1 Biomass and primary production of a 8-11 m depth meadow *versus* < 3 m depth
2 meadows of the seagrass *Cymodocea nodosa* (Ucria) Ascherson

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17

18 Abstract

19

20 Current knowledge about the abundance, growth, and primary production of the
21 seagrass *Cymodocea nodosa* (Ucria) Ascherson is biased towards shallow (depth < 3 m)
22 meadows although this species also forms extensive meadows at larger depths along the
23 coastlines. The biomass and primary production of a *C. nodosa* meadow located at a
24 depth of 8-11 m was estimated at the time of maximum annual vegetative development
25 (summer) using reconstruction techniques, and compared with those available from
26 shallow meadows of this species. A depth-referenced data base of values at the time of
27 maximum annual development was compiled to that end. The vegetative development
28 of *C. nodosa* at 8-11 m depth was not different from that achieved by shallow (depth <
29 3 m) meadows of this species. Only shoot density, which decreased from 1637 shoots
30 m⁻² to 605 shoots m⁻², and the annual rate of elongation of the horizontal rhizome,
31 which increased from 23 cm apex⁻¹ year⁻¹ to 71 cm apex⁻¹ year⁻¹, were different as depth
32 increased from < 3 m to 8-11 m. Depth was a poor predictor of the vegetative
33 development and primary production of *C. nodosa*. The biomass of rhizomes and roots
34 decreased with depth (g D.W. m⁻² = 480 (± 53, SE) - 32 (± 15, SE) depth (in m), R² =
35 0.12, F= 4.65, d.f. = 35, P = 0.0381) which made total biomass of the meadow to show a
36 trend of decrease with depth but the variance of biomass data explained by depth was
37 low. The annual rate of elongation of the horizontal rhizome showed a significant
38 positive relationship with depth (cm apex⁻¹ year⁻¹ = 18 (± 5.1, SE) + 5.0 (±1.33, SE)
39 depth (in m); R² = 0.50, F= 14.07, d.f. = 14, P = 0.0021). As shoot size and growth did
40 not change significantly with depth, the reduction of shoot density should drive any
41 changes of biomass and productivity of *C. nodosa* as depth increases. The processes by
42 which this reduction of *C. nodosa* abundance with depth occur remain to be elucidated.

43

44 Keywords: Seagrasses, *Cymodocea nodosa*, depth gradient, biomass, shoot density,

45 primary production, Mediterranean Sea

46 1. Introduction

47

48 Light availability sets the maximum depth of seagrass colonization (Duarte,
49 1991). Shoot density of seagrass meadows decreases as depth increases (Romero, 1989;
50 West, 1990; Romero et al., 1998; Krause-Jensen et al., 2000) while seagrass biomass
51 usually reaches a maximum at intermediate depths and decreases towards the depth
52 limit (Duarte, 1991; Krause-Jensen et al., 2000). Strong correlations between light
53 attenuation and the decline of shoot density and seagrass biomass with depth (Duarte,
54 1991; Krause-Jensen et al., 2000) suggest that light availability is the main factor
55 driving the depth distribution of seagrasses. *In situ* manipulation of light availability and
56 transplant experiments have shown that the leaf growth and biomass of the seagrass
57 *Zostera marina* L. at the deep limit of distribution is dependent on light availability
58 (Dennison and Alberte, 1985; Dennison and Alberte, 1986). The decline of shoot
59 density with depth is considered a plant response to reduce self-shading when light
60 availability is low (Olesen and Sand-Jensen, 1993; Krause-Jensen et al., 2000).
61 Additionally, the size of *Z. marina* shoots increases with depth, and more biomass is
62 allocated to leaves than to rhizomes and roots (Krause-Jensen et al., 2000). *Z. marina*
63 plants grown under an experimental light gradient showed that rhizome growth declined
64 faster than leaf growth as light was reduced (Olesen and Sand-Jensen, 1993). These
65 changes of biomass allocation are interpreted as a plant response to reduce respiration
66 costs and maintain growth under low light availability (Olesen and Sand-Jensen, 1993;
67 Krause-Jensen et al., 2000).

68

69 Shoot density, leaf biomass, and productivity of the Mediterranean seagrass
70 *Posidonia oceanica* (L.) Delile decrease from the shallow to the deep limit of

71 distribution of this species (Pirc, 1984; Romero, 1989; Romero et al., 1998; Olesen et al.,
72 2002; Gobert et al., 2003). Shoot size has been shown to remain fairly constant (Pirc,
73 1984; Olesen et al., 2002) or decrease (Gobert et al., 2003) as depth increases, but the
74 number of leaves per shoot and the growth rate of individual shoots do not change with
75 depth (Pirc, 1984; Romero, 1989; Olesen et al., 2002; Gobert et al., 2003). Hence, the
76 decline of leaf biomass and productivity of *P. oceanica* meadows with depth seems to
77 be driven by the decline of shoot density mainly.

78

79 *Cymodocea nodosa* (Ucria) Ascherson is a common seagrass species in the
80 Mediterranean Sea and the North-Atlantic coast of Africa, including the Canary Islands
81 (den Hartog, 1970), which shows an ample depth distribution from the intertidal
82 (Vermaat et al., 1993) to depths of 33-35 m (Drew, 1978; Reyes et al., 1995a; Canals
83 and Ballesteros, 1997). It can be found on a wide range of substrata too, from coarse
84 sand to muddy sediments (Peduzzi and Vuković, 1990; Pavón-Salas et al., 2000), and
85 forms extensive meadows in shallow, sheltered places such as lagoons (Terrados and
86 Ros, 1992; Ribera et al., 1997; Agostini et al., 2003), bays (Pérez and Camp, 1986;
87 Pérez and Romero, 1994) or harbor areas (Reyes et al., 1995b). Most part of the
88 knowledge about the magnitude and seasonality of biomass, growth and primary
89 production of *C. nodosa* has been produced in shallow (depth < 3 m) meadows (Caye
90 and Meinesz, 1985; Pérez et al., 1991; van Lent et al., 1991; Terrados and Ros, 1992;
91 Vermaat et al., 1993; Pérez and Romero, 1994; Pérez et al., 1994; Sfriso and Ghetti,
92 1998; Cancemi et al., 2002; Guidetti et al., 2002; Agostini et al., 2003). Logistical
93 reasons (i.e., shelter from wave action, diving time) might be behind this bias in
94 knowledge for *C. nodosa* also forms extensive meadows at larger depths along the
95 coastlines (Reyes et al., 1995a; Calvín et al., 1999).

96
97 The few analysis of the vegetative development of *Cymodocea nodosa* along
98 depth gradients (from 2 to 6 m: Peduzzi and Vuković, 1990; from 0.4 to 3.8 m: Olesen
99 et al., 2002) show that shoot density and leaf biomass decrease with depth, while the
100 rhizome plus roots to shoot biomass ratio either decreases or does not change, and shoot
101 size remains fairly constant.

102
103 In this study we estimated plant size, shoot density, biomass and primary
104 production of *Cymodocea nodosa* growing at depths of 8 m and 11 m, a common
105 location of the meadows formed by this seagrass species in the Mediterranean, and
106 compared the results obtained with those available from shallow (depth < 3m) meadows
107 of this species to evaluate if the vegetative development of deep *C. nodosa* meadows is
108 different from that of shallow meadows. By increasing the depth range from which
109 knowledge about the vegetative development of this species is available we could
110 evaluate if depth-related changes of plant size, shoot density, biomass and primary
111 production of *C. nodosa* were consistent with those observed in other seagrass species.

112

113

114 2. Methods

115

116 The study was performed at Sant Pol beach, Sant Feliu de Guixols, NE Spain
117 (41° 47.227'N, 3° 03.206'E), where *Cymodocea nodosa* forms a spatially homogeneous
118 meadow (100 % cover of the substratum) between depths of 7 m and 11 m. The
119 meadow is patchy at a depth of 18.5 m, and isolated plants can be found at a depth of 21
120 m. *C. nodosa* is not present at depths < 7m. The main sampling station was located at a

121 depth of 11 m with additional samples collected at a depth of 8 m. Sampling was
122 performed in July 2002, when the vegetative development of *C. nodosa* is near its
123 annual maximum (Terrados and Ros, 1992; Pérez and Romero, 1994; Cebrián et al.,
124 1997; Marbà et al., 1996; Rismondo et al., 1997; Sfriso and Ghetti, 1998; Guidetti et al.,
125 2002; Agostini et al., 2003). The vertical attenuation coefficient for downward
126 irradiance (K_d) was estimated by measuring scalar irradiance at noon just below the
127 surface of the sea and at the top of the leaf canopy at the depth of 11 m with a Li-Cor
128 spherical quantum sensor LI-193SB (Kirk, 1983; page 95). K_d at Sant Pol beach in July
129 2002 was 0.095 m^{-1} , a relatively low value for coastal waters (cf. Kirk, 1983; page 112).

130

131 The abundance of *Cymodocea nodosa* was quantified as shoot density (number
132 of shoots m^{-2}) and biomass (g dry weight m^{-2}). Shoot and flower densities were
133 estimated by counting the number of shoots and flowers present in 25 samples collected
134 using a 10.2 cm in-diameter stainless-steel corer that was inserted in the sediment to a
135 depth of 40 cm. The biomass of *C. nodosa* in 10 of the corer samples was sorted into
136 leaves, rhizomes, roots, and flowers, and dried at 60°C during 48 hours to estimate dry
137 weight. Nitrogen in the leaves was determined using a Carlo-Erba NA-1500 CHN
138 analyzer, and phosphorus was determined spectrophotometrically (Parsons et al., 1984)
139 after wet oxidation with boiling H_2SO_4 . Seed abundance was estimated from the number
140 of seeds that were collected in the corer samples.

141

142 Growth rates were estimated using reconstruction techniques (Duarte et al.,
143 1994) which are based on the estimation of the age of the shoots using the
144 "plastochrone" concept, the time elapsed between the appearance of two consecutive
145 structural modules (i.e., leaves) in plants (Erikson and Michelini, 1957). The age in

146 years of the shoots was estimated by counting the number of standing leaves and scars
147 left by fallen leaves on the vertical rhizome of each shoot and dividing the resultant
148 number by the average number of leaves produced by *C. nodosa* in one year. Such
149 average was estimated through the analysis of the sequence of lengths of the internodes
150 of large pieces of vertical rhizome (cf. Duarte et al., 1994) which were measured with a
151 stereomicroscope.

152

153 The length (cm) of the vertical rhizome of all the shoots collected in the corer
154 samples was measured, and the number of roots, root scars and flower scars present in
155 them was counted. These counts, when divided by the age of the shoots, provide an
156 estimate of the annual elongation of the vertical rhizome and the number of roots and
157 flowers produced by the shoots. Horizontal rhizome pieces of more than 30 cm in-
158 length were collected haphazardly throughout the meadow by hand to estimate their
159 growth rate. The number of standing leaves and leaf scars (i.e. an age estimate) of all
160 the shoots in each rhizome piece collected was counted, as well as the number of
161 internodes and distance (cm) between them. The age difference between shoots in a
162 rhizome piece allowed us to calculate the average elongation and number of internodes
163 produced per leaf plastochrone. The annual rates of elongation and internode production
164 of the horizontal rhizome were estimated by multiplying those per-leaf-plastochrone
165 estimates by the average number of leaves produced during one year. A subsample of
166 10 fragments of both vertical and horizontal rhizomes was haphazardly chosen and the
167 number of internodes of each fragment was counted and its length measured before
168 drying to estimate average mass and length of vertical and horizontal rhizome
169 internodes. Similarly, three subsamples of 10 small and 10 large unbroken roots were
170 chosen and weighed after drying to estimate the average mass per root. The abundance

171 of apexes of horizontal rhizome was estimated by counting the number of them present
172 in 24 quadrats (1600 cm²) haphazardly placed within the meadow. Knowledge of the
173 age of each shoot allows to build the age distribution of the population of shoots (cf.
174 Duarte et al., 1994). Mean and median shoot age were estimated, and the resultant age
175 distributions were compared with those available in the literature (Kolmogorov-
176 Smirnov two sample test, Sokal and Rohlf, 1981).

177

178 We searched the marine ecology and biology literature for studies describing the
179 vegetative development (plant size, shoot density, biomass, nutrient content of leaves,
180 growth, and productivity) of *Cymodocea nodosa* and compiled a depth-referenced data
181 base of values at the time of maximum annual development (listed in caption of Fig. 1).
182 To evaluate the differences of vegetative development between "shallow" and "deep"
183 meadows we compared the mean of the values obtained at the depths of 8 m and 11 m at
184 our study site with the mean of the corresponding values reported by those compiled
185 studies which were performed at depths < 3 m (two-sample t test, two-tailed). Graphing
186 of compiled values against depth suggested that vegetative development was highly
187 variable at shallow meadows and that declines with depth of shoot density, biomass and
188 other variables describing vegetative development occurred at depths > 3 m. We
189 calculated the coefficient of variation of the mean (V^* , Sokal and Rohlf, 1981) of
190 selected variables describing the vegetative development of *C. nodosa* in shallow (depth
191 < 3 m) and deep (depth > 3 m) meadows to evaluate the significance of the differences
192 in variability of the vegetative development between them (two-tailed Z test for
193 difference between two coefficients of variation, Zar, 1999). Further, we used linear
194 regression to examine depth-associated changes of the vegetative development of *C.*
195 *nodosa*.

196

197

198 3. Results

199

200 Shoot density of *Cymodocea nodosa* at 8-11 m depth in Sant Pol beach in July
201 2002 was lower than the annual maximum shoot density reported in most of the
202 meadows of this species studied previously (Fig. 1a). Leaf, rhizome plus roots, and total
203 biomass of *C. nodosa* at the annual maximum at 8-11 m depth were also at the low end
204 of the range of reported values (Fig. 1b). The annual maximum shoot density of *C.*
205 *nodosa* meadows at depth of 8-11 m was lower (two sample t-test, $P < 0.05$) than that of
206 meadows located at depths < 3 m (Table 1). The annual maximum of leaves, rhizome
207 plus roots, and total biomass tended to be also lower at 8-11 m depth than at depths < 3
208 m but the differences of the means were not significant (Table 1). The relative
209 allocation of biomass to leaves and rhizomes plus roots at 8-11 m depth was not
210 different than that of meadows located at depths < 3 m (Table 1, Fig. 1c).

211

212 The average number of leaves of *Cymodocea nodosa* shoots at the time of
213 maximum vegetative development at 8-11 m depth was not different from that of
214 meadows located at depths < 3 m (Table 1, Fig. 1d). The same occurred with the
215 average shoot mass (Table 1, Fig. 1e).

216

217 The annual average leaf plastochrone of *Cymodocea nodosa* at 8-11 m depth
218 was not different than that of meadows located at depths < 3 m (Table 1, Fig. 1f). The
219 annual leaf and total production of *C. nodosa* at 8-11 m depth were at low end of the
220 range of values published previously but was not different from the average values

221 estimated in meadows located at depths < 3 m (Table 1, Figs. 1g and 1h). The
222 elongation rate of the horizontal rhizome was, however, higher at 8-11 m depth than the
223 average value estimated in meadows located at depths < 3 m (Table 1). The nitrogen
224 and phosphorus content of *C. nodosa* leaves at 8-11 m depth was not different than the
225 average of values found in meadows located at depths < 3 m (Table 1).

226

227 The coefficient of variation of vegetative features and production estimates of
228 *Cymodocea nodosa* was not different (two-tailed Z test for difference between two
229 coefficients of variation, $P > 0.05$) between meadows located at depths lower and higher
230 than 3 m (Table 2). Depth was a poor predictor of the vegetative development of *C.*
231 *nodosa* for most of the linear regressions of the different variables used to describe the
232 vegetative development against depth were not significant neither considering the whole
233 range of data compiled nor only those data corresponding to depths > 3 m (Table 1).
234 Rhizome plus roots biomass decreased with depth ($- 32 \pm 14.6$ (SE) g D.W. m^{-1} , $P <$
235 0.05 , Table 1) which made total biomass to show a marginally significant trend of
236 decrease with depth too (Fig. 1b, Table 1). The rate of elongation of the horizontal
237 rhizome increased with depth ($P < 0.05$, Fig. 2a).

238

239 The average age of *Cymodocea nodosa* shoots varied between 2.5 years and 3.2
240 years while the median age varied between 2 years and 3 years in meadows located at
241 depths > 3 (Table 3), but were 1.7-1.8 years and 1 year only, respectively, in meadows
242 located at depths < 3 m. The comparison of the age distributions of living *C. nodosa*
243 shoots obtained in this study (Fig. 3a) with those available in the literature showed that
244 most shoot age distributions were different (Table 3). The age distributions of dead
245 shoots at 8 m and 11 m were different (Kolmogorov-Smirnov two sample test, $D = 0.186$

246 > $D_{0.05} = 0.169$, Fig. 3b), with average and median ages of 2.0 years and 2 years at the
247 depth of 8 m, and 1.7 years and 1 years, respectively, at the depth of 11 m.

248

249 The abundance of *Cymodocea nodosa* flowers at 8-11 m depth was not different
250 than that estimated in meadows located at depths < 3 m (Table 1) but likely mismatches
251 between peak flowering and sampling time make literature comparisons of flower
252 abundance difficult. The average and median age of shoots bearing a flower or flower
253 scars on the vertical rhizome were 3.4 years and 3 years at depth of 8 m, and 3.5 years
254 and 3 years, respectively, at depth of 11 m; the age distributions of these shoots were
255 not different at 8 m and 11 m (Kolmogorov-Smirnov two sample test, $D = 0.142 < D_{0.05}$
256 $= 0.290$, Fig. 3c). We found flowers in shoots of only 5, 7 and 8 leaf plastochrones in
257 age. Seed abundance tended to be lower at 8-11 m depth than at meadows located at
258 depths < 3 m but the difference of the means was not significant (Table 1).

259

260

261 Discussion

262

263 Our results show that the vegetative development of *Cymodocea nodosa* at 8-11
264 m depth is not different from that achieved by shallow (depth < 3 m) meadows of this
265 species. Only shoot density, which decreased by a factor of 2.7, and the annual rate of
266 elongation of the horizontal rhizome, which increased by a factor of 3, at 8-11 m depth
267 were different. Our results also show that depth is a poor predictor of the vegetative
268 development and primary production of *C. nodosa* for most of the linear regressions of
269 the variables describing the vegetative development of *C. nodosa* against depth were
270 non-significant.

271

272 The reduction of seagrass shoot density and biomass with depth has been
273 recognized before and attributed to the parallel decrease of light availability (Duarte,
274 1991). Further, the reduction of shoot density with depth has been considered the most
275 effective mechanism for seagrasses to acclimate to reductions of light availability as
276 depth increases (Olesen et al., 2002). A threshold light for rhizome branching to occur
277 has been proposed as a mechanism to explain the decrease of shoot density with depth
278 of *Zostera noltii* (Peralta et al., 2002) and *Posidonia oceanica* (Romero, 1989).

279

280 Previous studies that have examined the vegetative development of *Cymodocea*
281 *nodosa* along depth gradients (Peduzzi and Vuković, 1990; Olesen et al., 2002) show
282 that shoot density and leaf biomass decrease with depth. Given that the range of depths
283 examined by these studies is narrower (from 0.4 m to 6 m) than that examined by us
284 (from 0.3 m to 11 m) we expected to find stronger negative relationships of these
285 variables with depth than we did. The low predicting value that depth had to explain the
286 vegetative development and primary production of *C. nodosa* in our compiled data set
287 might be due to wide spatial variation of water turbidity and light availability among the
288 sites included in it, and apparently higher K_d values at shallow sites (0.57 m^{-1} to 0.35 m^{-1}
289 1 in the 0.4-1.5 m depth range, cf. Duarte, 1991; Terrados and Ros, 1995; Guidetti et al.,
290 2002) than at deep sites (0.19 m^{-1} to 0.095 m^{-1} in the 4-11 m depth range (Cancemi et
291 al., 2002; this study). Unfortunately, data about irradiance attenuation were not
292 available from most of the sites. Our results are consistent with previous studies on this
293 species (Peduzzi and Vuković, 1990; Olesen et al., 2002) for neither shoot mass and the
294 number of leaves per shoot nor the average annual leaf plastochrone and productivity of
295 individual shoots changed significantly with depth. Shoot density, leaf biomass and

296 productivity of *Posidonia oceanica* decrease significantly with depth while shoot size
297 and growth remain fairly constant (Pirc, 1984; Romero, 1989; Romero et al., 1998;
298 Olesen et al., 2002; Gobert et al., 2003). In the case of *Zostera marina*, shoot density
299 decreases exponentially with depth while shoot size increases linearly; as a result
300 biomass peaks at intermediate depths (Krause-Jensen et al., 2000).

301

302 We found that the rhizome plus roots to shoot biomass ratio of *Cymodocea*
303 *nodosa* did not change with depth. However, the biomass of rhizomes plus roots
304 decreased significantly with depth driving a marginally significant reduction of total
305 biomass of the meadow which suggests that a slight change of the mass allocation
306 pattern might take place. Previous studies on this species are inconclusive for the
307 rhizome plus roots to shoot biomass ratio either did not change (Olesen et al., 2002) or
308 decreased (Peduzzi and Vuković, 1990) as depth increased. The rhizome plus roots to
309 shoot biomass ratio of *Posidonia oceanica* does not seem to change with depth (Romero
310 et al., 1998; Olesen et al., 2002) but decreases in *Zostera marina* (Olesen and Sand-
311 Jensen, 1993; Krause-Jensen et al., 2000). Preferential allocation of biomass to leaves as
312 depth increases should not be considered, therefore, a general response of seagrasses to
313 reduce respiration costs and maintain growth under low light availability. Further, our
314 results do not provide support to the hypothesis that the variability of seagrass
315 vegetative development is higher at shallow depths where physical disturbance and light
316 availability are high than at deep meadows where physical disturbance and light
317 availability are low (Krause-Jensen et al., 2000; Middelboe et al., 2003).

318

319 Linear regression of the elongation rate of the horizontal rhizome of *Cymodocea*
320 *nodosa* against depth predicted a rate of increase rate of 5 cm apex⁻¹ year⁻¹ per m depth,

321 which is consistent with the results of Olesen et al. (2002). However, Peduzzi and
322 Vuković (1990) did not find any change of the rate of elongation the horizontal rhizome
323 of *C. nodosa* between depths of 2 m and 6 m. The rate of elongation of the horizontal
324 rhizome of *Posidonia oceanica* did not change between depths of 0.7 m and 15.6 m
325 (Olesen et al., 2002). It has been suggested that the elongation of the horizontal rhizome
326 of seagrasses might depend on shoot density (Caye and Meinesz, 1985; Marbà and
327 Duarte, 1998), likely through a self-shading constraint. Shoot density, however,
328 explained little of the variability of the rate of elongation of the horizontal rhizome data
329 included in our depth-referenced data base (Fig. 2b; $R^2 = 0.15$, $F = 2.34$, d. f. = 13, $P =$
330 0.1496).

331

332 The age distributions of *Cymodocea nodosa* shoots show exponential reductions
333 of shoot density since the ≤ 1 year class at shallow meadows (cf. Pérez, 1989; Terrados
334 and Ros, 1992) and since the 2 year class at deep meadows (Reyes et al., 1995b, and
335 this study). These results suggest that shoot turnover decreases with depth. Indeed, the
336 maximum age of shoots at 8-11 m depth was 11-12 years but only 4 to 8 years at
337 shallow meadows (Pérez, 1989; Terrados and Ros, 1992), and median shoot age was
338 lower at shallow than at deep meadows. The age distributions of dead shoots at 8-11 m
339 depth suggest, however, that most part of the shoots die within one year of being
340 produced.

341

342 In conclusion, the vegetative development of *Cymodocea nodosa* at 8-11 m
343 depth was not different from that achieved by shallow (depth < 3 m) meadows of this
344 species. Only shoot density, which decreased by a factor of 2.7, and the annual rate of
345 elongation of the horizontal rhizome, which increased by a factor of 3, were different as

346 depth increased from < 3 m to 8-11 m. Depth was a poor predictor of the vegetative
347 development and primary production of *C. nodosa*. As shoot size and growth did not
348 change significantly with depth, the reduction of shoot density should drive any changes
349 of biomass and productivity of *C. nodosa* as depth increases. The processes by which
350 this reduction of *C. nodosa* abundance with depth occur remain to be elucidated.

351

352

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545 Figure captions

546

547 Figure 1. Shoot density (a), total biomass (b), rhizome plus roots to shoot ratio (c),
548 number of standing leaves per shoot (d), and shoot mass (e) at the annual maximum of
549 vegetative development of *Cymodocea nodosa*, and annual average leaf plastochrone
550 interval (f), leaf (g), and total production (h) against depth. Triangles represent the 8-11
551 m depth meadow studied. Data sources: Pirc et al., 1983; Caye and Meinesz, 1985;
552 Pérez and Camp, 1986; Terrados, 1986; Duarte and Sand Jensen, 1990; Peduzzi and
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558 Cancemi et al., 2002; Guidetti et al., 2002; Olesen et al., 2002; Agostini et al., 2003;
559 Cunha and Duarte, 2005.

560

561 Figure 2. Linear regression of the annual rate of horizontal rhizome elongation against
562 depth (a) and shoot density (b) of *Cymodocea nodosa*. Triangles as in Fig. 1

563

564 Figure 3. Age distribution of living shoots (a), dead shoots (b), and of shoots that bore a
565 flower and/or a flower scar in the vertical rhizome (c) of *Cymodocea nodosa* at the
566 depths of 8 and 11 m at the study site.

567

Figure1 rev

Figure2 rev

Figure3 rev

1 Table 1. Vegetative features and production estimates of *Cymodocea nodosa* at depths of 8 m and 11 m in Sant Pol beach (NE Spain),
2 corresponding values reported previously of shallow (depth < 3 m) meadows of this species (see caption Fig. 1 for data sources), and results of
3 two-sample t-tests evaluating the significance of the difference between them, and of the linear regressions against depth considering the whole
4 range of depth data compiled and only those depths > 3 m. Bold face indicates significant differences.

Variable	This study Depth: 8 m Mean ± SE	This study Depth: 11 m Mean ± SE	Shallow meadows Depth < 3 m Mean ± SE, n	t-value, d.f., P-value	Linear regression whole depth range R ² , F-value, P-value	Linear regression depths > 3 m R ² , F-value, P-value
Annual maximum shoot density, shoots m ⁻²	623 ± 49	588 ± 38	1637 ± 106.5, 30	2.50, 30, 0.0179	0.06, 2.29, 0.1391	0.18, 1.35, 0.2892
Leaf biomass, g DW m ⁻²	46 ± 7.7	33 ± 3.6	188 ± 33.1, 29	1.18, 29, 0.2457	0.03, 1.17, 0.2852	0.07, 0.58, 0.4685
Rhizome and root biomass, g DW m ⁻²	186 ± 36.7	116 ± 16.3	454 ± 48.3, 28	1.68, 28, 0.1043	0.12, 4.65, 0.0381	0.07, 0.56, 0.4794
Annual maximum total biomass, g DW m ⁻²	235 ± 44.9	150 ± 20.6	664 ± 81.7, 25	1.64, 25, 0.1141	0.09, 4.13, 0.0504	0.08, 0.67, 0.4382
Rhizome and roots to shoot ratio	5.6 ± 2.11	3.8 ± 0.66	3.1 ± 0.37, 28	1.15, 28, 0.2594	0.01, 0.35, 0.5565	0.09, 0.68, 0.4369
Abundance of flowers, flowers m ⁻²	243 ± 60	153 ± 27	124 ± 33.7, 7	1.15, 7, 0.2876	0.03, 0.37, 0.5537	0.03, 0.10, 0.7767
Abundance of seeds, seeds m ⁻²	7 ± 5	65 ± 29	243 ± 62.8, 7	1.80, 7, 0.1148	0.04, 0.95, 0.3389	0.18, 2.63, 0.1307
Number of leaves per shoot	4.2 ± 0.1	3.6 ± 0.1	3.72 ± 0.265, 9	0.32, 9, 0.7539	0.01, 0.10, 0.7514	0.00, 0.00, 0.9422
Shoot mass, mg DW shoot ⁻¹	73.8 ± 10.2	58.3 ± 3.1	97 ± 12.4, 25	0.71, 25, 0.4867	0.02, 0.68, 0.4156	0.48, 2.77, 0.1944
Leaf plastochrone, days	38.5	40.1	31.4 ± 1.82, 15	1.58, 15, 0.1339	0.07, 1.52, 0.2321	0.21, 1.34, 0.2989
Elongation of horizontal rhizome, cm apex ⁻¹ year ⁻¹	88.2 ± 19.1	54.7 ± 8.0	23.3 ± 3.9, 12	4.40, 12, 0.0009	0.50, 14.07, 0.0021	0.37, 1.17, 0.3928
Leaf production, g DWm ⁻² year ⁻¹	104 ± 114.3	87 ± 93.4	594 ± 187.3, 10	1.21, 10, 0.2551	0.07, 1.23, 0.2850	0.14, 0.81, 0.4083
Total production, g DWm ⁻² year ⁻¹	275 ± 192.9	169 ± 128.3	998 ± 432.3, 5	1.20, 5, 0.2844	0.18, 1.58, 0.2489	0.50, 2.01, 0.2919
Leaf N, % of dry weight	2.00 ± 0.040	2.06 ± 0.045	1.99 ± 0.089, 22	0.04, 22, 0.9677	0.05, 1.31, 0.2635	0.54, 3.57, 0.1551
Leaf P, % of dry weight	0.206 ± 0.0016	0.149 ± 0.0016	0.138 ± 0.0115, 21	1.04, 21, 0.3085	0.11, 2.82, 0.1074	0.91, 10.80, 0.1880

1 Table 2. Coefficient of variation of vegetative features and production estimates of
2 *Cymodocea nodosa* in shallow (depth < 3 m) and deep (depth > 3 m) meadows, and Z
3 test to evaluate the significance of the difference between them. See caption Fig. 1 for
4 data sources.

Variable	Depth < 3 m	Depth > 3 m	Z value
	V* ± SE	V* ± SE	Critical Z _{0.05 (2)} = 1.960
Annual maximum shoot density, shoots m ⁻²	35 ± 5.1	55 ± 17.4	1.430
Leaf biomass, g DW m ⁻²	94 ± 20.3	61 ± 17.9	0.923
Rhizome and root biomass, g DW m ⁻²	56 ± 9.5	77 ± 26.6	0.891
Annual maximum total biomass, g DW m ⁻²	61 ± 11.3	67 ± 21.3	0.194
Rhizome and roots to shoot ratio	62 ± 11.0	64 ± 19.9	0.013
Abundance of flowers, flowers m ⁻²	69 ± 25.3	122 ± 65.1	0.864
Abundance of seeds, seeds m ⁻²	66 ± 23.5	219 ± 117.7	0.960
Number of leaves per shoot	21 ± 5.1	43 ± 14.5	1.739
Shoot mass, mg DW shoot ⁻¹	63 ± 12.0	22 ± 7.2	1.507
Leaf plastochrone, days	22 ± 4.2	19 ± 5.3	0.392
Elongation of horizontal rhizome, cm apex ⁻¹ year ⁻¹	58 ± 15.1	53 ± 22.7	0.217
Leaf production, g DWm ⁻² year ⁻¹	97 ± 36.2	79 ± 30.8	0.362
Total production, g DWm ⁻² year ⁻¹	91 ± 45.5	67 ± 31.9	0.381
Leaf N, % of dry weight	21 ± 3.2	17 ± 5.5	0.562
Leaf P, % of dry weight	38 ± 6.6	55 ± 27.6	0.573

5

Table 3

Table 3. Number of shoots aged, average and median shoot age (on main diagonal) of *Cymodocea nodosa* at the study location and other meadows of this species. D values and critical $D_{0.05}$ values (below main diagonal) of Kolmogorov Smirnov two sample tests performed to evaluate the significance of the difference between the age distribution of *C. nodosa* shoots at each meadow. Bold face indicates significant differences in shoot age distribution.

	11 m, this study	8 m, this study	6 m, Reyes et al., 1995a	1 m, Pérez, 1989	0.5 m, Terrados and Ros, 1992
11 m, this study	588, 2.5, 2				
8 m, this study	D = 0.141 >> $D_{0.05} = 0.078$	623, 3.2, 3			
6 m, Reyes et al., 1995a	D = 0.156 >> $D_{0.05} = 0.069$	$D = 0.054 < D_{0.05} = 0.068$	1112, 3.1, 3		
1 m, Pérez 1989	D = 0.223 >> $D_{0.05} = 0.064$	D = 0.320 >> $D_{0.05} = 0.062$	D = 0.347 >> $D_{0.05} = 0.051$	1920, 1.7, 1	
0.5 m, Terrados and Ros, 1992	D = 0.224 >> $D_{0.05} = 0.065$	D = 0.320 >> $D_{0.05} = 0.064$	D = 0.339 >> $D_{0.05} = 0.053$	D = 0.056 > $D_{0.05} = 0.046$	1641, 1.8, 1