

Article

Diversity and Distribution Patterns of Hard Bottom Polychaete Assemblages in the North Adriatic Sea (Mediterranean)

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Abstract: The knowledge on the hard bottom polychaete assemblages in the Northern Adriatic Sea, a Mediterranean region strongly affected by environmental pressures, is scarce and outdated. The objective of this paper was to update the information on polychaete diversity and depict their patterns of natural spatial variation, in relation to changes in algal coverage at increasing depth. Hard bottom benthos was quantitatively sampled by scraping off the substrate from three stations at Sveti Ivan Island (North Adriatic) at three depths (1.5 m, 5 m and 25 m). Polychaete fauna comprised 107 taxa (the majority of them identified at species level) belonging to 22 families, with the family Syllidae ranking first in terms of number of species, followed by Sabellidae, Nereididae, Eunicidae and Serpulidae. Considering the number of polychaete species and their identity, the present data differed considerably from previous studies carried out in the area. Two alien species, *Lepidonotus tenuisetosus*, which represented a new record for the Adriatic Sea, and *Nereis persica*, were recorded. The highest mean abundance, species diversity and internal structural similarity of polychaete assemblages were found at 5 m depth, characterised by complex and heterogeneous algal habitat. The DISTLM forward analysis revealed that the distribution of several algal taxa as well as some algal functional-morphological groups significantly explained the observed distribution patterns of abundance and diversity of polychaete assemblages. The diversity of the North Adriatic hard bottom polychaete fauna is largely underestimated and needs regular updating in order to detect and monitor changes of benthic communities in the area.

Keywords: Annelida; Polychaeta; benthos; community structure; algae

1. Introduction

The North Adriatic Sea is the northernmost sector of the Mediterranean Sea, with peculiar geomorphological, hydrographical and biogeographical characteristics. With an average depth of 35 m, this semienclosed basin represents the most extensive region of shallow water in the Mediterranean [1,2], being one of the most productive areas too, due to high amount of nutrients loaded by the Po River [3]. It is also the coldest Mediterranean sector, together with the Gulf of Lion and the North Aegean

Sea, thus inhabited by species of boreal affinity [4,5]. The Adriatic Sea and in particular its northern part, exhibits the highest species richness of invertebrates in the Mediterranean basin [6]. Being also densely populated and thus under high anthropogenic pressures, the North Adriatic is a sensitive area currently undergoing severe environmental changes (climate change, fishing impacts, destruction of habitats, pollution, introduction of non indigenous species) that affect the benthic communities [6–8]. Updating the knowledge about benthic diversity and understanding patterns of benthic assemblages' vertical and horizontal spatial variation are benchmarks for detecting and monitoring environmental changes, also according to the European Union Marine Strategy Framework Directive [9].

Polychaetes are among the most abundant and species-rich marine benthic groups, showing a wide functional diversity and adaptation to different environmental conditions [10,11]. Thus, they are often used as surrogates to estimate the state and dynamics of benthic communities [12–15]. North Adriatic soft bottom polychaete fauna is well known, e.g., [16–32], while the knowledge on hard bottom polychaete assemblages from natural substrates is scarce, i.e., [33–42] and their diversity might be largely underestimated as indicated by recent studies dealing with Syllidae and Sabellidae polychaete families [43,44].

The structure of hard bottom benthic assemblages is characterised by having high small- and middle-scale spatial variability (i.e., patchiness), both alongshore and at different depths, which is caused by various interplaying biological (e.g., predation, competition, recruitment) and physical-chemical (e.g., light intensity, temperature, salinity, hydrodynamics, sedimentation, habitat complexity) factors [45–50]. In particular, the distribution of hard-bottom polychaetes is strongly dependent on the bathymetric variation in algal composition and the associated changes in algal forms [51–53]. However, studies aiming at understanding the role of the above-mentioned factors in structuring polychaete assemblages have not been done in the North Adriatic so far.

The aims of our study were: (1) to update the knowledge of the faunal composition of the North Adriatic hard bottom polychaete assemblages, and (2) to assess variation of their spatial distribution in relation to changes in algal assemblages along a bathymetric gradient and according to substrate orientation.

2. Materials and Methods

The study area was in the vicinity of the city of Rovinj (Croatia, North Adriatic Sea) at Sveti Ivan Island. Islands of the Rovinj archipelago and the coastal area of up to 500 m from the coastline, were proclaimed by Rovinj Municipality a natural landscape reserve. The area is characterized by calcareous rocky shelf extending from 0 to about 25 m depth, with a gentle-medium slope. The submarine slopes of the Sveti Ivan Island are representing typical infralittoral environments of the North Adriatic Shelf.

Collecting surveys were carried out in June 2007, taking into consideration that the maximum development of macroalgal assemblages in the Northern Adriatic Sea occurs in the spring–early summer period [54,55]. Benthos was sampled using scuba diving at stations A (N 45° 02.69', E 13° 37.18') and B (N 45° 02.7', E 13° 37.48') on the southern side and station C (N 45° 02.87', E 13° 37.34') on the northern side of the Island (Figure 1). At each station, three depths (1.5 m, 5 m and 25 m) were appointed along a vertical transect and at each depth three replicates of 10 × 10 cm surface quadrats covered with macroalgae were randomly chosen (27 samples in total). Samples were collected by scraping off the substrate including the whole algal coverage present within the 10 × 10 cm quadrats using hammer and chisel. The scraped material was collected within plastic bags. Although ordinarily a sampling area of 20 × 20 cm is suggested for benthic studies in the Mediterranean [56], the small sample size (10 × 10 cm) in this research was chosen in order to minimize sampling impacts in the natural reserve. Furthermore, 10 × 10 cm replicate areas were already used in other studies dealing with polychaetes in the Mediterranean Sea and revealed to allow acceptable representation of polychaete diversity and distribution patterns [11,57,58]. Each replicate unit (10 × 10 cm surface

quadrats) was photographed underwater to facilitate the determination of the associated algal taxa and their percent coverage.

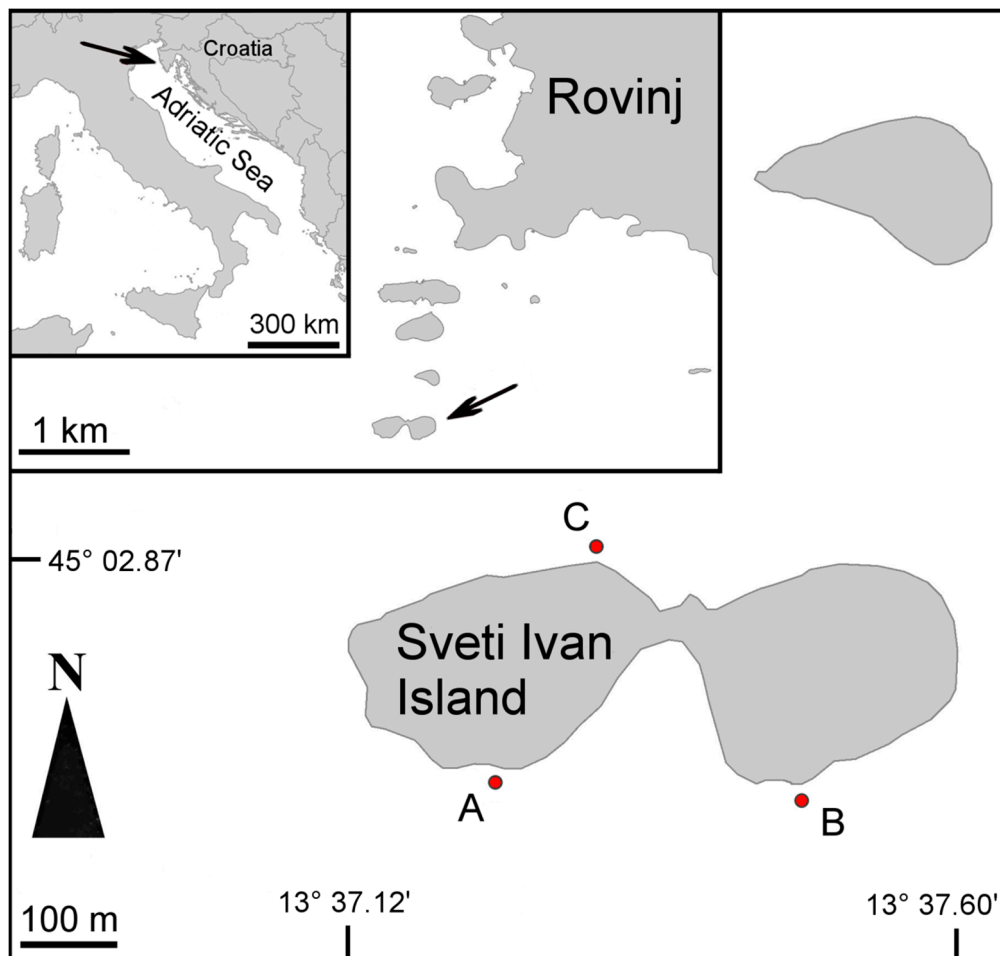


Figure 1. Location of the study area and sampling stations (A–C). Arrows indicating city of Rovinj area and Sveti Ivan Island.

In the laboratory, samples were fixed in 8% formaldehyde seawater solution and subsequently rinsed with fresh water and sieved through 0.5mm mesh and preserved in 70% ethanol. Polychaetes and algae were sorted and determined to the lowest taxonomic level possible using stereo- and light microscope. In some cases, it was not possible to identify the organisms at species level but only at higher taxonomic categories (e.g., genus, family). For this reason, we used the term taxa instead of species throughout the manuscript to indicate the recorded taxonomic entities. When specimens belonging to the same genus were clearly different from each other, however, we considered them belonging to different undetermined species (e.g., *Nereis* sp. 1 and *Nereis* sp. 2). The coverage of each algal thallus, representing the surface covered in an orthogonal projection, was determined according to Boudouresque [56] and Cormaci et al. [59]. Algae from each sample were placed on the surface that has an area equal to that sampled in situ (10×10 cm). Consequently, for each algal species the percentage of total quadrat area (100 cm^2) covered by the projection of all the thalli was estimated. Value for total algal coverage can reach more than 100% in the presence of multilayered assemblages (as canopy forming algae) or epibiosis [56]. Additionally, algae were grouped in the following functional-morphological groups: Articulated calcareous, Corticated, Encrusting, Filamentous, Foliose and Leathery [60,61], and coverage of each functional-morphological group per sample was calculated as well. The polychaete specimens were deposited at the Center for Marine Research of the Ruđer Bošković Institute in Rovinj (Croatia) (IRB-CIM) and in the collection of the Natural History Museum in Rijeka (Croatia) (PMR).

For each replicate sample, polychaete assemblages were characterised by their respective abundance (N), species richness (S), Hill's species diversity index ($N1$) and Hill's evenness index ($N10$) [62]. In order to graphically represent trends in the number of species found within the collected samples, species accumulation curve of observed species (Sobs) was created. Moreover, to estimate the number of species potentially present in the area, curves of estimated number of species were calculated using the Jackknife 1, Jackknife 2 and Bootstrap methods [63].

Nonparametric distance-based permutational analysis of variance by permutation of residuals under a reduced model (PERMANOVA) [64,65] was used to test for differences in univariate indices (based on Euclidean distances of untransformed data) and in multivariate structure (based on Bray–Curtis similarity of untransformed data) of the polychaete assemblages between stations and depths. PERMANOVA design included two crossed factors: station (3 levels, random) and depth (3 levels, fixed). Post-hoc pair-wise comparisons allowed detecting the source of significant variations. For significant terms, a permutational analysis of multivariate dispersions (PERMDISP) [66] was used to test the homogeneity of samples dispersion from their group centroids. When the number of permutations was low (less than 1000), Monte Carlo probability ($P(\text{MC})$) was considered instead of permutational probability ($P(\text{perm})$). To calculate p values for PERMANOVA and PERMDISP, 9999 permutations were used.

Multivariate patterns were visualised by nonmetric multidimensional scaling ordination (nMDS). The similarity percentage routine (SIMPER) [67] (70% cut off), was used to detect the taxa most responsible for within-depth similarity and between-depth dissimilarity and, at each depth, the between-station dissimilarity. When analysing dissimilarity between stations, taxa were considered important if they exceeded an arbitrarily chosen threshold of 4% of dissimilarity between stations at each depth.

Potential relationships among structuring algal taxa, algal functional-morphological groups, depth and orientation of the sampling station in respect to the island geography (south/north) and the variation of polychaete assemblages were assessed by nonparametric multiple regression analyses based on Bray–Curtis dissimilarities [65] exerting the distance-based multivariate analysis for a linear model using forward selection procedure (DISTLM), with 9999 permutations. Resemblance matrix produced by DISTLM analyses informed on the correlation among all pairs of explanatory variables to check for multicollinearity [68]. The predictor variables included depth, orientation and percent coverage of each structuring algal species (cut-off 5% of the total cover) in the first DISTLM analysis and depth, orientation and percent coverage of algal functional-morphological groups in the second one. Results of the forward selection procedure with the sequential tests (i.e., fitting each variable one at a time, conditional on the variables that are already included in the model) were presented. All analyses were done using PRIMER v.6 [69], with the add-on PERMANOVA+ [66].

3. Results

3.1. Algal Assemblages

Forty-eight algal taxa were recorded in the research area (Table S1). Total mean algal coverage was higher at 1.5 m and 5 m depths if compared to 25 m depth in all three stations (Figure 2). At 1.5 m depth, algal assemblages were characterised by the high coverage of the articulated calcareous algae (mostly *Corallina officinalis* Linnaeus, *Halimnion* sp. and *Jania* spp.), the corticated algae (mostly *Alsidium* sp. and *Laurencia obtusa* (Hudson) J.V.Lamouroux) and the foliose algae (mostly *Padina pavonica* (Linnaeus) Thivy, *Dictyota dichotoma* (Hudson) J.V.Lamouroux and *Dictyota dichotoma* var. *intricata* (C.Agardh) Greville) (Figure 3a). Filamentous algae (mostly *Ectocarpus* sp. and *Carradoriella elongata* (Hudson) A.M.Savoie & G.W.Saunders) and encrusting algae (mostly *Valonia utricularis* (Roth) C.Agardh and *Peyssonnelia rubra* (Greville) J.Agardh) were present with low percent coverage at 1.5 m depth. At 5 m depth, encrusting algae (mostly *P. rubra* and *P. heteromorpha* (Zanardini) Athanasiadis) dominated, followed by foliose algae (mostly *P. pavonica*, *Flabellia petiolata* (Turra) Nizamuddin, 1987

and *D. dichotoma*), while articulated calcareous algae (mostly *C. officinalis*, *Halimion* sp. and *Jania* spp) were present with a lower coverage (Figure 3b). Corticated algae (mostly *Gelidium spinosum* (S.G.Gmelin) P.C.Silva) and filamentous algae (mostly *Polysiphonia* sp. and *Cladophora* spp.) had very low percent coverage at 5 m depth. At 25 m depth, filamentous algae (mostly *Cladophora* spp., *Sphacelaria plumula* Zanardini, and *Polysiphonia* sp.) were prevalent and followed by encrusting algae (mostly *P. rubra* and an unidentified rose coloured encrusting algae) (Figure 3c). Foliose algae (mostly *Rhodymenia* sp., *D. dichotoma* and unidentified foliose algae) showed a very low coverage at 25 m depth. At this depth, corticated algae (mostly *Rodriguezella* sp.) were present only at stations B and C, while articulated calcareous algae (only *Halimeda tuna* (J.Ellis & Solander) J.V.Lamouroux) were found only at station C, all of them with very low coverage. *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, the single species of the leathery functional group, appeared with a 2% coverage only in one sample from 1.5 m depth (Figure 2).

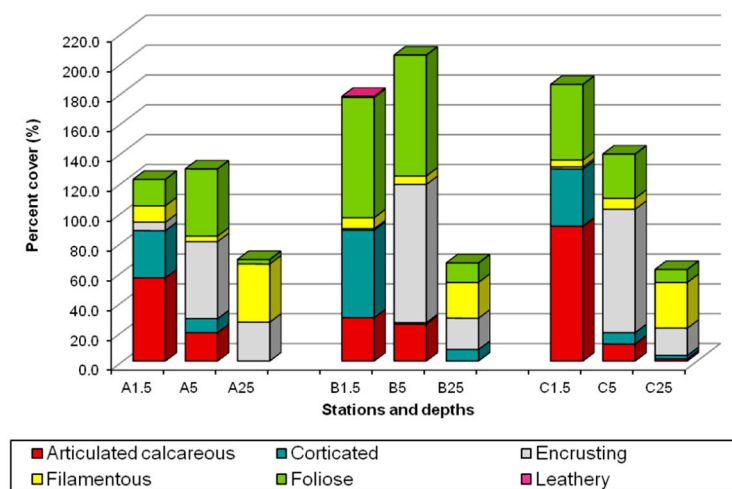


Figure 2. Mean total percent coverage of algae and algal functional-morphological groups, per stations and depths.

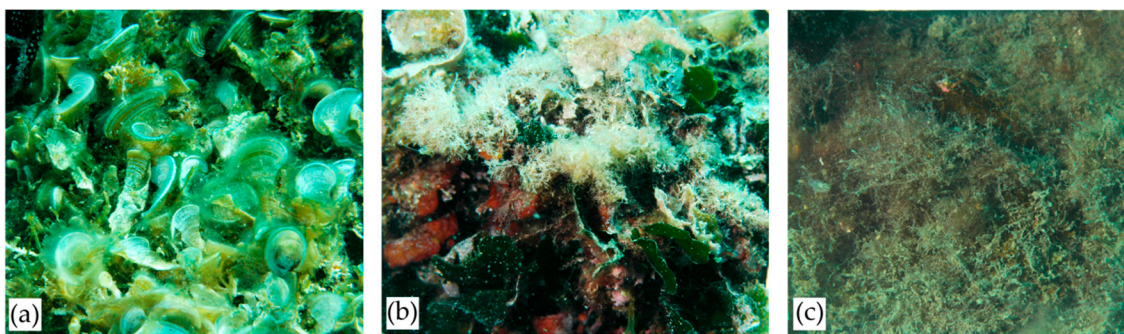


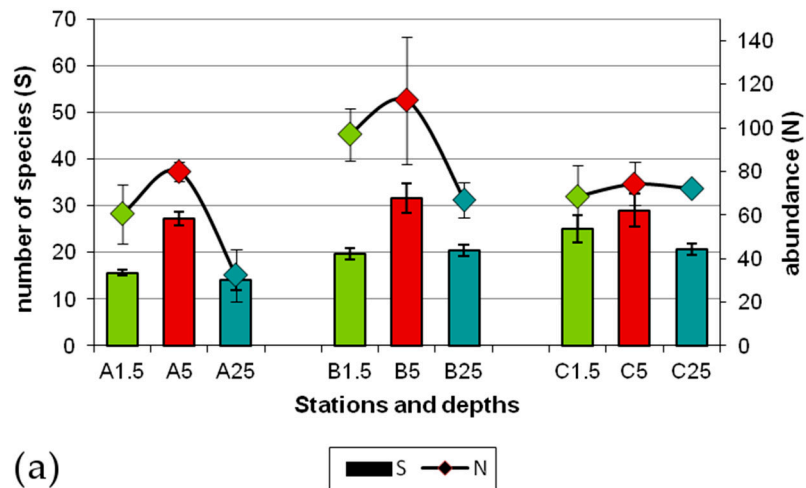
Figure 3. Representative algal assemblages at: (a) 1.5 m depth; (b) 5 m depth; (c) 25 m depth.

3.2. Composition and Diversity of Polychaete Assemblages

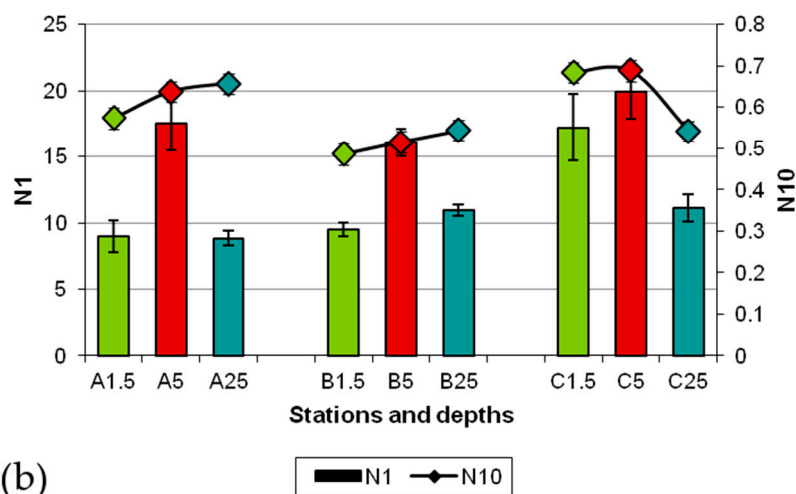
As a whole, 1993 polychaete specimens from 107 taxa (94 at species level) belonging to 22 families were found (Table S2). The richest families in terms of number of species were Syllidae (39 species), Sabellidae (13), Nereididae (12), Eunicidae (7) and Serpulidae (7), while eleven families were represented by only one species. Altogether 62 species from 16 families were found at 1.5 m depth, 73 species from 15 families at 5 m depth and 66 species from 15 families at 25 m depth.

The mean number of individuals and species, as well as the mean Hill's species diversity index, were the highest at 5 m depth, while no particular pattern could be observed in the Hill's evenness index (Figure 4a,b). Significant differences in species richness and Hill's species diversity index were revealed

both horizontally (between stations) and vertically (between depths), while differences in polychaete abundance and Hill's evenness index were significant only between stations (Table 1). In particular, the assemblages at 5 m depth had significantly higher species richness and Hill's species diversity index than those of 1.5 m and 25 m depths, at most of the stations (Table S3). Significant differences between stations in all univariate diversity descriptors were revealed mostly at 1.5m depth and to a lesser extent also at 25 m depth (Table S3).



(a)



(b)

Figure 4. (a) Mean (\pm SE) total abundance (N; lines) and species richness (S; bars); (b) Mean (\pm SE) Hill's species diversity index (N1; bars) and Hill's evenness index (N10; lines) at each studies station and depth.

Syllidae was the dominant family at all three depths, both in abundance and species richness, followed by Nereididae, Sabellidae and Eunicidae at 1.5 m depth and, at 5m depth, by Sabellidae, Nereididae and Eunicidae considering abundance, and Nereididae, Eunicidae and Sabellidae considering species richness (Figure 5). At 25 m depth, Syllidae were particularly dominant, and followed by Sabellidae, Nereididae, Serpulidae and Eunicidae, both considering abundance and number of species. Abundance and diversity of Serpulidae increased with depth. In fact, at 1.5 m only one specimen of *Vermiliopsis infundibulum* (Philippi, 1844) was found, while the Serpulidae were represented by 18 specimens belonging to 4 species at 5 m depth and 23 specimens belonging to 6 species at 25 m depth.

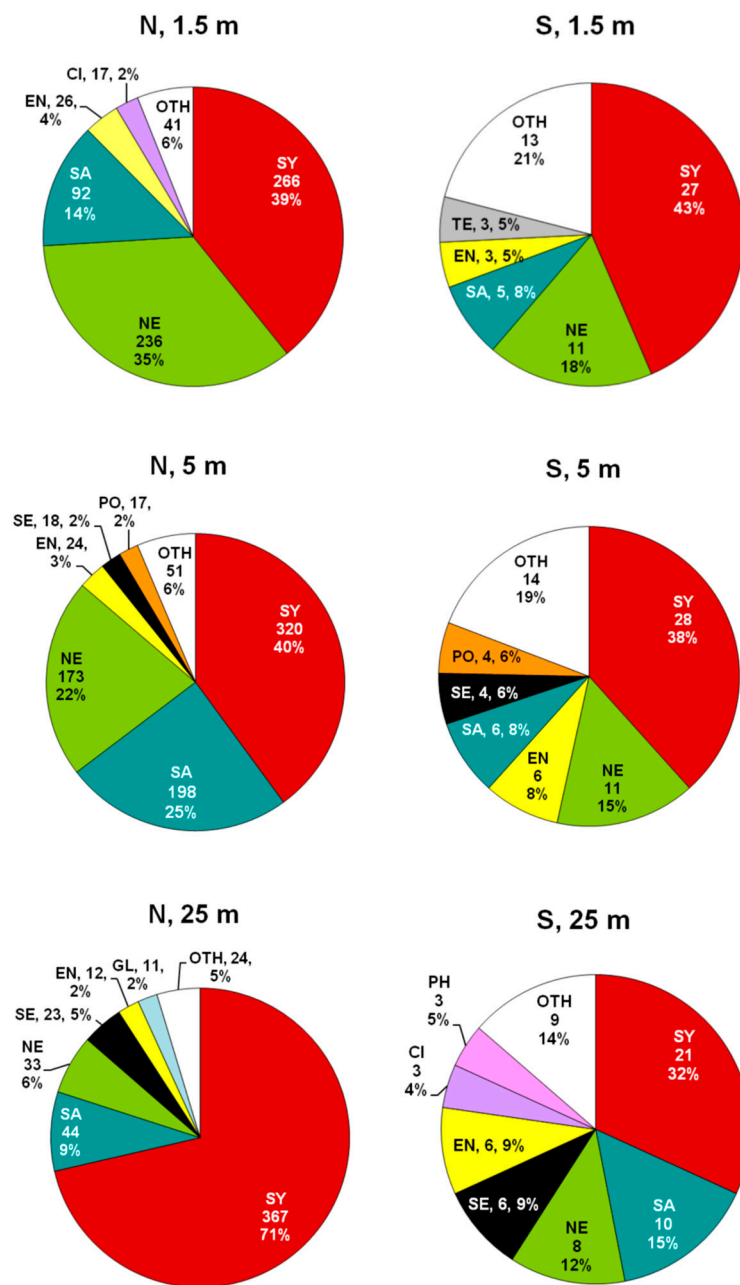


Figure 5. Cumulative abundance (N; on the left side) and species richness (S; on the right side) of polychaetes per family at the three studied depths. CI—Cirratulidae, EN—Eunicidae, GL—Glyceridae, NE—Nereididae, PH—Phyllodocidae, PO—Polynoidae, SA—Sabellidae, SE—Serpulidae, SY—Syllidae, TE—Terebellidae, OTH—other families.

Most species were locally rare, with 36 species found only in one sample (Figure S1), while only *Sphaerosyllis pirifera* Claparède, 1868, *Amphiglena mediterranea* (Leydig, 1851) and *Platynereis dumerilii* (Audouin & Milne Edwards, 1833) were widely distributed in the whole area in most of the samples (25, 21 and 21 respectively). Neither the accumulation, nor the estimator curves (Jackknife1, Jackknife2, Bootstrap) reached the asymptote (Figure S2), suggesting a potential higher number of species ranging from 121 to 167 in the area.

Table 1. Results of PERMANOVA and PERMDISP analyses (untransformed data) testing for differences in abundance (N), species richness (S), Hill's species diversity index (N1), Hill's evenness index (N10) and structure (Stru) of polychaete assemblages between stations (st) and depths (de). df, degrees of freedom; SS, sum of squares; MS, mean squares; Up, unique perms; F, F-ratio; P (perm), probability. Significant *p*-values (*p* < 0.05) are given in bold.

Source	df	PERMANOVA					PERMDISP				
		SS	MS	Pseudo-F	Up	P (perm)	df1	df2	F	P (perm)	
N	st	2	5442.3	2721.1	4.7539	9953	0.0192	2	24	2.4351	0.1038
	de	2	4607.2	2303.6	4.3316	6066	0.0995	-	-	-	-
	stxde	4	2127.3	531.81	0.92908	9956	0.4792	-	-	-	-
	Res	18	10303	572.41							
S	st	2	178.74	89.37	6.4866	9949	0.0076	2	24	0.3413	0.8379
	de	2	627.63	313.81	18.976	3825	0.0254	2	24	0.2366	0.7997
	stxde	4	66.148	16.537	1.2003	9950	0.3431	-	-	-	-
	Res	18	248	13.778							
N1	st	2	101.95	50.974	7.9594	9955	0.0028	2	24	1.0783	0.4925
	de	2	282.77	141.38	9.7949	6091	0.0459	2	24	3.2631	0.0885
	stxde	4	57.737	14.434	2.2538	9950	0.0995	-	-	-	-
	Res	18	115.28	6.4043							
N10	st	2	0.0809	0.0405	4.293	9950	0.0304	2	24	1.1321	0.4103
	de	2	0.0065	0.0032	0.25075	6086	0.7786	-	-	-	-
	stxde	4	0.0517	0.0129	1.3718	9937	0.2792	-	-	-	-
	Res	18	0.1696	0.0094							
Stru	st	2	7282.2	3641.1	2.5077	9900	0.0001	2	24	4.2513	0.0549
	de	2	26808	13404	4.3784	6114	0.0176	2	24	2.4735	0.1668
	stxde	4	12246	3061.4	2.1084	9843	0.0001	8	18	8.0471	0.0122
	Res	18	26136	1452							

3.3. Patterns of Variation of Polychaete Assemblages Structure

There were significant differences in the structure of polychaete assemblages both among stations and depths, but also for the interaction term station \times depth (Table 1), with the significant alongshore variation occurring only at 1.5 m depth, between stations B and A and between stations B and C (Table S3). Moreover, significant differences in the structure of polychaete assemblages were revealed at all stations between 1.5 m and 25 m depth, and between 5 m and 25 m depth, while differences between 1.5 m and 5 m depth were significant only at station B. Results of PERMDISP analyses confirmed that these differences were not barely due to differences in the dispersion of the samples (Table S3).

At all stations, difference in polychaete assemblages between three depths was clearly evident, with assemblages from 1.5 m and 5 m depths being more similar among each other and different from those at 25 m depth (Figure 6). Station A showed the highest scattered distribution among replicates at 25 m depth, and the most homogeneous assemblages were those at 5 m depth. In fact, the average similarity in species composition and abundance between samples was the highest at 5 m depth (45.22%), intermediate at 25 m depth (39.51%) and the lowest at 1.5 m depth (34.39%) (Table 2A). The most abundant species were the sabellid *Amphiglena mediterranea*, the nereidids *Platynereis dumerilii*, *Nereis usticensis* Cantone, Catalano & Badalamenti, 2003 and *Nereis pulsatoria* (Savigny, 1822) and the syllids *Syllis rosea* (Langerhans, 1879), *S. pirifera* and *Exogone dispar* (Webster, 1879) at 1.5 m depth; the sabellid *A. mediterranea*, the syllids *S. pirifera*, *E. dispar*, *Syllis variegata* Grube, 1860, *Syllis prolifera* Krohn, 1852 and *Syllis corallicola* Verrill, 1900, and nereidids *P. dumerilii*, *Nereis* sp. 1 and unidentified juvenile nereidids at 5 m depth; and the syllids *Syllis armillaris* (O.F. Müller, 1776), *Syllis gracilis* Grube, 1840, *Syllis gerlachi* (Hartmann-Schröder, 1960) and *S. pirifera* at 25 m depth (Table 2A). The lowest average dissimilarity in species composition and abundance was between 1.5 m and 5 m depth (68.67%), while it was higher between 1.5 m and 25 m (88.42%) and between 5 m and 25 m depth (79.59%) (Table 2B).

Differences in abundance of the most abundant species of Syllidae (i.e., *S. rosea*, *S. prolifera*, *S. armillaris*, *S. gracilis*, *S. gerlachi*, *S. pirifera*), Nereididae (i.e., *N. usticensis*, *P. dumerilii*, *Nereis* sp. 1, Nereididae juv. indet.) and Sabellidae (i.e., *A. mediterranea*) at different depths, were mainly responsible for these dissimilarities (Table 2B). *Amphiglena mediterranea* had the highest abundance at 5 m depth, was slightly less abundant at 1.5 m depth and poorly represented at 25 m depth. *Syllis armillaris* and *S. gracilis* were very abundant at 25 m depth and poorly represented at 1.5 and 5 m depth. Only low percentages of dissimilarities were due to the differences in taxonomic composition (i.e., presence/absence of species). Namely, the sabellid *Amphicorina rovigensis* Mikac, Giangrande & Licciano, 2013 characterised 1.5 m depth but was absent at 5 m depth, *N. usticensis* and *S. rosea* characterised 1.5 m depth but were absent at 25 m depth, the syllid *Odontosyllis ctenostoma* Claparède, 1868 characterised 5 m depth, but was absent at 25 m depth, and the sabellid *Hypsicomus stichophthalmos* (Grube, 1863) characterised 25 m depth but was absent at 5 m depth (Table 2B).

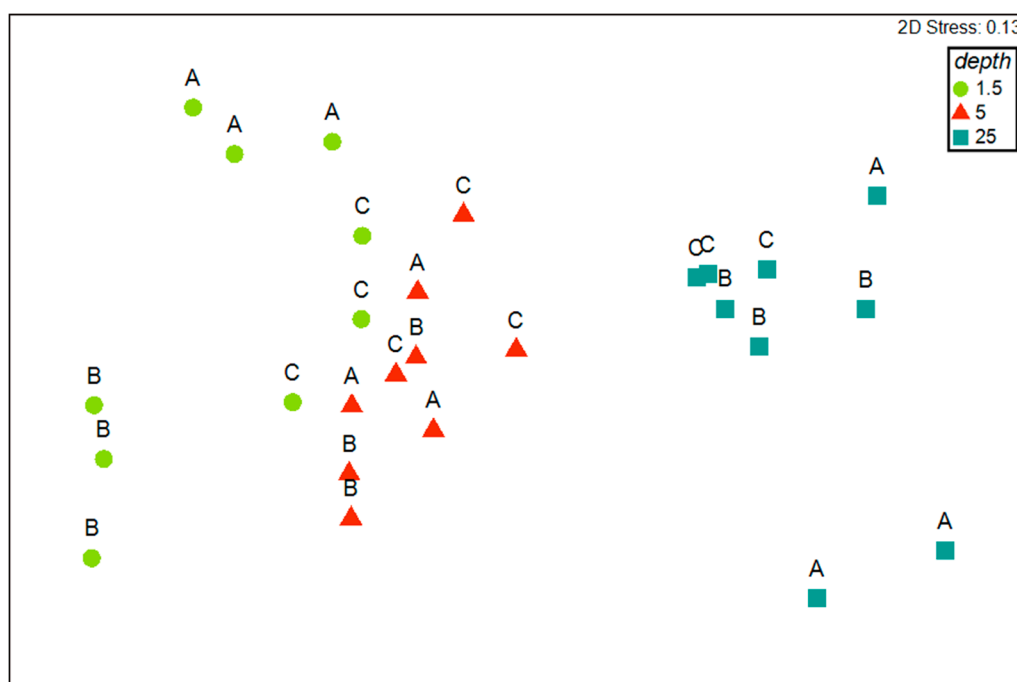


Figure 6. Nonmetric multidimensional scaling (MDS) ordination plot based on Bray–Curtis similarity of untransformed data, comparing structure of polychaete assemblages between samples. A–C = stations.

The species that contributed with 4% or more to the dissimilarity between stations at each depth, were mostly the same ones that characterised that depth (Table S4): *N. usticensis*, *N. pulsatoria*, *S. rosea*, *S. prolifera*, *A. mediterranea*, *S. pirifera*, *P. dumerilii*, *E. dispar*, *Ceratonereis (Composetia) costae* (Grube, 1840) and Nereididae juv. indet. at 1.5m depth; *A. mediterranea*, *A. rovigensis*, *S. prolifera*, *S. gerlachi*, *Nereis* sp. 1, *S. pirifera* and *P. dumerilii* at 5 m depth; and *S. armillaris*, *S. pirifera*, *H. stichophthalmos*, *S. gracilis*, *S. variegata*, *S. gerlachi* and *Spirobranchus triqueter* (Linnaeus, 1758) at 25 m depth.

Table 2. Results of SIMPER analyses (cut-off 70%) used to identify taxa that mostly contribute to (A) faunal similarity within depths, (B) faunal dissimilarity between depths. Abund = mean abundance, Sim% = mean similarity, Sim/SD = similarity/standard deviation, Contrib% = contribution relative to single taxon, Cum% = cumulative contribution, Av.Ab = mean abundance, Diss% = mean dissimilarity, Diss/SD = dissimilarity/standard deviation.

A		Group	Species	Abund	Sim%	Sim/SD	Contrib%	Cum%		
		Sim%								
A	1.5 m 34.39%	<i>Amphiglena mediterranea</i>	9.67	8.14	1.63	23.68	23.68			
		<i>Platynereis dumerilii</i>	6.11	5.41	1.48	15.72	39.40			
		<i>Nereis usticensis</i>	11.00	2.45	0.33	7.13	46.54			
		<i>Syllis rosea</i>	7.00	2.45	1.11	7.12	53.66			
		<i>Sphaerosyllis pirifera</i>	4.67	2.43	0.71	7.06	60.72			
		<i>Nereis pulsatoria</i>	3.11	2.35	1.05	6.85	67.56			
		<i>Exogone dispar</i>	2.89	1.62	0.65	4.70	72.26			
		5 m 45.22%	<i>Amphiglena mediterranea</i>	17.56	10.46	1.43	23.14	23.14		
	<i>Sphaerosyllis pirifera</i>		5.67	4.06	1.46	8.97	32.11			
	Nereididae juv. indet.		4.00	3.04	2.11	6.71	38.82			
	<i>Nereis</i> sp. 1		4.56	2.92	1.37	6.46	45.28			
	<i>Exogone dispar</i>		3.67	2.79	2.13	6.17	51.45			
	<i>Syllis variegata</i>		3.33	2.55	1.33	5.63	57.08			
	<i>Platynereis dumerilii</i>		3.56	2.50	1.17	5.52	62.60			
	<i>Syllis prolifera</i>		5.33	2.12	0.71	4.69	67.29			
	<i>Syllis corallicola</i>		3.11	2.08	2.29	4.59	71.88			
	25 m 39.51%	<i>Syllis armillaris</i>	13.22	13.05	1.55	33.04	33.04			
		<i>Syllis gracilis</i>	6.67	8.38	1.88	21.21	54.25			
		<i>Sphaerosyllis pirifera</i>	8.78	5.93	0.98	15.01	69.27			
		<i>Syllis gerlachi</i>	4.11	3.65	1.44	9.24	78.50			
	B		Groups	Species	Av. Ab	Av.Ab	Diss%	Diss/SD	Contrib%	Cum%
			Diss%		Group 1.5	Group 5				
	B	1.5 and 5 68.67%	<i>Amphiglena mediterranea</i>	9.67	17.56	7.34	1.35	10.69	10.69	
			<i>Nereis usticensis</i>	11.00	0.11	5.99	0.71	8.72	19.41	
			<i>Syllis rosea</i>	7.00	0.44	4.01	0.58	5.83	25.24	
			<i>Syllis prolifera</i>	5.44	5.33	3.89	1.16	5.66	30.91	
			<i>Sphaerosyllis pirifera</i>	4.67	5.67	3.38	1.13	4.92	35.83	
<i>Platynereis dumerilii</i>			6.11	3.56	2.53	1.21	3.68	39.51		
<i>Nereis</i> sp. 1			1.11	4.56	2.39	1.22	3.48	42.99		
Nereididae juv. indet.			2.00	4.00	2.16	1.47	3.15	46.14		
<i>Syllis variegata</i>			0.33	3.33	2.01	1.44	2.92	49.07		
<i>Exogone dispar</i>			2.89	3.67	1.95	1.29	2.84	51.90		
<i>Syllis corallicola</i>			1.00	3.11	1.89	1.69	2.75	54.65		
<i>Syllis gerlachi</i>			0.22	3.00	1.88	0.76	2.74	57.39		
<i>Nereis pulsatoria</i>			3.11	1.00	1.62	1.18	2.35	59.74		
<i>Amphicorina rovigensis</i>			0.00	3.33	1.46	0.41	2.13	61.87		
<i>Nereis rava</i>			0.78	2.67	1.46	1.30	2.13	64.00		
<i>Ceratonereis (Compositia) costae</i>			1.11	2.00	1.42	0.92	2.06	66.07		
<i>Odontosyllis ctenostoma</i>			0.33	2.44	1.36	0.86	1.98	68.05		
<i>Lysidice unicornis</i>			2.33	0.44	1.14	1.11	1.66	69.71		
<i>Syllis armillaris</i>			1.00	1.89	1.11	0.98	1.62	71.33		
1.5 and 25 88.42%			<i>Syllis armillaris</i>	1.00	13.22	9.06	1.75	10.24	10.24	
	<i>Amphiglena mediterranea</i>	9.67	0.33	7.29	1.53	8.24	18.48			
	<i>Nereis usticensis</i>	11.00	0.00	7.20	0.70	8.14	26.63			
	<i>Sphaerosyllis pirifera</i>	4.67	8.78	6.06	1.13	6.85	33.48			
	<i>Syllis rosea</i>	7.00	0.00	5.26	0.61	5.95	39.43			
	<i>Syllis gracilis</i>	0.22	6.67	5.09	1.73	5.75	45.18			
	<i>Platynereis dumerilii</i>	6.11	0.44	4.62	1.26	5.23	50.41			
	<i>Syllis prolifera</i>	5.44	1.00	3.82	0.86	4.32	54.73			
	<i>Syllis gerlachi</i>	0.22	4.11	2.87	1.31	3.24	57.97			
	<i>Hypsicomus stichophthalmos</i>	0.11	3.11	2.47	0.43	2.79	60.76			
	<i>Nereis pulsatoria</i>	3.11	0.11	2.45	1.26	2.77	63.54			
	<i>Exogone dispar</i>	2.89	0.11	2.34	0.99	2.65	66.18			
	<i>Lysidice unicornis</i>	2.33	0.11	1.62	1.25	1.83	68.01			
	Nereididae juv. indet.	2.00	0.11	1.43	0.74	1.62	69.63			
	<i>Dodecaceria concharum</i>	1.67	0.22	1.30	0.98	1.47	71.10			

Table 2. Cont.

B	Group Sim%	Species	Abund	Sim%	Sim/SD	Contrib%	Cum%	
5 and 25 79.59%		<i>Amphiglena mediterranea</i>	17.56	0.33	11.40	1.52	14.33	14.33
		<i>Syllis armillaris</i>	1.89	13.22	7.94	1.62	9.97	24.30
		<i>Sphaerosyllis pirifera</i>	5.67	8.78	5.07	1.23	6.37	30.66
		<i>Syllis gracilis</i>	0.67	6.67	4.29	1.65	5.40	36.06
		<i>Syllis prolifera</i>	5.33	1.00	3.24	1.13	4.08	40.14
		<i>Nereis</i> sp. 1	4.56	0.56	2.89	1.26	3.63	43.77
		Nereididae juv. indet.	4.00	0.11	2.73	1.57	3.43	47.20
		<i>Syllis gerlachi</i>	3.00	4.11	2.64	1.11	3.32	50.52
		<i>Exogone dispar</i>	3.67	0.11	2.59	1.38	3.25	53.77
		<i>Platynereis dumerilii</i>	3.56	0.44	2.46	1.12	3.09	56.86
		<i>Hypsicomus stichophthalmos</i>	0.00	3.11	2.17	0.41	2.72	59.59
		<i>Syllis variegata</i>	3.33	1.56	1.89	1.17	2.38	61.96
		<i>Syllis corallicola</i>	3.11	0.44	1.75	1.43	2.20	64.17
		<i>Amphicorina rovigensis</i>	3.33	0.22	1.67	0.44	2.10	66.26
		<i>Odontosyllis ctenostoma</i>	2.44	0.00	1.56	0.85	1.96	68.22
		<i>Nereis rava</i>	2.67	1.11	1.55	1.16	1.95	70.17

Table 3. Results of DISTLM-forward analysis. (A) Variables: percent coverage of each algal taxa (5% cut-off), depth and orientation), (B) Variables: percent coverage of algal functional-morphological groups, depth and orientation). Only variables significantly contributing to explain variation of polychaete assemblages ($p < 0.05$) are presented. Prop.: proportion of explained variation; Cumul.: cumulative proportion of explained variation.

	Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.
A	Depth	20617	9.9394	0.0001	0.28448	0.28448
	<i>Peyssonnelia rubra</i>	5681.7	2.9532	0.0005	0.078398	0.36287
	<i>Haloptilon</i> sp.	5214.1	2.9279	0.0008	0.071947	0.43482
	<i>Filamentous</i> sp. 1	4161.5	2.488	0.001	0.057423	0.49224
	<i>Padina pavonica</i>	2822.7	1.7447	0.0263	0.038949	0.53119
	<i>Codium efusum</i>	2954.4	1.9048	0.0208	0.040766	0.57196
B	Depth	20617	9.9394	0.0001	0.28448	0.28448
	Encrusting	5292.2	2.7277	0.0007	0.073024	0.3575
	Foliose	4853.6	2.6764	0.0011	0.066971	0.42447
	Articulated calcareous	3064.3	1.7444	0.0306	0.042282	0.46675

When considering the percent coverage of each algal taxon separately, 6 variables (over a total of 36) significantly explained 57.2% of the variation of the polychaete assemblages (Table 3A). Depth explained 28.4% of the variation, while the contribution of orientation was not significant. Overall, the percent coverage of algal taxa significantly explained 28.7% of the observed variation. *Peyssonnelia rubra* (7.8%), *Haloptilon* sp. (7.2%), one taxon of filamentous algae (5.74%), *Padina pavonica* (3.9%) and *Codium efusum* (4.1%) were significantly related to distribution of polychaete assemblages. When considering percent coverage of algal functional-morphological groups, 4 out of 9 variables significantly explained 46.7% of the variation of the polychaete assemblages (Table 3B). Among them, depth explained 28.4%, while algal functional-morphological groups all together explained 18.2% of the variation of polychaete assemblages. Orientation was again not significant. In particular, the encrusting (7.3%), foliose (6.7%) and articulated calcareous (4.2%) algae were significantly related to the distribution of polychaete assemblages.

4. Discussion

This is the first study of the hard bottom polychaete assemblages in the North Adriatic Sea over a wide bathymetric range also applying an experimental design that allows describing patterns of spatial distribution in relation to algal coverage. Previous hard bottom studies in the area considered mostly shallower benthic habitats, using qualitative sampling methods making results hardly comparable

to ours. In fact, Zavodnik [40,41] reported 38 polychaete species from *Cystoseira barbata* (Stackhouse) C. Agardh assemblages sampled at 0.5–1 m depth and 69 species examining different brown algae from 0–3 m depths. Later, Amoureux and Katzmann [34] reported 52 species from rocky bottom algal assemblages at 6–9 m depth, while Katzmann [37] collected 93 species from *Cystoseira* assemblages sampled at different depths ranging from 0–2.5 m. Amoureux [33] found 55 polychaete species among *Cystoseira* and *Peyssonelia* algae sampled at 3 m depth. In general, we can notice higher species richness (107 taxa from 22 families) in our study, which can be explained especially by the wider depth range considered herein. Indeed, if we consider only shallower habitats, species richness found herein (62 species at 1.5 m depth and 73 species at 5 m depth) is more similar to what was previously reported for the area. Moreover, our sampling covers the most representative hard-bottom vegetated habitats in the area, supposedly allowing collecting a larger number of polychaete taxa. The macroalgal assemblages found at Sveti Ivan Island are characteristic for the western coast of the North Adriatic Sea, with photophilic algae typically dominating in shallow, and sciaphilic in deeper waters [55,70], and they can be considered as representative habitats to study the diversity of rocky bottom polychaetes in the region. The ecological status of Sv. Ivan island was previously determined using macroalgae grouped in two ecological status groups (ESG I and ESG II) according to Orfanidis et al. [71], and categorized as overall high, while moderate at 1 m depth and good at 3 m depth [54,55]. The algal assemblages herein observed corresponded to those found in the above-mentioned studies. The articulated calcareous, corticated and foliose functional-morphological groups were dominant at 1.5 m depth, the articulated calcareous, encrusting and foliose ones at 5 m depth and the encrusting and filamentous ones at 25 m depth. Due to the strong exposure, the leathery macroalgal group was poorly developed [55,72], and represented only by *C. compressa*, with very low abundance. From a faunal point of view the present data on polychaetes differ considerably from previous studies, with 23 species recently reported for the first time in the North Adriatic region, including four new records for the Adriatic Sea, as described elsewhere [43,44,73], while the sabellid *Amphicorina rovignensis*, collected at Sveti Ivan during our survey, was recently described as new to science [44]. Peculiarly, some of the newly reported species were among the most abundant ones in the examined habitats, in particular *Nereis usticensis*, *Syllis corallicola*, *S. gerlachi*, *S. rosea* and, the most frequent species in this research, *S. pirifera*. Results of faunal and biogeographic analyses of Syllidae from Sveti Ivan Island indicated possible “meridionalization” of the North Adriatic fauna, i.e., the process of establishment of native Mediterranean warm-water species typical from southern sectors in colder northern areas [43,74]. Two species recorded during our survey are considered alien in the Mediterranean being considered Lessepsian migrants, *Nereis persica* Fauvel, 1911 and *Lepidonotus tenuisetosus* (Gravier, 1902) [73,75] and are deposited in the collection of the Natural History Museum in Rijeka (Croatia). *Nereis persica* was previously reported in the Adriatic Sea only twice, in its northern part [76,77]; however, those records were considered questionable [75]. Elsewhere in the Mediterranean, it was reported along the coast of Israel and Turkey [78,79]. If previous records of this species in the Adriatic Sea are eventually erroneous [75], our finding could represent a north-western widening of the species areal in the Mediterranean. *Lepidonotus tenuisetosus* was until now reported only in the Eastern Mediterranean, along the coasts of Israel, Egypt, Turkey and Greece [75,79–81]. Our finding of *L. tenuisetosus* represents the first report for the Adriatic Sea and might indicate a north-western widening of the species’ distribution. However, present data are not fully comparable with previous studies from a qualitative point of view, because the knowledge on polychaetes taxonomy is continuously evolving and new species are recorded and newly described also in the Adriatic Sea [73]. New records of some species in our research (such as *Nereis usticensis* Cantone, Catalano & Badalamenti, 2001 and several syllid species) might arise from the fact that former studies were carried out before those species were scientifically described [43]. Moreover, polychaete systematics changed a lot meanwhile and some taxa are not considered valid at present, such as for example previously reported *Pionosyllis serrata* Southern, 1914, *Syllis brevipennis* (Grube, 1863) and *Vermiliopsis richardi* Fauvel, 1909 [33,37], herein listed as *Nudisyllis pulligera* (Krohn, 1852), *Trypanosyllis (Trypanosyllis) coeliaca* Claparède, 1868 and *Vermiliopsis labiata*

(O. G. Costa, 1861) respectively. All this emphasizes the importance of research such as the present one, aiming at updating the knowledge of polychaetes biodiversity of an area.

Our data are more comparable to more recent studies conducted with similar experimental designs in the South Adriatic Sea by Giangrande et al. [82,83]. These authors found 152 polychaete taxa from 22 families in the first study [82] and 118 taxa from 18 families in the second one [83]. Species mostly characterising 5 m and 25 m depth in the south Adriatic [82] were mainly different from those characterising the same depths in our study, with only *P. dumerilii* being in common at 5 m and *S. armillaris* and *S. gerlachi* at 25 m depth. These differences could derive from local differences in the composition of algal assemblages, from different sampling periods (May and November in Giangrande et al. [82] and July in our research) and also from the biogeographic distribution of the Adriatic Sea polychaete fauna. Several species that characterised 5 m and 25 m depth in the south Adriatic were overall absent in our research, in particular *Syllis pulvinata* (Langerhans, 1881) that characterised 5 m and 25 m depth and *S. golfonovensis* (Hartmann-Schröder, 1962) and *Kefersteinia cirrhata* (Keferstein, 1862) that characterised 25 m depth. In fact, *S. pulvinata* and *S. golfonovensis* are species that were up to date found only in the southern part of the Adriatic, while it remains difficult to explain the absence of *K. cirrata* in our samples, since this species is reported as widely distributed in the whole Adriatic Sea [73]. This species is reported as *Psamathe fusca* Johnston, 1836 in the recent-most polychaete checklist of the Adriatic Sea, based on the synonymy proposed by Pleijel [84]. Although high, the number of taxa from Sveti Ivan Island is considerably lower than that reported in Giangrande et al. [82], possibly because of the smaller sampling surface and less extensive sampling period. Indeed, species area estimator curves suggested potentially higher species richness in our study area. Many species were rare and the analyses of distribution patterns indicating that additional sampling would probably yield more species, as well as it would presumably do sampling in different seasons, considering potential seasonal variability of hard bottom polychaete assemblages [51,58]. Thus, further studies encompassing different seasons and spatial scales should be done in order to upgrade the knowledge of diversity and spatial-temporal variation of hard bottom polychaete assemblages in the North Adriatic region.

The most abundant and species rich families found herein (particularly the Syllidae, but also the Sabellidae, Nereididae, Eunicidae and Serpulidae) are commonly reported as the most characteristic in the Mediterranean hard bottom polychaete assemblages [52,58,82,85,86].

The structure of the polychaete assemblages was highly variable alongshore, but only at the shallowest sites, while it clearly varied bathymetrically, which agrees with the most common trends previously identified, e.g., [11,82]. High variability in the shallowest habitats could be promoted by the high variability of environmental factors (temperature, salinity, hydrodynamics, light intensity etc.) [45]. However, the small sample size used herein might account for differences in presence/absence of several taxa that were represented by few individuals in the overall samples analysed and might have influenced the observed patterns of variation. The highest abundance and diversity and the highest similarity among polychaete assemblages was found at 5 m depth, as well as the highest number of species contributing to the similarity between samples, indicating that assemblages at this depth are the most diverse and structurally complex [87]. The high complexity of the algal coverage at intermediate depth, which can be considered an ecotone where photophilic and sciaphilic conditions coexist, together with a decrease in environmental variability, compared to the shallower habitat, possibly explain the observed increase in diversity [83,88]. In fact, at 5 m depth, the rich coverage of foliose algae (*Padina pavonica*, *Flabellia petiolata* and *Dictyota dichotoma*), structurally complex articulated calcareous algae (*Corallina officinalis* and *Jania* sp.) and encrusting algae (*P. rubra*, *P. heteromorpha*), with the last two forms being known to entrap considerable quantities of sediment, created altogether a complex and heterogeneous habitat suitable for diverse epifaunal and infaunal polychaete species. We expected the assemblages from 25 m depth to show the highest homogeneity as a consequence of the supposedly more stable environmental conditions. However, the within group similarity was lower at 25 m depth than at 5 m depth, likely because the sites at 25 m depth were situated at the end

of the rocky slope, very close to the soft bottom, which may give rise to occasional sedimentation. Enhanced sedimentation combined with reduced light intensity, together with the simplification of the algal-habitat structure, could also contribute to explaining the lower abundance and species richness at 25m depth [48,89]. However, we cannot exclude an effect of the limited sampling effort in the observed patterns of polychaete assemblage variation.

There was a common trend along the whole research area, characterised by differences in the structure of polychaete assemblages between the shallower depths (1.5 m and 5 m) and 25 m depth as already reported elsewhere [82]. The assemblages from 1.5 m and 5 m depths showed significant differences in their structure only at one station. In fact, assemblages from these shallower habitats were commonly characterised by species typical of shallow photophilic environments reported within a variety of algal assemblages (such are *Amphiglena mediterranea*, *Platynereis dumerilii*, *Syllis prolifera* and *Exogone dispar*) [52,53,90,91], while those from 25 m depth were characterised by species usually also found in sciaphilic habitats (such are *S. armillaris* and *S. gerlachi*) [82,90]. The increase of species richness of Serpulidae with depth observed in our research was already reported elsewhere in the Mediterranean [57,92]. It is probably related to the combining effects of the increment of hard (both lithic and organogenic) substrata, low hydrodynamic energy and shadowing, which favour the development of underlying biogenic concretions hosting species with coralligenous affinity [92]. As a whole, the distribution pattern of polychaete assemblages in the examined area appeared related mostly to depth, which, per se, covaries with different environmental variables (temperature, light intensity, hydrodynamics, sedimentation, etc). It was also shown to be related to the algal distribution, being correlation higher considering single algal species than algal functional-morphological groups. The encrusting calcareous alga *P. rubra* and the articulated calcareous alga *Halimtion* sp. ranked first among algal predictor variables of polychaete distribution, followed by one filamentous and two foliose species. The high percent coverage of articulated calcareous and encrusting algae certainly contributed to explain the high abundance and diversity of polychaetes, due to their high structural complexity that provided wide panoply of suitable microhabitats [88,93]. However, taxa with less complex morphology, i.e., the unidentified filamentous species, also contributed to significantly explain the variability of the polychaete assemblages. In particular, filamentous algal species characterised the habitat at 25 m depth. The spatial complexity of algal thalli surely represents an important factor influencing polychaete distribution, but other non-three-dimensional algal features (e.g., production of antagonistic metabolites, epiphyte colonization, palatability, capability to entrap sediment, etc.) could also be important and deserve further investigations [51,94].

In recent years, the North Adriatic macroalgal assemblages have suffered severe changes (such as reduction in algal coverage, variation in richness and species composition and simplification of the community structure), due to different natural and human driven impacts [95,96], which could likely provoke alterations of the resident polychaete assemblages. However, our algal-based predictor variables explained only part of the observed variability of the polychaete assemblages. We assume that other environmental variables, as well as biotic interactions among polychaetes and between them and other benthic invertebrates (e.g., competition for food or space, recruitment, predation, etc.) may contribute to explain part of the unexplained variability and, thus, should be considered in future studies [11,51,82].

Our results suggest that the diversity of the North Adriatic hard bottom polychaete fauna may be largely underestimated. Further faunal and ecological studies over larger spatial and temporal scale are thus welcome in order to implement our knowledge on diversity and distribution of polychaete assemblages in the area, which will serve as a necessary base to detect changes and predict consequences of natural and anthropogenic disturbances on benthic communities in this important and sensitive Mediterranean sector.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/12/10/408/s1>, Table S1: Mean coverage (MC) (%) (\pm standard error (\pm SE), in italics) of algal species and functional-morphological groups per station and depth. Most characteristic species are marked with asterisk (*); Table S2: Mean abundance (MA) (\pm standard error (\pm SE), in italics) of Polychaete taxa per station and depth. In bold values of most abundant taxa on each station and depth; Table S3. Results of PERMANOVA pair-wise and PERMDISP analyses testing for differences in abundance (N), species richness (S), Hill's species diversity index (N1), Hill's evenness index (N10) and structure (Stru) of polychaete assemblages: between stations, separately for each depth and between depths, separately for each station. Up, unique perms; t, t-test; P (perm), probability; P (MC), Monte Carlo probability. Significant P-values ($p < 0.05$) are given in bold; Table S4. Results of SIMPER analyses (cut-off 70%) used to identify taxa that mostly contribute to faunal dissimilarity between stations at each depth. Species contributing to dissimilarity with more than 4% are marked with asterisk. Av.Ab = mean abundance, Diss = mean dissimilarity, Diss/SD = dissimilarity/standard deviation, Contrib% = contribution relative to single taxon, Cum% = cumulative contribution; Figure S1. Distribution of species according to their frequency in the studied samples; Figure S2. Species area accumulation curve (Sobs, Species observed) and estimator curves (Jackknife1, Jackknife2 and Bootstrap).

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