# Temporal and spatial variation of the algal community in a southern Mediterranean shallow system

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**Abstract** – The algal community of a shallow system located in Western Sicily (Mediterranean Sea) has been investigated over 1 year in eight ponds (*fredde*). The spatiotemporal variation in total algal coverage, species richness and coverage in relation to environmental variables has been analysed. The algal community was very species-poor. A total of 50 taxa, mainly detached algae, was identified. A dominance of filamentous and foliose taxa was observed. Significant spatio-temporal differences in total algal coverage, species richness and coverage among the ponds of group A and those of group B were found. The separation into groups A and B was made *a priori* on the basis of different levels of proximity to the sea and anthropogenic pressure. In particular, the algal assemblage of ponds of group B, closer to the sea and subjected to a lower anthropogenic pressure, showed a good structure and the highest species richness. The separation of the ponds was successively confirmed by PERMANOVA. This study confirmed the effectiveness of using species as descriptors of spatio-temporal responses of the algal community to environmental variations.

algal community / Mediterranean Sea / Ruppia meadow / shallow systems / species composition / spatio-temporal variation

Résumé – Variation temporelle et spatiale de la communauté algale d'un système de basfonds de la Méditerranée méridionale. Le peuplement algal d'un système de bas-fond de la Sicile occidentale (Mer Méditerranée) a été analysé. En particulier, les fluctuations spatiotemporelles de la couverture totale algale, de la diversité et de la couverture spécifique par rapport aux caractéristiques du milieu ont été étudiées. L'étude a été menée pendant un an dans huit bassins (fredde). En général, le peuplement algal était très pauvre en espèces, avec 50 taxons au total, dont la majorité était détachée. Une dominance d'espèces foliacées et filamenteuses a été observée. Des différences spatio-temporelles significatives au niveau de la couverture totale algale, de la diversité et de la couverture spécifique ont été observées entre les bassins du groupe A et ceux du groupe B. Cette séparation en groupes A et B a été effectuée *a priori* sur la base des différents degrés de proximité de la mer et de la pression anthropique. En particulier, le peuplement algal des bassins du groupe B, plus proches de la mer et caractérisés par une faible pression anthropique, était mieux structuré et présentait une plus grande diversité spécifique. La séparation des bassins à été confirmée successivement par l'analyse PERMANOVA. Cette étude a confirmé l'efficace de l'analyse au niveau spécifique dans la description des réponses spatio-temporelles de la communauté algale aux variations des caractéristiques du milieu.

communauté algale / composition spécifique / Mer Méditerranée / prairies à *Ruppia* / systèmes de bas-fonds / variations spatio-temporelles

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## INTRODUCTION

Along the Mediterranean coasts, highly variable shallow systems with higher salinities than in marine environments are widespread. Shallow waters act as an interchange buffer between open sea and land, and aquatic macrophytes forming extended meadows or beds represent key species regulating fluxes of energy and matter (Wetzel, 1975; Short & Coles, 2001). Macrophytes play an important ecological role in these systems, providing shelter for many organisms (Borowitzka *et al.*, 2006; Gillanders, 2006) and creating a physical multidimensional framework for algal epiphytes that represent an important and palatable food source for a wide range of invertebrates (from benthos to insects) and small fishes.

In shallow systems, *Ruppia* species (Verhoeven, 1979; Calado & Duarte, 2000; Menéndez, 2002; Malea *et al.*, 2004; Mannino & Sarà, 2006; Pergent *et al.*, 2006) form extended beds also inhabited by algae. These play a fundamental role in determining the biomass, production of organic matter and biodiversity of the system. Within the algal community, epiphytes (which are important primary producers in macrophyte communities) and detached algae may shade light available to plant leaves, and therefore reduce plant productivity (Pergent *et al.*, 2006 and references therein). Moreover, a strong relationship between algae and macrophytes has been highlighted recently in the *Ruppia* system: macroalgae could have a negative effect on plant coverage, causing higher algal coverage and lower plant coverage. Higher levels of suspended matter at lower plant coverage could allow algal biomass to reach high coverage values (particularly in summer), producing a notable shading effect and limiting plant development (Mannino & Sarà, 2006; Pergent *et al.*, 2006).

Since algal communities are an important part of benthic ecosystems and are considered as good descriptors, they are widely used to characterise and monitor aquatic systems (Rodríguez-Prieto & Polo, 1996; Eriksson *et al.*, 1998; Cormaci & Furnari, 1999; Piazzi *et al.*, 2001; Orfanidis *et al.*, 2003; Rindi & Guiry, 2004). Algal assemblages, which are affected by numerous physical, chemical and biological factors, are usually described by species composition and abundance. Indeed, species richness and composition, which are more sensitive to the detection of community shifts, have allowed an accurate description of the structure of macroalgal assemblages (Phillips *et al.*, 1997; Piazzi *et al.*, 2002), and thus they are now used in the assessment of water quality under the European Water Framework Directive (Wells *et al.*, 2007). Moreover, diatoms, owing to their sensitivity to eutrophication, are commonly used as short-term indicators to assess the trophic status of aquatic systems (Kelly, 1998; Kitner & Poulíčková, 2003; Dell'Uomo, 2004).

In recent years the morphological group approach has also been used in ecological studies to detect shifts in community structure (Littler, 1980; Littler & Littler, 1980; Hay, 1994; Steneck & Dethier, 1994), as it is rapid and inexpensive. However, it has been highlighted that small scale shifts in community structure are not well separated if the morphological group approach is used, and therefore this method may result in significant loss of information (Phillips *et al.*, 1997; Piazzi *et al.*, 2004).

Studies on *Ruppia* systems in the Mediterranean Sea have dealt mostly with the growth, dynamics, productivity and reproduction of the macrophyte (Santamaria & Hootmans, 1998; Menéndez, 2002; Agostini *et al.*, 2003; Malea *et al.*, 2004; Gesti *et al.*, 2005; Mannino & Sarà, 2006; Pergent *et al.*, 2006). Less attention has been paid to the associated algal community (Verhoeven, 1980;

Sortino *et al.*, 1981; Calvo *et al.*, 1982; Mannino & Sarà, 2006). The aim of the present work is to characterise the algal community colonizing the *Ruppia* system in the Natural Reserve "Saline di Trapani e Paceco" (Western Sicily, Italy), a shallow Mediterranean system dominated by *Ruppia cirrhosa* (Petagna) Grande, using species as descriptors and focussing on the spatio-temporal variation in total algal coverage, species richness, and coverage in relation to environmental variables.

### **MATERIAL AND METHODS**

#### Study area

The Natural Reserve "Saline di Trapani e Paceco" is located in Western Sicily (Mediterranean Sea, 37°52'N; 12°28'E; Fig. 1). The area hosts the most important saltworks system of the Western Mediterranean, composed by about 25 ponds which cover a total surface area of about 1000 ha. There are two main types of ponds in a saltworks system: the "*fredde*" (hereafter referred to as ponds), which receive water directly from the sea, are permanently flooded and of variable salinity; and the "*crude*", which are fed by ponds.

The *Ruppia* system of the study area is among the largest in the Mediterranean Sea, functioning as the main ecological corridor in the central-western Mediterranean for avifauna migrating from Africa to Europe (Sorci *et al.*, 1991).

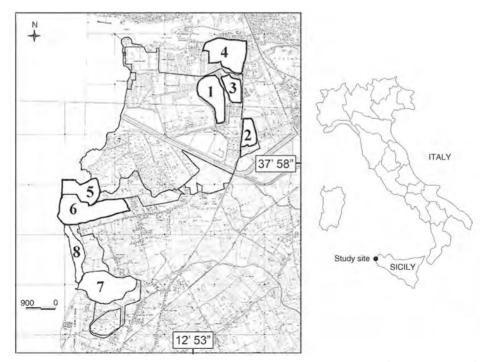


Fig. 1. Map of the saltworks system showing the location of the studied ponds (1, 2, 3, 4, 5, 6, 7, 8).

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In total 8 ponds were studied, representing about 60% of currently active ponds for salt production in the area. Different anthropogenic pressures, including agriculture, urbanisation and aquaculture, affect the functioning of this ecosystem. Environmental variables (temperature, salinity and water depth, which all showed very similar values in the studied ponds), the proximity to the sea and the degree of anthropogenic pressure were investigated. The latter two features made it possible to separate *a priori* the ponds into two main groups: group A (ponds 1, 2, 3 and 4, far from the sea and subjected to a higher anthropogenic pressure), and group B (ponds 5, 6, 7 and 8, closer to the sea and subjected to a lower anthropogenic pressure). The studied ponds were characterised by a muddy-sand bottom and water depths of 0.4-0.5m, which showed little variation during the study period (Table 1). Mean salinity ranged from 21 psu in winter to 40 psu in summer. Mean temperature ranged from  $12^{\circ}$ C in winter to  $26^{\circ}$ C in summer (Table 1).

## Sample collection and processing

Sampling took place over the whole of 2006 in spring, summer, autumn and winter. A single sampling was carried out in each period. Samples ( $400 \text{ cm}^2$ ) were randomly chosen and three replicates were collected at each pond in each period. After collection, *Ruppia* plus algae 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 analysis. Samples were examined by light microscopy; all collected taxa were identified and reproductive conditions were assessed.

Period	Pond	Т	S	D	Unv	RC	AC	AT	Period	Pond	Т	S	D	Unv	RC	AC	AT
Sp		21.3	33.2		27.0	50.7	22.3	16.0	Sp		20.8	33.4		11.7	78.0	10.3	28.0
Su	1	26.4	40.1	0.4	15.0	55.0	30.0	13.0	Su	5	26.0	39.8	0.5	5.0	80.0	15.0	19.0
Au	1	15.4	28.0	0.4	79.7	0.0	20.3	12.0	Au	5	15.3	28.2	0.5	14.0	74.3	11.7	16.0
Wi		12.2	20.7	7	85.0	0.0	15.0	12.0	Wi		12.1	20.8		20.0	70.0	10.0	12.0
Sp		21.4	33.6		28.5	50.0	21.5	16.0	Sp		21.2	33.4		12.5	75.5	12.0	27.0
Su	2	25.9 40.0		16.0	53.0	31.0	14.0	Su	6	26.1	40.1	0.5	5.5	79.2	15.3	19.0	
Au	2	15.2	27.8 <sup>0.4</sup>	81.0	0.0	19.0	13.0	Au		15.4	28.3		15.5	73.5	11.0	14.0	
Wi		12.1 21.0		85.5	0.0	14.5	13.0	Wi		12.2	21.1		20.0	69.0	11.0	12.0	
Sp		21.4	33.5		27.0	52.5	20.5	18.0	Sp		21.3	33.5		13.5	74.0	12.5	24.0
Su	2	26.1	39.8		18.0	55.0	27.0	16.0	Su	-	26.0	39.7	0.4	5.5	77.0	17.5	16.0
Au	3	15.2	28.3	0.4	82.0	0.0	18.0	12.0	Au	7	15.3	27.8	0.4	19.0	69.3	11.7	14.0
Wi		12.4 20.8		90.0	0.0	10.0	12.0	Wi		12.0	21.1		24.0	65.7	10.3	14.0	
Sp		21.4 33.6		30.3	44.4	25.3	17.0	Sp		21.2	33.4		11.7	75.3	13.0	22.0	
Su	4	26.2	39.7	0.4	17.0	49.0	34.0	14.0	Su	0	26.0	39.7	0.4	6.3	77.7	16.0	15.0
Au	4	15.4	28.2	0.4	80.5	0.0	19.5	11.0	Au	8	15.4	27.8	0.4	19.3	68.7	12.0	14.0
Wi		12.3	21.1		90.0	0.0	10.0	11.0	Wi		12.3	21.0		23.5	65.5	11.0	14.0

Table 1. Mean values of main chemical, physical and biological variables in the studied ponds (Sp = Spring; Su = Summer; Au = Autumn; Wi = Winter; T,  $^{\circ}C$  = Temperature; S = Salinity; D, m = Depth; Unv,  $^{\circ}\%$  = Unvegetated substrate; *RC*,  $^{\circ}\%$  = *Ruppia cirrhosa* coverage; AC,  $^{\circ}\%$  = Algal coverage; AT, n = Algal taxa)

Taxonomy and nomenclature follow Anagnostidis & Komárek (1985, 1988), Komárek & Anagnostidis (1986, 1989, 1998), Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot & Genkal (1999), Furnari *et al.* (2003) and Hayden *et al.* (2003).

## **Community parameters**

The spatio-temporal variations of 3 parameters were analysed: total algal coverage (%), species richness, and species coverage, the latter two used as measures of community structure. Species richness was measured as species number, whereas species coverage (i.e. the surface covered in vertical projection by the species) was expressed as a percentage (Boudouresque, 1971). The Rhodophyta/Chlorophyta index (Sfriso *et al.*, 2002; Curiel *et al.*, 2004), suggested for water quality evaluation of lagoon habitats, and the Shannon-Weaver diversity index (DI; the mean diversity index represents an indicator of environmental conditions), were calculated.

#### Statistical Analyses

In order to test for differences in species richness and coverage between ponds and time-periods, a Permutational Multivariate Analysis of Variance (PERMANOVA: Anderson, 2001a; Sarà et al., 2007) was carried out using PRIMER (release 6, licensed to Chiara Romano). PERMANOVA combines traditional test-statistics (ANOVA designs) and multivariate nonparametric methods. It is based on symmetric dissimilarity and provides probability values (p, significant if <0.05) using permutation methods. Groups of ponds (Group 1 including ponds 1-4 and Group 2 including ponds 5-8) and periods of the year (spring, summer, autumn and winter) were used as fixed factors. Pond was treated in the analysis as random factor and nested in Group. Quadrates in each pond represented replicates (n = 3). All variables were transformed to y' = ln(y+1) in all analyses in order to retain information on relative abundances and reduce differences in scale among the variables. The Bray-Curtis dissimilarity measure was used for all analyses, and all P-values were calculated using 9999 permutations of the residuals under a reduced model (Anderson, 2001b). Non-metric multidimensional scaling (nMDS) was used to describe PERMANOVA results.

#### RESULTS

The total *Ruppia* coverage showed significant spatial and temporal differences (Table 1; Fig. 2). It was negatively related to total algal coverage, but positively related to species richness, in agreement with existing data (Mannino & Sarà, 2006).

Total algal coverage and species richness varied significantly according to the pond and the sampling period, showing the same trend in all ponds. It showed a positive relation to salinity and temperature, especially increasing from winter to summer when the maximum values had been reached. Species richness, also positively related to salinity and temperature, peaked in spring (Table 1; Fig. 3).

In the ponds of group A, species richness values were lower than in the ponds of group B (Table 1; Fig. 3), whereas total algal coverage was higher

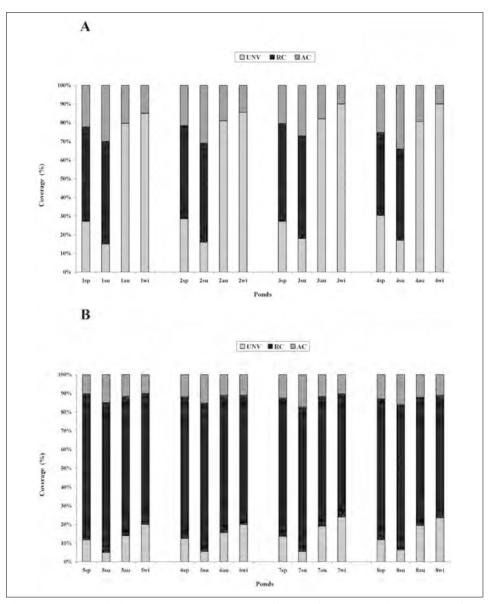


Fig. 2. Temporal variations of Unvegetated substrate (UNV, %), *Ruppia cirrhosa* coverage (RC, %) and Algal coverage (AC, %) in the studied ponds (sp = spring; su = summer; au = autumn; wi = winter): (A) ponds 1, 2, 3, 4 and (B) ponds 5, 6, 7, 8.

(Table 1; Fig. 2). A total of 50 algal taxa (Table 2) was identified: 17 Chlorophyta, 14 Rhodophyta, 9 Bacillariophyta, 6 Ochrophyta and 4 Cyanophyta. Only 11 taxa occurred in all ponds. Reproductive structures were observed in Rhodophyta only (both attached and epiphytic) and detected only in the ponds of group B. The dominant algae (Rhodophyta and Chlorophyta) were essentially filamentous and

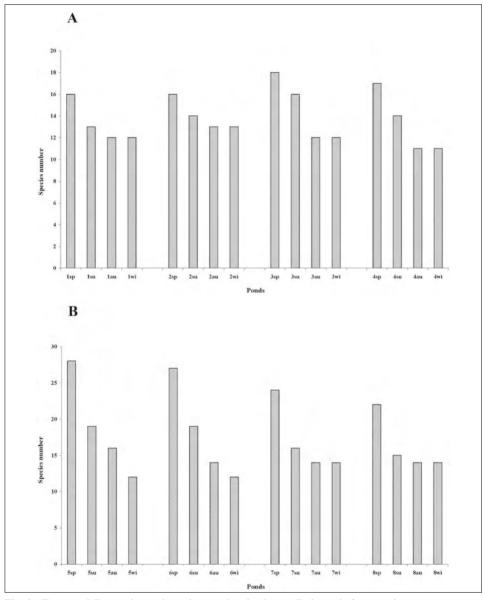


Fig. 3. Temporal fluctuations of species number in the studied ponds (sp = spring; su = summer; au = autumn; wi = winter): (A) ponds 1, 2, 3, 4 and (B) ponds 5, 6, 7, 8.

single-layered foliose species characterized by thin thalli, fast nutrient uptake and growth rates, and ephemeral life forms (i.e. opportunistic species). Only few perennial taxa with thick, corticated or calcareous thalli and relatively slow growth rates were recorded, and then only in the ponds of group B.

Algae species could be grouped into attached (important in stabilizing sediments), detached (pleustophytic) and epiphytic (attached to macroalgae and

Table 2. List of taxa recorded in the studied ponds (+ = presence; - = absence; m = male gametophyte; f = female gametophyte; t = tetrasporophyte)

Pond	1	2	3	4	5	6	7	8
Algae								
Cyanophyta								
<i>Blennothrix lyngbyacea</i> (Kützing <i>ex</i> Gomont) Anagnostidis <i>et</i> Komárek	+	+	+	+	+	+	+	+
Calothrix crustacea Thuret	-	-	-	-	+	+	+	+
Chroococcus turgidus (Kützing) Nägeli	+	+	+	+	-	-	-	-
Cyanothece aeruginosa (Nägeli) Komárek	+	+	+	+	+	+	+	+
Rhodophyta								
Acanthophora nayadiformis (Delile) Papenfuss	-	-	-	-	+	+	+	+
Boergeseniella fruticulosa (Wulfen) Kylin	-	-	-	-	-	+	-	-
Chroodactylon ornatum (C. Agardh) Basson	+	+	+	+	+	+	+	+
Dasya baillouviana (S.G. Gmelin) Montagne	-	-	+	+	+m	+	+	+
Jania rubens (Linnaeus) J.V. Lamouroux var. rubens	-	-	-	-	+	+	-	-
Laurencia obtusa (Hudson) J.V. Lamouroux	-	-	-	-	+	+	+	+
Spyridia filamentosa (Wulfen) Harvey	-	-	-	-	+	-	-	-
Stylonema alsidii (Zanardini) K.M. Drew	+	+	+	+	+	+	+	+
Ochrophyta								
Phaeophyceae								
Dictyota dichotoma (Hudson) J.V. Lamouroux var. dichotoma	-	-	-	-	+	+	+	+
Dictyota dichotoma (Hudson) J.V. Lamouroux var. intricata (C. Agardh) Greville	-	-	-	-	+	+	+	+
Ectocarpus sp.	-	-	-	-	+	+	-	+
Feldmannia paradoxa (Montagne) Hamel	-	-	-	-	+	+	+	+
Chrysophyceae								
Dynobrion sp.	+	-	-	-	-	-	-	-
<i>Vaucheria</i> sp.	-	-	-	-	-	-	+	+
Bacillariophyta								
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	+	+	+	+	+	+	+	+
Cocconeis placentula Ehrenberg	+	+	+	+	+	+	+	+
<i>Fragilaria capucina</i> var. <i>capitellata</i> (Grunow) Lange-Bertalot	+	+	+	+	-	-	-	-
Gyrosigma acuminatum (Kützing) Rabenhorst	-	-	-	-	+	+	+	+
Nitzschia fonticola Grunow	-	-	-	-	+	+	+	+
Surirella sp.	-	-	-	-	+	+	+	+
Surirella ovalis Brébisson	+	+	+	+	-	-	-	-
Chlorophyta								
Acetabularia acetabulum (Linnaeus) P.C. Silva	-	-	-	-	+	+	-	-
Caulerpa prolifera (Forsskål) J.V. Lamouroux	-	-	-	-	+	+	-	-
Chaetomorpha linum (O.F. Müller) Kützing	+	+	+	+	+	+	+	+

Table 2. List of taxa recorded in the studied ponds ( $+ = $ presence; $- = $ absence; $m = $ ma	le
gametophyte; $f = female gametophyte; t = tetrasporophyte)$ (continued)	

Pond	1	2	3	4	5	6	7	8
Chaetomorpha mediterranea (Kützing) Kützing var. mediterranea	+	+	+	+	+	+	+	+
Cladophora sericea (Hudson) Kützing	+	+	+	+	+	+	+	+
Cladophora socialis Kützing	-	+	+	-	+	-	-	+
Entocladia leptochaete (Huber) Burrows	+	+	+	+	+	+	+	+
Entocladia viridis Reinke	-	-	-	-	+	+	+	+
<i>Gayralia oxysperma</i> (Kützing) K.L. Vinogradova <i>ex</i> Scagel <i>et al.</i> f. <i>oxysperma</i>	-	-	+	+	-	-	-	-
Phaeophila dendroides (P. et H. Crouan) Batters	+	+	+	+	-	-	-	-
Pringsheimiella scutata (Reinke) Höhnel ex Marchewianka	+	+	-	-	+	-	+	-
Rhizoclonium tortuosum (Dillwyn) Kützing	+	+	-	-	-	-	-	+
Ulva compressa Linnaeus	+	+	+	+	+	+	-	-
Ulva intestinalis Linnaeus	+	+	+	+	-	-	-	-
Ulva laetevirens Areschoug	-	+	+	+	-	-	-	-
Ulva prolifera O.F. Müller	-	-	-	-	+	+	+	+
Ulvella lens P. et H. Crouan	-	+	-	-	-	+	+	-
Ruppia cirrhosa epiphytes								
Rhodophyta								
Ceramium deslongchampsii Chauvin ex Duby	-	-	-	-	+	+	+m	+
Ceramium diaphanum (Lightfoot) Roth	-	-	-	-	+ f	+ f	+ f	+
Chondria capillaris (Hudson) M.J. Wynne	-	-	-	-	+ t f	+ t	+ t	+ t f
Lophosiphonia obscura (C. Agardh) Falkenberg	+	+	+	+	-	-	-	-
Polysiphonia elongata (Hudson) Sprengel	-	-	-	-	+ t f	-	+	-
Polysiphonia sertularioides (Grateloup) J. Agardh	-	-	-	-	+ t f	+ t	-	+ t
Bacillariophyta								
Fragilaria ulna (Nitzsch) Lange-Bertalot	+	+	+	+	-	-	-	-
Nitzschia dissipata (Kützing) Grunow	+	+	+	+	+	+	+	+
Species richness (n)	22	24	23	22	36	34	30	31

macrophyte leaves) species. A dominance of detached, opportunistic Chlorophyta species (Fig. 4) belonging to the genera *Chaetomorpha* Kützing, *Cladophora* Kützing and *Ulva* Linnaeus was observed in all ponds.

The Rhodophyta/Chlorophyta (R/C) index was low in all samples: < 1.0 (bad) for the ponds of group A, and 1.0 (insufficient) for those of group B, confirming the conditions of high environmental stress in these ponds (particularly due to salinity values), where only opportunistic species (mainly green algae) may survive. The DI values were higher in the ponds of group B (Fig. 5); both indices confirmed the separation of the ponds into two groups A and B.

The algal community of the ponds of group A was dominated by Chlorophyta, both in terms of species richness and coverage; in particular, *Chaetomorpha linum* (O.F. Müller) Kützing and *Ulva intestinalis* Linnaeus were

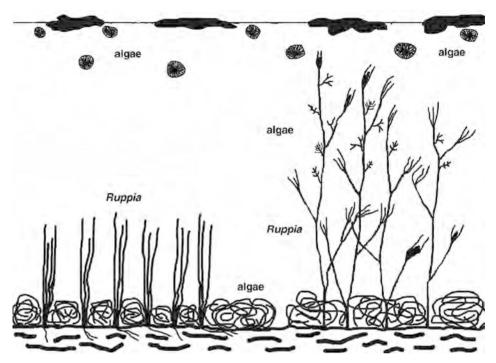


Fig. 4. Drawing showing the vertical structure of *Ruppia* system (from Verhoeven, 1979 modified).

present throughout 2006 with high coverage values. *Ulva compressa* Linnaeus showed an increase from spring to autumn, and *Chaetomorpha mediterranea* (Kützing) Kützing var. *mediterranea* showed a peak in summer.

In the ponds of group B both green and red algae were abundant but high coverage values were observed only for red algae. In particular, *Chondria capillaris* (Hudson) M.J. Wynne was recorded throughout 2006 with high coverage values, while *Chaetomorpha linum* was observed only during spring and summer, and *Laurencia obtusa* (Hudson) J.V. Lamouroux occurred only in spring. The presence of brown and coralline red algae was also noticed in these ponds.

The epiphytic community inhabiting macroalgae was characterised by Cyanophyta, particularly *Cyanothece aeruginosa* (Nägeli) Komárek and *Blennothrix lyngbyacea* (Kützing *ex* Gomont) Anagnostidis *et* Komárek, and Bacillariophyta, particularly *Planothidium lanceolatum* (Brébisson *ex* Kützing) Lange-Bertalot and *Cocconeis placentula* Ehrenberg. Cyanophyta showed the highest specificity for *Chondria* and *Chaetomorpha* thalli, whereas Bacillariophyta were recorded mostly on *Chaetomorpha* thalli. The epiphytic community showed clear spatio-temporal variations in composition and abundance, with highest coverage values recorded in spring and autumn. No significant differences were observed between the two groups of ponds A and B. Based on Van Dam *et al.* (1994), Bacillariophyta species were mainly eutraphentic (indicators of high intensity of primary production) and mesosaprobic (indicators of moderate or high pollution). Particularly, in the ponds of group B Bacillariophyta were

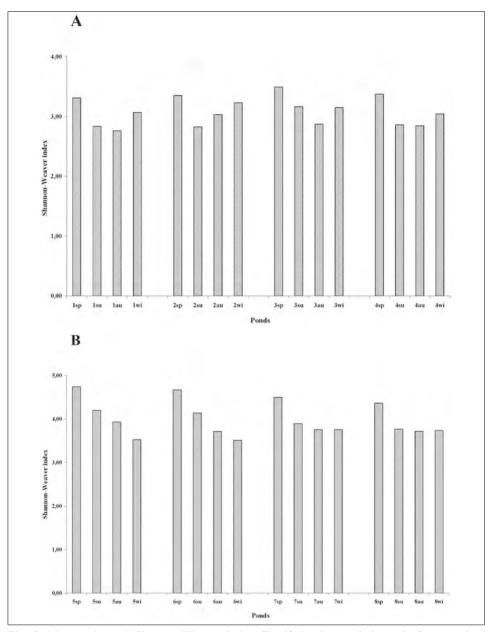


Fig. 5. Mean values of Shannon-Weaver index (D. I.) in the studied ponds (sp = spring; su = summer; au = autumn; wi = winter): (A) ponds 1, 2, 3, 4 and (B) ponds 5, 6, 7, 8.

essentially  $\beta$ -mesosaprobic (indicating moderate pollution) whereas in the ponds of group A diatoms were mainly  $\alpha$ -mesosaprobic (indicating high pollution).

The epiphytic community inhabiting *Ruppia* leaves was very speciespoor, with only 8 taxa recorded (Table 2). It was dominated by Rhodophyta of the

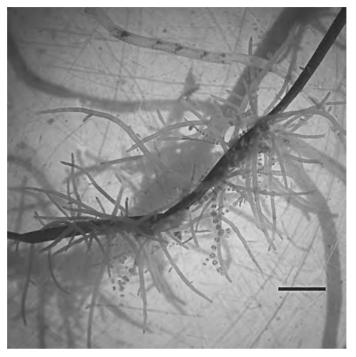


Fig. 6. Epiphytes of Ruppia cirrhosa (Petagna) Grande leaves. Scale bar: 3.5 mm.

genera *Ceramium* Roth, *Chondria* C. Agardh, *Lophosiphonia* Falkenberg and *Polysiphonia* Greville (Fig. 6), and two Bacillariophyta: *Fragilaria ulna* (Nitzsch) Lange-Bertalot and *Nitzschia dissipata* (Kützing) Grunow. Significant variations were found according to the pond and the sampling period. The highest species richness values were recorded in spring, and the highest number of species was always recorded in the ponds of group B (Table 2). Thus, the epiphytic flora of *Ruppia* leaves showed the same spatio-temporal trend found for attached and detached macroalgae. Moreover, species richness and species coverage values were highest on the oldest leaves and near their apices, a pattern already known for most macrophytes (Buia *et al.*, 1985; Cullinane *et al.*, 1985; Reyes *et al.*, 1998; Borowitzka *et al.*, 2006).

Plant coverage was significantly different among ponds throughout the studied period (PERMANOVA, p<0.05; Fig. 7; Table 3), supporting the *a priori* separation of the ponds into two groups A and B (p<0.05). PERMANOVA highlighted significant differences linked to sampling periods both for coverage and general environmental features (Table 3). Analyses on presence data also revealed significant differences (PERMANOVA, p<0.05) between the two groups of ponds A and B (Fig. 8).

#### DISCUSSION

The *Ruppia* system present in the study area showed a simple vertical structure as observed in other Mediterranean *Ruppia* meadows, with two vegetation layers (macrophyte plus algae: Verhoeven, 1980). The number of algal

			Coverage		Environmental setup				
Source	df	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)		
Group	1	88000.0	156.6	***	167.6	1471.2	***		
Period	3	7427.1	67.6	***	49.0	1363.0	***		
Pond (Group)	6	561.9	2488.9	***	0.1	39.4	***		
Group x Period	3	8410.4	76.5	***	30.6	851.7	***		
Period x Pond (Group)	18	109.9	487.0	***	0.0	12.5	***		
Residual	64	0.2		***	0.0				

Table 3. Results of PERMANOVA testing differences among Groups of ponds and periods for a) general coverage and b) environmental setup.

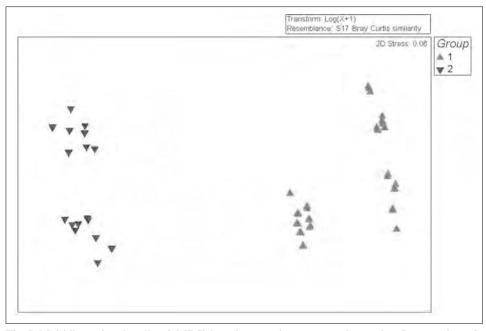


Fig. 7. Multidimensional scaling (nMDS) based on species coverage data, using Groups of ponds as fixed factor.

taxa was not very high, reflecting high environmental stress which appears to interfere with the settling of a well structured algal community.

The species composition of the algal community was similar to that described previously for *Ruppia* beds, whereas the species richness values were higher than those observed in other Mediterranean *Ruppia* systems (27 taxa in Verhoeven, 1980; 32 taxa in Sortino *et al.*, 1981; 29 taxa in Calvo *et al.*, 1982). Total algal coverage, species richness and species coverage showed significant spatial and temporal variations, highlighting a relationship to environmental variables. The separation of the ponds into two main groups (A and B), made *a priori* on the basis of different levels of proximity to the sea and anthropogenic pressure, was confirmed by PERMANOVA. Present results support the existence of

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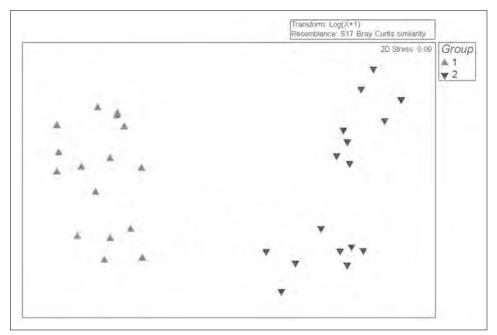


Fig. 8. Multidimensional scaling (nMDS) based on species presence data, using Groups of ponds as fixed factor.

significant differences among temporal periods due to differences in salinity and temperature (Sarà, 2009).

Filamentous and foliose algae (mainly Chlorophyta and Rhodophyta) characterised by short-lived and fast-growing thalli (i.e. opportunistic, r-strategist algae), were well represented in all samples. The dominance of r-taxa and the very small number (in ponds of group B) or absence (in ponds of group A) of k-taxa appears to confirm the conditions of high environmental stress in this system, where only species characterised by high tolerance to environmental variations and fast turnover may survive and reproduce. The dominance of opportunistic algae also confirms the presence of the anthropogenic pressure in these ponds. Indeed, the ratios between annual and perennial macroalgae is considered a potential indicator of eutrophication, as high nutrient concentrations favour the growth of ephemeral algae (Möller et al., 2009 and references therein). The dominance of mesosaprobic diatoms (indicators of moderate or high pollution) also confirms anthropogenic pressure in this system. The reduced number of perennial species could be also related to highly mobile substrata, which tend to inhibit the attachment and growth of macroalgae and favour thin and fast-growing opportunistic species (mainly Chlorophyta and filamentous Rhodophyta: Wallenstein et al., 2009).

In the studied ponds the recruitment of algae appears to be linked to the proximity to the sea, as highlighted by the highest species richness values recorded for the ponds of group B (closer to the sea and thus with a greater recruitment). When macroalgae reach the ponds and settle in, they develop floating forms on the surface ("acropleustophytic": *Cladophora* and *Ulva*) and/or lying and rolling forms on the sandy bottom ("bentopleustophytic": *Chaetomorpha*), which may

allow them to adapt well to the peculiar environmental conditions. Therefore, the dominance of detached algae (mainly filamentous and foliose Chlorophyta) recorded in all ponds suggests that these peculiar environmental conditions enhance the competitiveness of detached forms, that dominate on the other ones (attached and epiphytic).

The dominance of Chlorophyta and Rhodophyta, together with the very small number of brown algae, confirms the effectiveness of using the R/C index instead of the R/P (Rhodophyceae/Phaeophyceae) index to describe the water quality of shallow systems. Species composition and coverage appears to be strongly related to anthropogenic pressure. Especially during the summer, in the ponds of group A (which are subjected to a higher anthropogenic pressure) *Ulva* species typical of disturbed habitats with a high availability of nutrients reached the highest coverage values, causing significant detrimental effects (e.g. shading) on the *Ruppia* bed, limiting plant growth. By contrast, in the ponds of group B (which are subjected to a lower anthropogenic pressure) the highest total coverage of *Ruppia*, were observed.

The epiphytic community inhabiting *Ruppia* leaves was very speciespoor, with a smaller number of species compared to the epiphytic community of other aquatic macrophytes (Borowitzka *et al.*, 1990; Mazzella *et al.*, 1994; Rindi *et al.*, 1999; Piazzi & Cinelli, 2000).

The structure of the epiphytic community of macrophyte leaves is known to be significantly related to several factors including availability of propagules, leaf morphology and turnover, and the surrounding environment (Dalla Via *et al.*, 1998; Piazzi *et al.*, 2004; Borowitzka *et al.*, 2006). The results of this study suggest that leaf structure and turnover of *Ruppia* plants, together with environmental conditions, are not favourable to the attachment and settling of a structured epiphytic community.

Moreover, these results suggest that the algal community inhabiting this *Ruppia* system is not a result of a random aggregation of species and is significantly affected by environmental conditions. Temperature and salinity appear to be significantly related to temporal variations of the algal variables under study, whereas spatial variations appear to be strongly affected by the proximity to the sea and anthropogenic pressure. Proximity to the sea, which influences the recruitment of algae, would affect species richness; anthropogenic disturbances, which favour the settling of opportunistic, *r*-strategist algae characterised by high resistance and resilience capacity (*sensu* Connell & Sousa, 1983), would affect species composition. Overall this study supports the use of species as indicators to detect community shift in relation to environmental variations.

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