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Spatio-temporal variability of borer polychaetes in *Posidonia oceanica* beds and its relation to meadow structure

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Abstract

Posidonia oceanica forms an extremely productive and complex ecosystem in the coastal Mediterranean Sea, providing a suitable habitat for hundreds of plant and animal species. Among motile invertebrates associated to *Posidonia* meadows, polychaete borers of the plant sheaths represent a unique group that exploits a peculiar microhabitat. They belong to the family Eunicidae with three dominant species, *Lysidice collaris*, *L. ninetta* and *L. unicornis*. Due to their strong association with *Posidonia* shoots, these animals are particularly suitable to study the plant and animal spatial relationships and their pattern of variability. The aim of this work was to evaluate the frequency of occurrence of these animals (Index of Borer, IB) at different spatial and temporal (summer *vs* winter) scales in two *Posidonia* meadows off Ischia Island (Gulf of Naples, Italy), which are exposed to different degrees of human impact and hydrological conditions. Results showed significantly different IB values of *L. collaris* (the most abundant species) between meadows and at scales from 10s to 100s of meters, as well as between summer and winter. The IB of *L. ninetta* showed significant differences only at scales of 10s of meters while, on the contrary, the IB of *L. unicornis* (the less abundant species) did not show variability at any spatial or temporal scale. Most of the variance was in the most impacted meadow and less exposed to water movement, suggesting a higher level of small and intermediate scales of patchiness of borers in this meadow. These variation patterns are discussed in relation to local environmental differences between the studied *Posidonia* beds.

Keywords: *Posidonia oceanica*, seagrass, spatial variability, Eunicidae, borer polychaetes, Index of Borer, nested ANOVA, spatial variability, Mediterranean Sea.

Introduction

Posidonia oceanica (L.) Delile is an endemic seagrass species of the Mediterranean Sea that forms one of the most productive and complex ecosystems in coastal areas, occurring from less than 1 to more than 40 m depth (Hemminga & Duarte, 2000; Procaccini *et al.*, 2003). The relatively high shoot density of healthy *Posidonia* meadows, as well as the complex plant architecture creates several microhabitats providing shelter, food availability and a suitable substrate for settlement of hundreds of plant and animal species (Gambi *et al.*, 1992; Mazzella *et al.*, 1992; Attrill *et al.*, 2000). In the last few years it has been demonstrated that *P. oceanica* shows a high spatial variability both in shoot density and plant morphology at different spatial scales that potentially affects the structure and functioning of the associated biotic communities (Balestri *et al.*, 2003; Kendrick *et al.*, 2005; Gambi *et al.*, 2006; Zupo *et al.*, 2006a,b; Montefalcone *et al.*, 2008; Vasapollo, 2009; Vasapollo & Gambi, 2012). Therefore, to address the issue of spatial variability in this complex seagrass system is very challenging.

Except for a relatively few investigations, information on the relationships between meadow and plant features, and distribution of the associated organisms remains scarce and rarely takes into account an appropriate spatial scale, or a comparison of spatial scales. Most of the previous investigations on the spatial distribution pattern of *Posidonia oceanica* meadows and their associated faunal assemblages, have mainly focused on variations at a geographical scale, or within a meadow across depth (shallow *vs* deep stands) (Buia *et al.*, 2000), showing none or inappropriate spatial replication. The main constraint of this lack of replication is often not only the effort of sampling but also of data processing and taxonomic impediment (Giangrande, 2003). The relatively few studies that have focused on spatial variations within a meadow at a given depth or along a depth gradient, are those mainly dealing with leaf and rhizome epiphytes (Balestri *et al.*, 2003; Piazzi, 2004; Pardi *et al.*, 2006; Balata *et al.*, 2007; Montefalcone *et al.*, 2008). In addition, recent findings demonstrate that *P. oceanica* beds are characterized by high anisotropy at medium (hundreds of m) and small (tens of m) spatial scales with peculiar growth patterns forming nest-like structures (Zupo *et al.*, 2006a,b). Such differences in the spatial distribution are mirrored by the distribution of some of the associated organisms, such as the polychaete borers, living in the seagrass sheaths (Gambi *et al.*, 2006).

Among the highly diverse organisms associated to the plant, the sheath borers (bases of old leaves persisting on the rhizome), represent a unique group of invertebrates, which exploits quite a peculiar microhabitat. They are represented in *Posidonia oceanica* by polychaetes and isopods (Guidetti *et al.*, 1997; Gambi, 2002). The polychaetes, all belonging to the family Eunicidae, are represented by *Lysidice collaris* Grube, 1870, *L. ninetta* Audouin and Milne Edwards, 1833, *L. unicornis* (Grube, 1840) (previously as *Nematonereis unicornis* and recently moved to the genus *Lysidice*, Zanhol *et al.,* 2013), and *Marphysa fallax* Marion & Bobretzky, 1875 (Gambi, 2002). However, Cinar (2013) pointed out that another recently re-erected species of the genus *Lysidice*, *L. margaritacea* Claparède, 1868 (Kurt Sahin & Cinar, 2009), could also have the sheath boring potential. These species burrow sinuous galleries inside the mesophyll of the sheaths (Guidetti, 2000; Gambi, 2002). The major component of the rhizome layer is represented by the lignified tissue of the sheaths, which together account for a large fraction of total biomass in *P. oceanica* systems, acting as a sink of C and persisting along the rhizomes for several years (Romero *et al.*, 1994; Mateo & Romero, 1997; Cebrian & Duarte, 2001).

Although borer polychaetes might not feed on sheath detritus exclusively (Vizzini *et al*., 2009), their burrowing activity could play a significant role in the detritus pathway of the *Posidonia* system, thus enhancing the decay and fragmentation of a resource that is scarcely exploited by other metazoans (Guidetti, 2000; Gambi, 2002). These polychaetes are particularly suitable for studying the scale of variability and the spatial relationships between plant and animal, since borers are linked to individual rhizome/shoot, sometimes in the proportion of one or up to max 3 individuals per shoot (Gambi & Cafiero, 2001). Furthermore, polychaete borers show a relatively constant abundance in time (Gambi & Cafiero, 2001; Gambi, 2002). Various studies were performed to evaluate borer distribution, which consider different spatial resolution and temporal frame, and have highlighted quite different patterns of distribution and frequency of such organisms according to various factors such as meadow depth, shoot density, sheath age and rhizome epiphytes, as well as shoot density anisotropy (Guidetti, 2000; Gambi & Cafiero, 2001; Gambi *et al.*, 2006; Cigliano *et al.*, 2007). Any common pattern has yet been evidences, but borer distribution seems to depend on different patterns of spatial resolution, especially when the distribution of different species is considered (Gambi *et al.*, 2005). In addition, certain studies have also addressed the hypothesis that borer organisms could be used as bioindicators of meadow health and good environmental conditions (Di Maida *et al.*, 2003; Gambi *et al.*, 2005). The aims of the present work are: 1) to evaluate the variability in polychaete borer abundance and distribution at different spatial scales (from kilometres to meters) utilizing a hierarchical sampling design (Underwood, 1997), never applied previously to these organisms, and their possible relation to meadow structure. In this respect, we considered two *Posidonia oceanica* meadows subjected to different degrees of human impact and hydrological conditions (Vasapollo, 2009; Vasapollo & Gambi, 2012); 2) to verify the pattern of variability of borer occurrence over time, considering two contrasting periods of canopy development, which mirrors the pattern of plant features, discussed in a related study (Vasapollo & Gambi, 2012) and, therefore, the possibility of using borer frequency to estimate meadow health; 3) to compare the patterns observed by this hierarchical design and fine spatial scale examination with those derived from previous studies, performed in the same meadows or on other beds previously studied at different spatial scales and time resolution.

Material and Methods

Study area

The study was conducted around the island of Ischia (Gulf of Naples, Italy; Fig. 1) in two *Posidonia oceanica* meadows.

The Lacco Ameno meadow

It is located on the north-west coast of Ischia Island, from the promontory of Punta Vico to the town of Casamicciola. This bed presents a continuous meadow from 1 to 30 m depth although recent studies show that the meadow is experiencing a reduction of the lower limit and shoot density, and also an increase in fragmentation in the upper areas (5 – 15 m), mainly due to the effect of pleasure

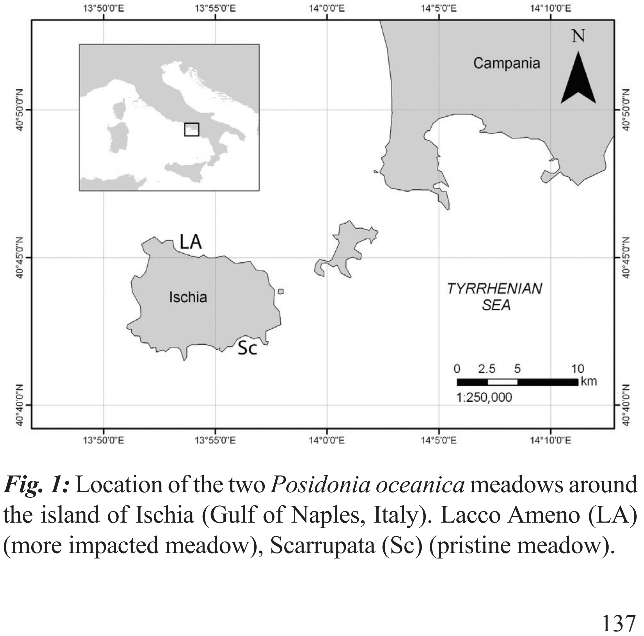

Fig. 1: Location of the two *Posidonia oceanica* meadows around the island of Ischia (Gulf of Naples, Italy). Lacco Ameno (LA) (more impacted meadow), Scarrupata (Sc) (pristine meadow).

boat anchoring during the summer season and to the influence of waste waters discharged through the two sewage pipes of the two adjacent towns of Lacco Ameno and Casamicciola (Buia *et al.*, 2003; Zucco, 2003; Zupo *et al.*, 2006a,b; Lopez y Royo *et al.*, 2011). A restricted part of this meadow (in front of "delle Monache" beach) is an Italian LTER station (Long term monitoring site) (http://data.lter-europe.net/deims/site/LTER_EU_IT_060; http://www.lteritalia.it/siti12/19.pdf) studied since 1989. In this specific area, borer polychaetes were previously studied both along depth (Gambi & Cafiero, 2001) and at finer spatial scale with the Kriging technique in relation to the shoot density pattern (Gambi *et al.*, 2006; Zupo *et al.*, 2006b). More recently, shoot density and plant features, according to a hierarchical design, have been analysed by Vasapollo & Gambi (2012), and have shown high variability of all plant variables at the smallest scale investigated (1-3 m).

The Scarrupata meadow

It is located on the south-east coast of Ischia Island, from Cape Grosso to Cape San Pancrazio. This meadow is exposed to relatively strong wave action, and is considered a pristine meadow since it is quite far from the towns, and neither direct sewage nor mooring (restricted to the shallower bottom areas during the summer season) affect the meadow (Buia *et al.*, 2003; Lopez y Royo *et al.*, 2011). The Scarrupata meadow starts from 8 - 10 m to around 35 m with a relatively continuous and gentle slope except for a steeper discontinuity between 15 and 20 m and between 25 and 30 m where the lower limit is characterized by a high and eroded matte testifying the presence of strong bottom currents (Buia *et al.*, 2003). Recently, shoot density and other plant features were studied at different spatial scales thus also highlighting a high variability at fine scale (1-3 m; Vasapollo & Gambi, 2012). Borer polychaetes have been studied in a previous investigation along depth and in time, demonstrating a pattern of segregation of different borer species according to depth: *Lysidice collaris* occurring mainly in shallow stands (10-15 m) and *L. ninetta* in deep stands (28 m) (Gambi *et al.*, 2003).

General physical parameter settings of both studied areas are reported in Lorenti *et al.* (2005).

Sampling strategy

The sampling followed a hierarchical nested design (Fig. 2) to test the variability of the polychaete borer frequencies and *Posidonia oceanica* shoot density and other plant features at different spatial scales, ranging from meters to kilometres. For each meadow (location), three randomly selected sites (A, B and C) were chosen at a distance of about 180 m apart; for each site, two stations (1 and 2) were randomly selected about 25 m apart; for each station, three plots (a, b and c) were randomly selected at 3 m apart; finally, two quadrats (x and y) of 40 x 40 cm per plot were randomly selected one meter apart.

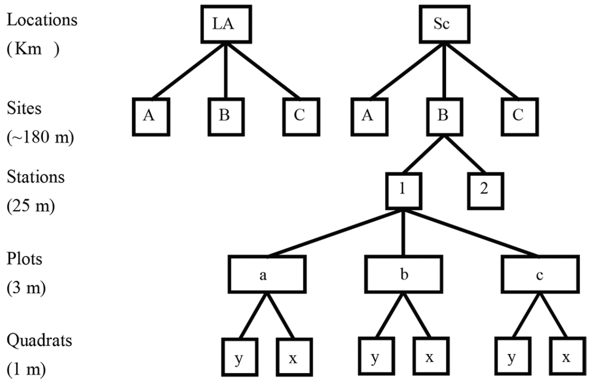

Fig. 2: Hierarchical sampling design used in this study. 2 random 40 x 40 quadrats (y and x; 1 meter apart) nested in each of 3 random plots (a, b and c; 3 meters apart), nested within each of two random stations (1 and 2; 25 meters apart), nested within each of 3 random sites (A, B and C; about 180 meters apart), nested within each of 2 fixed locations (LA and Sc; several kilometers apart). Depth of sampling fixed at 15-17 m.

The sampling was performed at a fixed depth range of 15 - 17 m to avoid further variability due to depth, which could potentially mask the effects of horizontal spatial variability. This depth range was selected since previous studies showed that shoot density variability was relatively high and that the most typical, abundant and rich vagile fauna, as well as borer organisms occurred at this depth (Gambi *et al.*, 1992; Gambi & Cafiero, 2001; Zupo *et al.*, 2006a). In addition, this range was logistically feasible to perform SCUBA diving sampling safely during relatively long and repetitive dives.

The collection of samples was repeated during two different periods: late spring/summer 2007 and late autumn/winter 2007–2008, representing the maximum and minimum in plant features and canopy development, respectively. This did not represent seasonality *tout court* but only the analysis of the plant features in two contrasting temperature and seasonal conditions. For each time period and meadow, the sampling was repeated on two different dates, spaced at least 1 month apart: May and July 2007, and November/December 2007 and February 2008 in Lacco Ameno; June and September 2007, and December/January and March 2008 in Scarrupata. For each 40 x 40 quadrat (plot), shoot density was evaluated by counting all the shoots, and 20 vertical (ortothropous) shoots were collected as deep in the sediment as was necessary to encounter the horizontal insertion of the vertical shoot for the analyses and stored in 4% buffered seawater formalin. Morphological features were measured in 10 *Posidona oceanica* shoots per quadrat following standard protocols (see, e.g. Buia *et al.*, 2004; Vasapollo, 2009; Vasapollo & Gambi, 2012). The rhizomes of all the 20 shoots of each quadrat were used for the analysis of borer occurrence and evaluation of borer frequency. In order to be consistent with other studies, species were named as in previous investigations. However, some taxonomic investigations are in progress since *L. ninetta* is a complex of at least two sibling species (Iannotta *et al.*, 2007; 2009), which currently cannot be distinguished morphologically. *L. collaris* is very close to the recently re-erected species *Lysidice margaritacea* Claparède, 1868 (Kurt Şahin & Çinar, 2009). However, re-examination of random samples of this species in our collection (approx 20%), according to the paper by Kurt Şahin & Çinar (2009), confirmed its taxonomic identity as *L. collaris*; we thus assume that only this species occurs in our study area.

Polychaete borer frequency was calculated for each plot as the Index of Borer (IB: percentage of shoots hosting living polychaetes with respect to the whole examined) (Gambi & Cafiero, 2001). IB was calculated both for the total number of polychaete borers and for individual species.

Data Analysis

Cochran's C test was performed as an *a priori* test to evaluate the homogeneity of variance of borer frequency (IB) between the samples. A fully-nested Analysis of Variance (ANOVA) was performed to test the null hypothesis of no differences both for shoot density and for borer frequencies, among meadows and sampling periods at any scale. The Student - Newman - Keuls (SNK) test was performed *a posteriori* whenever a significant difference was revealed by ANOVA. Multivariate analysis of variance based on permutations (PERMANOVA, Anderson, 2001a) was performed for single species of borer frequencies as well as for the variability of the guild. The analysis was based on Bray-Curtis dissimilarities. Each term in the analysis was tested using 4999 permutations to test an α significance level of 0.05 (Anderson, 2001a,b). The Monte Carlo procedure was used to calculate the probability when possible permutations were not sufficient to obtain a reasonable test (Anderson, 2001b). Single species IB were represented in a non-metric multidimensional scaling (nMDS) as centroids based on location - site - season - date to visualize the pattern of index distributions. Centroids were calculated based on scores of Principal Coordinate Analysis (PCoA) and then represented in the Euclidean space in the nMDS. Spearman's rank correlation was performed to test for relationships between shoot density and other morphological features of the *Posidonia* shoots, and polychaete borer frequencies.

Results

Shoot densities were significantly different ($F_{1, 144} = 90.7$, $p < 0.01$) among meadows. At Lacco Ameno, an average value of 255.7 ± 12.8 shoot m^{-2} was recorded (mean ± SE), while at the Scarrupata 411.5 ± 19.7 shoot m^{-2}.

A total of 1,252 borer polychaete individuals were found: *Lysidice collaris* (790 individuals), *L. ninetta* (337) and *L. unicornis* (125).

The percentages of the relative abundance for each species (Table 1) showed that *L. collaris* was the most abundant species for both meadows and seasons (especially in winter), while *L. ninetta* represented the second most abundant species, and *L. unicornis* was always the less abundant species.

Table 1: Percentages of three borer polychaetes found in two *Posidonia oceanica* meadows off the island of Ischia, over the two seasons considered.

	Lacco Ameno		Scarrupata	
	Summer	Winter	Summer	Winter
L. collaris	66.3%	52.5%	59.7%	70.8%
L. ninetta	24.4%	30.0%	30.1%	23.6%
L. unicornis	9.3%	17.5%	10.2%	5.7%

Borer polychaetes presented an elevated variability both between meadows and within each meadow. Total IB (all species together; Fig. 3, Table 2) varied at several spatial scales (from location to site and station). The mean frequency of the borer polychaetes was slightly higher at Scarrupata (22.8 ± 1.0%) than at Lacco Ameno (16.3 ± 1.0%), although at the latter location, some higher peaks were reached (e.g. at Site C, Stations 1 and 2 during the summer season); moreover, the SNK test showed that site C at station 1, in the summer, differed significantly from sites A and B (Table 3). Concerning the stations, the SNK test showed that differences for both dates of sampling were only apparent at Lacco Ameno, in the summer,. As regards the temporal scales, no significant differences were recorded.

When considering the IB of the single species, different patterns of variability were observed. The most abundant species, *L. collaris* (Fig. 4), showed significant differences at almost all the spatial scales considered except for the plot scale (Table 2). The interaction between location and season showed that at Lacco Ameno, in winter, the IB of that species reached the smallest values, being different from all the others (Table 3). Concerning the sites, most of the differences concerned Lacco Ameno, in summer, as well as the stations in summer (Table 3). The ANOVA also showed differences between the seasons, with higher values in summer than in winter (Table 3). Overall, *L. collaris* showed a more patchy distribution at Lacco

Ameno than at Scarrupata, and this was more evident during summer, while in winter its distribution appeared more homogeneous (as is also evident in Figure 4). The highest *L. collaris* IB values were recorded at Scarrupata, although two main peaks were observed at Lacco Ameno during summer at station 1, site C, on both sampling dates. The mean IB values of *L. collaris* were significantly different between the two meadows: 16.4 ± 1.0% at Scarrupata and 10.9 ± 0.9% at Lacco Ameno.

The other abundant borer polychaete species *L. ninetta* (Fig. 5), showed a higher patchiness in summer at Lacco Ameno while, during winter, its distribution pattern was more homogeneous. *L. ninetta* showed significant

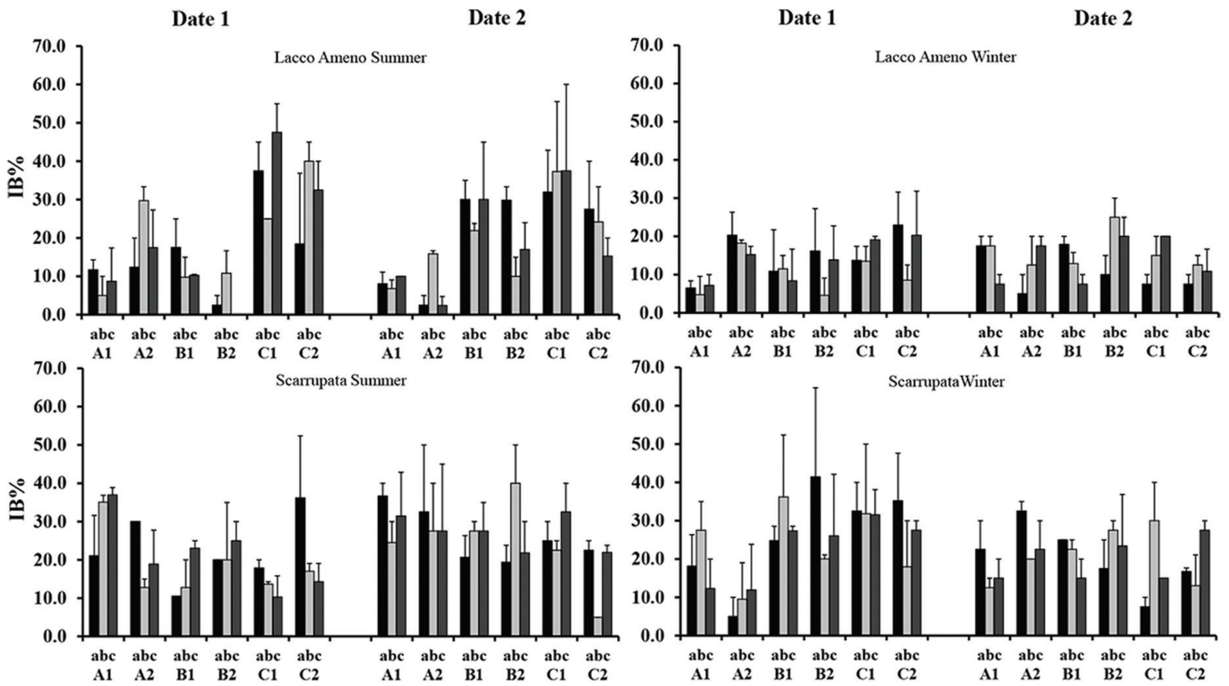

Fig. 3: Total Index of Borer (IB) of three borer polychaete species pooled together (*L. collaris*, *L. ninetta* and *L. unicornis*) measured in the two meadows (Lacco Ameno and Scarrupata) over two seasons (summer and winter) and two dates of sampling in each season.

Table 2: ANOVA and Cochran's C test results for total IB and single species IB (*Lysidice collaris*, *L. ninetta* and *L. unicornis*) at the different spatial and temporal scales: L = Location; S = Season; D = Date of sampling; Si = Site; St = Station; Pl = Plot. (F = Fixed factor; R = Random factor). Significant values (α = 0.05) in bold.

			IB tot			IB *L. collaris*			IB *L. ninetta*			IB *L. unicornis*		
	Effect	df	MS	F	p	MS	F	P	MS	F	p	MS	F	p
L	F	1	3068	15.94	**0.016**	2199.8	45.85	**0.002**	302.25	2.23	0.210	3.44	0.22	0.666
S	F	1	901.25	4.68	0.096	542.02	11.30	**0.028**	190.82	1.41	0.301	12.33	0.78	0.428
LS	F	1	429.67	2.23	0.209	1289.4	26.88	**0.007**	23.13	0.17	0.701	56.36	3.54	0.133
D(LS)	R	4	192.49	0.30	0.871	47.97	0.08	0.987	135.57	1.00	0.436	15.91	0.62	0.653
Si(D(LS))	R	16	633.86	4.14	**0.001**	592.97	5.17	**0.000**	135.47	1.94	0.068	25.56	1.68	0.121
St(Si(D(LS)))	R	24	152.98	1.64	**0.048**	114.67	1.71	**0.035**	69.65	2.07	**0.007**	15.19	1.41	0.124
Pl(St(Si(D(LS))))	R	96	93.14	0.78	0.900	66.94	0.62	0.993	33.630	1.10	0.295	10.79	0.84	0.812
Error		144	118.84			107.58			30.49			12.77		
Cochran's C				0.06	0.99		0.07	0.99		0.10	1.00		0.10	1.00
Transformation				none			None			none			none	

differences only at station scale (Table 2) and the SNK test showed that most of the differences were observed at Scarrupata (Table 3). Scarrupata showed the highest frequency of this borer species, in both seasons, although a large peak was evident at Lacco Ameno, at station 1 of site C, on the first date of sampling in summer (Fig. 5).

The IB of the less abundant species *L. unicornis* did not show any particular pattern of distribution with no significant differences at any scale or season (Table 2).

The PERMANOVA results (Table 4) clearly show the spatial variability between the two meadows (i.e. location scale), site and station scales, but it also shows that the single species IB presented a variability in the interaction location x season. This means that a certain variability due to season existed for each meadow, and that was consistent with the results obtained by the ANOVA and SNK tests in Tables 2 and 3 that clearly show how *L. collaris* varied between seasons at Lacco Ameno and, since it was the most abundant species, it also caused the significant differences with season in the PERMANOVA (Table 4).

The ordination obtained by plotting the centroids in the nMDS analysis (Fig. 6), showed a certain degree of

Table 3: SNK test results of the significant factors of the ANOVA analysis for total IB and single species IB (*Lysidice collaris* and *L. ninetta*) at the different spatial and temporal scales: D1= Date 1; D2= Date 2; A1= site A station 1 (same interpretation for the rest of the capital letters followed by number 1 and 2). Only significant values (α = 0.05) are reported.

Total IB				IB *L. collaris*				IB *L. ninetta*				
Lacco Ameno		A1<C1				Lw<Ls=Ss=Sw		Lacco Ameno	D1	all stations<C1	Summer	Stations
		B1<C1	Summer									
		A2>C2	Sites	Lacco Ameno		A1=B1>C1	Summer	Scarrupata	D2	A2<B2>C1	Summer	Stations
		A2>B2				A2<B2=C2	Sites		D1	A2<B1	Winter	
		A1<B1	Winter									
		A1<C1	Sites	Scarrupata		A2=C2>B2	Summer Sites					
	D1	A1<C1		Lacco Ameno	D1	B2<A1=A2=B1=C1=C2	Summer					
		B2<C1=C2	Summer		D2	A1=A2<C1	Stations					
			Stations									
	D2	A1<C1										
		A2<C1										

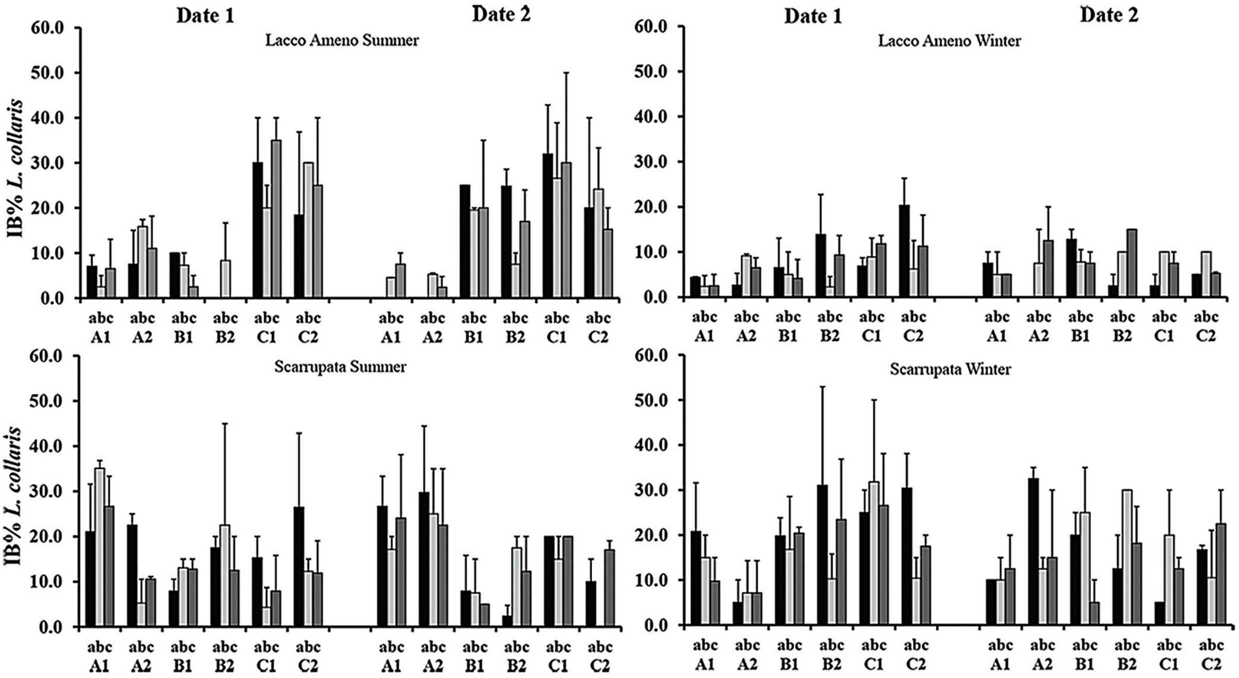

Fig. 4: *Lysidice collaris* Index of Borer (IB) measured in the two meadows (Lacco Ameno and Scarrupata) over two seasons (summer and winter) and two dates of sampling in each season.

separation of the two meadows, and also between sites and stations, although the separation between seasons in both meadows was not so evident. The nMDS showed a higher dispersion and scattering of the centroids (sample points) in the more impacted meadow at Lacco Ameno, compared to the more pristine location at Scarrupata. The analysis appeared to highlight the fact that at less disturbed and more pristine conditions of the meadows, there were more homogeneous distributions and frequencies of borer polychaetes.

Finally, the Spearman rank correlation (Table 5) between borer frequency, and *Posidonia* shoot density and morphological features showed significant positive correlations. However, the coefficients were all relatively low (range 0.22-0.29). Total IB showed modest correlation with Sheath Biomass (SB; 0.27), Leaf Standing Crop (LSC; 0.26) and Shoot density (ShD; 0.22); while *L. collaris* IB was better corre-

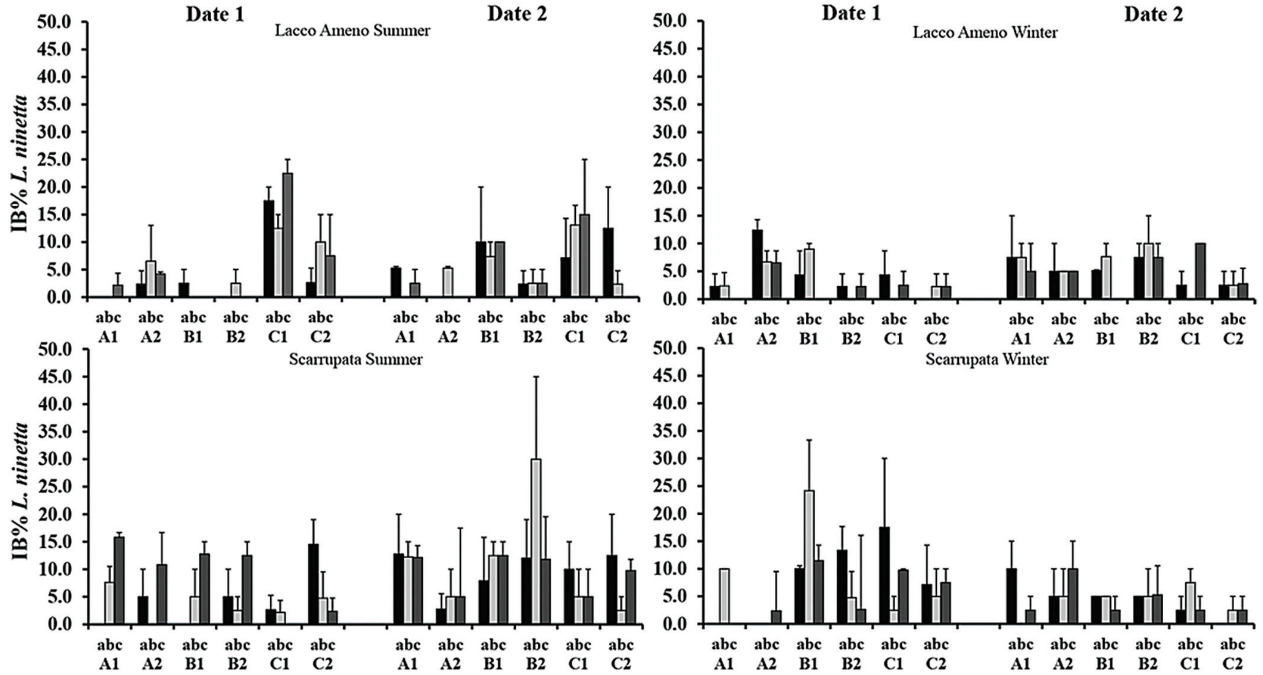

Fig. 5: *Lysidice ninetta* Index of Borer (IB) measured in the two meadows (Lacco Ameno and Scarrupata) over two seasons (summer and winter) and two dates of sampling in each season.

Table 4: PERMANOVA results of single species IB. P(perm) are values calculated by permutations; P(MC) are values calculated by Monte Carlo simulations. Significant values (α = 0.05) in bold.

				Borer Frequencies		
	df	SS	MS	pseudo-F	P(perm	P(MC)
L	1	17299.34	17299.3	6.69	**0.0028**	**0.0042**
S	1	5114.12	5114.12	1.98	0.1612	0.1506
LS	1	8355.89	8355.89	3.23	**0.0416**	**0.0498**
D(LS)	4	10350.02	2587.51	0.58	0.8422	0.8744
Si(D(LS))	16	71771.00	4485.69	1.70	**0.0244**	**0.0142**
St(Si(D(LS)))	24	63262.25	2635.93	1.68	**0.0008**	**0.0002**
Pl(St(Si(D(LS))))	96	150580.53	1568.55	0.93	0.7620	0.7552
Residual	144	242674.24	1685.24			
Total	287	569407.39				

lated with Leaf Area Index (LAI; 0.28), Leaf Biomass (LB; 0.29), Leaf Standing Crop (0.29) and Shoot density (0.25).

Discussion

It is widely accepted that studying more than a single spatial scale in complex habitats, such as seagrass meadows, may resolve several problems in interpreting distribution variability of the associated organisms (Wiens, 1989; Levin & Dec, 1992; Underwood, 1992; Fraschetti *et al.*, 2005). If a sampling design fails to consider the appropriate spatial scale, an inadequate and low representative description of the state of a particular meadow may occur. Moreover, a wrong selection of spatial and temporal scale may avoid comparisons among meadows because differences may be confused with natural variability (Balestri *et al.*, 2003; Vasapollo & Gambi, 2012). Following these considerations, we tested the hypothesis that heterogeneity across scales of *Posidonia oceanica* meadow features (as described by Vasapollo & Gambi, 2012) may influence the distribution of the associated animals, represented in this case by the borer polychaetes. In fact, as many authors have found for several plant features, spatial variability is high at scales of a few meters to centimetres (Balestri *et al.*, 2003; Borg *et al.*, 2005; Montefalcone *et al.*, 2008; Vasapollo & Gambi, 2012). But the question is: to what extent do organisms associated with *Posidonia* follow the same patterns of plant spatial variability? This is the first approach of multiple spatial scales sampling design applied to the study of the distribution of the borer polychaetes of *Posidonia* sheaths.

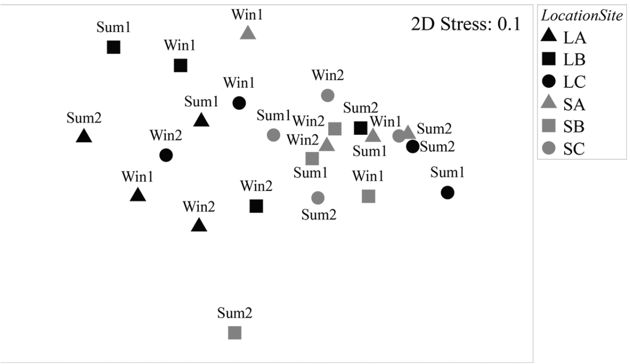

Fig. 6: Non-metric multidimensional scaling (nMDS) ordination on the basis of Euclidean distances of centroids of the three borer polychaete species: *Lysidice collaris*, *L. ninetta* and *L. unicornis*. LA = Lacco Ameno site A; LB = Lacco Ameno site B; LC = Lacco Ameno site C; SA = Scarrupata site A; SB = Scarrupata site B; SC = Scarrupata site C; Win1 = Winter date 1; Win2 = Winter date 2; Sum1 = Summer date 1; Sum2 = Summer date 2.

The borer species recorded here and their respective dominance are consistent with previous studies in the same area (Guidetti *et al.*, 1997; Gambi, 2002), as well as in various geographic areas investigated (Di Maida *et al.*, 2003; Gambi *et al.*, 2005). In various studies, in fact, *L. collaris* was the dominant species, while *L. unicornis* is always the less abundant species. *L. unicornis* is smaller in size, compared to the other two species, especially as regards its body diameter (Gambi & Cigliano, 2006), and therefore it may be more constrained by size in colonizing a tough tissue such as *Posidonia* sheaths. The mean IB values obtained in this investigation in the same meadows differed from previous studies and are not fully comparable due to different depths, sampling design and periods considered (Gambi & Cafiero, 2001, Gambi *et al.*, 2003, 2006).

Regardless of the actual IB values, the hierarchical sampling design revealed differences in borer polychaete distribution at different spatial scales in the two studied meadows (from a few km to 100s and 10s of metres), and also allowed an assessment of the random variability of these organisms. Most of the variance associated to the IB indices (all borer species together and *L. collaris*) was determined by the Lacco Ameno meadow and only marginally by the Scarrupata one (*L. ninetta*). This was an important finding since it seems that in a highly dense and pristine meadow (Scarrupata), polychaete borers tend to occupy the space available (*Posidonia* shoots) in a more homogeneous way. While, in a more disturbed meadow (Lacco Ameno), resulting in a higher patchy habitat, their variability at small scale increases and their distribution is less homogenous. Therefore, the IB values *per se* do not provide an indication of the “environmental health” status of the meadows, as hypothesized by a number of previous studies (Di Maida *et al.*, 2003; Gambi *et al.*, 2005), but it is the scale of spatial variability (i.e. the patchiness) of borer frequency that could be a descriptor of the general conditions of the meadow.

Table 5: Spearman’s Rank Correlations between IBs and the *Posidonia oceanica* features. NL: number of leaves; LS: leaf surface; SL: sheath length; LL: leaf length; LW: leaf width; LAI: leaf area index; LB: leaf biomass; SB: sheath biomass; LSC: leaf standing crop; ShD: shoot density. Significant correlations (α = 0.05) in bold.

	Spearman’s Rank Correlations			
	IB tot	IB *L. collaris*	IB *L. ninetta*	IB *L. unicornis*
NL	0.1	-0.01	0.08	0.07
LS	0.14	**0.21**	0.08	-0.14
SL	0.02	0.06	-0.04	-0.01
LL	0.14	**0.22**	0.05	-0.12
LW	-0.12	-0.09	-0.09	-0.03
LAI	**0.22**	**0.28**	0.09	**-0.16**
LB	**0.23**	**0.29**	0.14	-0.14
SB	**0.27**	**0.26**	**0.17**	-0.02
LSC	**0.26**	**0.29**	0.12	-0.06
ShD	**0.22**	**0.25**	0.09	-0.16

One may expect that the higher patchy distribution of borers at Lacco Ameno was due not only to the patchiness of the habitat (*Posidonia* shoots), as above stated and as a result of human activities such as boat anchoring that increases gaps and patchiness in the distribution of the plant (Zupo *et al.*, 2006a), but also the actual shoot density ranges and spatial variability (Vasapollo & Gambi, 2012). However, although the overall shoot density was much lower at Lacco Ameno than at Scarrupata (Vasapollo & Gambi, 2012), the values of borer frequency are not as low at Lacco Ameno, as expected by simply considering shoot density ranges. Some of the previous investigations have hypothesized that polychaete borers could be somehow related to shoot density (Guidetti, 2000; Gambi *et al.*, 2005), also by applying spatial autocorrelation techniques (Gambi *et al.*, 2006). In this latter study, polychaete borers, similarly to *Posidonia* shoots (Zupo *et al.*, 2006a,b), formed nest-like structures where shoot density as well as borer frequencies increased toward the centre of the “nests”. However, in that study, only a single nest structure overlap was found; the mismatching of the other nests clearly shows that the two patterns of distribution are driven by different factors acting at different spatial scales. In a study at regional scale, along the Sardinia coast, borer frequency was found to increase with depth, with higher IB at deeper stands (25-30 m) than at shallow ones (5-10 m), so that borers seemed to be somehow negatively related to shoot density (Gambi *et al.*, 2005). However, when the correlation was tested, shoot density raw values did not show any correlation with IB. On the other hand, the IB values were positively correlated, although with low values, when density-ranges were normalized to the respective depth of collection (according to

Pergent-Martini & Pergent, 1996) and when the IB of single species was analysed (Gambi *et al.*, 2005).

The relationship between shoot density and IB values recorded here was positive, but again relatively weak, and due to the fact that we considered a single depth range, we could not test correlation with density depth-related categories. Thus, this relationship requires further support and clarification.

Therefore, in addition to higher meadow patchiness, other reasons should be considered to explain the higher IB variability at Lacco Ameno. Borer distribution has been proven to be influenced by local and small scale factors such as sheath age (Gambi & Cafiero, 2001), as well as epiphytic covering of the rhizomes (Cigliano *et al.*, 2007). Therefore, factors such as the age of the individual shoots and plant patches, and the level of epiphyte colonization on the rhizomes, or the degree of mud content and burial of the rhizomes in the sediment may further influence the scale of spatial variability of these organisms, at a level that they can mask and alter the effects of other factors acting at larger scale. Thus, the use of IB as an indicator of the condition of a meadow should be considered with caution since the variability of plant features mentioned above, as well as other local factors could be more relevant than environmental differences between meadows at larger scales.

Although borer polychaetes represent a particular group in the *Posidonia* ecosystem, exploiting a low palatable source of food (sheath detritus) and favouring the fragmentation and recycling of refractory organic matter, they are not strictly linked to the plant, and the borer species are also common in other coastal habitats. Thus, other factors related to the distribution of borer species in other habitats that may supply their occurrence on the meadows, could be considered. These factors may act at different spatial scale and somehow independently to the presence and structure of *Posidonia oceanica* meadows. These results highlight the complicated and unsolved relationships between habitat features and the distribution of organisms, especially in systems as spatially complex as the seagrass meadows. However, designing an appropriate spatial and temporal sampling program, and considering all the different variables at the same scale is the only way to generate and test further hypotheses and explanations. Analyses carried out for other motile fauna taxonomic components are in progress to integrate the data presented here and shed more light on this complex topic.

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