ratio</th>
<th>Type F-ratio</th>
<th>Treatment × Type F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lengthwise growth of the horizontal rhizome (cm)</td>
<td>0.99, p = 0.4112</td>
<td>50.63, p &lt; 0.0001</td>
<td>3.13, p = 0.0419</td>
</tr>
<tr>
<td>New horizontal rhizome mass (g DW)</td>
<td>0.73, p = 0.5427</td>
<td>26.43, p = 0.0003</td>
<td>2.40, p = 0.0942</td>
</tr>
</tbody>
</table>
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LITERATURE CITED


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