

## ORIGINAL ARTICLE

# Temporal variations in the spatial distribution of shoot density in a *Posidonia oceanica* meadow and patterns of genetic diversity

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## Keywords

Kriging; meadow structure; *Posidonia oceanica*; shoot density; genetic diversity.

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## Abstract

The meadows of the Mediterranean endemic seagrass *Posidonia oceanica* exhibit relatively high variations of structural and biometric features at various spatial scales. An investigation performed in 1992 in the meadow off Lacco Ameno (Island of Ischia, Gulf of Naples, Italy) detected peculiar spatial patterns of plant distribution, characterized by nestlike structures with radially increasing or decreasing shoot densities. Eight years later (2000), geo-referenced collections at selected points were repeated to trace the temporal variations of shoot density and investigate the recurrence of the density cores previously detected. In addition, shoots for molecular analyses were collected to check the hypothesis that nestlike patterns exhibit highest levels of genetic variability, due to the confluence of several genetically distinct stolons. The 2000 survey confirmed the presence of the main density cores detected in 1992, although their spatial distribution was slightly shifted and a general decrease of spatial anisotropy was observed, probably due to an increased disturbance, mainly due to pleasure boat anchoring. Patterns of genetic diversity showed a more complex picture, well related to the shoot density spatial pattern, especially when compared with the previous 1992 survey. Patterns of genetic diversity confirmed our previous hypotheses on the genesis of shoot density cores, suggesting they are produced over long time, due to a slow stolonization process and a convergence of different genotypes. Regression of the meadow and decrease of density may lead, in short periods, to a homogenization of the density patterns, while genetic diversity cores represent a long-term 'memory' of their previous distribution.

## Problem

*Posidonia oceanica* (L.) Delile is an endemic seagrass of the Mediterranean, where it forms dense meadows, often continuous along bathymetric gradients, which may be considered as 'climax' habitats for several associated plant and animal species (Mazzella *et al.* 1992a,b; Ott & Mazzella 1992). Its stands may occur in patches of various sizes or in continuous meadows (Buia *et al.* 2004; Borg *et al.* 2005). Their extension and architectural features, determined by both internal regulative mechanisms and environmental factors (*i.e.*, irradiance, seafloor morphology,

sedimentation rate, water quality, *etc.*), dramatically influence the structure and dynamics of the whole seagrass ecosystem (Mazzella & Buia 1989; Buia *et al.* 2000). Previous investigations demonstrated that several attributes of meadows (*i.e.*, shoot density, leaf morphology, epiphyte biomass, plant growth rate, genetic structure, *etc.*) exhibit variations at different spatial scales (Procaccini *et al.* 2001; Oliva 2002; Gobert *et al.* 2003; Balestri *et al.* 2004; Kendrick *et al.* 2005a, b; Zupo *et al.* 2006) and may also be influenced by human impacts (Mazzella & Buia 1989; Ruiz & Romero 2003; Balestri *et al.* 2004; Piazzi *et al.* 2004). Increasingly, the detailed mapping of seagrass meadows is

improving our ability to forecast the levels of biodiversity (Jordan *et al.* 2005).

Several methods were used to map, at various spatial scales, the structure of seagrass beds, including side-scan sonar monitoring (Panayotidis *et al.* 1981; Colantoni *et al.* 1982), aerial photogrammetry (Meinesz & Laurent 1982) and satellite imagery (Pasqualini *et al.* 2005). The use of these mapping techniques allowed for the detection of spatial patterns at a large/medium scale (e.g., the well documented decrease of shoot density along the depth gradient in *P. oceanica* meadows; Pasqualini *et al.* 2005; Ralph *et al.* 2005). However, density, morphology and growth can vary on a very small scale, even along the same isobath (Balestri *et al.* 2003). Small-scale variations of the meadow structure can be due to the seafloor morphology, the patterns of sexual reproduction and the genetic makeup characterising each single meadow (Buia & Mazzella 1991; Migliaccio *et al.* 2005; Arnaud-Haond *et al.* in press).

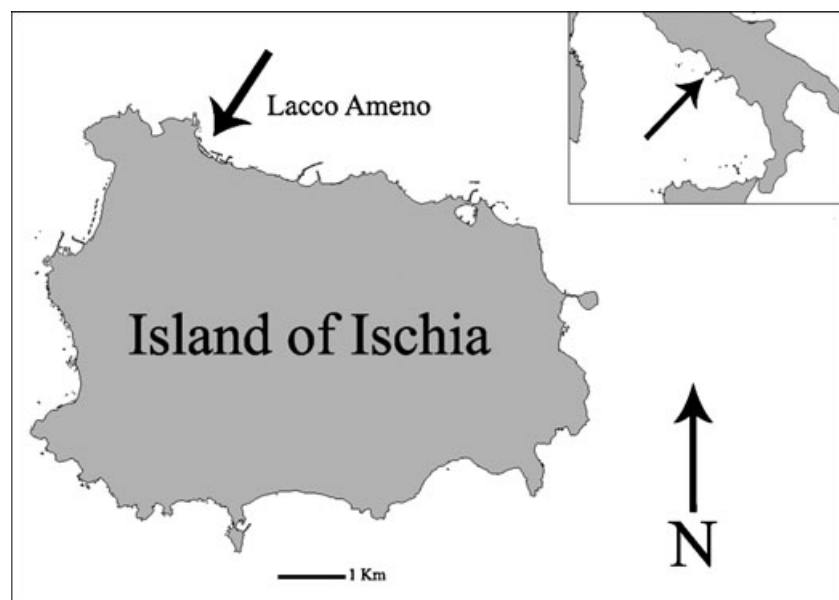
Detailed maps of seagrass beds (Scardi & Fresi 1986; Scardi *et al.* 1989; Pergent 1990; Pergent *et al.* 1995) and of related biotic variables (Oliva 2002; Gobert *et al.* 2003; Gambi *et al.* in press) were obtained by the Kriging technique, a cartographic method based on a stochastic interpolator, developed for geostatistical purposes (Matheron 1969, 1970). In 1992, this technique was applied to the *Posidonia* meadow of Lacco Ameno d'Ischia (Gulf of Naples, Italy; Zupo *et al.* 2006). Interestingly, this study permitted to demonstrate that shoot density of *P. oceanica*, on a small scale (metres), was influenced by the seafloor morphology, with areas of radially decreasing density located on the top of depth climbs, and areas of radially increasing density located at the centre of bottom depressions (Zupo *et al.* 2006). The high number of sto-

lons co-occurring in the high-density patches could either belong to the same clone or represent distinct clones. In the latter case, higher densities should be characterized by higher genetic variability (Migliaccio *et al.* 2005). The answer is not trivial, as sexual reproduction in *P. oceanica* is variable both in space and time (Balestri 2004). Although a recent analysis demonstrated that genetic variability in *P. oceanica* is higher than previously thought (Arnaud-Haond *et al.* 2005), in most cases asexual reproduction seems to be still the predominant way of meadow expansion (Migliaccio *et al.* 2005; Arnaud-Haond *et al.* in press). The analysis of patterns of genetic diversity could be of help in the interpretation of the spatial and temporal changes of shoot density within a meadow. Hence, some questions remained open: (1) Do the cores of density persist in the same position, or do they displace in time? (2) Are the patterns of density described in 1992 stable in time? (3) Should we expect a consistent picture in the distribution of genetic variability in relation to the density of nestlike structures?

To answer these questions, an investigation on the *P. oceanica* meadow off Lacco Ameno was repeated in 2000. Collections were performed at 21 points (selected among those considered in the previous investigation) in order to follow the temporal evolution of the density cores and to compare their distribution with the patterns of genetic diversity.

## Material and Methods

This study was conducted in the *Posidonia oceanica* meadow off Lacco Ameno (Island of Ischia, Naples, Italy; Fig. 1), that is settled on 'matte' and extended, almost

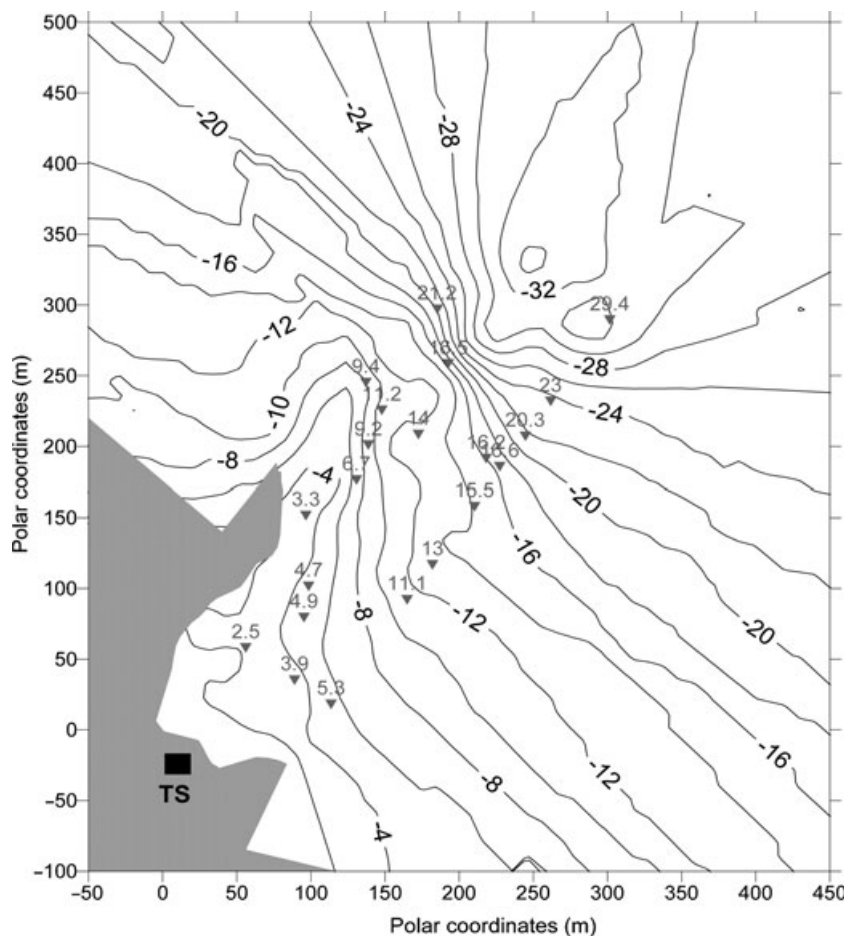


**Fig. 1.** Location of the studied *Posidonia oceanica* meadow off Lacco Ameno in the Island of Ischia (Gulf of Naples, Italy).

continuously, between 1 and 33 m depth (Colantoni *et al.* 1982). Environmental features of the site varied rather uniformly with depth (Buia *et al.* 2003). Therefore, this meadow represented an ideal site to investigate, on a long term, the stability of the small-scale spatial distribution, influenced by seafloor morphology (Zupo *et al.* 2006). To re-analyse the spatial anisotropy of the *P. oceanica* meadow in Lacco Ameno, the same 64,000 m<sup>2</sup> (400 m × 160 m) surface area considered in 1992 (Zupo *et al.* 2006) was taken into account, with a sampling grid of 10 m (640 nodes). The previous investigation was based on 105 randomly selected points (16% of the total number of grid nodes) and took into account the depth and the shoot density measured at each point, according to the technique described by Scardi *et al.* (1989). This number of samples was considered sufficient to obtain a detailed and representative map of the area, because the Kriging technique allows for detailed reconstructions based on a relatively low number of field measurements (Scardi & Fresi 1986; Wackernagel 1994). In the present investigation, conducted in May 2000, a reduced set of 21 nodes

was selected within the sampling grid. The chosen nodes corresponded to the position of specific patterns and nest-like structures. Each point was geographically located using a topographical station (Nikon NTD-2) positioned on the coast and three optical prisms located on a boat (Fig. 2). Once the boat was correctly positioned on a sampling node (detected by means of the topographical station and communicated to the operator using a portable radio), a concrete block was cast. On this spot, divers recorded depth and shoot density on a 40 × 40 cm fixed surface. Two replicate measures of shoot density were obtained at each node, and the average value was taken into account for the Kriging reconstruction. At each of the 21 nodes, nine shoots were collected on a predefined small-scale grid (Migliaccio *et al.* 2005) for the analysis of degree of genetic variability. Analysis on genetic structure, in fact, was not performed in the 1992 survey.

Genomic DNA was extracted from each single shoot, following a modified CTAB method (Procaccini *et al.* 1996). Individual multilocus genotypes were assessed by means of 13 microsatellite loci (Procaccini & Waycott



**Fig. 2.** Location of the selected 21 points (dots) sampled in 2000 with the indication of their depth (metres), superimposed to the depth map obtained in 1992 (Zupo *et al.* 2006). The map is reported in polar coordinates with the Y axis oriented towards the geographical north. TS = topographical station.

1998; Alberto *et al.* 2003). Detailed protocols for DNA isolation and microsatellite scoring are according to Migliaccio *et al.* (2005). The genetic diversity was calculated for each sampling point (plotted in the Kriging graphs) as the ratio between distinct genotypes (G, genets) over total number of shoots (N, ramets) (G/N).

Geo-referenced data collected in 2000 such as depth (m), shoot density (no. shoots m<sup>-2</sup>) and genetic diversity (G/N), were plotted over the maps obtained in 1992, to detect temporal variation and relationships among the considered variables. A Kriging analysis of shoot density and molecular data was performed by means of a Surfer 7 package (Golden Software Inc.) (Zupo *et al.* 2006), although the small number of samples collected did not allow for a fine description of the spatial patterns (Scardi *et al.* 1989). To investigate the spatial anisotropy of shoot density, a variogram based on linear and spherical functions was fitted, in accordance with the previous investigation (Zupo *et al.* 2006). Finally, geo-referenced data of shoot density and genetic diversity were superimposed on the detailed maps of shoot density obtained, to check the consistency of spatial distributions and to assess the single *versus* multi-clonal contribution to the dynamics of shoot density along time.

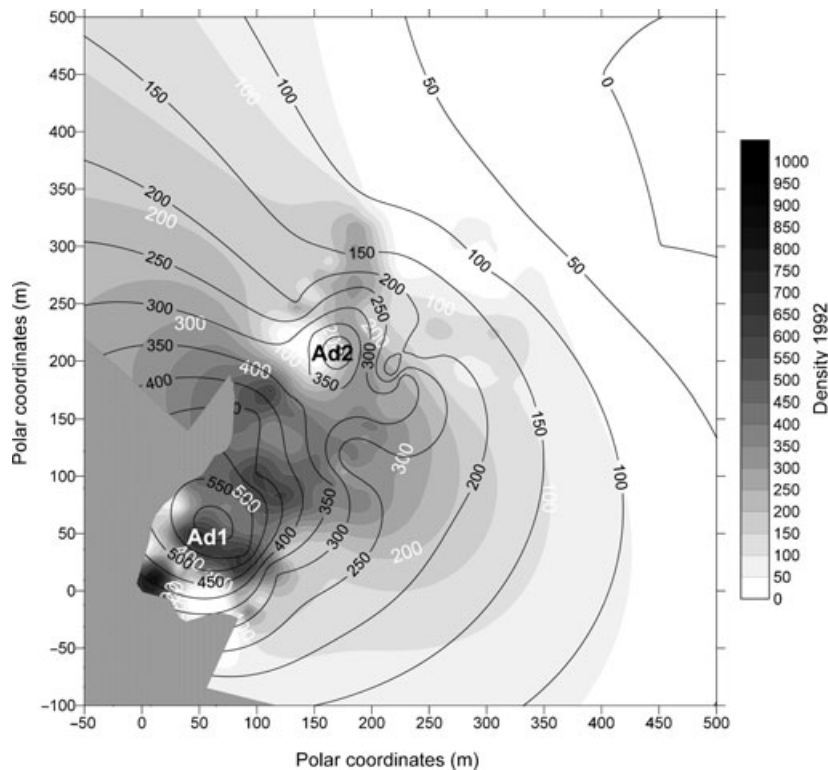
## Results

Seafloor morphology, as inferred by measured depths (Table 1), was almost identical to that observed in 1992 by Zupo *et al.* (2006). Therefore, no Kriging of the depth data was undertaken. The shallowest point was at 2.5 m depth, while the deepest point was at 29.4 m, in correspondence with a seafloor depression (Fig. 2). Several measurement points in the western side of the sampling area were located along an elongated hill following the profile of the coast, and the deepest ones (9.4 and 11.2 m depth, respectively) corresponded to the centre of previously identified cores of shoot density. Two points were located in the centre of a sharp depth cline that followed the hill (16.5 and 21.2 m depth, respectively), while the remaining points were regularly distributed along the depth gradient, in the eastern side of the sampling area (Fig. 2).

Shoot density data are reported in Table 1. Variability among the two replicates in the same point of the grid was relatively low, as indicated by the standard deviations (Table 1), except in a few points located in the shallower stand of the meadow, characterized by the highest patchiness and densities. The comparison between the shoot density values measured in this study (2000) and those

**Table 1.** Comparison of the shoot density (no. shoots m<sup>-2</sup>) values measured in 1992 and 2000, respectively, at the same collection points. The first column refers to the sampling node, indicated by a letter (ordinate) and a number (abscissa). The following two columns indicate the x–y absolute coordinates (metres from the origin, measured by means of a topographical station located on the beach, see Fig. 2). The other columns contain the mean shoot densities (no. shoots m<sup>-2</sup>) measured at each point in 1992 and in 2000, and the depths (m) measured at each point in the two periods. Variation between the two sampling periods are also indicated as no. shoots m<sup>-2</sup> and % variation for density, and metres for depth.

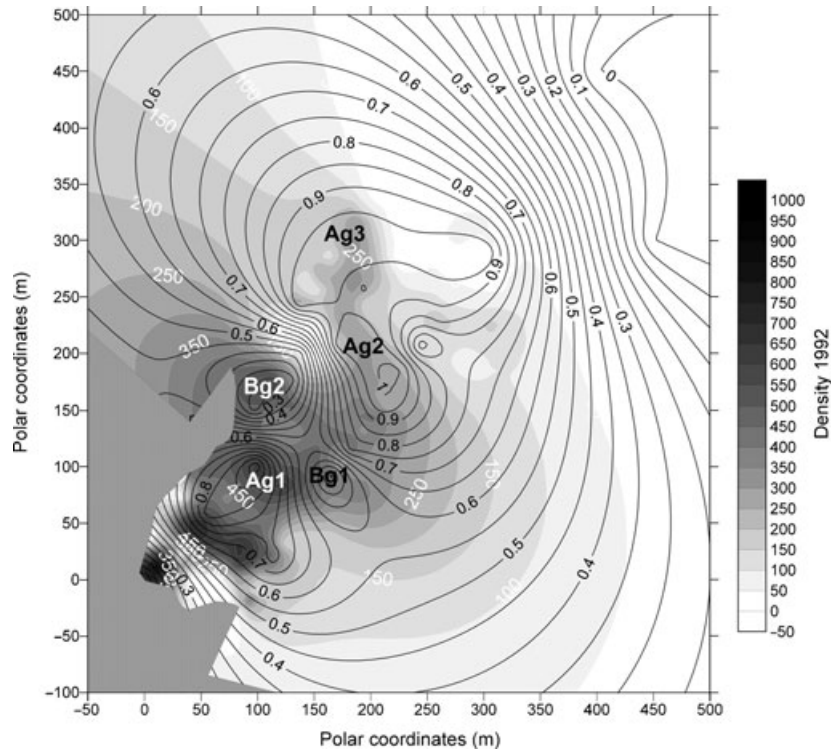
station	X	Y	1992 density (mean)	2000 density (mean ± SD)	variation	% variation	1992 depth (m)	2000 depth (m)	variation
I-7	56.07	57.88	600	631 132.6	31	5.2	2.2	2.5	0.3
G-17	96.58	151.47	380	468 35.4	88	23.2	3	3.3	0.3
O-7	88.95	35.1	662	581 26.5	-81	-12.2	3.6	3.9	0.3
L-13	98.46	101.51	650	446 75.1	-203	-31.2	5	4.7	-0.3
M-11	95.29	79.37	543	546 92.8	3	0.6	4.6	4.9	0.3
R-7	113.61	18.02	493	306 17.7	-187	-37.9	5.5	5.3	-0.2
G-20	130.67	176.13	310	334 163.5	24	7.7	7.1	6.7	-0.4
G-23	138.76	200.79	280	321 4.4	41	14.6	9.5	9.2	-0.3
E-27	137.1	245.06	200	200 17.7	0	0	8.6	9.4	0.8
R-16	164.87	91.99	456	356 8.8	-100	-21.9	11	11.1	0.1
G-26	147.84	225.45	343	343 75.1	0	0	11.2	11.2	0
R-19	181.95	116.65	487	190 13.3	-296	-60.8	12.6	13	0.4
L-26	172.5	208.36	300	475 8.8	175	58.3	13.2	14	0.8
R-24	210.43	157.75	243	331 35.4	87	35.8	14.9	15.5	0.6
O-26	218.16	191.28	260	137 48.6	-122	-46.9	15.6	16.2	0.6
G-30	192.62	258.33	260	275 79.5	15	5.8	16.3	16.5	0.2
P-26	227.38	185.58	290	343 61.9	53	18.3	15.9	16.6	0.7
R-30	244.6	207.07	118	178 30.9	59	50.0	21.1	20.3	-0.8
F-34	185.19	296.9	350	132 84.0	-217	-62.0	21	21.2	0.2
R-33	261.69	231.73	193	200 17.7	6	3.1	23	23	0
R-40	301.56	289.26	62	87 35.4	25	40.3	30	29.4	-0.6



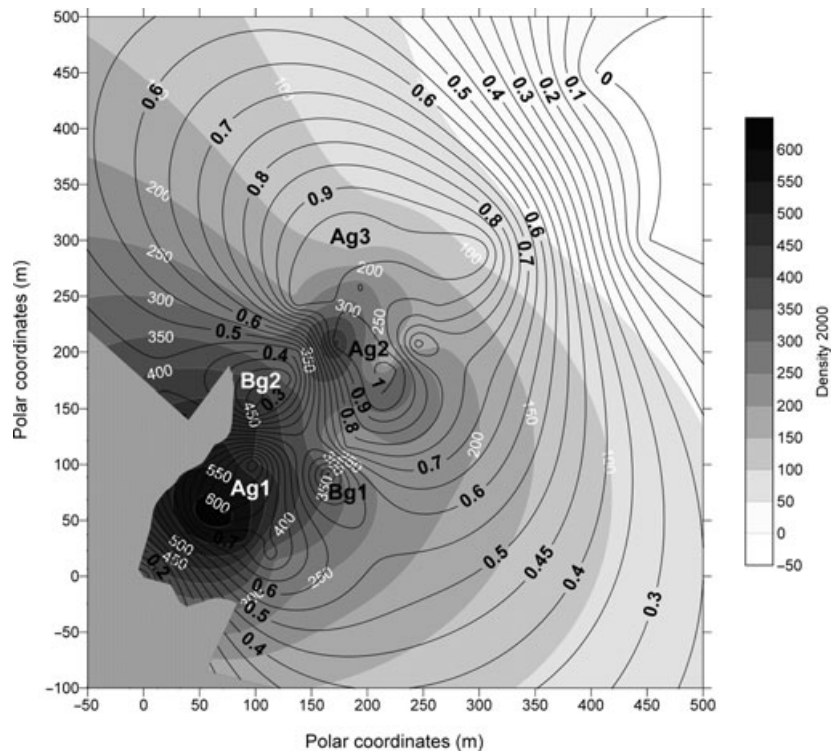
**Fig. 3.** Kriging representation of shoot density (no. shoots  $\text{m}^{-2}$ ) measured in 2000, superimposed on the map of density (grey scale) obtained in 1992. Ad1 and Ad2 are the centres of nestlike structures exhibiting an increase of shoot density towards the centre in the 2000 survey.

recorded in 1992 (Table 1) indicated, on the whole, a similar spatial distribution. Slight differences (only five points demonstrated variations higher than 100 shoots  $\text{m}^{-2}$ ) were mainly observed in the shallower sector, characterized by the highest patchiness, and corresponding with the main nestlike cores of density. For instance, in the centre of the nestlike core indicated as Ad1 (Fig. 3) the density dropped from 650 (in 1992) to 447 (present investigation) shoots per square metre. However, the data obtained suggested a general agreement, over a decadal scale, of the shoot density patterns. In fact, the Kriging reconstruction (black lines in Fig. 3), albeit based on a reduced set of data, almost corresponded to the previous patterns of shoot density (grey levels) obtained in 1992. Yet, a more uniform distribution and a lower spatial anisotropy was found with respect to the first study (Zupo *et al.* 2006), especially in areas characterized by the highest patchiness, as demonstrated by the presence of a larger nested structure (Ad1, in Fig. 3) in the centre of the bay, in place of various cores of density detected in 1992. Another density core (Ad2 in Fig. 3), located in the deeper meadow about 50 m east of the inverse nestlike structure observed in the previous investigation, exhibited a density pattern similar to that of core Ad1. Therefore, the inverse nestlike core recorded in 1992 was no longer present, while a new density core (Ad2) appeared in the close vicinity (Fig. 3).

The analysis of microsatellite diversity yielded a total of 136 diverse genotypes (G) over 181 ramets (N) analysed ( $G/N = 0.75$ ). Fifty-six alleles were scored, in total, with an average of 4.58 alleles per locus (Migliaccio *et al.* 2005). The patterns of G/N values were largely coherent with the shoot density patterns reconstructed in 1992. In fact, most nestlike cores of diversity (black lines in Fig. 4) followed the patterns of shoot density (grey levels) identified by Zupo *et al.* (2006). The genetic diversity was low in the shallow stand of the meadow (up to 3–4 m depth), increased from 12 to 29 m depth ( $0.7 > GN > 1$ ), and uniformly decreased in the deepest meadow (>30 m). The maximum diversity was recorded in two cores closely located, indicated as Ag2 and Ag3 in Fig. 4. Another core of high diversity was located in the shallow meadow (Ag1 in Fig. 4). Two inverse cores (*i.e.*, diversity decreasing towards the centre, named Bg) were located in the eastern and in the western sides of the sampling area, respectively (Bg1 and Bg2 in Fig. 4). The map of genetic diversity was only partially over-imposable to the density map reconstructed by the present data (Fig. 5). Ag1 corresponded well to the main density core (Ad1), in the shallow meadow, and Ad2 was within two density cores located in the middle stand. Interestingly, the core of genetic diversity named Ag3 in Figs 4 and 5, corresponded to a density core present in 1992, which was apparently absent in the present investigation. The



**Fig. 4.** Kriging representation of the distribution of genetic diversity (G/N ratio) (black isolines), superimposed on the shoot density (no. shoots  $m^{-2}$ ) structure (grey scale) recorded in 1992 (Zupo *et al.* 2006). 'Ag' and 'Bg' are the cores of nestlike structures with increasing and decreasing of genetic diversity towards the centre, respectively.



**Fig. 5.** Kriging representation of the distribution of genetic diversity (G/N ratio) (black numbers), superimposed on the map of shoot density (no. shoots  $m^{-2}$ ) (white numbers) obtained by Kriging of the 21 points of density measured in 2000 (grey scale). 'Ag' and 'Bg' are the cores of nestlike structures with increasing and decreasing of genetic diversity towards the centre, respectively.

inverse cores of genetic diversity Bg1 and Bg2 (Fig. 5) were located on the eastern and western limits of the dense meadow and corresponded to average densities of 400 shoots per square metre.

## Discussion

The agreement between the depth values measured at each point in the present investigation and those measured in 1992 confirms the exact positioning of the grid points in the field. The absence of variation in the depth profile is not unexpected, given the absence of dramatic events that could have altered the local topography (Mateo & Romero 1997; Gambi *et al.* 2005). The position of the sampling points corresponds to 'critical' zones identified in the previous investigation (*i.e.*, centres of nestlike cores, abrupt depth increases, clines of shoot density), and allows for an evaluation of the temporal stability of the density patterns. In fact, most points are located in areas characterized by higher spatial anisotropy (clustered isolines).

The highest and the lowest shoot density values recorded in the previous survey (1992) are not confirmed, suggesting that the meadow has been subjected to a reduction of the spatial anisotropy during the last decade. Several Mediterranean meadows exhibited similar evolution, apart from local differences due to anthropogenic pressure (Leriche *et al.* 2004). A decrease in shoot density was recorded in the shallowest stands, although the reduced set of sampling points and the lack of measures over 2.5 m depth provided a minor resolution in this area. The density decline may be due to various impacts which occurred in the last decade (Warnau *et al.* 1998), including anchoring by pleasure boating (Buia *et al.* 2003).

With respect to the previous representation, the patterns of shoot density appear more homogeneous. Most nestlike cores of high density, characterizing the shallow stand, merge and homogenize in the central area, producing a single core with maximum density of 600 shoot  $m^{-2}$  in the centre (Ad1 in Fig. 3). Similarly, most nestlike cores characterizing and diversifying the structure of the meadow in 1992, merged in a single core located 120 m off the coast, with maximum density of 450 shoot  $m^{-2}$  in the centre (Ad2 in Fig. 3). The reduced number of samples of the present investigation in respect to the 1992 data could have an effect on the observed changes in the level of anisotropy within the meadow. Nevertheless, the Kriging reconstruction may be considered as a good representation of the actual patterns of shoot density and nested structures as a real feature observed at a small scale.

In some cases, circular structures may be produced as a 'bias' of the interpolation technique at a higher spatial

scale, when the analysis is based on a low number of sampling points (tendency of Kriging to connect isolines in areas where no data are available). At a low scale, however, this interpolation effect should be excluded (Borg *et al.* 2005). Similar nested structures, partially matching those of shoot density, were observed when some plant-associated organisms (borer polychaetes) were analysed on the same grid (Gambi *et al.* in press).

It is interesting to observe that the spatial structure of the meadow was confirmed after 8 years, as well as the typical decrease of density along depth, detectable even applying the Kriging technique to the reduced set of data. Depth-related patterns of density are a stable feature of *P. oceanica* meadows (Augier & Boudouresque 1973; Panayotidis *et al.* 1981; Colantoni *et al.* 1982; Pergent & Pergent-Martini 1988) as a result of large variations in irradiance and temperature (Pirc 1986; Zimmerman *et al.* 1994; Lorenti *et al.* 1995; Zupo *et al.* 1997). The density structure, in contrast, can vary at a scale of metres (Duarte & Sand-Jensen 1990; Alcoverro *et al.* 1995; Balestri *et al.* 2004). Cores of density might be produced by the confluence of several branches (plagiotropic rhizomes) towards the same area. They may represent zones of higher resource availability (Zupo *et al.* 2006), as it is reported that rhizomes tend to expand towards these areas (Duarte 1991; Dalla Via *et al.* 1998; Marbà & Duarte 1998). Following this hypothesis, patterns of density can be related to the seabed topography: areas of radially decreasing density are located on the top of depth increments (higher irradiance), while areas of radially increasing density are located at the centre of bottom depressions (lower irradiance). Nevertheless, the present investigation indicates that the seafloor geomorphology may not be the only factor influencing the shoot density patterns (Ruiz & Romero 2003; Bensoussan *et al.* 2004) and its temporal dynamics. In fact the seafloor morphology was unchanged during the last 8 years, while the density structure changed as previously discussed.

The study of the Kriging map of genetic diversity provides useful insights for the comprehension of this problem. Five main cores of genetic diversity were detected: three (Ag1, Ag2, Ag3) with increasing genetic diversity towards the centre (centripetal), and two (Bg1, Bg2) with an opposite trend and a decreasing genetic diversity towards the centre (centrifugal) (Figs 4 and 5). Interestingly, their overall positions well agree with the density cores detected in 1992. In contrast, only the position of two cores Ag1 and Ag2 corresponds to the shoot density cores detected in the present investigation (Migliaccio *et al.* 2005). These nested structures may have a different origin. The presence of centrifugal structures may indicate a transition period, during which the plant stolons move towards new areas (Duarte 2002) and could be produced

by degeneration of the aggregates previously defined or by the incipient coalescence of neighbour patches. The presence of nestlike structures with increasing density in the centre may correspond to areas of abundant resources, triggering (over a long period) the convergence of stolons from neighbour areas. The consistent spatial correlation recorded between such structures of density and similar nestlike structures of genetic-diversity observed in the present study suggests strong inter-genet competition for the resource, as stolons coming from neighbour areas may represent different genotypes.

Several density cores characterized the Lacco Ameno meadow in 1992, due to an active process of expansion and growth (Ballesta *et al.* 2000). In the last decade, increasing levels of disturbance acting on the meadow may have led to a homogenization of the previous complex density structure. However, such homogenization does not seem to select among genotypes and we can hypothesize that the patterns of genetic structure is steady and, in contrast, the density structure is changeable at least on a decadal scale. In this perspective, a comparison of these two variables may allow for detecting both the genesis of the meadow structure and the occurrence of recent alterations.

Density cores were confirmed to be vital nuclei of the meadow (Zupo *et al.* 2006): in optimal conditions and during long-term processes of growth, they were originated by genetically distinct stolons, moving from the surrounding areas. In this case, their location is well correlated to the seafloor morphology, because light may be the limiting factor (Enriquez *et al.* 2004; Runcie & Durako 2004). In stress conditions (*e.g.*, mechanical impacts, higher level of sedimentation, altered nutrient availability; Ruiz *et al.* 2001; Cancemi *et al.* 2003), their structure becomes inconspicuous.

Taking into account an increase of the human impact on this meadow in the last decade (Buia *et al.* 2003), we suggest that the relatively high density values and the complex patterns of shoot density recorded in 1992 (Zupo *et al.* 2006) indicated a meadow in a good state of health. In contrast, a lower anisotropy in the plant distribution may indicate a condition of increased stress and impacts affecting the meadow off Lacco Ameno. In this view, we may also expect that a restoration of the ideal conditions of plant growth will produce new nuclei of density at the lateral edges of the meadow. The presence of expansion nuclei, in fact, may be crucial to survive through dramatic fluctuations of density due to occasional mass mortality (Duarte 2002). Regression processes acting on the meadow will produce a progressive decrease of density in most areas and only few cores may maintain higher density (and high genetic diversity), responsible for subsequent long-term recovery.

## Summary

Previous investigations (1992) on the shoot density distribution of the *Posidonia oceanica* meadow off Lacco Ameno (Island of Ischia, Bay of Naples, Italy) detected complex spatial patterns and indicated the presence of nestlike structures with a rounded shape, and with density increasing ('A' type) or decreasing ('B' type) towards the centre. Their presence and distribution was correlated to the seafloor geomorphology and to the growth strategy of the plant. The present investigation (2000) was aimed at checking the time stability of nestlike structures and at testing the hypothesis formulated on their genesis, taking into account also molecular data (spatial distribution of genetic diversity). A Kriging stochastic interpolator was used to follow the spatial distributions of density along time and the genetic diversity within the meadow. The results indicated a general loss of spatial anisotropy which occurred in the last decade, probably due to anthropic influences. Patterns of genetic diversity confirmed our previous hypotheses on the genesis of shoot density cores, suggesting they are produced over long time, due to a slow stolonization process and the convergence of different genotypes. Regression of the meadow and decrease of density may reduce their presence and lead, in short periods, to a homogenization of the density patterns, while genetic diversity cores represent a 'memory' of their distribution over longer periods.

## Acknowledgements

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## References

- Alberto F., Correia L., Arnaud-Haond S., Billot C., Duarte C.M., Serrão E. (2003) New microsatellite markers for the endemic Mediterranean seagrass *Posidonia oceanica*. *Molecular Ecology Notes*, **3**, 253–255.
- Alcoverro T., Duarte C.M., Romero J. (1995) Annual growth dynamics of *Posidonia oceanica*: contribution of large-scale versus local factors to seasonality. *Marine Ecology Progress Series*, **120**, 203–210.

- Arnaud-Haond S., Alberto F., Teixeira S., Procaccini G., Serrão A., Duarte C.M. (2005) Assessing genetic diversity in clonal organisms: low diversity or low resolution? Combining power and cost efficiency in selecting markers. *Journal of Heredity*, **96**(3), 1–7.
- Arnaud-Haond S., Migliaccio M., Diaz-Almela E., Teixeira S., Vliet M., Alberto F., Procaccini G., Duarte C.M., Serrão E. (in press). Vicariance patterns in the Mediterranean Sea: East–West cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. *Journal of Biogeography*.
- Augier H., Boudouresque C.F. (1973) Dix ans de recherches dans la zone marine du parc national de Port-Cros (France). Première partie. *Annales de la Société de Sciences naturelles et Archéologiques de Toulon*, **25**, 119–138.
- Balestri E. (2004) Flowering of the seagrass *Posidonia oceanica* in a north-western Mediterranean coastal area: temporal and spatial variations. *Marine Biology*, **145**(1), 61–68.
- Balestri E., Cinelli F., Lardicci C. (2003) Spatial variation in *Posidonia oceanica* structural, morphological and dynamic features in a northwestern Mediterranean coastal area: a multi-scale analysis. *Marine Ecology Progress Series*, **250**, 51–60.
- Balestri E., Benedetti-Cecchi L., Lardicci C. (2004) Variability in patterns of growth and morphology of *Posidonia oceanica* exposed to urban and industrial wastes: contrasts with two reference locations. *Journal of Experimental Marine Biology and Ecology*, **308**(1), 1–21.
- Ballesta L., Pergent G., Pasqualini V., Pergent-Martini C. (2000) Distribution and dynamics of *Posidonia oceanica* beds along the Alberes coastline. *Comptes Rendus de l'Académie des Sciences series III-Sciences de la vie-Life Sciences*, **323**(4), 407–414.
- Bensoussan N., Romano J.C., El-Haikali B., Lafont M.G. (2004) Relevant time scales in studying coastal marine ecosystems functioning. *Comptes Rendus Geoscience*, **336**(10), 909–918.
- Borg J.A., Attrill M.J., Rowden A.A., Schembri P.J., Jones M.B. (2005) Architectural characteristics of two bed types of the seagrass *Posidonia oceanica* over different spatial scales. *Estuarine Coastal and Shelf Science*, **62**(4), 667–678.
- Buia M.C., Mazzella L. (1991) Reproductive strategies of the Mediterranean seagrasses: *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers., and *Zostera noltii* Hornem. *Aquatic Botany*, **40**, 343–362.
- Buia M.C., Gambi M.C., Zupo V. (2000) Structure and functioning of Mediterranean seagrass ecosystems: an overview. In: Pergent G., Pergent-Martini C., Buia M.C., Gambi M.C. (Eds), *Proceedings 4th International Seagrass Biology Workshop. Biologia Marina Mediterranea*, **7**(2), 167–190.
- Buia M.C., Gambi M.C., Lorenti M., Dappiano M., Zupo V. (2003) Aggiornamento sulla distribuzione e sullo stato ambientale dei sistemi a fanerogame marine (*Posidonia oceanica* e *Cymodocea nodosa*) delle isole Flegree. In: Gambi M.C., De Lauro M., Jannuzzi F. (Eds), *Ambiente marino costiero e territorio delle isole flegree (Ischia Procida Vivara – Golfo di Napoli)*. Risultati di uno studio multidisciplinare. Liguori Editore, Napoli. *Memorie dell'Accademia di Scienze Fisiche e Matematiche*, **5**, 163–186.
- Buia M.C., Gambi M.C., Dappiano M. (2004) The seagrass systems. In: Gambi M.C., Dappiano M. (Eds), *Mediterranean Marine Benthos: a Manual of Methods for Its Sampling and Study. Biologia Marina Mediterranea*, **11**(Suppl. 1): 133–184.
- Cancemi G., De Falco G., Pergent G. (2003) Effects of organic matter input from a fish farming facility on a *Posidonia oceanica* meadow. *Estuarine Coastal and Shelf Science*, **56**(5–6), 961–968.
- Colantoni P., Gallignani P., Fresi E., Cinelli F. (1982) Patterns of *Posidonia oceanica* (L.) Delile beds around the island of Ischia (Gulf of Naples) and in adjacent waters. *P.S.Z.N.I.: Marine Ecology*, **3**, 53–74.
- Dalla Via J., Sturmbauer C., Schonweger G., Sotz E., Mathekwitsch S., Stifter M., Rieger R. (1998) Light gradient and meadow structure in *Posidonia oceanica*: ecomorphological and functional correlates. *Marine Ecology Progress Series*, **163**, 267–278.
- Duarte C.M. (1991) Seagrass depth limits. *Aquatic Botany*, **40**, 363–377.
- Duarte C.M. (2002) The future of seagrass meadows. *Environmental Conservation*, **29**(2), 192–206.
- Duarte C.M., Sand-Jensen K. (1990) Seagrass colonization: patch formation and patch growth in *Cymodocea nodosa*. *Marine Ecology Progress Series*, **65**, 193–200.
- Enriquez S., Marba N., Cebrian J., Duarte C.M. (2004) Annual variation in leaf photosynthesis and leaf nutrient content of four Mediterranean seagrasses. *Botanica Marina*, **47**(4), 295–306.
- Gambi M.C., Dappiano M., Lorenti M., Iacono B., Flagella S., Buia M.C. (2005) “Chronicle of a death foretold” – features of a *Posidonia oceanica* bed impacted by sand extraction. In: Özhan E. (ed), *Proceedings of the Seventh International Conference on the Mediterranean Coastal Environment MEDCOAST 05, 25–29 October 2005*, Kusadasi, Turkey: 441–450.
- Gambi M.C., Trani B., Cigliano M., Zupo V. (in press) The “Kriging” approach to the study of the relationships between *Posidonia oceanica* meadows structure and distribution of the associated fauna: an example with borer polychaetes. *Biologia Marina Mediterranea*, **13**(4) (2006).
- Gobert S., Kyramarios M., Lepoint G., Pergent-Martini C., Bouquegneau J.M. (2003) Variations at different spatial scales of *Posidonia oceanica* (L.) Delile beds; effects on the physico-chemical parameters of the sediment. *Oceanologica Acta*, **26**, 199–207.
- Jordan A., Lawler M., Halley V., Barrett N. (2005) Seabed habitat mapping in the Kent Group of islands and its role in marine protected area planning. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **15**(1), 51–70.
- Kendrick G.A., Duarte C.M., Marbà N. (2005a) Clonality in seagrasses, emergent properties and seagrass landscapes. *Marine Ecology Progress Series*, **290**, 291–296.

- Kendrick G.A., Duarte C.M., Marbà N. (2005b) Modelling formation of complex topography by the seagrass *Posidonia oceanica*. *Estuarine Coastal and Shelf Science*, **65**, 717–725.
- Leriche A., Boudouresque C.F., Bernard G., Bonhomme P., Denis J. (2004) A one-century suite of seagrass bed maps: can we trust ancient maps? *Estuarine Coastal and Shelf Science*, **59**(2), 353–362.
- Lorenti M., Mazzella L., Buia M.C. (1995) Light limitation of *Posidonia oceanica* (L.) Delile growth at different depths. *Rapports Commission Internationale Exploration Scientifique de la Mer Méditerranée*, **34**, 34.
- Marbà N., Duarte C.M. (1998) Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series*, **174**, 269–280.
- Mateo M.A., Romero J. (1997) Detritus dynamics in the seagrass *Posidonia oceanica*: Elements for an ecosystem carbon and nutrient budget. *Marine Ecology Progress Series*, **151**(1–3), 43–53
- Matheron G. (1969) Le krigeage universel. *Cahiers Centre Morphologie Mathématique*, **1**, 83.
- Matheron G. (1970) La théorie des variables régionalisées et ses applications. *Cahiers Centre Morphologie Mathématique*, **5**, 212.
- Mazzella L., Buia M.C. (1989) Variazioni a lungo termine in alcuni parametri strutturali di una prateria a *Posidonia oceanica*. *Nova Thalassia*, **10**, 533–542.
- Mazzella L., Buia M.C., Gambi M.C., Lorenti M., Russo G.F., Scipione M.B., Zupo V. (1992a) Primary production of *Posidonia oceanica* and the vagile fauna of leaf stratum: a spatio temporal analysis in a meadow off the island of Ischia (Gulf of Naples, Italy). In: Keegan B.F. (Ed.), *Space and Time Series Data Analysis in Coastal Benthic Ecology*. Commission of the European Communities, Environmental Research Programme, Galway, Ireland: 519–539.
- Mazzella L., Buia M.C., Gambi M.C., Lorenti M., Russo G.F., Scipione M.B., Zupo V. (1992b) Plant–animal trophic relationships in the *Posidonia oceanica* ecosystem of the Mediterranean Sea: a review. In: John D.M., Hawkins S.J., Price J.H. (Eds), *Plant–Animal Interactions in the Marine Benthos*. *Systematics Association Special*, Volume 46. Clarendon Press, Oxford: 165–187.
- Meinesz A., Laurent R. (1982) Cartes de la végétation sous-marine des Alpes Maritimes (Côtes françaises de la Méditerranée). 1-Limite inférieure de l'herbier de *Posidonia oceanica* dans le golfe Juan et à l'est des Iles de Lérins. *Annales Institut Océanographique*, **58**, 103–112.
- Migliaccio M., De Martino F., Silvestre F., Procaccini G. (2005) Meadow-scale genetic structure in *Posidonia oceanica* L. (Delile). *Marine Ecology Progress Series*, **304**, 55–65.
- Oliva S. (2002) Analisi spatio-temporale di variabili strutturali e funzionali di una prateria di *Posidonia oceanica* attraverso l'utilizzo della tecnica del Kriging. Master Thesis, University "Parthenope" Naples: 88 pp (in Italian).
- Ott J., Mazzella L. (Eds) (1992) Functional approach of the *Posidonia oceanica* ecosystem of the Mediterranean. *Special Issue P.S.Z.N.I: Marine Ecology*, **13**, 83 pp.
- Panayotidis P., Boudouresque C.F., Marcot-Coqueugniot J. (1981) Microstructure de l'herbier de *Posidonia oceanica* (Linnaeus) Delile. *Botanica Marina*, **24**, 115–124.
- Pasqualini V., Pergent-Martini C., Pergent G., Agreil M., Skoufas G., Sourbes L., Tsirika A. (2005) Use of SPOT 5 for mapping seagrasses: an application to *Posidonia oceanica*. *Remote Sensing of Environment*, **94**(1), 39–45.
- Pergent G. (1990) Utilisation de la technique du krigeage et cartographie benthique: intérêt et limites. *Rapports Commission Internationale Exploration Scientifique de la Mer Méditerranée*, **32**, 6.
- Pergent G., Pergent-Martini C. (1988) Phénologie de *Posidonia oceanica* (Linnaeus) Delile dans le bassin méditerranéen. *Annales Institut Océanographique Paris*, **64**(2), 79–100.
- Pergent G., Barbier J.C., Pergent-Martini C., Soullard M. (1995) Microstructure de l'herbier à *Posidonia oceanica* de la baie de Calvi (Corse). *Rapports Commission Internationale Exploration Scientifique de la Mer Méditerranée*, **34**, 39.
- Piazzi L., Balata D., Cinelli F., Benedetti-Cecchi L. (2004) Patterns of spatial variability in epiphytes of *Posidonia oceanica* – differences between a disturbed and two reference locations. *Aquatic Botany*, **79**(4), 345–356.
- Pirc H. (1986) Seasonal aspects of photosynthesis in *Posidonia oceanica*: influence of depth, temperature and light intensity. *Aquatic Botany*, **120**, 9–15.
- Procaccini G., Waycott M. (1998) Microsatellite loci identified in the seagrass *Posidonia oceanica* (L.) Delile. *Journal of Heredity*, **89**, 562–568.
- Procaccini G., Alberte R.S., Mazzella L. (1996) Genetic structure of the seagrass *Posidonia oceanica* in the Western Mediterranean: ecological implications. *Marine Ecology Progress Series*, **140**, 153–160.
- Procaccini G., Orsini L., Ruggiero M.V., Scardi M. (2001) Spatial patterns of genetic diversity in *Posidonia oceanica*, an endemic Mediterranean seagrass. *Molecular Ecology*, **10**, 1413–1421.
- Ralph P.J., Macinnis-Ng C.M.O., Frankart C. (2005) Fluorescence imaging application: effect of leaf age on seagrass photokinetics. *Aquatic Botany*, **81**(1), 69–84.
- Ruiz J.M., Romero J. (2003) Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photosynthesis of the seagrass *Posidonia oceanica*. *Marine Pollution Bulletin*, **46**(12), 1523–1533.
- Ruiz J.M., Perez M., Romero J. (2001) Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. *Marine Pollution Bulletin*, **42**(9), 749–760.
- Runcie J.W., Durako M.J. (2004) Among-shoot variability and leaf-specific absorptance characteristics affect diel estimates of *in situ* electron transport of *Posidonia australis*. *Aquatic Botany*, **80**(3), 209–220.

- Scardi M., Fresi E. (1986) Interpolation techniques in benthological mapping: a comparison between trend-surface analysis and kriging. *Rapports Commission Internationale Exploration Scientifique de la Mer Méditerranée*, **30**, 18.
- Scardi M., Fresi E., Ardizzone G.D. (1989) Cartographic representation of sea-grass beds: application of a stochastic interpolation technique (Kriging). In: Boudouresque C.F., Jeudy de Grissac A., Olivier J. (Eds), *Second International Workshop on Posidonia oceanica Beds. GIS Posidonie Publ. Fr.*, **2**, 19–27.
- Wackernagel H. (1994) Cokriging versus kriging in regionalized multivariate data analysis. *Geoderma*, **62**, 83–92.
- Warnau M., Biondo R., Temara A., Bouquegneau J.M., Jangoux M., Dubois P. (1998) Distribution of heavy metals in the echinoid *Paracentrotus lividus* from the Mediterranean *Posidonia oceanica* ecosystem: seasonal and geographical variations. *Journal of Sea Research*, **39**(3–4), 267–280.
- Zimmerman R.C., Cabello-Pasini A., Alberte R.S. (1994) Modeling daily production of aquatic macrophytes from irradiance measurements: a comparative analysis. *Marine Ecology Progress Series*, **114**, 185–196.
- Zupo V., Buia M.C., Mazzella L. (1997) A production simulation model for *Posidonia oceanica* based on temperature. *Estuarine Coastal and Shelf Science*, **44**, 483–492.
- Zupo V., Mazzella L., Buia M.C., Gambi M.C., Lorenti M., Scipione M.B., Cancemi G. (2006) A small-scale analysis of the spatial structure of a *Posidonia oceanica* meadow off the Island of Ischia (Gulf of Naples, Italy): relationship with the seafloor morphology. *Aquatic Botany*, **84**(2), 101–109.