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## Differences in the growth cycle of *Ruppia cirrhosa* (Petagna) Grande in a Mediterranean shallow system

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### Abstract

*Ruppia cirrhosa* growth cycle was analysed in a southern Mediterranean shallow system throughout 1 year. We examined the temporal variation in *R. cirrhosa* cover percentage, shoot density, biomass, leaf length, no. flowers m<sup>-2</sup> and no. fruits m<sup>-2</sup> in two groups of pond characterized by differences in some environmental parameters. Ponds were comparable for salinity and temperature but they differed for other environmental parameters such as water depth, level of suspended organic matter and chlorophyll *a* (CHL *a*). Biological parameter values were higher in B ponds, characterized by lower values of water depth, suspended organic matter and CHL *a*. A seasonal trend for all considered biological parameters in both typologies of ponds with maximum values in summer was also observed. Moreover, differences were observed between the two groups of ponds in relation to the reproductive strategy adopted by the plant, with populations subjected to a higher organic input and a lower water depth displaying an annual cycle. Results showed how *R. cirrhosa* is able to resist and to adapt to variations in environmental conditions because of the plasticity and flexibility in the growth cycle and in the reproductive effort.

**Keywords:** Aquatic macrophytes, growth cycle, Mediterranean Sea, salt works systems, *Ruppia cirrhosa*

**Abbreviations:** DW, dry weight; TSM, total suspended matter; CHL *a*, chlorophyll *a*

### Introduction

Species that belong to the genus *Ruppia* L. are common in shallow systems where they often form dense and monospecific beds (Verhoeven 1979; Calado & Duarte 2000; Menéndez 2002; Malea et al. 2004; Mannino & Sarà 2006; Pergent et al. 2006; Nikolić et al. 2013). *Ruppia* plants show annual/perennial growth cycles, according to the permanency of the water body (Verhoeven 1979; Kantrud 1991; Malea et al. 2004). In temporarily flooded areas, the growth cycle of *Ruppia* plants is completed before the lagoon dries up, and that led to the interruption of growth and then to the death of these plants; instead, in permanently flooded areas, plants maintained their vegetative structures throughout the year, although maximum development takes place in summer (Verhoeven 1979; Brock 1982; Gesti 2000; Malea et al. 2004; Gesti et al. 2005). In the Mediterranean region, three species, *Ruppia*

*maritima* L., *Ruppia cirrhosa* (Petagna) Grande and *Ruppia drepanensis* Tineo (considered by Zhao & Wu 2008 as synonyms of *R. cirrhosa*), are now recognized, the last species occurring only in the south-western Mediterranean (Comín et al. 1993; Triest & Sierens 2009, 2010).

These species, forming extended meadows or beds, are common in shallow systems (e.g. saltworks basins). Saltmarsh ponds and saltworks, widespread along the Mediterranean coast, represent peculiar and stressed habitats characterized by high salinity, poorly oxygenated sediments and wind-exposed waters. Few macrophytes can grow and reproduce in systems with these features. *R. cirrhosa*, due to its wide tolerance limits to salt variations, is able to survive in habitats from oligo- to hyperhaline, showing a competitive advantage at higher salinity (Verhoeven 1979, 1980). *R. cirrhosa* is a facultative annual/perennial species found especially in permanent lagoons (Verhoeven 1975, 1979; Menéndez & Peñuelas 1993; Ribera et al.

1997; Viaroli et al. 1997; Duarte et al. 2002; Menéndez 2002; Menéndez et al. 2002; Agostini et al. 2003; Mannino & Sarà 2006; Obrador & Pretus 2010), although it has also been found in temporary lagoons in coastal areas of the north-western Mediterranean (Verhoeven 1979; Gesti 2000).

*R. cirrhosa* shows extremely high resistance to variations in environmental conditions: salinity, water turbidity, the redox status of surface sediments, light availability and water level that are considered the main factors controlling the development and abundance of this plant (Verhoeven 1979, 1980; Menéndez & Comín 1989; Azzoni et al. 2001; Menéndez et al. 2002; Obrador et al. 2007; Sfriso et al. 2009; Obrador & Pretus 2010; Sfriso 2010). The enrichment in nutrients may have effects on *R. cirrhosa* plants improving their development (Pergent et al. 2006) even though Mannino and Sarà (2006) showed that *R. cirrhosa* cover and suspended organic matter were negatively correlated.

In spite of relevant ecological and structuring role of *R. cirrhosa*, only recent studies have focused on temporal dynamics of this species (e.g. Calado & Duarte 2000; Menéndez 2002; Gesti et al. 2005; Mannino & Sarà 2006; Pergent et al. 2006; Casagrande & Boudouresque 2007; Obrador et al. 2007; Shili et al. 2007; Obrador & Pretus 2010). Many studies have shown the *Ruppia* capacity to adopt a different growth cycle (annual or perennial) in response to the permanency of the water body (Verhoeven 1979; Brock 1982; Kantrud 1991; Gesti

2000; Malea et al. 2004), suggesting a considerable plasticity in the allocation of resources to seed production or vegetative growth.

In this study, the hypothesis of differences in the growth cycle was formulated in relation to different environmental conditions and different anthropic pressures evaluated as organic inputs. To achieve this aim, we analysed the *R. cirrhosa* growth cycle and described the temporal variation in some plant parameters collecting specimens from two different typologies of ponds (A and B groups), mainly differing for water depth, suspended organic matter and chlorophyll *a* (CHL *a*) conditions, located at the Natural Reserve “Saline di Trapani e Paceco” (Western Sicily, southern Mediterranean).

Results from previous studies conducted in this saltworks system have indeed highlighted that these two groups of ponds were well separated on the basis of their abiotic and biotic features (Mannino & Sarà 2006; Mannino 2010).

## Materials and methods

### Study area

The study was carried out in the Natural Reserve “Saline di Trapani e Paceco” (western Sicily, 37°52'N, 12°28'E; Figure 1). The area, composed of about 25 ponds, has an extension of about 1000 ha. The studied *R. cirrhosa* system can be considered one of the largest in the Mediterranean Sea, functioning as the main ecological corridor in

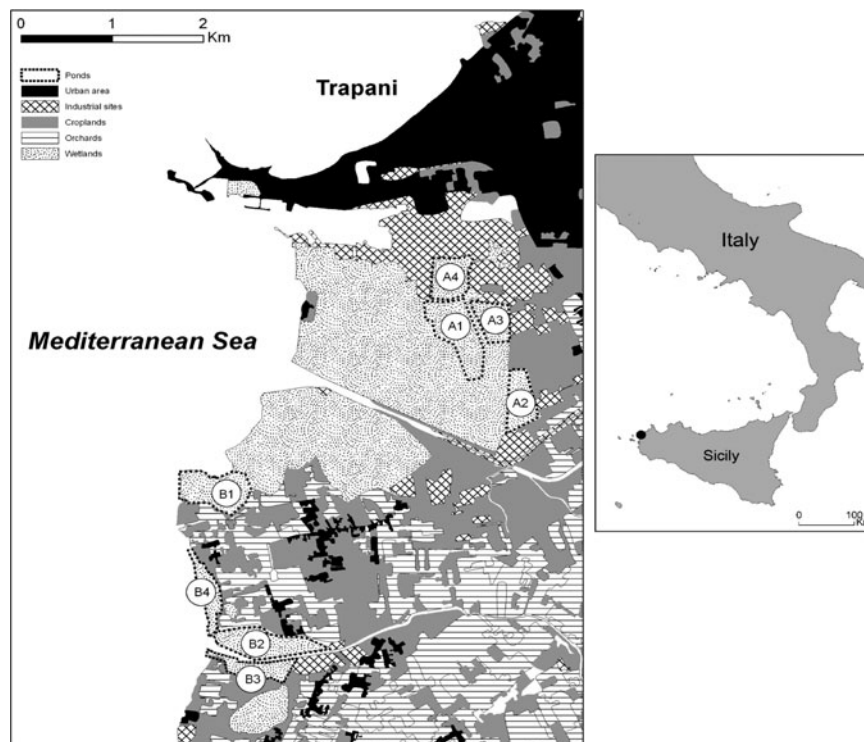


Figure 1. Map of the saltworks system: A1–A4; B1–B4.

the central-western Mediterranean for the avifauna migrating from Africa to Europe (Sorci et al. 1991).

In total, eight ponds, characterized by a muddy-sand bottom and affected by different anthropogenic pressures, including agriculture, urbanization and aquaculture, were studied. The ponds were split into two main groups: A group (ponds subjected to a higher anthropogenic pressure) and B group (ponds subjected to a lower anthropogenic pressure) using biotic and abiotic characteristics (Mannino & Sarà 2006; Mannino 2010).

*Environmental parameters*

Water temperature, salinity and depth were measured monthly *in situ* and estimated in triplicates. For the estimation of the nutrient inputs, in terms of total suspended matter (TSM) and CHL *a*, we refer to Mannino and Sarà (2006). These two parameters were considered as an expression of the human activities affecting the basins system. In this study, we referred to the TSM and CHL *a* data reported by Mannino and Sarà (2006), assuming that human pressure remained similar in the different time periods.

*Sample collection*

The studied ponds were monitored every month for 1 year and sampling campaigns were carried out four times during 2007 (T1, Winter; T2, Spring; T3, Summer; T4, Autumn), collecting all samples on the same day for each time period. *R. cirrhosa* cover percentage, plant parameters (leaf length, no. flowers m<sup>-2</sup> and no. fruits m<sup>-2</sup>), shoot density (no. shoots m<sup>-2</sup>) and biomass (g DW m<sup>-2</sup>) were assessed using a quadrat sampler of 400 cm<sup>2</sup> (20 cm × 20 cm). Three replicates for each variable were randomly collected for

each combination of factors (group of ponds and time). *Ruppia* cover was estimated by visual census *in situ*.

In the laboratory, *R. cirrhosa* shoots were sieved and washed with tap water to remove sediment and large debris, stored in 4–5% formalin in seawater, and kept at 4°C until subsequent analysis. Before biomass determination, leaves and rhizome plus roots were separated and a careful scraping by hand to remove epibiotic organisms and washing in 0.03% (v/v) HCl to remove encrustations were done. Biomass was estimated by weighting after oven drying (70°C to constant weight). To calculate the total biomass, we added aboveground biomass (shoots and leaves) to belowground biomass (rhizomes and roots). The leaf length was measured with a digital caliper (accuracy 0.02 mm).

*Experimental design and statistical analyses*

We tested for differences in percentage cover of *R. cirrhosa*, shoots density and total biomass using the Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001; PRIMER 6 and PERMANOVA +B20 package, Clarke & Gorley 2006) in a univariate context.

The analyses were performed on Bray–Curtis measures of square root-transformed data, using 9999 permutations of the appropriate units. The experimental design adopted for each variable was a two-factorial design: time (Ti; four levels, fixed) and groups (Gr; two levels, fixed).

**Results**

*Environmental parameters*

Water depth ranged from 0.3 to 0.7 m and showed little temporal variation during the study period but differed between the two groups of ponds (Table I).

Table I. Mean values of chemical, physical and biological variables in the studied ponds.

Period	Group	T	S	D	RC	TB	SD	Period	Group	T	S	D	RC	TB	SD
T1		12.2	20.7		0	0	0	T1		12.1	20.8		37	111	990
T2		20.3	33.2		49	172	1309	T2		20.8	33.4		67	361	2383
T3	A1	26.4	40.1	0.4	60	269	1851	T3	B1	26.0	39.8	0.6	85	537	3180
T4		15.8	28.0		10	41	305	T4		15.8	28.2		50	230	1502
T1		12.1	21.0		0	0	0	T1		12.2	21.1		38	111	992
T2		20.4	33.6		49	174	1312	T2		20.2	33.4		66	360	2391
T3	A2	25.9	40.0	0.3	61	270	1853	T3	B2	26.1	40.1	0.7	84	530	3178
T4		15.7	27.8		8	42	308	T4		16.0	28.3		51	229	1500
T1		12.4	20.8		0	0	0	T1		12.0	21.1		38	113	995
T2		20.4	33.5		49	172	1312	T2		20.3	33.5		67	361	2377
T3	A3	26.1	39.8	0.3	60	269	1850	T3	B3	26.0	39.7	0.6	84	535	3183
T4		15.8	28.3		10	42	304	T4		15.7	27.8		50	229	1509
T1		12.3	21.1		0	0	0	T1		12.3	21.0		38	112	994
T2		20.4	33.6		48	174	1305	T2		20.2	33.4		67	360	2391
T3	A4	26.2	39.7	0.4	59	272	1857	T3	B4	26.0	39.7	0.6	84	534	3169
T4		16.0	28.2		10	42	306	T4		16.0	27.8		50	231	1502

T1, Winter; T2, Spring; T3, Summer; T4, Autumn; T (°C), temperature; S, salinity; D (m), depth; RC (%), *Ruppia cirrhosa* coverage; TB (g DW m<sup>-2</sup>), total biomass; SD (no. shoots m<sup>-2</sup>), shoot density.

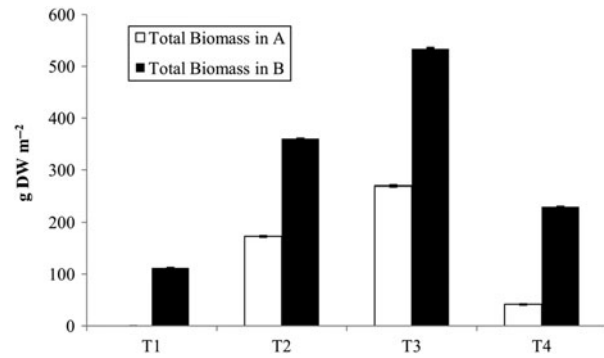


Figure 2. Total biomass (mean  $\pm$  SD) of *Ruppia cirrhosa* sampled at different ponds groups (A, white and B, black) on four sampling dates.

Ponds presented comparable values of temperature and salinity (according to the season) (Table I). Temperature and salinity showed a clear seasonal trend with maximum values in summer (on average 26.1 and 39.8, respectively) and minimum values in winter (on average 12.2 and 20.9, respectively) (Table I).

#### *R. cirrhosa meadow*

In the study area, the dominant plant was *R. cirrhosa*, but macroalgae were also present (Mannino & Sarà 2006; Mannino 2010). Sparsely small patches of *Cymodocea nodosa* (Ucria) Ascherson among *R. cirrhosa* beds were also observed in B ponds (Mannino & Sarà 2006). *R. cirrhosa* percentage cover, shoot density m<sup>-2</sup> and total biomass varied significantly according to the two different typologies of ponds and among the sampling times (Figures 2–4; Table II) (Table III;  $T_i \times Gr: P(\text{perm}) < 0.001$ ).

In both typologies of ponds, all parameters showed increasing values from spring to summer when the maximum values were observed (T3)

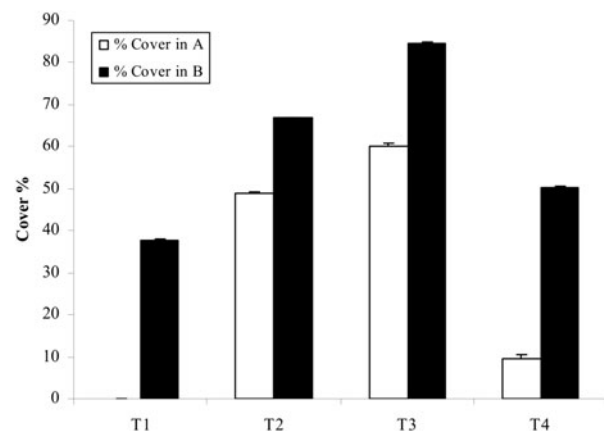


Figure 3. Cover percentage (mean  $\pm$  SD) of *Ruppia cirrhosa* sampled at different ponds groups (A, white and B, black) on four sampling dates.

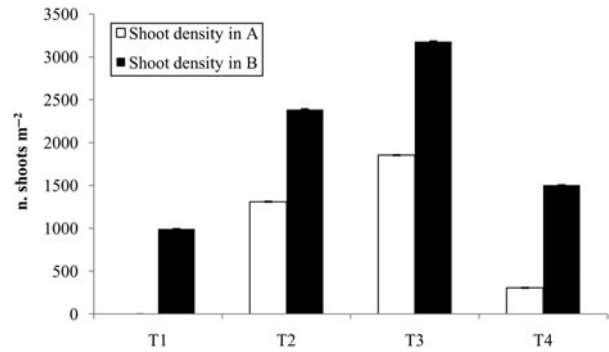


Figure 4. Shoots density (mean  $\pm$  SD) of *Ruppia cirrhosa* sampled at different ponds groups (A, white and B, black) on four sampling dates.

(Table I). After the summer, these values decreased reaching the low values in winter when the plant totally disappeared in A ponds.

#### Plant parameters

Growth cycle of *R. cirrhosa* began in spring (T2), with an initial horizontal expansion followed by a rapid vertical expansion in summer (T3), characterized by an intense branching process and an active leaves production.

In all ponds, *Ruppia* plants showed continuous life cycles but the strategies were different (Figure 5). Trends showed plants collected at the A ponds depicted an annual growth cycle. The plant, in fact, totally disappeared in winter (T1), leaving a huge quantity of seeds as dormant organs on the bottom. The growing period was observed during the next spring.

Instead, plants collected at the B ponds maintained their vegetative plant structures throughout the year and rhizomes stayed alive in the period of quiescence (T1), showing a perennial growth cycle. Flowering took place in spring and fruits were observed in summer (Table IV).

#### Discussion

*R. cirrhosa* is the dominant macrophyte in the studied shallow system, where it forms dense and quite monospecific beds, according to its ability to survive and reproduce between 1.5 and 80 psu as reported in the literature (Brock 1979; Verhoeven 1980; Shili et al. 2007 and references within). *R. cirrhosa* can be considered more competitive and resistant than the other submerged macrophytes for its physiological features in stressed environmental conditions.

Indeed, low macrophyte diversity is usually reported for brackish systems (Remane & Schlieper 1971). Here, *R. cirrhosa* meadow showed a simple vertical structure as observed in other Mediterranean *Ruppia* systems, with two vegetation layers, macro-

Table II. Analysis of variance on shoots density, total biomass and percentage cover data of *Ruppia cirrhosa*.

Source	df	Shoots density			Total biomass			Percentage cover					
		SS	MS	Pseudo-F	P(perm)	SS	MS	Pseudo-F	P(perm)	SS	MS	Pseudo-F	P(perm)
Ti	3	46,041	15,347	4.1048E6	***	38,771	12,924	7.1999E5	***	33,161	11,054	23,324	***
Gr	1	11,218	11,218	3.0006E6	***	9376.2	9376.2	5.2236E5	***	17,106	17,106	36,095	***
TixGr	3	55,691	18,564	4.9651E6	***	52,121	17,374	9.6789E5	***	24,206	8068.7	17,026	***
Res	88	0.32901	3.7388E-3			1.5796	1.795E-2			41.704	0.47391		
Total	95	1.1295E5				1.0027E5				74,515			

Notes: SS, sum of squares; MS, mean square. \*\*\*P(perm) < 0.001.

Table III. Pair-wise *t* test.

	T1	T2	T3	T4
Shoots density	883.99***	8640.2***	464.33***	1499.2***
Total biomass	362.48***	2711.2***	365.36***	669.4***
Percentage cover	60.146***	141.52***	89.783***	1007.7***

Notes: Term “TixGr” for pairs of levels of factor “Group” (A,B). \*\*\*P(perm) < 0.001.

phyte plus algae (Verhoeven 1980; Mannino 2010). In all studied ponds, *R. cirrhosa* showed the maximum development at the third date of sampling, corresponding to the summer, as observed in other permanent brackish Mediterranean habitats (Verhoeven 1979; Menéndez 2002). Afterwards, plants decline progressively as a result of vertical stems decomposition at their bases and of epiphytes growth (particularly abundant in summer), the main cause of stem weakening (Verhoeven 1979).

The growth rate of *R. cirrhosa* was similar to that described previously for *Ruppia* species in other coastal lagoons (Verhoeven 1979; Menéndez 2002; Malea et al. 2004).

By contrast, the mean leaf length was higher than that reported previously for *Ruppia* species (Malea et al. 2004). As expected, meadow and plant parameters showed significant differences among sampling times and results showed two different reproductive strategies in relation to the different typologies of ponds. Even though samples were collected at the same water permanency conditions, all the variables considered (cover percentage, shoot density, biomass, leaf length, no. flowers m<sup>-2</sup> and no. fruits m<sup>-2</sup>) have confirmed the hypothesis of the occurrence of two different reproductive strategies in the specimens collected in the two groups of ponds (A and B). Plants in A ponds adopted an annual growth cycle with a total disappearance in winter, whereas plants in B ponds showed a perennial life cycle with winter quiescence and a vegetative growth from rhizomes in spring. This result is in contrast with Malea et al. (2004) and Gesti et al. (2005), who observed that in permanent water bodies perennial strategy seems to be favoured. However, we know that apart from water permanency, other factors (e.g. water temperature and salinity and light availability) are involved in the dynamics of this submerged macrophyte (Verhoeven 1979; Azzoni et al. 2001; Obrador & Pretus 2010).

The lowest flower and fruit number recorded for perennial populations (B ponds) highlighted low resources allocation into reproduction, as observed previously in other perennial *Ruppia* populations

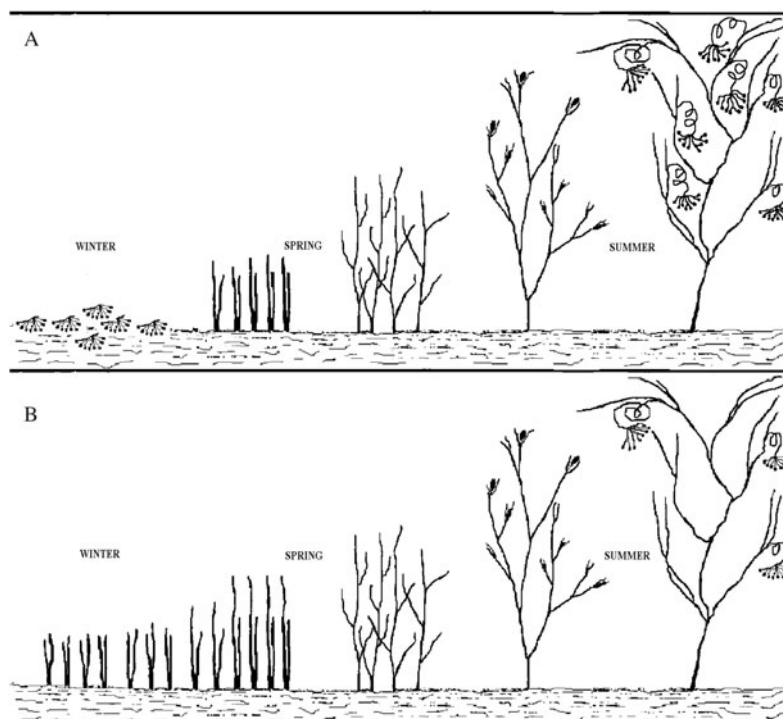


Figure 5. Annual (A) and perennial (B) growth cycles (from Verhoeven 1979 modified) of *Ruppia cirrhosa* sampled at ponds A and B.

(Verhoeven 1979; Brock 1983; Cancemi et al. 2002; Malea et al. 2004). By contrast, in the annual populations, a higher resource allocation was observed as indicated by the highest flower and fruit numbers.

Macrophyte descriptors also differ between the two groups of ponds with values higher in B than in A ponds. Biomass outcomes were comparable with values registered previously (Pérez & Camp 1986; Menéndez & Comín 1989; Calado & Duarte 2000; Menéndez 2002; Pergent et al. 2006; Casagrande & Boudouresque 2007), but were higher than values reported in Ballester (1985) and lower than values reported in Obrador et al. (2007) and Obrador and Pretus (2010).

Results showed a positive relationship between plant development and water level and also suggested that *R. cirrhosa* reproduction efficiency and growth cycle seemed to be negatively related to different TSM and CHL *a* conditions. This is in agreement

with Mannino and Sarà (2006) and Mannino (2010) who described that, at higher levels of suspended matter (group A of ponds), algal biomass reaches notably in summer higher values which produces detrimental effects (e.g. shading and anoxic events generated by algal decomposition) on *R. cirrhosa* growth. The turbidity due to the algal proliferation that is responsible for light attenuation may also negatively affect *R. cirrhosa* (Menéndez & Comín 1989).

Moreover, algal growth may limit the photosynthetic oxygen production by the *Ruppia* plants and consequently the oxygen transport to the roots, with a high accumulation of sulphide concentrations, resulting in the death of the roots and rhizomes (Azzoni et al. 2001). Furthermore, algal decomposition led to significant fluxes of free sulphide (Viaroli et al. 1996). In addition, different physiological requirements of algae and phanerogams led to mutual exclusion (Giusti & Marsili-Libelli 2005).

Table IV. No. flowers  $m^{-2}$  and no. fruits  $m^{-2}$  and leaf length (mean  $\pm$  SD) of *Ruppia cirrhosa* sampled at ponds A and B on four sampling dates.

		T1	T2	T3	T4
A group	No. flowers $m^{-2}$	–	511.5 $\pm$ 31.20	2362.67 $\pm$ 162.49	–
	No. fruits $m^{-2}$	–	–	436.33 $\pm$ 20.91	–
	Leaf length (cm)	–	9.2 $\pm$ 0.3	25.78 $\pm$ 0.7	18.69 $\pm$ 0.3
B group	No. flowers $m^{-2}$	–	145.92 $\pm$ 4.01	611.33 $\pm$ 23.53	–
	No. fruits $m^{-2}$	–	–	47.08 $\pm$ 3.06	–
	Leaf length (cm)	6.1 $\pm$ 0.2	12.3 $\pm$ 0.2	35.4 $\pm$ 0.1	25.2 $\pm$ 0.2

Menéndez et al. (2002) instead found a positive correlation between healthy conditions of *R. cirrhosa* beds and polluted water inputs.

Relationship between plant and TSM is not univocal as the TSM concentration is driven not only by external input but also by the plant production–decomposition cycle.

To date, several studies have been focused on the effects of water pollution and eutrophication on macrophytes (Azzoni et al. 2001; Menéndez et al. 2002; Pergent et al. 2006; Zuccarini & Kampuś 2011; Manolaki & Papastergiadou 2012; Azzella et al. 2013; Liendo et al. 2013).

The occurrence of two different patterns of growth would suggest that *R. cirrhosa* has an internal regulating mechanism and a resilience capacity (Connell & Sousa 1983) that allow to adapt to different environmental conditions.

Nevertheless, the present results were still insufficient to depict more general conclusions on this plant, and further studies are necessary to understand well its dynamics. Correlations among environmental factors and *R. cirrhosa* parameters should be evaluated through long-term time-series analysis. Considering that shallow aquatic ecosystems are continuously threatened by human activities (Croce et al. 2012), taking into account the effects of *R. cirrhosa* organic matter on the detritus cycle and the crucial role of *R. cirrhosa* in providing shelter and food for many organisms, the species and its habitat should be worthy of attention and protection.

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