Transitional Waters Bulletin
TWB, Transit. Waters Bull. 4 (2010) n. 1, 35-52
ISSN 1825-229X, DOI 10.1285/i1825229Xv4n1p35
http://siba-ese.unisalento.it

RESEARCH ARTICLE

Factors affecting changes in phanerogam distriburion patterns of Orbetello lagoon, Italy

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Abstract

1 - In this study recent changes of the distribution of three phanerogam species (*Ruppia cirrhosa, Cymodocea nodosa*, and *Nanozostera noltii*) were studied in the Orbetello lagoon, a meso-eutrophic and human stressed ecosystem.

2 - The aim was: I) to produce SURFER distributions maps, and II) to statistically evaluate significant changes in the spatial distribution taking into account two different factors (*basin* and *year*). For these reasons, percentage of coverage (CP) of each species was estimated in 38 sites equally distributed inside the Eastern and Western basins both in summers 2003 and 2009.

3 - The geostatistical maps evidenced changes in phanerogams distribution during the studied period but only variations observed for the *N. noltii* species at basin level resulted significant on a statistical basis. Significant differences at basin level were also obtained by the multivariate analysis. These results might be explained by the different ecological characteristics existing between Western and Eastern basins.

Keywords: phanerogams, transitional water, eutrophication, coverage (%).

Introduction

Coastal lagoons are naturally stressed ecosystems which suffer from frequent environmental disturbances and fluctuations (Barnes, 1980; Kjerfve, 1994). High biological productivity rates (Whittaker, 1975) are related both to the geomorphologic characteristics of these systems and to external factors (i.e. wind intensity, sea-lagoon water exchanges, human pressure) which influence general hydrodynamics, abiotic and biological relationships and which determine the large physical and ecological boundaries and gradients characterizing lagoons (Kormas *et al.*, 2001; Hung and Kuo, 2002; Muslim and Jones, 2003; Newton *et al.*, 2003; Zaldivar *et al.*, 2003). If nutrient inputs are moderate, pelagic primary production show a low development of macroalgae and phanerogams dominance, on the contrary, if concentrations of bioavailable nutrients are high, primary production can be based on macroalgae and/or microphyes supporting the whole trophic webs (Terrados and Ros, 1992; Knoppers, 1994; Scheffer, 1998; Souza *et al*., 2003, Viaroli *et al*., 2008). Excessive increases in nutrient loads, both from natural and/or human origin, determine in water ecosystems the occurrence of eutrophication consequences (Morand and Briand, 1996) which produce changes in abiotic matrices such as the column water and surface sediments (Chessa *et al*., 2005), zoological, and phytosociological assemblages or communities (Orfanidis *et al*., 2008; Viaroli *et al*., 2008). Relationships among sediment characteristics (i.e. pH, ORP, Eh, grain–size, nutrients, sulphide) and phanerogams distribution were observed in many studies (Giusti *et al*, 2010; Renzi *et al*., 2007; Van Katwijk and Wijgergangs, 2004; Chau, 2002; Azzoni *et al*, 2001; Miller and Sluka, 1999; Viaroli *et al*, 1996; Goodman *et al*, 1995; Short, 1987; Ferrari *et al*., 1972;) evidencing sediment as a key element for the plants establishment, presence and recolonization after the occurrence of environmental crises (Plus *et al*., 2003). Furthermore, phanerogams actively contribute to the regulation of the oxidation level in sediments by spreading the oxygen produced by photosynthesis from the rhizosphere (Sand-Jensen *et al*., 1982; Pedersen *et al*., 1998) and to the reduction of system turbidity (Mannino and Sarà, 2006).

A recent study performed in 12 coastal systems, evidenced the progressive declines of about the 65% of phanerogams and of the 48% of other submerged aquatic vegetation (SAV) *taxa* during the past 150-300 years (Lotze *et al*., 2006). The observed decrease is referable to the occurrence of different factors such as chemical pollution, water eutrophication, physical impacts, modifications of the trophic structure, and impacts produced by urban settlements (Duarte, 2002; Orth *et al*., 2006; Short *et al*., 2006). Worldwide, significant management efforts are underway to restore the extent and the water quality of transitional waters with the aim to recover productivity and habitat value of these important ecosystems (Lirman *et al*., 2008). Phytosociological dominance is a complex and not jet well understood phenomenon which depends on multifactor levels of interaction between abiotic and biological factors and the relative importance among each variable actually is not completely explained. Furthermore, fluctuations on yearly basis produce, as ecological effect, rapid changes in population settlements, and in phanerogams distribution (Orfanidis *et al*., 2008).

The Orbetello lagoon represents a coastal ecosystem widely studied by scientific communities during the latest thirty years. The main abiotic characteristics of the study area, such as sediment grain-size, water fluxes, bottom morphology, etc, were detailed characterized and constantly monitored such as human hot-spot pollution sources (Focardi *et al*., 2009; Specchiulli *et al*., 2008; Renzi *et al*., 2007, 2009). Macrophytes dominance assessment of this ecosystem evidenced frequent changes during the time principally related to water eutrophication phenomena as documented by the literature (Lenzi *et al*., 2003; Bombelli and Lenzi 1996). Even if management strategies performed in this ecosystem have notably reduced water eutrophication, macroalgae and phanerogam dominances always shows frequent changes both in spatial distributions and in quantity (Lenzi *et al*., 2003). Relationships among phanerogams distributions and ecological factors which characterize lagoon systems have poorly been explored by the literature. Choosing the Orbetello lagoon as study area, in this paper authors aimed: I) to produce SURFER distribution maps for three phanerogam species (*Ruppia cirrhosa*

(Petagna) Grande, *Nanozostera noltii* (Horneman) Tomlinson Posluzny, *Cymodocea nodosa* Ucria), II) to evaluate statistically significant changes in the spatial distribution of species at two different factors (*basin* and *year*).

Materials and methods

The Orbetello lagoon

The Orbetello lagoon is located in Southern Tuscany (Italian West Coast), between 42°25' and 42°29' Lat. North and 11°10' and 11°17' Long. East (Figure 1).

The wetland covers a total surface of 25.25 km^2 and it is divided by a dam in two communicating basins known as the Western (W) and Eastern (E) ones of about 15.25 and 10.00 km^2, respectively. The mean water depth is 1.20 m, ranging from 0.30 to 1.70 m with maxima reported for the lagoon centre of both basins (Specchiulli *et al.*, 2008). Its geomorphology and the presence of a dam drastically reduce water circulation. Exchanges with the sea occur by artificial pumping of sea water throughout two canals located in W basin and named Fibbia and

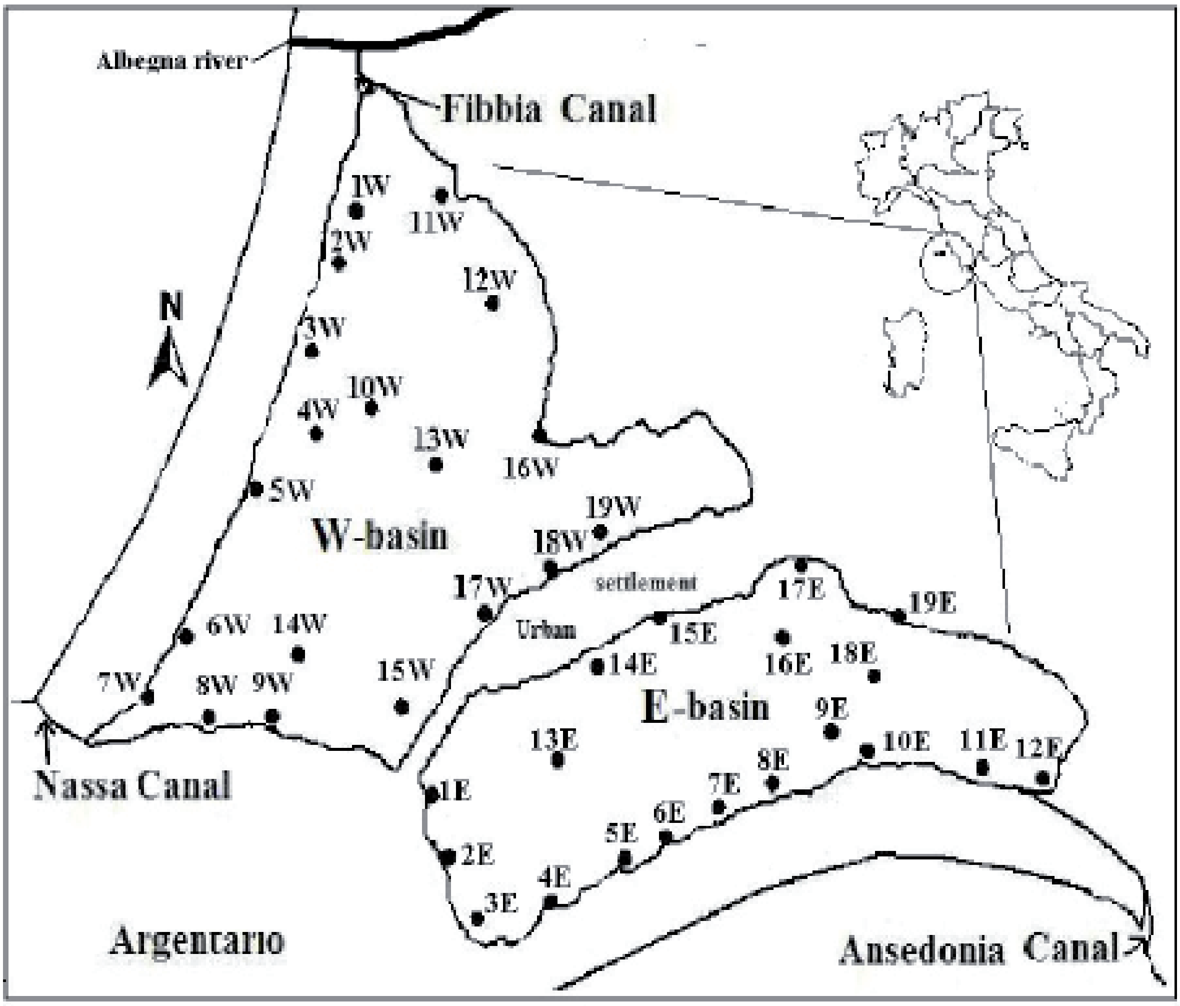


Figure 1 - Sampling sites inside the Orbetello lagoon. W basin = Western basin, E basin = Eastern basin.

Nassa. The E basin receives water throughout the dam and discharges its outflow water in the sea by the Ansedonia canal. Winds show marked seasonal variability concerning both intensity and direction with a dominance of NW and SSE during the winter and frequencies of 7 and 10% respectively (Aminti *et al.*, 2003). Minima in water temperatures and the increase of water oxygenation and turbidity during the winter are aspects associated to the occurrence of the strongest winds (>15.4 ms^{-1}). Previous studies evidenced as the Albegna river which constitutes the principal freshwater input (15 $m3s^{-1}$), could represents for the Orbetello lagoon also a significant contribute of nitrogen, phosphorus and total organic charge. In fact, chemical analyses performed in the Albegna water showed nitrogen and phosphorous levels of 2,934 and 1,478 tonnes *per* year respectively with a total organic charge of 220,219 inhabitants equivalents *per* year (ARPAT, 2007). The meso-eutrophic characters of this ecosystem has been evidenced by previous studies and further general data concerning hydrodynamics, winds, and the structure of this ecosystem are available in Lenzi *et al.* (2003), Giusti and Marsili-Libelli (2006), and Specchiulli *et al.* (2008). From May to September, to face the eutrophication phenomena, exchanges among the lagoon water and the sea are forced by pumping to flux from the Nassa and Fibbia canals towards the Ansedonia ones (Lenzi *et al.*, 2003; Renzi *et al.*, 2007). During this flux, both the evaporation and the nutrient release phenomena from sediments towards the water column, determine a general increase of nutrient and salinity levels in the E basin (Specchiulli *et al.*, 2008), on the other hand without the forced circulation the water quality could be even worse (Lenzi, 1992). Effluents of various human activities such as municipal wastewater treatment plants (Renzi *et al.*, 2009), industrial activities (Focardi *et al.*, 2009), urban settlement (Specchiulli *et al.*, 2010), and intensive aquaculture farms (Porrello *et al.*, 2005) heavily stress the whole ecosystem from well localizable hot spot sources (Lenzi *et al.*, 2003). A synthesis

Table 1 - Main human pollution sources which affected the study area.

Description	**Localization inside the Orbetello lagoon**	**Reference**
iron-manganese mines	Basin: Eastern *sampling replicates*: 1E, 2E, 3E	Focardi et al., 2009
urban settlement	Basin: Eastern and Western *sampling replicates*: 15W, 17W, 18W, 14E, 15E, 17E	Perra et al. (in press) Renzi et al., 2009 Specchiulli et al. (in press)
municipal wastewater treatment plant effluents	Basin: Western *sampling replicates*: 19W	Renzi et al., 2009
effluents from fish farm activities	Basin: Eastern and Western *sampling replicates*: 6W, 7W, 8W, 11E, 12E	Porrello et al., 2005 Renzi et al., 2007

Table 2 - Basic abiotic factors of two different lagoon basins. Principal characteristics related to water and sediment matrices in both sediments are reported as range of variation. Data are extracted from Specchiulli et al., 2008 (*) and Renzi et al., 2007 (**). Notes: Water variables, T = temperature, S = salinity, DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorous, Chl-a = chlorophyll-α, Sediment variables, pH = pH, Eh = redox potential, TP = total phosphorous, silt = particles with diameter inferior than 63 µm, nr = not reported, # = maximum value recorded in correspondence of the Municipal Waste Water treatment plant effluent.

Basin	Water (*)			Sediment (**)	
	Variables	Winter	Summer	Variables	year 2003
Eastern Basin	T (°C)	4-11	nr	pH	7.4-8.2
	S (‰)	30-35	nr	Eh (mV)	<-280, -210
	DIN (µM)	25-75#	2-12	TP (mg kg^{-1})	0.015-0.050
	SRP (µM)	0.2-0.4	0.5-0.7	silt (%)	0-90
	Chl-a (µgL^{-1})	5-7	2-7		
Western Basins	T (°C)	6-7	nr	pH	<7-8.6
	S (‰)	20-35	nr	Eh (mV)	-250, -170
	DIN (µM)	25-45	2-12	TP (mg kg^{-1})	<0.005-0.04
	SRP (µM)	0.2-0.4	0.4-0.7	silt (%)	0-70
	Chl-a (µgL^{-1})	4-6	2-5		

concerning the principal human pollution sources which could represent a significant impact in this ecosystem is reported in Table 1, whereas principal physico-chemical characteristics of both lagoon basins obtained by the literature are reported in Table 2.

Samplings

Thirty eight sites equally divided among W and E basins were sampled in summers 2003 and 2009 (Figure 1). Samplings were performed according to a logic model to reduce type I errors (Underwood, 1992; 1993; Benedetti-Cecchi, 2004) and based on two factors: *basin* (two levels, fixed), *year* (two levels, fixed) (Figure 2). Total observations performed were 76. Geographical locations of sampling replicates were randomly extracted from a squared subsample grid of 1x1 km^2 length. This method was selected to reduce the sampling error (Cochran, 1977). Stations characterized by high turbidity levels were excluded due to the impossibility to perform visual samplings and, as consequence, sampling sites localized in deeper areas were underestimated. Extracted coordinates were in situ localized using a Global Positioning System (Garmin, mod. *e*-trex legend) and georeferenced to a topographic map of the same area from the regional cartography system (2 m of resolution) on a system of geographic reference (UTM), with international ellipsis WGS84. In a previous phytosociological study performed by Lenzi (1984) in the Orbetello lagoon, a 1m^2 surface was selected as sampling unit. This surface, in lagoon ecosystems, could not constitute the minimal area of sampling, according to Boudouresque (1970). To equilibrate the low extension of the sampling unit adopted, the number of replicates in that study was very high, upper than 300 (about one sample every 8.7 ha). A sampling strategy equilibrated by a high frequency (one sample every 4 ha) was also chosen by Ferrari *et al*. (1972) for the characterization of the SAV in the Comacchio lagoons. In this study, an opposite criteria was selected: the

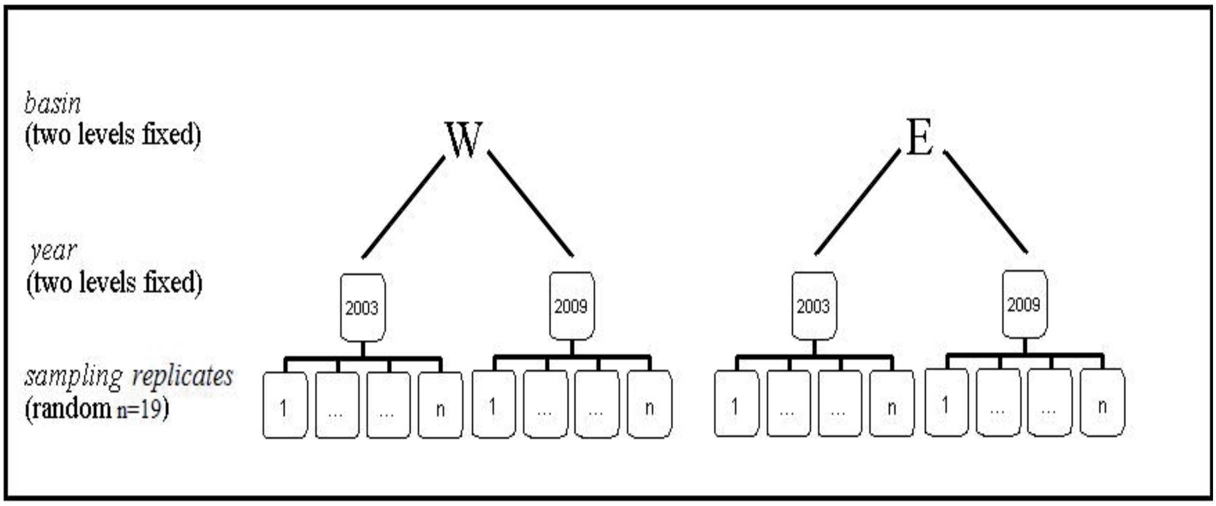


Figure 2. Logical model applied for the sampling design.

number of sampling replicates was reduced instead the samplings surface was increase, as adopted by other studies performed in lagoon ecosystems (Giaccone and Piccoli, 1974; Giaccone, 1974; Cognetti *et al*, 1978). For this reason, a sampling surface of 9 m^2 (3x3 m), was chosen. This sampling strategy allowed to include the minimal sampling area and to better individuate the blended patches of the three phanerogams which otherwise would have been difficult to assess using a 1 m^2 surface. At each sampling replicates, the percentages of coverage (CP) of *C. nodosa.*, *R. cirrhosa.* and *N. noltii* expressed on the whole sampling surface, were visually estimated according to Boudouresque (1970). Phanerogams samples were collected in plastic bottles and carried to the laboratory with the aim to verify acquired filed data. Species determinations were performed using an Optika SZR10 microscopy. Observations considered of particularly ecological significance which were acquired closed but not inside the sampled sites were evidenced in SURFER distributions but excluded from the statistical analyses.

Statistical analyses

Spatial distributions were obtained using a geostatistical gridding method, the kriging (Matheron and Armstrong, 1987) which produces visually appealing contour and surface plots from spaced data. Contours were constructed from data using computer package SURFER v8.0. The multivariate analyses performed to evaluated the statistical significance of observed distributions in a multivariate dimension, were developed using Primer E Software package v6.0 (Plymouth Marine Laboratory, UK) according to Clarke and Warwick (2001). Bray-Curtis distances resemblance matrix was calculated after log(x+1) and successively square root (√) transformations of field data; the aim of this procedure was to reduce the dataset variability and, in particular, to estimate the statistical effects due to the rarest species (*C. nodosa*) (Clarke and Green, 1988). Logarithmic transformation using the log(x+1) was necessary to eliminate the zeros which are present in abundances acquisitions. The significance of distributions relating to selected factors were tested by the application of the ANOSIM (Analysis of similarities) test statistic R one-way which was performed associated to the Pairwise test and imposing the run of 9,999 permutations. This procedure allows testing hypothesis for differences between groups of samples, using permutation/randomisation methods on resemblance matrix. Statistical analyses were performed to explore significance of distributions concerning considered the factors *a priori* defined (Table 1). To explain results obtained by the ANOSIM test performed on the factor year, the non-metric multi dimensional scaling (*nm*MDS) technique was applied to explore variables dissimilarities performing runs by the application of the Kruskal stress formula 1, imposing minimum stress of 0.01 and restarting the process 50 times. The diagram obtained was superimposed to the results obtained from the cluster analysis in order to evidence the level of significance of observed segregations. The ANOVA one-way analysis of variance were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, *www.graphpad.com*) to explore the effect of the factors *basin* and *year* on the distribution of each species separately.

Results

The spatial distributions of the considered species, obtained for both the sampling campaigns (2003, 2009), were reported in Figures 3-5. The distribution of *C. nodosa* didn't show a significant variation during the observation period (Figures 3a, b, Table 3). However, new localized settlements were observed closed but not inside the sampling sites selected for this study (9W; 10E) in 2009. Due to the importance of this observation, acquired data were noticed with a circle (Figure 3b).

On the contrary, the SURFER distribution maps evidenced the occurrence of changes in *N. noltii* and *R. cirrhosa* distributions.

In particular, a significant contraction of the surface covered in W basin and the disappearance of settlements in E ones, were evidenced for *N. noltii* in 2009 (Figures 4a, b, Table 3). *R. cirrhosa* (Figures 5a, b, Table 3) showed a non significant decrease of covered surface in both basins with the disappearance of meadows localized near the dam. Nevertheless, higher CP were reported near the E sandbar (5E-10E) and in the lagoon area major influenced by the Albegna river inlet (1W; 2W). Furthermore, a new low-density meadow localized near the urban settlement (15E-17E) was reported in E basin.

Table 3 - Comparisons between basin and year of phanerogams using (one way) Anova. ns = not significant.

Species	*basin*	*year*
Ruppia cirrhosa	ns	ns
Cymodocea nodosa	ns	ns
Nanozostera noltii	F=0.053 p=0.009	ns

The significance of the observed phanerogams distributions was explored in relation to different factors (*basin*, *year*) according to a multivariate procedure. Concerning the factor *basin*, the ANOSIM test produced a global R value of 0.03 (with a level of significance of sample statistic, indicated as *p*, of 4.1%, and a number of permuted statistics greater than or equal to global R, indicated as NPS, of 412) (Table 4). On the contrary, results obtained on the factor *year* evidenced no significance (global R value of 0.001; *p*=38.3%). The *nm*MDS performed on variables and superimposed to the cluster

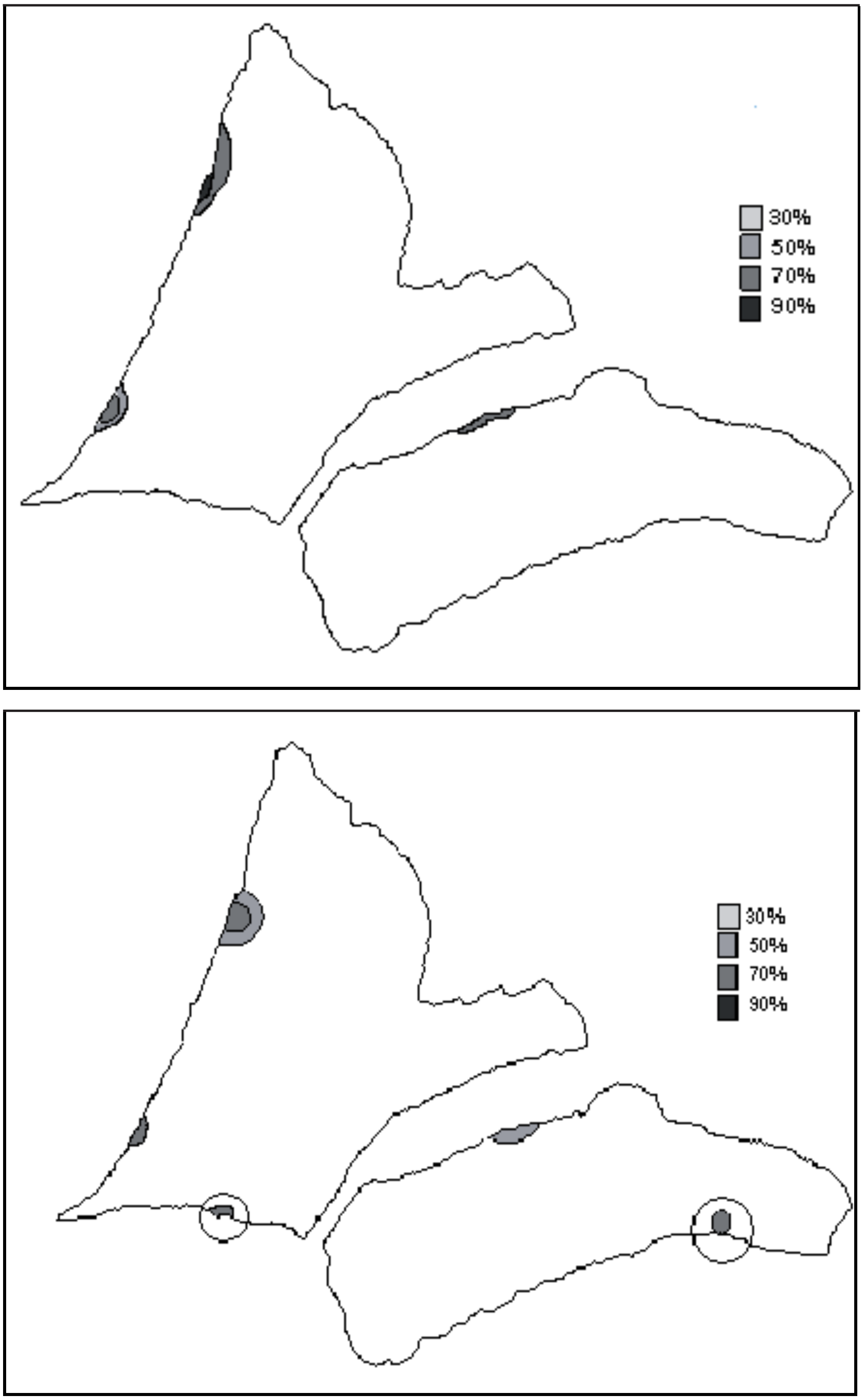
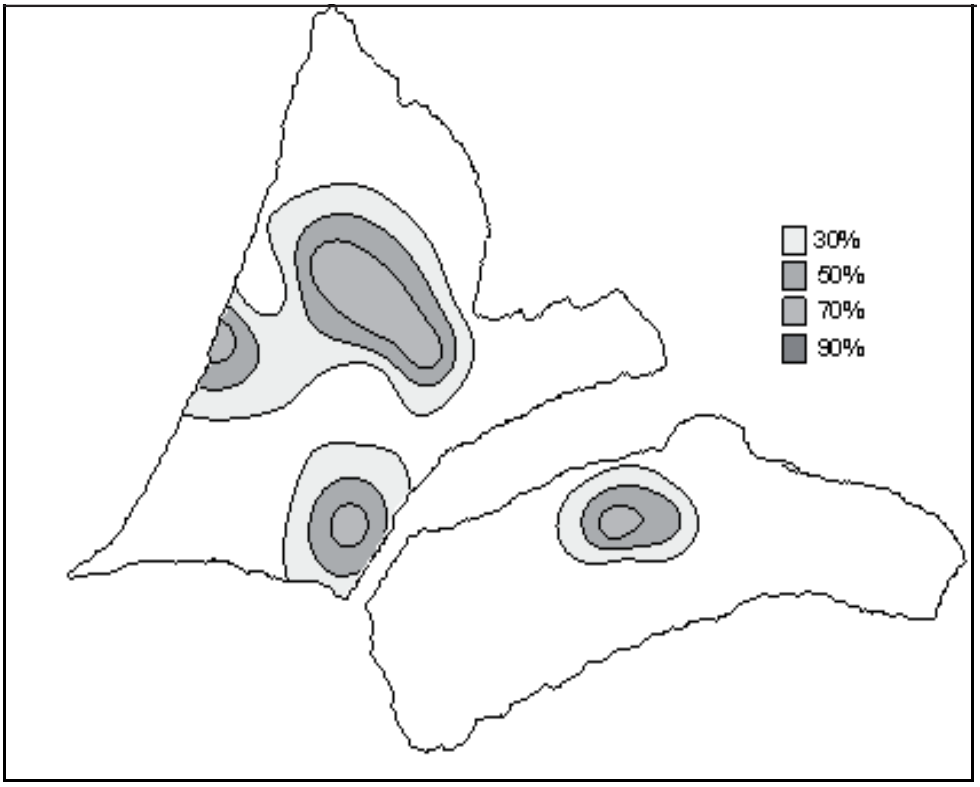


Figure 3. Surfer distribution map of *C. nodosa* in the years 2003 (a) and 2009 (b). Results are represented as percentage of coverage (CP).



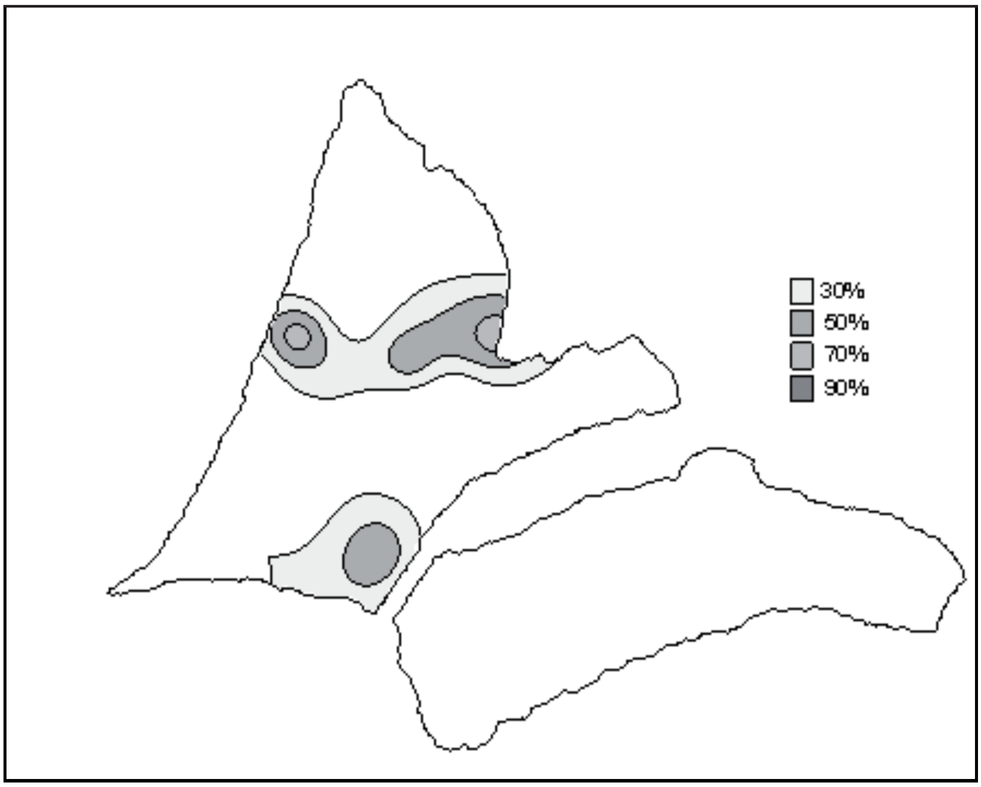
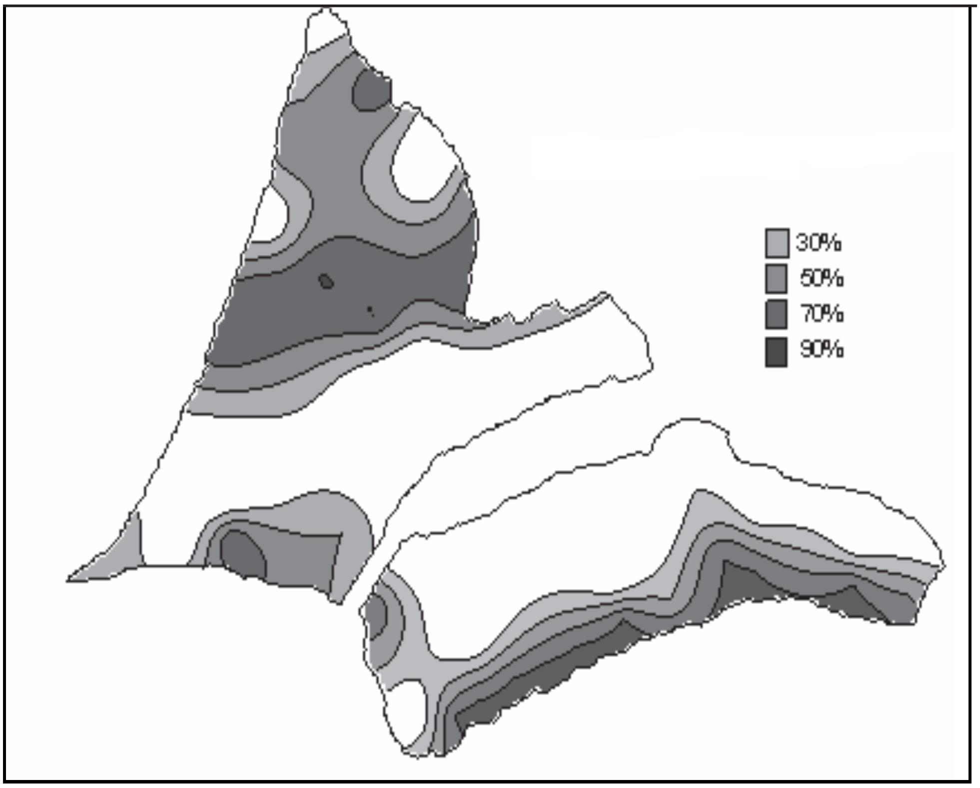


Figure 4. Surfer distribution map of *N. noltii* in the years 2003 (a) and 2009 (b). For more information see Figure 3.



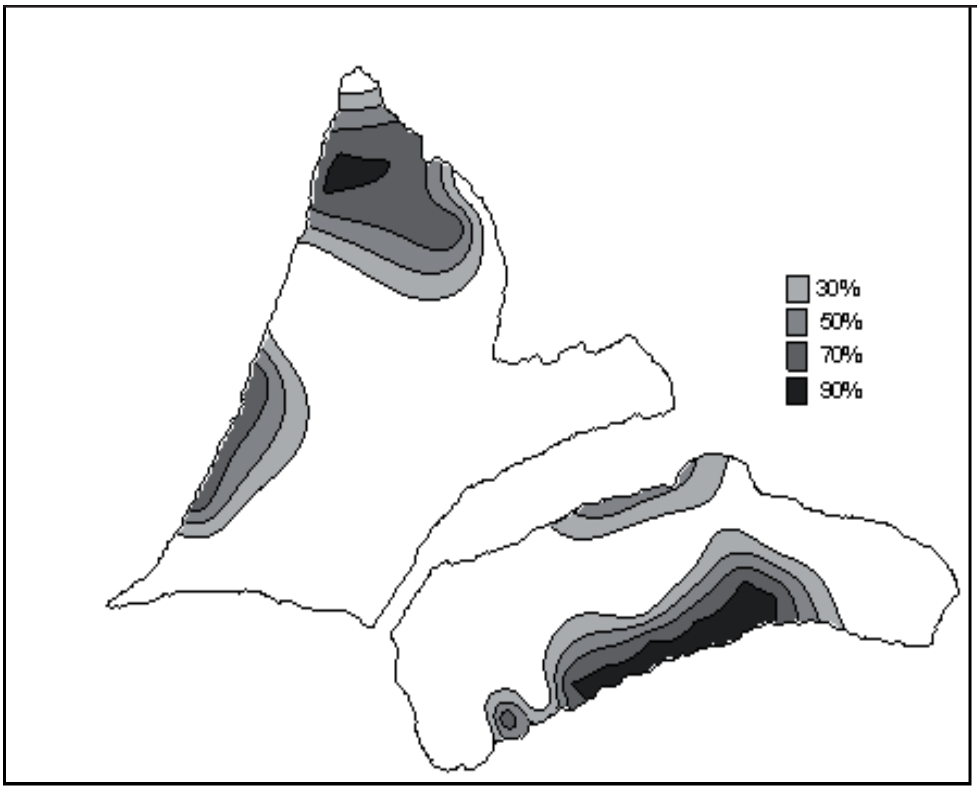


Figure 5. Surfer distribution map of *R. cirrhosa* in the years 2003 (a) and 2009 (b). For more information see Figure 3.

Table 4 - Comparisons between basin and year of phanerogams using multivariate statistical analyses.

Factor	R	*p* (%)	NPS
basin	0.030	4.1	412
year	0.001	38.3 (ns)	-

analyses were reported at different levels of similarity (Figure 6). Comparing projections obtained for each species related to each year, results evidenced the absence of differences for *C. nodosa* and *R. cirrhosa* (both within the 60% of similarity). Concerning *N. noltii*, distributions among *years* were within the 30% of similarity.

On a multivariate basis, the presence of different trend related to the *year* of each species produced a not significant global effect when the whole database was considered. To support the significance of obtained results, collected data were explored considering factors *basin* and *year* by the application of the univariate approach on each species separately.

In Figure 7, results obtained comparing

Figure 6. nmMDS performed on variables superimposed to the cluster analysis. Number = year, C = *C. nodosa*, N = *N. noltii*, R = *R. cirrhosa*.

averages ± standard deviations data were reported for each species and both years. Even if some differences were observed, results obtained by the coupled comparison among *year* of sampling, were within the standard deviation ranges for each species.

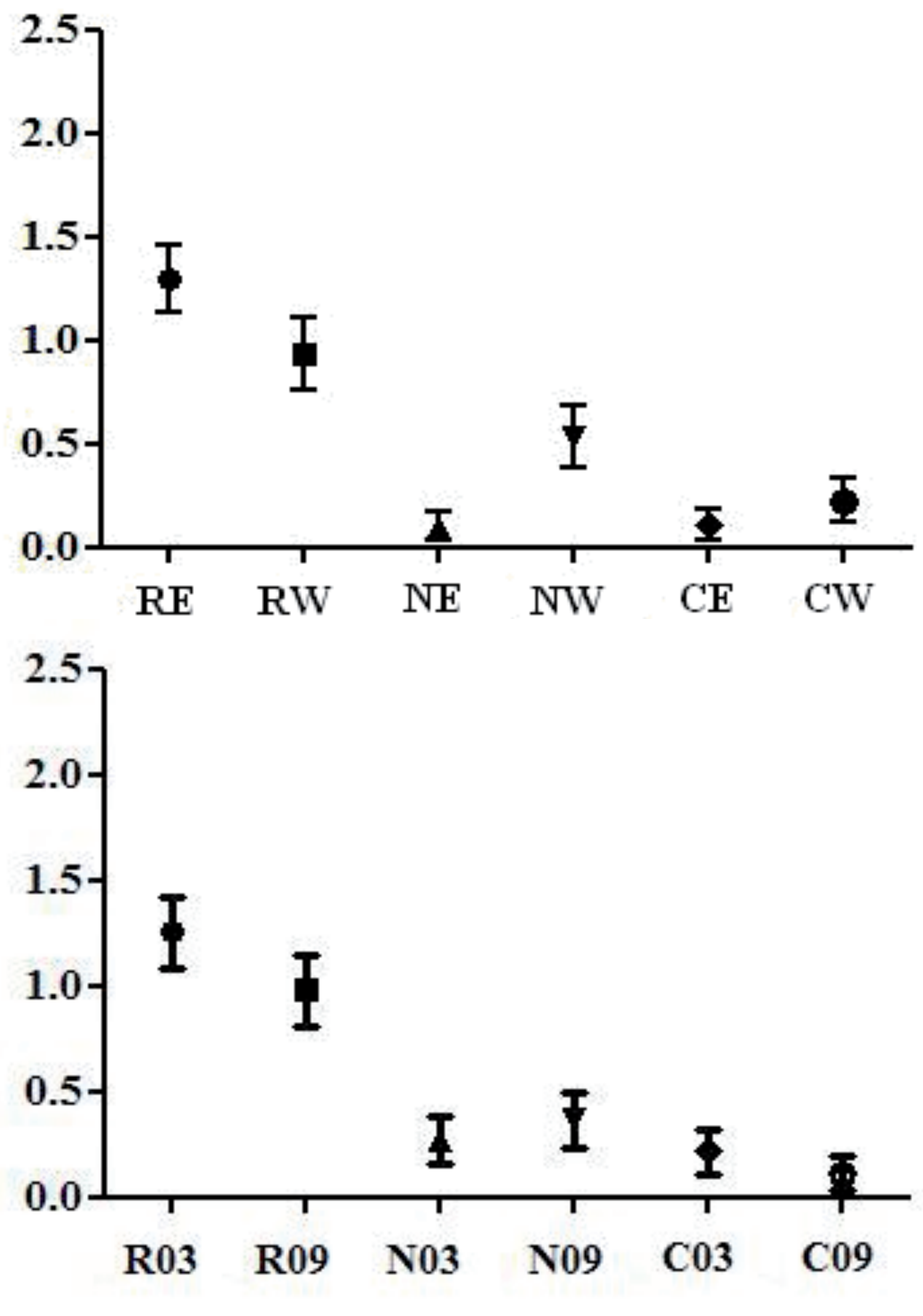


Figure 7. Average values of the percentage of coverage(CP) for each species related to the factors basin (a) and year (b). Notes: E = eastern basin, W = Western basin. Number = year, C = *C. nodosa*, N = *N. noltii* R = *R. cirrhosa*. For more information see Figure 6.

Discussion

The major difference in phanerogam spatial distribution among the two studied years, was observed for *N. noltii*, which evidenced a general reduction and a regression from the central areas of the E basin. A reason for this observation could be due to the ecology of this species and to the different ecological characteristics of the Orbetello lagoon basins. In previous studies, as show in Table 2, the E basin evidenced a higher salinity and nutrients concentration in water column than the W ones, as a consequence of the water circulation. As showed by Hootsmans *et al*. (1987) and Loques *et al*. (1990), the best germination efficiency occur when salinity ranges within 1-30 ‰. The spread difficulty encounter by *N. noltii* in the Orbetello lagoon is probably due to the water salinity, which remains during the year higher than the optimum range observed for this species. Furthermore, recent studies evidenced that this species tends to enhance its canopy related to the increase of nutrients concentrations in sediments, whereas higher concentrations in water column determine an opposite response, promoting the shoot mortality and/or reducing the shoot recruitment (Cabaço *et al*, 2008). In W basin, a general trend towards the reduction was observed even if the effluents of the municipal waste water treatment plants, that discharged near the station 19W, were drastically reduced from the end of 2006. This phenomenon was similar to the general trend of *N. noltii* observed by Bernard *et al*. (2007) in the Berre lagoon where nutrient inputs are liable for settlement regression even when urban and domestic pollution were remove. According to the authors, a possible competition with other phanerogams species can't be excluded, in fact, in E basin the reduction of the *N. noltii* was observed in 2009 whereas, a contextual increase in correspondence of the same sites, was recorded for *R. cirrhosa*.

Nevertheless, also *R. cirrhosa* seems to be affected by a general decrease in W basin, because it was sampled during 2009 in less sites than in 2003. In the E basin, on the contrary, *R. cirrhosa* showed a significant increase of CP in 2009, where it was partly replaced by *N. noltii*. These results agree to the prediction model proposed by Giusti and Marsili-Libelli (2006) and Giusti *et al*. (2010), for which it is provided an expansion of this species towards the central E basin from the meadows previously located near the Feniglia sandbar. According to the authors, this development was sustained by a closed water circulation which favours seeds deposition. Another possible reason of this observation may be related to the ecology of *R. cirrhosa* which is known to be able to tolerate higher overall salinity range than *N. noltii* (Verhoeven 1975; Den Hartog, 1970). This observation was also supported by the "confinement concept" for lagoon environment and the relative zonation, proposed by Guelorget and Perthuisot (1983). According to these authors the species *R. cirrhosa* was assigned to the IV zone, typical of lagoon ecosystem, where the other marine species disappear.

Furthermore in Orbetello lagoon, previous studies (Renzi *et al*., 2007; Bombelli and Lenzi, 1996) detected that in meso-eutrophic conditions *R. cirrhosa* tends to colonize mainly close sandbar sandy bottoms, characterized by lower nutrient levels and higher redox potential values than those observed in the central lagoon silty sediments. According to these observations, the phanerogams settlements could vary also on yearly scale, in relation to sediments conditions.

C. nodosa prefers lagoon areas characterized by frequent exchanges with sea water, a stronger influence of tidal effects, and bottoms constituted by coarse and sandy sediments characterized by lower nutrient levels, as observed also by Sfriso and Ghetti (1998) in the Venice lagoon. In the Orbetello lagoon, such characterized areas cover a lower surface if compared to low water circulation ones dominated by silts in bottoms (Renzi *et al.*, 2007). Is probably due to this reason that *C. nodosa* colonizes only a little and quite constant surface inside the Orbetello lagoon, where, during the relative short-term considered, it has showed a not significant variation of CP. Available literature evidenced that this species shows a prevalence of the sexual reproduction towards the asexual ones, with a high seeds mortality associated (>70% during the first year). Furthermore, the growth rates of new colonized areas are reported to be low and increase with the increasing of the patch dimensions (Duarte and Sand-Jensen, 1990). The diffusion of *C. nodosa* meadows inside the lagoon system is probably, for such a reason, limited by the existing conditions.

The statistical analyses evidenced that the factor *basin* was able to determine different phanerogams assemblage, probably related to the different substrate composition and ecological characteristics of the two basins (Cognetti *et al.*, 1978; Verhoen, 1979; Mannino and Sarà, 2006; Renzi *et al*, 2007). However, the phanerogam settlements recorded during the monitoring period showed not significant changes.

Conclusions

Results obtained in this study evidenced that the phanerogam settlement inside the Orbetello lagoon is significantly affected by the different ecological characteristics among basins. On the contrary, not significant changes were recorded concerning the year of sampling. On the basis of the results obtained in this study, the discrimination among factors which could have determined this apparent stability could not be performed. In fact, this result could be due to an effective stability of the trophic level of this system obtained by the management actions performed to face the eutrophication phenomena which could have contribute significantly to establish constant trophic levels inside this lagoon, determining a relative consequent stability of the phanerogam distributions observed in 2003 and 2009. Another hypothesis is that changes could be occurred at a frequency higher than the temporal scale adopted in this study. Further monitoring programmes performed on these species at a lower time intervals associated to the measurement of nutrients in water could be useful to clarify if fluctuations related to the time are occurring.

Acknowledgement

Authors are grateful to the Orbetello Pesca Lagunare society to their support in field activities.

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