

# Recruitment of Serpuloidea (Annelida: Polychaeta) in a marine cave of the Ionian Sea (Italy, central Mediterranean)

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This paper is the first attempt to study the recruitment of Serpuloideans in a Mediterranean marine cave through the use of artificial substrates placed in three different positions, from the entrance to the interior of the cave. This study provides qualitative and quantitative data concerning Serpuloidea recruitment on panels removed successively after one, three, six and 12 months of permanence in the cave. A homogeneous distribution of juveniles Spirorbidae throughout the cave axis had already been detected after one month of panel immersion. Spirorbids were recorded also on panels removed after three months as well as serpulids, which began to be detected during this sampling time, even though represented by just one species. A significantly different pattern of distribution throughout the cave axis was observed after only six months, while other serpulids were detected for the first time. The pattern of species distribution seemed to reflect the biotic and environmental conditions of the cave. The highest serpuloidean species abundance and diversity was found on panels placed in the intermediate position within the cave. After only one year of panel immersion, a total of 20 taxa (11 Serpulidae and 9 Spirorbidae) was recorded, including five species listed for the first time in the marine caves of the Salento Peninsula.

## INTRODUCTION

Serpuloidea (Annelida: Polychaeta) are pioneer organisms able to colonize a wide variety of substrates including man-made structures (Watson & Barnes, 2004). Many studies have been carried out on the timing and factors influencing Serpuloidean settlement and recruitment in their natural environments (Qian, 1999; Kupriyanova et al., 2001; Cotter et al., 2003). However, there is not much literature concerning the recruitment pattern of these polychaetes within marine caves. With reference to the Mediterranean basin, all the available information refers to mature Serpuloidea assemblages already representing a component of complex benthic communities within caves.

With the improvement and spreading of diving techniques, investigations on serpulid distribution in Mediterranean marine caves became possible.

Recently, Bianchi & Sanfilippo (2003) reviewed the last fifty years of research on serpulids and spirorbids in Italian submarine caves. In 1959, Banse analysed material collected in shallow marine caves along the Tyrrhenian Italian coast, then other studies on the benthic communities in three marine caves within the Peninsula Sorrentina (Tyrrhenian Sea), demonstrated that serpulid species usually occupy the darkest inner caves (Balduzzi et al., 1989). On the other hand, in the submarine caves the light and water movement and exchange are reduced and the sessile benthic fauna living in these conditions is mainly represented by filter-feeders (Gili et al., 1986; Balduzzi et al., 1989; Bianchi & Sanfilippo, 2003).

All the above mentioned studies refer to mature Serpuloidea assemblages. In this framework, the present paper constitutes a first attempt to characterize the recruitment of Serpuloideans within a marine cave, and provides the spatio-temporal distribution through the use of artificial substrates. The qualitative data obtained after one year of study on the Serpuloidea recruited on experimental bare panels placed in the Mediterranean marine cave 'Grotta di Ciolo' (Ionian Sea) are provided in this study. Moreover quantitative data concerning the species zonation along the cave axis are also presented.

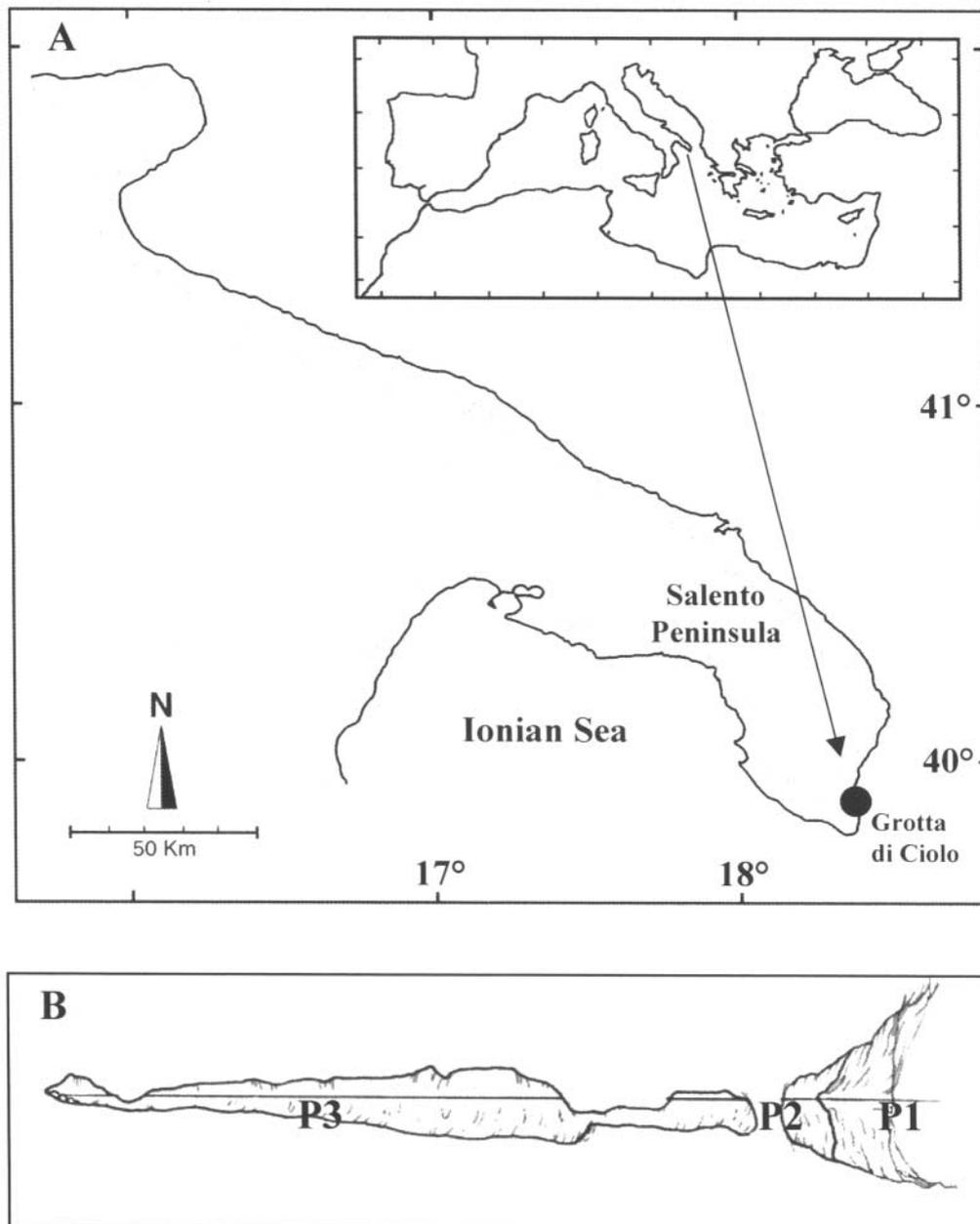
## MATERIALS AND METHODS

### *Biotope*

The site (Figure 1) is the shallow marine cave, 'Grotta di Ciolo', located near Capo di Leuca (southern Apulia, south-eastern Italy, central Mediterranean, 39°50'38"N–18°23'11"E). It is a horizontal 'blind cave' of more than 120 m long with a single semi-submerged entrance and air chambers along the greater part of it. The entrance floor is 5.5 m deep, while the inner recesses are only 0.5 m deep. The cave system is constantly supplied by hypogean freshwater, which generates sharp haloclines and thermoclines in the inner portion.

### *Experimental methods*

Seventy-two baked clay 225 cm<sup>2</sup> panels (15×15 cm) (according to Cotter et al., 2003) were placed by SCUBA



**Figure 1.** (A) Map of the study area and (B) side view of the marine cave 'Grotta di Ciolo' with location of each position within the cave: P1, entrance; P2, intermediate position; P3, inner position.

divers in March 2002 in three different positions of the cave: at the entrance (P1), and at an intermediate (P2) and inner position (P3) at 20 and 80 m from the entrance. The choice of the panel material was made according to its non toxicity, its rough surface, and its easy tractability. In each cave position, two metallic grids of 1.5 m<sup>2</sup> ('Areas' in the experimental design) were placed on the cave wall (left side of the cave axis). In order to avoid both interferences on early succession from the existing benthos and differences in recruitment derived from surface orienting, the panels were fixed on the metallic grids by two nylon ties through two holes made in the opposite edges of each panel.

Three panels were retrieved from each area (corresponding to six panels from each position) after one

(T1), three (T2), six (T3) and 12 months (T4). The panels were preserved in neutralized (pH 7.3) formalin solution at 4% in seawater and examined under a dissecting microscope in the laboratory. All of the Serpuloidean recruits on each panel surfaces were coloured by rose Bengal solution, classified and counted. Some of the Serpuloideans remained unidentified because the specimens had not reached the size for which unequivocal species identification could be made. In this case, the unidentified individuals were classified at genus level only and herein reported as *Genus* sp. indet. The species were mostly identified according to the classification made by Bianchi & Morri (2000) who considered the Serpuloidea as a superfamily comprised of the two families Serpulidae *sensu strictu* and Spirorbidae.



**Figure 2.** Non-metric multidimensional scaling ordinations (nMDS plots) on the basis of the Bray–Curtis dissimilarities on  $\ln(x+1)$  transformed data testing the differences among Serpuloidea assemblages on panels collected in each sampling Time: T1 (one month), T2 (three months), T3 (six months) and T4 (one year). Each point represents the centroid of the  $N=3$  observations from the two areas within each position (see Materials and Methods).

#### Statistical analysis

The experimental design consisted of three factors: Time (T) has 4 levels, is orthogonal and fixed; Position (P) has 3 levels, is orthogonal and fixed; Area (A) has 2 levels, is nested in P and random,  $N=3$  per combination of factors, for a total of 72 observation units. Analysis of variance (ANOVA) was used to assess significant differences in the mean abundance of Serpuloidea among 'Positions', among 'Areas within Position' and among 'Times'. Prior to the analysis, the homogeneity of variance was tested using the Cochran's test and, when necessary, data were  $\ln(x+1)$  transformed to remove heterostochasticity. Whenever transformations did not produce homogeneous variance, ANOVA was used nevertheless after setting  $\alpha=0.01$  to compensate for the increased likelihood of Type I Error. The Student–Newman–Keuls test (SNK) was used for post-hoc comparisons among means. The analysis was done using GMAV 5 computer program (University of Sydney, Australia).

Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001) was used to assess differences in the Serpuloidea assemblages according to the full experimental design.

The Bray–Curtis similarity matrix on untransformed data was used to generate 2-dimensional ordination plots by the non-metric Multidimensional Scaling (nMDS) technique (Clarke 1993).

## RESULTS

#### Qualitative data

A total of 20 taxa (11 Serpulidae and 9 Spirorbidae) recruited on the 72 panels were recorded after one year of study (Table 1). The taxa for which the identification at species level was not always possible, belong to the genera *Hydroides*, *Protula*, *Salmacina* and *Vermiliopsis* among Serpulidae and *Spirorbis* among Spirorbidae.

One month after the panel immersion (T1), only small indeterminate specimens belonging to the genus *Spirorbis* had settled on panels within all the three considered positions.

At T2, the Serpulidae *Pomatoceros triqueter* (Linnaeus, 1767) and the Spirorbidae *Janua pagenstecheri* (Quatrefages, 1865) and *Neodexiospira pseudocorrugata* (Bush, 1904) were found for the first time on the panels; 11 out of the

**Table 1.** Taxonomic list of serpuloideans detected on the panels collected throughout one year of study from each position.

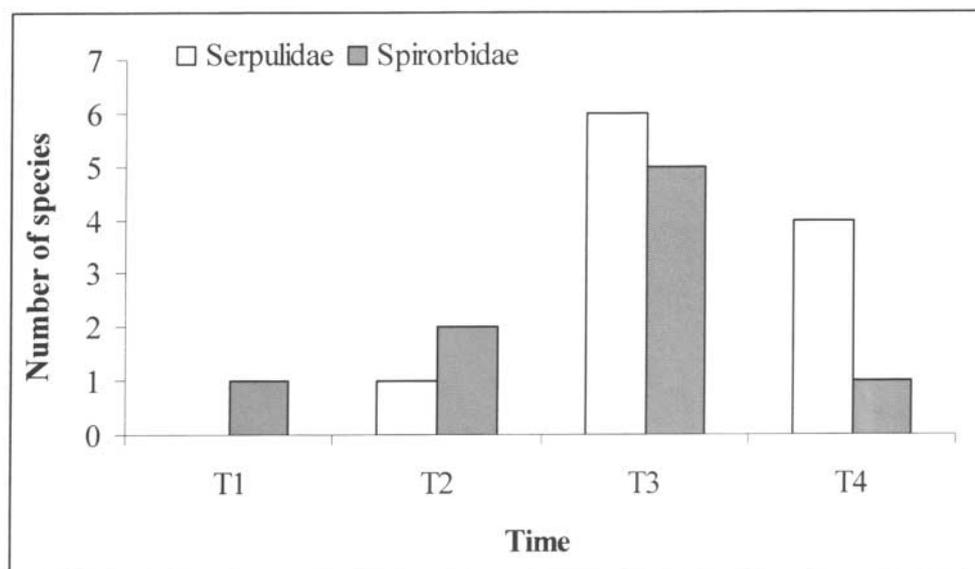
	T1 April 2002			T2 June 2002			T3 September 2002			T4 March 2003		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
<b>SERPULIDAE</b>												
<i>Hydroides pseudouncinatus</i> (Zibrowius, 1968)	-	-	-	-	-	-	-	+	-	-	+	+
<i>Hydroides niger</i> Zibrowius, 1971	-	-	-	-	-	-	-	+	+	+	+	-
<i>Hydroides</i> sp. indet.	-	-	-	-	-	-	-	-	+	-	-	-
<i>Vermiliopsis striaticeps</i> (Grübe, 1862)	-	-	-	-	-	-	-	-	-	-	+	+
<i>Vermiliopsis</i> sp. indet.	-	-	-	-	-	-	-	+	+	-	-	+
<i>Pomatoceros triqueter</i> (Linnaeus, 1767)	-	-	-	-	+	-	-	+	-	-	+	-
<i>Serpula concharum</i> Langerhans, 1880	-	-	-	-	-	-	-	-	-	-	+	-
<i>Spirobranchus polytrema</i> (Philippi, 1844)	-	-	-	-	-	-	-	-	-	+	+	-
<i>Salmacina</i> sp. indet.	-	-	-	-	-	-	-	+	-	-	+	-
<i>Protula</i> sp. indet.	-	-	-	-	-	-	-	+	+	-	+	-
<i>Semivermilia cribrata</i> (O.G. Costa, 1861)	-	-	-	-	-	-	-	-	-	-	+	-
<b>SPIRORBIDAE</b>												
<i>Spirorbis infundibulum</i> Harris & Knight-Jones, 1964	-	-	-	-	-	-	-	+	-	-	-	-
<i>Spirorbis cuneatus</i> Gee, 1964	-	-	-	-	-	-	+	+	-	-	-	-
<i>Spirorbis</i> sp. indet.	+	+	+	+	-	+	+	-	-	-	-	-
<i>Protolaeospira striata</i> (Quiévreux, 1963)	-	-	-	-	-	-	-	+	-	-	+	-
<i>Pileolaria heteropoma</i> (Zibrowius, 1968)	-	-	-	-	-	-	-	-	-	+	-	-
<i>Vinearua koehleri</i> (Caullery & Mesnil, 1897)	-	-	-	-	-	-	+	+	+	+	+	+
<i>Janua pagenstecheri</i> (Quatrefages, 1865)	-	-	-	-	+	+	-	+	-	+	+	-
<i>Janua</i> sp.	-	-	-	-	-	-	-	+	-	-	+	-
<i>Neodexiospira pseudocorrugata</i> (Bush, 1904)	-	-	-	-	+	-	+	-	-	+	+	+

+, present; -, absent.

20 Serpuloideans detected throughout one year of observation, were firstly found at T3: six Serpulidae (*Hydroides pseudouncinatus* Zibrowius, 1968, *H. niger* Zibrowius, 1971, *Salmacina* sp. indet., *Vermiliopsis* sp. indet., *Protula* sp. indet., *Hydroides* sp. indet.) and five Spirorbidae (*Spirorbis cuneatus* Gee, 1964, *S. infundibulum* Harris & Knight-Jones, 1964, *Protolaeospira striata* (Quiévreux, 1963), *Vinearua koehleri* (Caullery & Mesnil, 1897) and *Janua* sp.). Finally, at T4 the remaining five species, the Serpulidae

*Spirobranchus polytrema* (Philippi, 1844), *Vermiliopsis striaticeps* (Grübe, 1862), *Serpula concharum* (Langerhans, 1880), *Semivermilia cribrata* (O.G. Costa, 1861), and the Spirorbidae *Pileolaria heteropoma* (Zibrowius, 1968), appeared on the panels.

Data relating to the Serpuloidea distribution throughout the cave axis during the study period, are reported in Table 1. In T1 and T2, only *Spirorbis* juveniles were recruited on the panels deployed at P1. Since T3,

**Figure 3.** Number of Serpulidae and Spirorbidae newly recruited on panels at each sampling time.

**Table 2.** Summaries of ANOVAs testing for spatial and temporal differences in average Serpuloidea abundances settled within the marine cave. Terms already involved in significant higher order interactions were not analysed.

Source of variation	DF	MS	F	P	F versus
T	3	244780			
P	2	573469			
A(P)	3	196.19	0.01	n.s.	Residual
T P	6	185957	864.28	***	T×A(P)
T A(P)	9	215.16	0.02	1	Residual
Residual	48	13946.17			
Total	71				

Cochran's test (Transform=None)	SNK test
n.s.	T1
	T2
	T3
	T4

T, Time; P, Position; A, Area; \*\*\*,  $P < 0.001$ ; n.s., not significant.

most of the Spirorbidae taxa were mainly recorded at P2. By contrast, only four Spirorbidae taxa (*Spirorbis juveniles* indet., *Janua pagenstecheri*, *Vinearia koehleri* and *Neodexiospira pseudocorrugata*) were able to colonize the inner section of the cave (P3). Moreover, *Janua* sp., *Protolaeospira striata* and *Spirorbis infundibulum* were detected only at P2.

Among Serpulidae, *Pomatoceros triqueter* (tube length of up to 30 mm) was the first species identified on panels collected after three months from the intermediate position (P2). Almost all serpulid taxa listed in T3 and T4, were mainly recorded once again at the intermediate position (P2).

Moreover, Serpulidae *Pomatoceros triqueter*, *Salmacina* sp. indet., *Serpula concharum* and *Semivermilia cribrata* were always detected in the intermediate position.

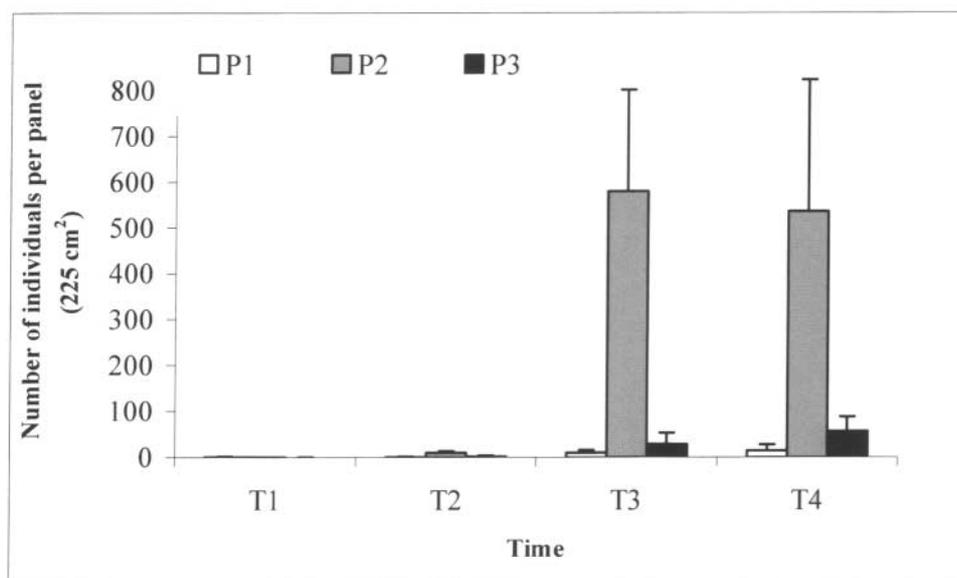
The PERMANOVA reveals a significant 'Time × Position' interaction ( $F_{6,9}=3.20$ ,  $P < 0.001$ ), thus suggesting different patterns of assemblage development depending on the

different positions. Such differences are portrayed by nMDS ordinations (Figure 2) and become graphically clearer considering the plots relating to T3 and T4.

#### Quantitative data

Data concerning the newly recruited taxa for each Time are reported in Figure 3, showing that the highest number of species was recorded in T3 for both Serpulidae and Spirorbidae (6 and 5 respectively). By contrast, the lowest number of newly recruited spirorbids was recorded in T1 and T4 (1 species). Serpulids reached the minimum recruitment number in T2 (only 1 species) and were absent in T1.

Considering all three positions within each sampling time, a total of 12 specimens (0.003 ind/cm<sup>2</sup>) was reported in T1, 83 (0.02 ind/cm<sup>2</sup>) in T2, 3717 (0.92 ind/cm<sup>2</sup>) in T3 and 3648 (0.90 ind/cm<sup>2</sup>) in T4.



**Figure 4.** Spatial and temporal distribution of the whole Serpuloidea group within the marine cave. Each column represents the mean total number ( $\pm$  Standard Deviation,  $N=6$ ) of individuals detected on panels in each sampling time (T1, T2, T3 and T4) at each of the three positions (P1, P2 and P3).

In Figure 4 quantitative data concerning the spatial and temporal distribution are presented for overall Serpuloidean recruitment per panel (225 cm<sup>2</sup>). In T1 and T2, a very low level of recruitment was observed for all the three positions with the maximum value recorded in T2 at P2 (10.83 ± 3.49). The highest abundances were found within the intermediate position (P2) after six (T3) and 12 (T4) months (580.33 ± 221.84 and 535.67 ± 288.77) respectively.

The ANOVA performed on quantitative data enable a significant 'Time × Position' interaction to be detected (Table 2), indicating that differences in time are not consistent across the different positions. The SNK test, reveals significant differences between P2 and P3 in T2, T3 and T4. By contrast, no significant differences are evident in T1 among the three positions.

## DISCUSSION

In the marine coastal waters the light intensity and the hydrodynamism-depending factors such as temperature, salinity and dissolved oxygen have measurable effects on Serpuloidean settlement and recruitment (Kupriyanova et al., 2001). These environmental factors are particularly relevant within marine caves where they drastically change along the horizontal axis (Gili et al., 1986). It is also well known that recruitment of serpulids and spirorbids is affected by the immersion time of experimental bare panels in the marine environment (e.g. Watson & Barnes, 2004).

### T1 – first month

The homogeneous distribution of *Spirorbis* throughout the cave axis together with the constant presence of spirorbids during the whole year of study, can be explained by the ecological and reproductive features of the Spirorbidae family. The continuous reproduction is the general rule in spirorbids (Kupriyanova et al., 2001) and the life cycle strategy within this group involves a short larval period in the plankton after which the larvae creep or swim from a few minutes to a day before settling (Gee & Knight-Jones, 1962). The small size and fragility of the *Spirorbis* specimens settled in this first time did not allow identification at species level which was possible only from T3, when *S. infundibulum* and *S. cuneatus* were clearly recognized. However, small *Spirorbis* specimens newly recruited and unidentified at species level were still detected on panels retrieved in T2 and T3.

### T2 – third month

The record of *Pomatoceros triqueter* in June, corresponding to the beginning of the summer period in the Mediterranean basin, suggests that the unsuccessful recruitment of this species in April (T1) could be related to the low temperature recorded in the surrounding environment. This hypothesis is also supported by experimental studies carried out by Castric-Fey (1983), reporting that *Pomatoceros* settlement is influenced by temperature, and by Cotter et al. (2003) mentioning that there is a single annual peak abundance in *Pomatoceros* recruitment during the summer in Bantry Bay (Ireland). Moreover,

laboratory studies have demonstrated that *P. triqueter* larvae are planktonic from ten days to two months before their settlement (Bianchi, 1981). Bianchi (1981) also refers that *P. triqueter* spawns throughout the whole year. This assumption leads us to believe that *P. triqueter* larvae would have been present in the water column since T1, but the unfavourable condition of the low sea-temperature inhibited their settlement and recruitment on the first set of retrieved panels.

As regards Spirorbidae, our results concerning *Janua pagenstecheri* agreed with data reported by Terlizzi et al. (2000) from the harbour of Ischia (Tyrrhenian Sea), where this species settles mainly during the June–July period. Similarly, *Neodexiospira pseudocorrugata* has been reported to settle in the summer period by Bianchi (1981), thus confirming their reproductive seasonality.

### T3 – sixth month

A significantly different pattern of distribution throughout the cave axis was observed after only six months (T3) of panel immersion, when other serpulid species were detected for the first time and the distribution pattern of newly recruited species of Serpuloidea seemed to reflect the biotic and environmental conditions of the cave. It is well known that the decrease in light and food availability from the entrance to the interior of the caves, has a major influence on the communities leading to decreased abundances and densities, and changes in composition (Gili et al., 1986).

In this study, the comparative analysis of the three positions within the cave 'Grotta di Ciolo', evidenced the highest diversity at the intermediate one (P2), and the lowest at the inner position (P3). The intermediate zone of the cave could offer the most favourable conditions for the establishment of a more diversified assemblage, probably because of the relatively higher trophic supply, other than the protection from potentially harmful water dynamics and the absence of light-dependent competitors (e.g. macroalgae) which instead are both relevant at P1 (Denitto, 2004; Denitto et al., in press).

On the contrary, the low colonization of the dark, confined cave position, may be due to severe limitations in food resources leading to sterile pseudopopulations or a sink population, deficiency in larval dispersal from both allochthonous and autochthonous sources, with a particular effect of sedimentation and finally predation by filter-feeding invertebrates on larvae released and moving nearby them (Balduzzi et al., 1989).

The reduced hydrodynamism in the inner position could affect the Serpuloidean dispersal within the cave (Bianchi & Sanfilippo, 2003). Most of the Serpulidae are, in fact, free spawners releasing planktonic larvae which are able to swim and reach the naked rocky walls located at the inner part of the caves, whilst Spirorbidae are brooders producing larvae freely swimming for only a few hours (Kupriyanova et al., 2001). However, patterns of gregarious settlement in environments characterized by reduced hydrodynamism could also be observed after the occasional settlement and following reproduction of spirorbids. Indeed the spirorbids are able to reach these confined positions during periodical events such as sea-storms and tidal currents (Bianchi & Sanfilippo, 2003).

Among the species newly recruited on panels collected in T3, the spirorbid *Spirorbis cuneatus* mainly chose the outer position whilst *Vinearia koehleri* showed no preferences in colonizing specific positions within the cave on the basis of its homogeneous distribution along the cave axis. The findings of these two species in this sampling time are in accordance with Bianchi's results (1981), attesting to a settlement in the warm season. At P2 two species belonging to the genus *Janua* were found: *J. pagenstecheri* and *J. sp.* which had been detected in the following and last sampling time. The latter species, quite different from the former regarding the opercular shape, shows morphological features similar to *Spirorbis gnomonicus* Bailey, 1969 from Chios (Greece), characterized by an opercular plate with an eccentric distal process. This species was later synonymized with *Janua pagenstecheri* (Quatrefages, 1865) by Knight-Jones et al. (1975). In addition, specimens from the Aegean Sea (Milos, Greece) showing similar morphological features, were described by Bianchi & Morri (2000) as belonging to *J. pagenstecheri gnomonica*, considered a subspecies of *J. pagenstecheri* for having distinct ecological requirements. With regard to our finding, the co-occurrence of *J. pagenstecheri* and *J. sp.*, could suggest that they are two distinct species and further investigations would be needed to clarify this issue.

#### T4—twelfth month

The analysis of the panels retrieved after one year of exposure shows the highest species abundance and diversity within the cave. With the exception of *Hydroides niger* and *Pomatoceros triqueter* reaching in March the maximum tube length of approximately 50 mm, a very high abundance of small-sized specimens has been reported also in T4. The rate of post-settlement growth of juvenile Serpuloidea has been studied well for some species (for a recent review see Kupriyanova et al., 2001), among which *P. triqueter* and *J. pagenstecheri* are part of this study. The tube length reported for *P. triqueter* seems to be in accordance with the growth rates recorded for this species and whose growth has also been observed in another Mediterranean coastal habitat. Indeed in this case, values ranged from 0.2 in winter to 7.0 mm/month in summer (Sentz-Braconnot, 1968). Moreover, Klöckner (1976) demonstrated that *P. triqueter* tube growth rate reaches the maximum at temperatures ranging between 6°C and 22°C, as a result of the function of the worm body size (the larger the worm, the slower it grows). In our study, *P. triqueter* has always been detected at P2, thus suggesting that this species does not suffer from trophic depletion thanks to the relative strong water movement still detectable at this intermediate position. Therefore, the favourable environment allows this species to increase at the maximum growth rate.

In conclusion, after only one year of exposure of the panels, a total of 20 taxa was recorded, including five species listed for the first time in the Salento marine caves: *Pomatoceros triqueter*, *Serpula concharum*, *Spirobranchus polytrema*, *Spirorbis infundibulum* and *Vinearia koehleri*. Moreover, for the first time, we note the presence of living *Spirorbis infundibulum* specimens within an Italian marine cave biotope. Previously, this species was recorded only as empty tubes in north-western Sicily caves (Bianchi &

Sanfilippo, 2003). In addition, Bianchi & Sanfilippo (2003) reported 23 species of Serpuloidea inhabiting the marine caves along the Salento Peninsula, traditionally considered as a biogeographical-crossroads between the western and eastern Mediterranean basins and Adriatic Sea. Our results contribute to improve the taxonomic knowledge which is currently available and add new ecological data on the spatio-temporal distribution of early Serpuloidean assemblages in the submarine caves.

The numerous species and the high number of specimens found on the test panels confirm their role as pioneer organisms in primary successions of evolving assemblages in a submarine cave (Denitto, 2004; Denitto et al., in press). Moreover, the data gathered in this study can be used as a background to conduct further investigation aimed at a better appreciation of the mimic assemblage patterns on natural substrata and on a larger temporal scale. In this context, they could be useful to fully approach the ecological role of the Serpuloidea within more complex cave communities.

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