

2 **Responses of two Mediterranean seagrasses to experimental**
3 **changes in salinity**4 **Yolanda Fernández-Torquemada ·**
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8 **Abstract** The aim of this study is to examine the
9 effects of variations in salinity levels on growth and
10 survival of two fast-growing Mediterranean seagrasses,
11 *Cymodocea nodosa* and *Zostera noltii*. We also
12 tested the capacity of *C. nodosa* to acclimate to a
13 gradual increase in salinity and to discover how it
14 responds to a sharp rise in salinity in combination
15 with other factors, such as increases in temperature,
16 seasonality and different plant-population origins.
17 Several short-term (10 days) experiments were con-
18 ducted under controlled conditions. For each exper-
19 iment, ten marked shoots were placed in 5-l aquaria,
20 where they were exposed to different salinity treat-
21 ments (ranging from 2 to 72 psu). Growth and
22 survival of both species were significantly affected
23 by salinity. A significant effect between salinity and
24 temperature on the shoot growth rate of *C. nodosa*
25 was also detected, but not on shoot mortality. When
26 *C. nodosa* plants were acclimated by gradually
27 increasing the salinity level, it was observed that
28 acclimatisation improved tolerance to salinity
29 changes. A different response to salinity variations,
30 depending on the origin of the plants or the season of

the year, was also detected. These results indicated 31
that *Z. noltii* plants tolerate conditions of hyposalinity 32
better than *C. nodosa*, and that the tolerance range of 33
C. nodosa may change depending on the temperature, 34
the season or the population. 35

Keywords Salinity effects · Salinity tolerance · 36
Desalination impact · *Cymodocea nodosa* · 37
Zostera noltii 38

Introduction 40

Seagrasses have evolved from continental angio- 41
sperms that have returned and adapted to life in 42
completely submerged saline environments (den 43
Hartog, 1970), which they are able to tolerate as a 44
result of various biochemical, physiological and 45
morphological adaptations (Jagels, 1973; Tyerman, 46
1989; Arai et al., 1991; Pak et al., 1995; Fukuhara 47
et al., 1996; Fernández et al., 1999; Touchette, 2007). 48
These mechanisms have allowed seagrasses to occur 49
naturally in various aquatic environments with 50
different salinity values, including brackish and 51
oceanic waters (Walker, 1985; Adams & Bate, 52
1994; Tomasko & Hall, 1999), and from almost 53
stable to a more fluctuating environment. 54

To date, studies examining salinity influence or 55
tolerance in seagrasses have focused mainly on 56
estuarine species, which can be exposed to a wide 57
range of salinity levels, due to seasonal and natural 58

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59 variations occurring in their habitat (Wortmann et al.,
60 1997; Benjamin et al., 1999; Kamermans et al.,
61 1999). Results from these studies have shown that
62 alterations to salinity levels can affect plant metabo-
63 lism, growth, reproduction, survival and distribution
64 (McMillan & Moseley, 1967; Zieman, 1975; Walker
65 & McComb, 1990; Montague & Ley, 1993; Hillman
66 et al., 1995; Ramage & Schiel, 1998; Vermaat et al.,
67 2000; Chesnes & Montague, 2001). However, in
68 marine environments, salinity is considered an
69 approximately constant factor, so any alteration to
70 salinity levels may lead to significant disturbances to
71 open water seagrasses that are more stenohaline
72 species. Salinity values in the Mediterranean Sea are
73 not naturally subjected to large and rapid fluctuations.
74 However, some Mediterranean coastal areas are
75 currently exposed to anthropogenic alterations in
76 salinity levels caused by the discharge of brine
77 effluents from desalination plants (Fernández-Tor-
78 quemada et al., 2005a, 2009; Gacia et al., 2007).
79 These brine discharges have high salinity levels
80 (40–70 psu), and sometimes a relatively high tem-
81 perature and low pH (Lattemann & Höpner, 2003), so
82 these and other environmental conditions occurring at
83 the same time may have an interaction with increased
84 salinity levels.

85 Previous studies have shown that the endemic
86 species *Posidonia oceanica* is quite sensitive to
87 increases in salinity levels (Fernández-Torquemada
88 & Sánchez-Lizaso, 2005; Gacia et al., 2007; Sánchez-
89 Lizaso et al., 2008), but little information exists with
90 regard to the response and tolerance of other Med-
91 iterranean seagrasses, such as *Cymodocea nodosa* and
92 *Zostera noltii*. *C. nodosa* and *Z. noltii* are relatively
93 small and fast-growing seagrasses with similar
94 behaviour and ecology, but different geographical
95 distribution. Whereas *C. nodosa* is a common species
96 in the Mediterranean and the eastern Atlantic, from
97 south Portugal to Senegal and around the Canary
98 Islands (den Hartog, 1970), *Z. noltii* is widespread
99 along the European Atlantic coastline from Sweden
100 to Mauritania, but is not common in the Mediterra-
101 nean Sea. *C. nodosa* may also show a wider depth
102 distribution (ranging from the intertidal to 33–35 m
103 deep) than *Z. noltii*, which has a narrower vertical
104 distribution (Drew, 1978; Vermaat et al., 1993;
105 Reyes et al., 1995). On the other hand, both species
106 have a high tolerance to a variety of environmental
107 conditions, such as irradiance changes, seasonal

temperature fluctuations and different nutrient con- 108
centrations (Phillips & Meñez, 1988; Marbà et al., 109
1996), and can become established on a wide range of 110
substrata, from sandy to muddy pristine as well as 111
degraded coastal sites (Peduzzi & Vukovič, 1990; 112
Pavón-Salas et al., 2000; Charpentier et al., 2005). As 113
the habitat preferences and environmental limits of 114
C. nodosa and *Z. noltii* are very broad, these species 115
can be expected to have higher salinity tolerance 116
levels than *P. oceanica*. Throughout their distribu- 117
tion, both seagrasses can be found forming mixed 118
meadows in areas where salinity values differ from 119
marine conditions, such as estuaries and coastal 120
lagoons (den Hartog, 1970; Mazzella et al., 1993; 121
Vermaat et al., 2000; Greve & Binzer, 2004). Based 122
on their field distribution, *C. nodosa* and *Z. noltii* 123
have been classified as euryhaline species (den 124
Hartog, 1970), although it is expected that individuals 125
of both species that currently occur in coastal 126
Mediterranean waters will have a different salinity 127
tolerance to those from other populations occurring in 128
habitats with greater fluctuations in salinity. 129

130 On the other hand, salinity tolerance in both
131 species cannot be easily inferred from these field
132 data, as the salinity range of normal growth and
133 development for a species is usually narrower than its
134 real tolerance limits (Kinne, 1964), and there can be
135 multiple stressors in the field affecting the behaviour
136 and distribution of a species. As a result, controlled
137 experimental studies are needed to gather significant
138 information about the environmental tolerance of a
139 species. There are some experimental studies that
140 refer to the effects of salinity variations on these
141 seagrasses (Caye & Meinesz, 1986; Hootsmans et al.,
142 1987; Loques et al., 1990; Caye et al., 1992; Vermaat
143 et al., 2000; Pagès et al., 2010). Most of these studies
144 have focused on the role that reduced salinity levels
145 play in seed germination. Caye & Meinesz (1986)
146 demonstrated that they could induce *C. nodosa* seed
147 germination at any time of the year by reducing the
148 salinity and using a temperature between 20 and
149 25°C. These same authors observed that seeds
150 generally do not germinate at a salinity level of 38
151 psu, and the few that germinated did so very slowly
152 (over months). Furthermore, seedlings that had
153 germinated at lower salinity levels (15–30 psu) were
154 the only ones that continued to develop. Other studies
155 have demonstrated that low salinity levels also
156 stimulate *Z. noltii* seed germination (Hootsmans

157 et al., 1987; Loques et al., 1990). Vermaat et al.
 158 (2000) carried out an experiment with adult *Z. noltii*
 159 plants from two separate populations that were
 160 exposed to 15 and 35 psu and the day-length regimes
 161 of two seasons, and observed that both populations
 162 suffered high mortality at 35 psu. In a recent paper,
 163 Pagès et al., (2010) estimated that *C. nodosa* was
 164 tolerant to moderate salinity increases (44 psu), while
 165 apparently only sub-lethal effects appeared at 54 psu,
 166 but they only studied four salinity treatments.

167 The first aim of the present study is to redress the
 168 lack of existing information on the tolerance of
 169 *Cymodocea nodosa* and *Zostera noltii* to salinity
 170 changes and to establish their possible range of
 171 salinity tolerance. Several experiments of a short
 172 duration (10 days) were conducted under controlled
 173 conditions to estimate shoot growth rate and survival
 174 in both species when exposed to different levels of
 175 salinity. A more thorough study of *C. nodosa* was
 176 also carried out to prove the effect of other factors
 177 associated with the discharge of desalination brine on
 178 this species. We thus tested the capacity of *C. nodosa*
 179 to acclimate to gradual increases in salinity and its
 180 response to sharp rises in salinity combined with
 181 other factors or conditions, such as temperature
 182 increases, seasonality and different plant populations.

183 Materials and methods

184 Plant material

185 *Cymodocea nodosa* and *Zostera noltii* shoots were
 186 carefully collected by scuba diving from a mixed
 187 shallow meadow (−2 m) at Almadraba Beach
 188 (Alicante, SE Spain). Plants of a similar size and
 189 vitality were transported to the laboratory in a cooler
 190 containing ambient seawater from the sampling site
 191 (average salinity of 37.5 psu). Once transferred to the
 192 laboratory, the shoots were marked using a modified
 193 Zieman method (1974) and placed in plastic 5-l
 194 aquaria with sediment at different treatments for
 195 10 days.

196 Experimental design

197 Six sets of experiments were conducted in aquaria
 198 under environmentally controlled conditions, with a
 199 daylight regime of 12 h of light and 12 h of darkness,

and an average daytime underwater photon flux of 200
 30 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (measured with a LiCor 193 201
 SA spherical quantum sensor), that is approximately 202
 the environmental light intensity observed in the 203
 plants sampling site (Almadraba Beach, Alicante; 204
 38°22'N, 0°26'W). For each experiment, three aer- 205
 ated aquaria (replicates) were assigned per treatment. 206
 With the exception of experiment VI, ten plants were 207
 placed in each aquarium with no acclimation period, 208
 in order to simulate sudden increases in salinity. In all 209
 the experiments carried out, temperature and salinity 210
 were measured daily using a conductimeter (model 211
 1230; Thermo Orion), and salinity values were 212
 adjusted when necessary. Control-treatment salinity 213
 was the level measured in the area where the plants 214
 were collected (with a range of 36.8–38.1 psu, and an 215
 average of 37.5 psu). Increased salinity treatments 216
 were similar to hypersalinity values associated with a 217
 desalination brine discharge (Fernández-Torquemada 218
 et al., 2005a, 2009) and were prepared by adding 219
 natural salt produced by Santa Pola saltworks from 220
 seawater concentrate to ambient coastal seawater, 221
 while lower salinities were obtained by diluting 222
 seawater with freshwater. Once the aquaria were 223
 filled with treatment water, they were placed in a 224
 larger water-filled container, to keep temperatures as 225
 constant as possible during each experiment. 226

227 Leaf growth was measured, by using a hypodermic 227
 needle for *C. nodosa* or by using a water-insoluble 228
 marking pen for *Z. noltii*, as a daily elongation rate 229
 ($\text{cm}^2 \text{shoot}^{-1} \text{day}^{-1}$), and mortality as a percentage 230
 of dead shoots per aquaria at the end of each 231
 experiment. Shoots were considered dead when 232
 leaves died back and rhizomes and roots were totally 233
 degraded. In order to compare the results from 234
 selected experiments, some leaf growth rates were 235
 reported as a percentage relative to the growth 236
 obtained for the control plants. 237

238 The following section details the objectives, 238
 experimental design and methodology of the different 239
 experiments carried out. 240

241 *Experiment I: salinity tolerance of Zostera noltii*

242 To evaluate the effect of salinity on shoot growth rate 242
 and survival of *Z. noltii*, three consecutive and 243
 different tests of a short duration (10 days) were 244
 carried out during the summer period (July and 245
 August, temperature $\approx 25\text{--}27^\circ\text{C}$), working with a 246

247	wide range of salinities (21 different treatments	<i>Experiment V: differences between Cymodocea</i>	288
248	between 2 and 72 psu, each one replicated in three	<i>nodosa</i> populations	289
249	independent aquaria).		
250	<i>Experiment II: salinity tolerance of Cymodocea</i>		
251	<i>nodosa</i>		
252	This study was designed to evaluate the effect of	This experiment was performed during 10 days in	290
253	different salinity levels on shoot growth rate and	August (temperature $\approx 27^{\circ}\text{C}$) to determine whether	291
254	survival of <i>C. nodosa</i> . For this experiment, five short-	the response of this species to changes in salinity can	292
255	term (10 days) assays were carried out during spring	vary between individual plants from two populations	293
256	and summer months (April–July, tempera-	adapted to different conditions. The survival rates and	294
257	ture $\approx 22\text{--}25^{\circ}\text{C}$), with 27 different salinity levels	shoot growth rate among two <i>C. nodosa</i> populations	295
258	ranging from 2 to 72 psu (each salinity treatment	were compared: one from the western Mediterranean	296
259	replicated in three aquaria).	Sea (Alicante; $38^{\circ}22'\text{N}$, $0^{\circ}26'\text{W}$) and another from a	297
		near coastal lagoon located in southeast Spain (Mar	298
260	<i>Experiment III: seasonal response of C. nodosa</i>	Menor, Murcia; $37^{\circ}48'\text{N}$, $0^{\circ}46'\text{W}$) with a higher	299
261	<i>to increases in salinity</i>	salinity level (42–47 psu). The salinity levels applied	300
262	The aim of this experiment was to detect a possible	were 37 psu (the ambient level at the Mediterranean	301
263	seasonal variation of the response by <i>C. nodosa</i> to	sampling site), 44 psu (the level at the lagoon when	302
264	hypersalinity (control, 42, 47 and 52 psu). Increased	and where the plants were collected), 47 and 50 psu.	303
265	salinity experiments (10 days) were carried out at two		
266	different times of the year. Winter time (20–30	<i>Experiment VI: acclimation versus acute increases</i>	304
267	January, temperature $\approx 15^{\circ}\text{C}$) was selected because	<i>in C. nodosa</i>	305
268	of the reduced growth rate of <i>C. nodosa</i> during this		
269	season, compared with summer (5–15 July, tempera-	The aim of this experiment, also performed during	306
270	ture $\approx 25^{\circ}\text{C}$), when, due to the accumulation of	August (temperature $\approx 26^{\circ}\text{C}$), was to determine	307
271	sucrose and starch and increases in irradiance and	whether the acclimation of <i>Cymodocea nodosa</i> to	308
272	temperature, the species shows a higher rate of	gradual salinity variations allows for a greater	309
273	development.	tolerance to osmotic stress than an instantaneous	310
		transfer, as occurs with other seagrass species (Ralph,	311
274	<i>Experiment IV: salinity and temperature interaction</i>	1998). In some treatments, plants were acclimated in	312
275	<i>effects on C. nodosa</i>	their respective aquaria to three different salinity	313
276	This experiment of 10 days was carried out to	levels (42, 47, and 52 psu) in a stepwise manner	314
277	examine the synergistic effects of increases in	(2.5 psu per day) up from the habitat salinity of 37	315
278	temperature and salinity. Control aquaria were	psu, reaching the maximum salinity after 2 (42 psu),	316
279	maintained at 20°C seawater temperature during that	4 (47 psu) and 6 days (52 psu). In other treatments,	317
280	period (spring, March), while, for the other aquaria, a	plants were subjected to sudden increases in salinity	318
281	commercial heater was used with a thermostat to	(also 42, 47 and 52 psu), as performed in previous	319
282	increase the temperature to 25°C , representing sum-	experiments. Finally, all plants were subjected to	320
283	mer temperatures in their natural environment.	these salinity treatments during 10 days.	321
284	Within each temperature regime (20 and 25°C), four		
285	salinity treatments were tested to represent an	Statistical analyses	322
286	increase in this variable: 37 (control), 43, 48 and 53		
287	psu.	One-way ANOVA was used in experiments I and II	323
		to test for differences in mortality among the different	324
		salinity treatments used (including one as control).	325
		Nested ANOVA was used in the same experiments to	326
		test differences in shoot growth rate, with salinity	327
		considered as a fixed factor replicated in three aquaria	328
		(random factor and nested within salinity) and	329
		with ten marked shoots (replicates). In the rest of	330
		the experiments, an orthogonal factor was added	331

332 (temperature, original population, seasonality or
333 acclimation) to determine possible interactions
334 between salinity and these factors. Homogeneity of
335 variance had been previously verified using the
336 Cochran test (Underwood, 1997). The data were
337 log-transformed if normality was not verified.

338 When analysis of variance identified a significant
339 difference for any factor, the multiple comparison
340 post-hoc test SNK (Student–Newman–Keuls) was
341 applied to determine specific treatment differences.
342 All calculations were performed using the GMAV.5
343 program (University of Sydney; Underwood &
344 Chapman, 1997), with a minimum significance level
345 established at $P < 0.05$.

346 Results

347 The growth and survival of *Zostera noltii* were both
348 significantly affected by water salinity (Fig. 1). Shoot
349 growth rate was considerably affected at higher
350 salinities, but not at reduced salinities (Fig. 1). SNK
351 test results revealed that elongation rates were similar

and maximal at 2–41 psu, decreasing significantly
353 from 42 to 52 psu ($P < 0.05$). No growth was
354 observed at 57 psu and higher. Hypersalinity also
355 affected the mortality rate of this species (Fig. 1).
356 *Z. noltii* plants sustained considerable mortality at
357 salinity levels above 43 psu ($>16.7\%$), reaching
358 50% at ~ 50 psu and 100% mortality at 57 psu and
359 higher.

360 Salinity also had a strong effect on leaf elongation
361 rate and mortality in *Cymodocea nodosa* (Fig. 2). The
362 relationship between shoot growth rate and salinity
363 was bell-shaped (Fig. 2), with a distinct peak at 30–39
364 psu, and a significant reduction at salinity levels
365 higher than 41 psu or lower than 16 psu. Mortality
366 showed an opposite pattern (Fig. 2), with a minimum
367 value at the control salinity level. Shoot mortality
368 remained lower than 50% at salinity levels of less than
369 50 psu, but increased sharply above this level. All
370 plants died when exposed to freshwater (0 psu) and to
371 the highest salinity concentrations (≥ 57 psu).

372 The *C. nodosa* response to salinity increases during
373 the two seasons studied (winter and summer) showed a
374 significantly different behaviour (Fig. 3). The life

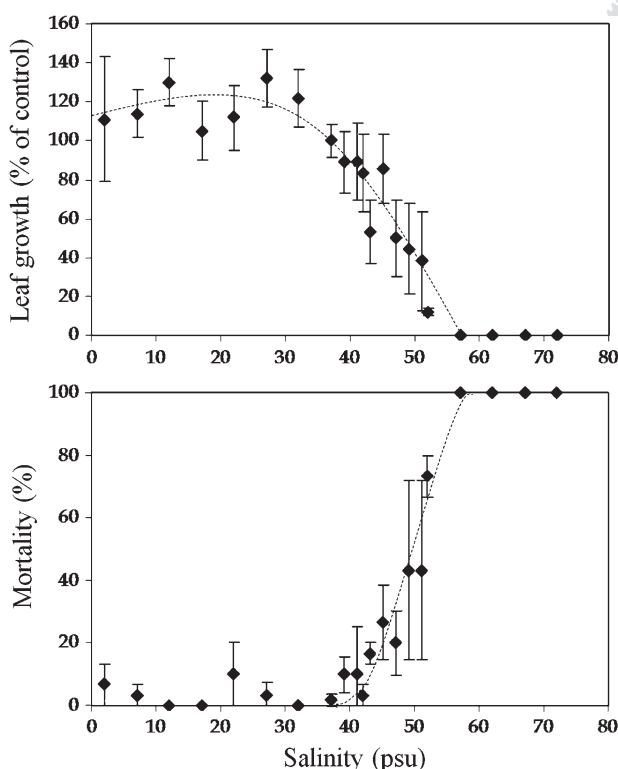


Fig. 1 *Zostera noltii* leaf growth (relative to the growth of the control plants) and mortality percentage at different salinity levels (bars represent standard errors)

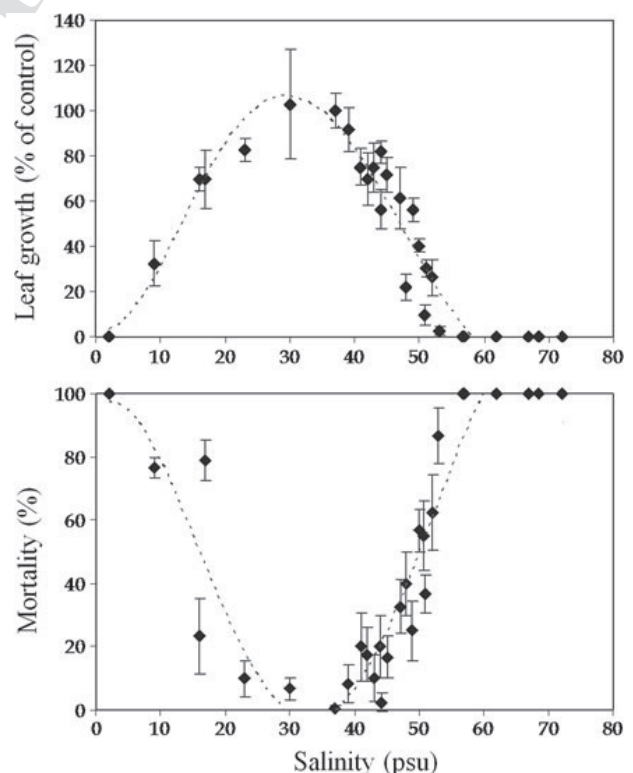


Fig. 2 *Cymodocea nodosa* leaf growth (relative to the growth of the control plants) and mortality percentage at different salinity levels (bars represent standard errors)

375 cycle of this species is strongly marked by seasonality
 376 (Pérez & Romero, 1992), with the result that in sum-
 377 mer, the plants presented a significantly ($P < 0.001$)
 378 higher leaf growth ($0.1963 \text{ cm}^2 \text{ day}^{-1} \text{ shoot}^{-1}$) than in
 379 winter ($0.0352 \text{ cm}^2 \text{ day}^{-1} \text{ shoot}^{-1}$). To compare the
 380 data obtained from the two seasons, the results were
 381 expressed as a percentage relative to the growth
 382 obtained at the control treatment (Fig. 3). Here, two-
 383 way ANOVA showed a significant interaction between
 384 salinity and the season of the year in which the
 385 experiment was carried out (Table 1). Subsequent
 386 SNK post-hoc tests indicated that, in summer,
 387 *C. nodosa* shoot growth rate was significantly higher
 388 at the control salinity level than at the other salinity
 389 treatment levels, whereas in winter, higher salinity
 390 (51 psu) is the only level that differs from the rest
 391 (Fig. 3). It was also observed that the effects of
 392 increased salinity on *C. nodosa* mortality was more
 393 evident in summer than in winter (Fig. 3), although no
 394 significant interaction was found between seasonality
 395 and salinity (Table 1). Shoot mortality was generally

396 lower ($\leq 20\%$) in winter, with higher mortality rates
 397 observed in summer (53.3% at 51 psu).

398 ANOVA revealed a significant effect of the inter-
 399 action between temperature and increased salinity on
 400 the leaf growth of this species ($P < 0.01$). Plants
 401 subjected to salinity levels between 37 and 48 psu
 402 showed greater shoot growth rates at the highest
 403 temperature (25°C), but when salinity was increased to
 404 53 psu, the increase in temperature did not affect leaf
 405 growth (Fig. 4). Although no interaction was found for
 406 mortality data (Table 1), mortalities associated with
 407 increases in salinity were lower at 25°C (Fig. 4). In
 408 fact, in this experiment, only salinity had a significant
 409 effect on the survival of *C. nodosa* shoots. Among the
 410 four salinity treatments, the highest mortality rates
 411 occurred at 53 psu (63.3–86.7%), followed by 48 psu
 412 (20–40%), with significantly lower values than 37 and
 413 43 psu (3.3–6.7%).

414 Analysis of variance also showed significant differ-
 415 ences in response to salinity among the populations
 416 studied (Table 1). Individuals from the Mar Menor
 417 presented greater shoot growth and survival rates than
 418 those from the Mediterranean Sea (Fig. 5). Similar
 419 shoot growth and survival was observed in plants
 420 subjected from 37 to 47 psu, with significantly lower
 421 values reported at 50 psu. On the other hand, although
 422 no significant interaction was observed, plants from the
 423 Mediterranean Sea exposed to 50 psu clearly showed
 424 lower growth and survival rates than those from the
 425 Mar Menor at the same salinity treatment (Fig. 5).

426 On the other hand, *C. nodosa* shoots acclimated to
 427 gradual increases in salinity (2.5 psu per day) showed
 428 higher shoot growth rates at the four salinity treat-
 429 ments studied than those not subjected to acclimation
 430 (Fig. 6). No dead shoots were found at the control
 431 salinity level. However, at high salinity levels, the
 432 mortality rate was higher for plants exposed to abrupt
 433 salinity changes, although ANOVA did not detect
 434 any significant interaction.

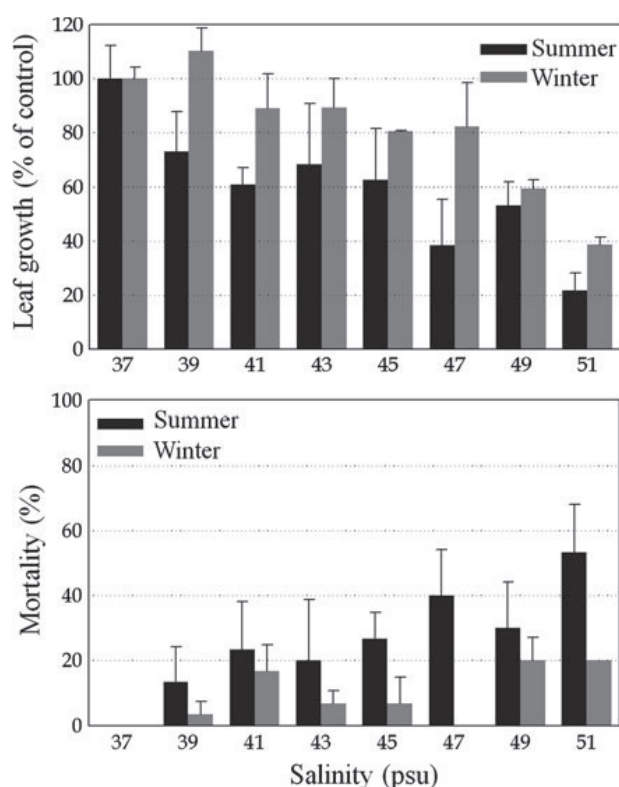


Fig. 3 Leaf growth per shoot, relative to the growth of the control plants, and mortality percentage of *C. nodosa* plants at different salinity levels during summer and winter (bars represent standard errors)

435 Discussion

436 Effects of salinity on shoot growth rate
 437 and survival of *C. nodosa* and *Z. noltii*

438 Under these laboratory conditions, *Zostera noltii* was
 439 found to be more tolerant of conditions of hyposali-
 440 nity than *Cymodocea nodosa*, but both species were

Table 1 Summary of the two-way ANOVAs testing the effects of different salinity treatments combined with other factors or conditions on *C. nodosa* shoot growth rate and shoot mortality

Experiment	Source of variation	Leaf elongation			Mortality		
		df	MS	F	df	MS	F
Exp. III	Salinity	7	0.0329	5.08***	7	723.81	3.66**
	Season of year	1	0.7594	117.47***	1	3,333.33	16.84**
	Salinity × season	7	0.0179	2.77*	7	280.95	1.42 ^{ns}
	Aquaria (salinity × season)	32	0.0065	2.31***	32	197.91	
	Residual	432	0.0028				
Exp. IV	Salinity	3	0.0404	94.32***	3	6,700.00	41.23***
	Temperature	1	0.0318	74.24***	1	600.00	3.69 ^{ns}
	Salinity × temperature	3	0.0031	7.16**	3	277.78	1.71 ^{ns}
	Aquaria (salinity × temperature)	16	0.0004	0.75 ^{ns}	16	162.50	
	Residual	216	0.0006				
Exp. V	Salinity	3	0.0003	5.73**	3	1,181.94	5.56**
	Population	1	0.0010	21.75***	1	2,204.17	10.37**
	Salinity × population	3	0.0001	1.32 ^{ns}	3	604.17	2.84 ^{ns}
	Aquaria (salinity × population)	16	0.0000	1.41 ^{ns}	16	212.50	
	Residual	216	0.0000				
Exp. VI	Salinity	3	0.0602	7.40**	3	1,693.06	8.13**
	Acclimation	1	0.0490	6.02*	1	2,204.17	10.58**
	Salinity × acclimation	3	0.0060	0.73 ^{ns}	3	293.06	1.41 ^{ns}
	Aquaria (salinity × acclimation)	16	0.0081	2.46**	16	208.33	
	Residual	216	0.0033				

ns non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$

441 sensitive to increases in salinity. These experiments
 442 showed significant decreases in shoot growth rates in
 443 both seagrasses at salinity levels higher than 41 psu.
 444 A decrease in leaf growth was also observed at
 445 salinity levels lower than 15 psu for *Cymodocea*
 446 *nodosa*, whereas *Z. noltii* plants were found to be
 447 highly tolerant to salinity levels as low as 2 psu,
 448 which was the lowest salinity treatment tested for the
 449 10-day period. This tolerance to low levels of salinity
 450 has also been found for other seagrasses, such as
 451 *Amphibolis antarctica* and *Posidonia sinuosa*, which
 452 can be highly tolerant to short-term reductions in
 453 salinity, even as low as 0–1 psu (Westphalen et al.,
 454 2005). The relationship between leaf growth and
 455 salinity was well described by a bell-shaped curve for
 456 *C. nodosa*, and by a sigmoid curve for *Z. noltii*, with
 457 maximum values recorded around ambient salinity
 458 levels. Similar responses, but with different relation-
 459 ships between salinity and plant growth, have
 460 been observed in several seagrasses (McMillan &
 461 Moseley, 1967; Walker, 1985; Walker & McComb,

1990; Kamermans et al., 1999) probably due to adverse
 effects on the meristematic tissues, interferences with
 carbon metabolism or negative alterations in the
 photosynthetic and respiratory rates (Ogata & Matsui,
 1965; Biebl & McRoy, 1971; Kraemer et al., 1999).

Mortality values for *C. nodosa* were significantly
 lower for salinity levels between 17 and 48 psu, with
 all plants dying at salinity levels of more than 56 psu.
 For *Z. noltii*, mortality was significantly lower at
 salinity levels below 47 psu, with 100% mortality at
 salinity levels above 56 psu. Previous studies have
 confirmed that salinity variations may affect seagrass
 survival under experimental conditions (Biebl &
 McRoy, 1971; Pinnerup, 1980; Vermaat et al.,
 2000; Fernández-Torquemada & Sánchez-Lizaso,
 2005; Fernández-Torquemada et al., 2005b), as well
 as in their natural habitats (Robblee et al., 1991;
 Wortmann et al., 1997; Kamermans et al., 1999; van
 Katwijk et al., 1999). Water salinities outside the
 tolerance range of a species may alter its metabolism
 and therefore cause direct death of the plant at very

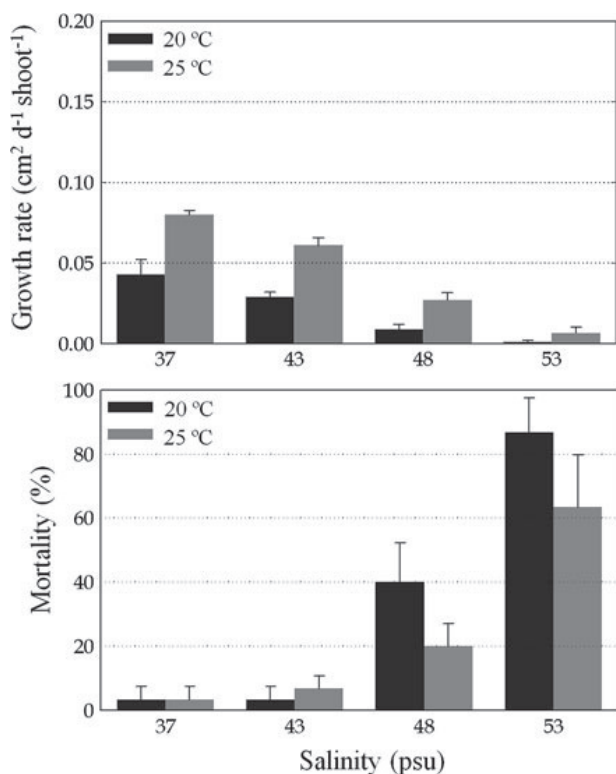


Fig. 4 *Cymodocea nodosa* shoot growth rate ($\text{cm}^2 \text{day}^{-1} \text{shoot}^{-1}$) and shoot mortality (%) at different salinity levels and temperatures (bars represent standard errors)

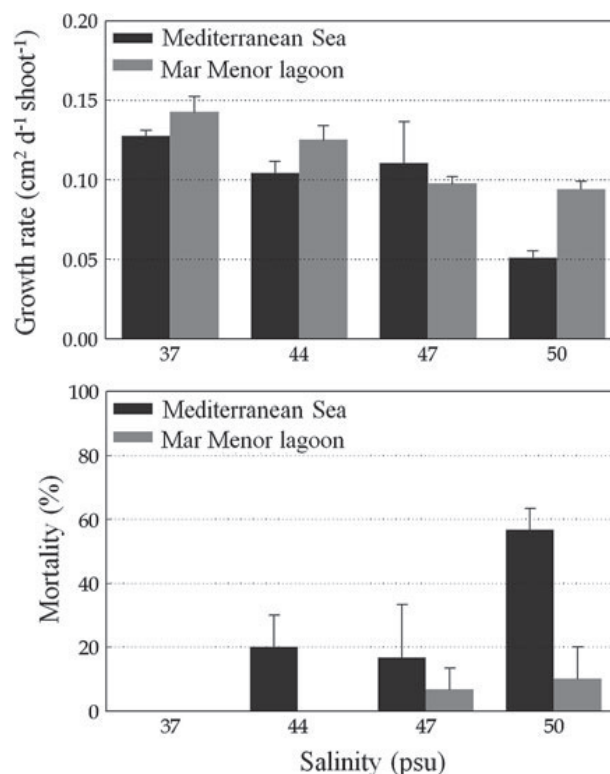


Fig. 5 Shoot growth rate ($\text{cm}^2 \text{day}^{-1} \text{shoot}^{-1}$) and mortality percentage of *C. nodosa* plants from two different populations exposed to different salinity levels (bars represent standard errors)

483 extreme levels (McMillan & Moseley, 1967; Adams
484 & Bates, 1994), or death in the long term if causing
485 starch-reserve depletion (Biebl & McRoy, 1971; Kerr
486 & Strother, 1985).

487 These results are also in accordance with the field
488 distribution of both seagrasses. *Z. noltii* is known to
489 colonise estuary and delta areas with a higher
490 influence of freshwater inputs than *C. nodosa*
491 (Vermaat et al., 2000; Greve & Binzer, 2004). Our
492 data are also coherent with the known colonisation of
493 these species in the Mar Menor, a coastal lagoon
494 located in the southeast of Spain. Until the end of the
495 nineteenth century, this lagoon contained very high
496 salinity levels (60–70 psu) that prevented it being
497 colonised by macrophytes. However, a subsequent
498 connection was established between the lagoon and
499 the Mediterranean Sea, causing salinity to drop to the
500 current levels (42–47 psu), allowing for the existing
501 seagrass meadows to become established (Pérez
502 Ruzafa et al., 1987).

503 It should be emphasised that all these experiments
504 were carried out under controlled aquarium conditions
505 and in short periods of time (10 days), so the response

506 observed here could differ to the one observed in the
507 natural environment. However, Walker & McComb
508 (1990) compared the effect of salinity variations on
509 the growth of a tropical seagrass, both in situ and in
510 aquaria, and found that its tolerance was similar in
511 both cases. In the present study, aquaria experiments
512 were also performed under low light levels ($30 \mu\text{mol}$
513 $\text{quanta m}^{-2} \text{s}^{-1}$), but higher environmental light
514 levels could imply a lower mortality or higher growth
515 of plants under stressing salinity treatments, because
516 those plants could be subjected to an additional co-
517 stressor, the low light level. Moreover, we only
518 worked with growth and survival of those species, and
519 no information about photosynthesis or other descrip-
520 tors is given. On the other hand, this study focussed
521 solely on the effects of increases in salinity on the
522 growth and survival of adult shoots, and the sensitivity
523 to environmental variations may differ considerably
524 between seedlings and adult plants, with young stages
525 typically being more sensitive to stressors than
526 adult ones. Future studies should therefore investigate
527 the effects of increases in salinity, together with

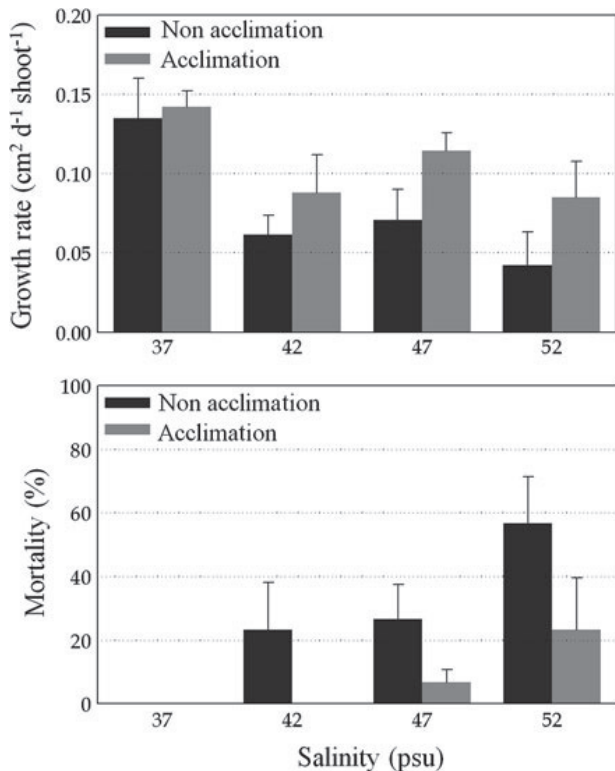


Fig. 6 Shoot growth rate ($\text{cm}^2 \text{day}^{-1} \text{shoot}^{-1}$) and shoot mortality (%) of *C. nodosa* plants acclimated to salinity increases (2.5 psu day^{-1}) versus plants subjected to sudden salinity increases (bars represent standard errors)

528 variations in other conditions, on seedling develop-
529 ment and seed germination of these species.

530 Nevertheless, if we compare the results obtained
531 here for *C. nodosa* and *Z. noltii* with those obtained
532 for *P. oceanica* (Fernández-Torquemada & Sánchez-
533 Lizaso, 2005), it can be observed that these species
534 were able to tolerate a wider range of salinity levels
535 than *P. oceanica*. This is coherent with the fact that
536 *C. nodosa* and *Z. noltii* are smaller and much more
537 plastic species, with a higher capacity to adapt to
538 environmental changes and recover from slight
539 disturbances (Pérez et al., 1994; Kraemer & Mazzella,
540 1999).

541 Interactions of salinity with other factors
542 on *Cymodocea nodosa*

543 Previous experiments have demonstrated the sensi-
544 tivity of *C. nodosa* to salinity variations in the short
545 term (10 days), but tolerance to salinity may also
546 depend on other environmental or intra-specific

factors. Other studies have demonstrated that osmotic 547
stress could modify the sensitivity of some seagrasses 548
to other environmental conditions, such as tempera- 549
ture or pH increases, light levels or eutrophication 550
(Biebl & McRoy, 1971; Ralph 1999; van Katwijk 551
et al., 1999; Vermaat et al., 2000). 552

Mediterranean seawater temperatures vary consid- 553
erably between seasons, but rapid temperature 554
increases may be linked to brine discharges from 555
certain desalination plants. For example, brine dis- 556
charges from multi-stage flash desalination plants can 557
cause increases of 15°C in the seawater temperature 558
in the receiving environment (Lattemann & Höpner, 559
2003). On the other hand, brine discharges from 560
reverse osmosis desalination plants do not imply 561
temperature increases, though it has been observed 562
that, in some of these facilities and during certain 563
periods of the year, the temperature of the effluent 564
can produce a slight increase in that of the receiving 565
environment (Fernández-Torquemada et al., 2005a). 566

Temperature is considered an important abiotic 567
factor that controls the production, growth and 568
survival of a species (Drew, 1979), and it has been 569
demonstrated that its influence on the metabolism of 570
marine plants modifies their capacity to adapt to 571
changes in other environmental factors, such as 572
illumination or salinity (Zieman, 1975). Plant growth 573
is most affected by light and temperature (Philippart, 574
1995; Marbà et al., 1996), and when these parameters 575
are close to a species' optimum values, this species 576
will tolerate a broader range of salinity levels (Kirst, 577
1989). It is known that, in several species of algae, 578
such as *Macrocystis integrifolia*, *Alaria esculenta* and 579
Cladophora rupestris, tolerance to saline stress is 580
reduced at extreme levels within their temperature 581
tolerance range (Druehl, 1981; Dring, 1992; Thomas 582
et al., 1988). In seagrasses, Biebl & McRoy (1971) 583
found an increase in plasmatic heat resistance with 584
increasing levels of salinity for subtidal forms of 585
Zostera marina, and Vermaat et al. (2000) observed 586
increased mortality in *Z. noltii* when the temperature 587
reached 20°C at higher experimental levels of 588
salinity. Thorhaug & Marcus (1981) indicated that 589
four seagrass species were less tolerant of low salinity 590
levels at temperature above 29°C . The present work 591
has also observed a significant interaction between 592
the two factors. For salinities ranging from 37 to 48 593
psu, *C. nodosa* plants possessed a higher growth rate 594
at the highest temperature (25°C), but when the 595

596 salinity was increased to 53 psu, the increase in
597 temperature did not have a positive effect on leaf
598 growth. This is probably because the increase in
599 metabolic activity at high temperatures is insufficient
600 for coping with the stress caused by high salinity
601 levels.

602 *Cymodocea nodosa* is also affected by seasonality,
603 showing minimum growth in winter due to lower
604 irradiance and temperatures. In this study, it was
605 observed that the salinity tolerance range of this
606 species may also vary throughout the year. This could
607 be related to maximum growth and, therefore, the
608 higher energy and metabolic consumption that occur
609 in summer due to the species' life cycle that makes
610 more evident salinity effects. In winter, plants survive
611 with smaller metabolic levels and are more able to
612 resist salinity-related stress.

613 On the other hand, tolerance can differ consider-
614 ably at one level of salinity depending on how such a
615 level is reached. Other studies report that gradual
616 increases in salinity are usually better resisted than a
617 sudden or rapid rise (Ralph, 1998). In this study,
618 *C. nodosa* plants exposed to slower increases of
619 salinity (2.5 psu per day) were able to tolerate
620 changes in salinity better than plants that had not
621 been acclimated, showing higher rates of growth and
622 survival.

623 The capacity of *C. nodosa* to improve its tolerance
624 to slow increases in salinity can be explained by an
625 acclimation of plants, and some structural or phys-
626 iological reversible changes that occur when plants
627 are exposed to stress. But when studying other
628 stresses, it has been observed that some plant
629 populations exposed to these same conditions during
630 a longer period may be able to adapt by means of
631 some genetic and irreversible changes (Peralta et al.,
632 2005).

633 In fact, when conducting one of the experiments
634 comparing the response of *Cymodocea nodosa* plants
635 from the Mar Menor lagoon with plants from the
636 Alicante (Mediterranean) near-shore coast, different
637 behaviours to salinity variations were observed for
638 both populations. For the Mediterranean population
639 studied, salinity tolerance was narrower than for the
640 Mar Menor population, as indicated by the high rates
641 of mortality and the significant reduction in shoot
642 growth rate at 50 psu. Plants from the Mar Menor,
643 meanwhile, showed a lower sensitivity to increases in
644 salinity, and similar rates of mortality and leaf growth

inside the salinity range studied. Salinity in the Mar
Menor lagoon currently ranges between 42 and 47
psu, and western Mediterranean coasts are at levels of
~37 and 38 psu. So these differences in tolerance to
salinity could be explained because, as occurs with
other widespread species, individuals adapt to the
different local conditions that occur naturally in their
habitats (Doering & Chamberlain, 1998; Benjamin
et al., 1999; Kamermans et al., 1999; van Katwijk
et al., 1999; Vermaat et al., 2000). For example,
Kamermans et al. (1999) and van Katwijk studies
performed with *C. nodosa*, significant differences
have also been found in the behaviour of the seeds of
this species in populations from the Golfe Juan Bay
in the French Mediterranean (Caye et al., 1992) and
from the Island of Ischia in Naples (Pirc et al., 1986),
although these differences were attributed to the
genetic variability of this species inside the Mediter-
ranean Sea. Consequently, it may be possible that the
wide geographical distribution of a species could
imply different tolerances among its populations.

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