

Article

Polychaetes Associated with Calcareous Red Algae *Corallina officinalis* in the Northern Adriatic Sea

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Abstract: Polychaetes are important marine invertebrates that contribute to sediment bioturbation, nutrient recycling, and food web dynamics. This study examines the diversity and structure of the polychaete assemblages associated with the red algae *Corallina officinalis* in areas with different levels of anthropogenic pressures of the Northeastern Adriatic Sea. Sampling was performed in the intertidal zones. Altogether, 54 species from 13 families were found, with Syllidae being the most abundant. Polychaete richness, relative abundance and diversity at sampling locations with and without anthropogenic pressures showed no significant difference. Multivariate analyses revealed significant differences in species composition between sites under anthropogenic pressures and those without, with key species such as *Sphaerosyllis pirifera*, *Syllis rosea*, *Syllis prolifera*, *Syllis gerundensis*, and *Platynereis dumerilii* playing significant roles. *Syllis rosea* was the most abundant in locations without anthropogenic pressures, while *S. pirifera* was the most abundant in locations under anthropogenic pressures. These results suggest that while polychaete communities are resilient, anthropogenic pressures are causing shifts in species composition. This pattern is consistent with the results of related studies, indicating a broader ecological trend. The shifts observed here should raise concern among conservation ecologists, underscoring the importance of long-term monitoring to understand and mitigate the impacts of human activities on coastal ecosystems.

Keywords: Polychaeta; Syllidae; Nereididae; intertidal area; coastal biodiversity; hard bottom; anthropogenic pressure



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1. Introduction

Polychaetes are mostly marine invertebrates inhabiting a wide range of habitats, from the intertidal zone to deep-sea sediments. More than 9000 species are currently known in the world, while 764 species within 360 genera and 62 families have been recorded in the Adriatic so far [1]. They have various feeding habits; some are predators, some feed on algae, while others feed on microorganisms, the remains of decayed organisms, organic matter, or detritus [2,3]. Active predators like *Glycera* spp. are equipped with powerful jaws for capturing prey, while others such as Spionidae are suspension feeders and deposit

feeders, and species like *Sabella spallanzanii* are filter feeders that trap suspended particles using feathery branchiae [4–6]. These feeding habits position polychaetes at various trophic levels, linking their microbial processes to higher-order predators, such as fish and crustaceans, which rely on polychaetes as a food source [3]. Polychaetes play a valuable role in bioturbation, oxygenating and processing sediment, making organic matter and nutrients available for other organisms through remineralization [7]. Burrowing species like *Arenicola marina* mix and oxygenate the sediment, enhancing nutrient cycling and promoting microbial activity [8]. In addition, their role is also important in the food web, considering that different organisms feed on polychaetes and vice versa [9,10]. On hard bottoms, they contribute by creating habitat structures, modifying substrates through bioerosion, filtering organic matter, and engaging in symbiotic interactions, all of which enhance biodiversity and ecosystem stability [11–14]. The calcareous dwelling tubes of species such as *Serpula vermicularis* contribute to habitat complexity by forming biogenic reefs, which support diverse assemblages of invertebrates and algae [15].

Polychaetes were often considered as opportunistic species benefiting from increased organic matter and were thus indicators of pollution. However, later research indicated that polychaetes are not solely opportunistic and that more sensitive species can also serve as environmental indicators [16,17]. In the Mediterranean Sea, several indices assess the quality of coastal waters by examining the ratio between sensitive and opportunistic groups and species [18,19]. Various indices use polychaetes as target organisms, such as AMBI [20], M-AMBI [21], BENTIX [22] and BQI [23]. To comply with international directives and conventions that require identifying disturbances using polychaete assemblage data, it is important to first establish a comprehensive database on the composition of natural assemblages [24]. Dauvin and Ruellet [25] proposed using the ratio of opportunistic polychaetes and amphipod crustaceans as a measure of assessing the ecological status of coastal waters (the BOPA index). Their model revised the previously proposed models in such a way as to reduce the effort in identifying individual taxa, which reduces the time and cost of assessing the condition.

Polychaete distribution on hard-bottoms correlates predominantly with algal structure and zonation, but their presence is more influenced by algal cover and epiphytes rather than specific macroalgal species [24]. They are one of the most diverse groups of invertebrates within *C. officinalis* settlements, significantly contributing to the fauna within the algae [26,27]. Investigating locations exposed to different wave actions—above, below, and across the zero level—revealed a distinct boundary between midlittoral and infralittoral polychaete assemblages. Physical disturbances such as emersion and wave action, along with predation pressure, were identified as key factors shaping their composition and structure, with algal cover also playing a significant role [28]. Further studies revealed that their spatial variability is influenced by a complex mix of biological factors (such as predation, competition, and recruitment) and physical–chemical factors (including light, temperature, salinity, hydrodynamics, sedimentation, and habitat complexity), with significant reliance on changes in algal composition [17].

A recent study conducted on Northern Adriatic hard-bottom assemblages revealed a diverse polychaete fauna comprising more than a hundred taxa, primarily identified at the species level and spanning 22 families. Syllidae emerged as the most species-rich family, followed by Sabellidae, Nereididae, Eunicidae, and Serpulidae [17]. Previous research focused on the polychaete fauna of soft and hard bottoms has consistently highlighted this taxonomic group as significantly understudied [17,29–31]. The discovery of a 31% increase in known sabellid taxa from just six stations underscores the importance of regular taxonomic updates for polychaete fauna in the Northern Adriatic [32].

Polychaete species composition in *Corallina* assemblages (*C. officinalis*, *C. elongata*, *C. granifera*) has been previously studied in different locations worldwide. In a study conducted along the Norwegian coast, serpulids and spirorbids were the most common polychaetes, except in sites impacted by wave action affecting their habitat. Additionally, errant polychaetes and several tube-dwelling taxa were detected but were not identified to the species level [33]. Another study off the coast of Livorno in the Tyrrhenian Sea showed Syllidae as the dominant polychaete family, followed by Nereididae, Eunicidae, Sabellidae, and Serpulidae. At the surface level, the lowest values for diversity, species count, and abundance were also recorded, likely due to emersion from wave action negatively affecting polychaete colonization [34]. The dominance of the Syllidae family was also confirmed at Southern Tyrrhenian coastal locations, with an abundant presence of individuals from the families Nereididae, Lumbrinereidae, and Eunicidae [35]. Similarly, along the Moroccan coast in the Western Mediterranean, the same dominance pattern was recorded, with Syllidae, Nereididae, and Sabellidae being the most abundant families. Prevalent species throughout the community were *Odontosyllis ctenostoma*, *Sphaerosyllis hystrix*, *Syllis prolifera*, *Perinereis cultrifera*, *Platynereis dumerilii*, and *Amphiglena mediterranea* [36]. Given that Syllidae were reported almost unanimously as the most dominant family in *Corallina* assemblages, a study along the North Atlantic coast of Spain focused on this family, and a total of 38 species belonging to 19 genera were recorded. The most common and abundant species was *Syllis armillaris*, followed by *Syllis gracilis* and *Syllis variegata* [37].

Considering the undervaluation of North Adriatic hard-bottom polychaete fauna diversity and the need for regular updates to monitor changes in benthic communities, our study aimed to improve our understanding of the faunal composition of these assemblages, specifically within *C. officinalis* turfs. The Mediterranean Sea, including the Adriatic, faces significant stressors like fisheries, tourism, temperature rises, acidification, pollution, and urbanization, which heavily impact hard-bottom benthic communities, especially near urban and industrial zones [38,39]. Additionally, a key objective was to assess differences in species composition and abundances between stations under anthropogenic pressure (A.P.) and those without it, providing valuable insights into how human activities influence the structure and diversity of these benthic ecosystems.

2. Materials and Methods

2.1. Study Area

The research was conducted in the Northeastern Adriatic Sea, specifically in the coastal areas of Southwest Istria and Brijuni National Park. The intertidal area of Southern Istria, dominated by carbonate sedimentary rocks, provides an ideal substrate for the red alga *Corallina officinalis* [40]. Sampling was carried out during the summer of 2018, from June to August, in four areas: Pula, Banjole, Premantura, and Brijuni National Park. Each area included at least one location under anthropogenic pressure and one location without A.P. Within each location, two sites spaced a minimum of 100 meters apart were identified, resulting in a total of 9 locations and 18 sites (Figure 1, Table S1). The main human activities characterizing the study areas include urban tourism and recreational facilities in Pula (notably at Saccorgiana), small harbors and seasonal tourism infrastructure in Banjole (Cintinera), intensive camping and sewage discharge near Premantura (Stupice), and localized tourism pressure in Brijuni National Park (Verige). Other sites within these areas, such as Verudela, Bumbište, Kamenjak, Javorika, and Dražice, were selected due to their minimal or absent direct anthropogenic pressure, serving as reference locations.

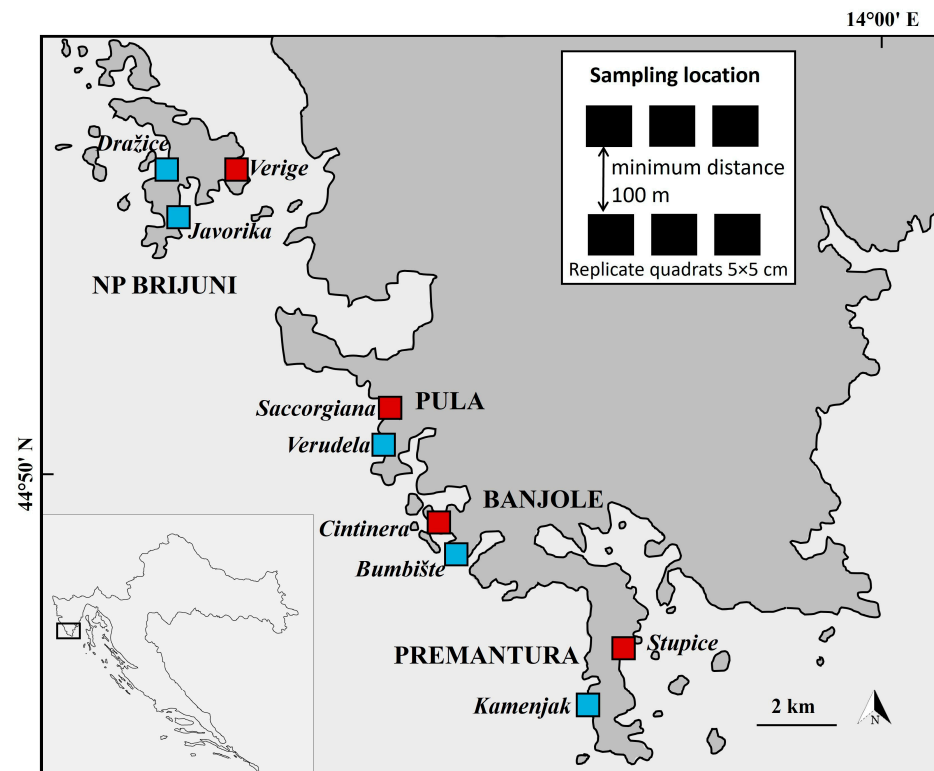


Figure 1. Four sampling areas (uppercase) along the Southern Istrian coast. Sampling locations under A.P. (red squares) and without A.P. (blue squares) are indicated in italics. Each location includes two sites with a total of six replicate 5×5 cm quadrats (black squares).

2.2. Sampling Methods and Processing

Prior to collecting samples, the distribution of *C. officinalis* along the Southern Istrian shore was mapped. The percentage of algal coverage within a coastal belt that was roughly 50 cm broad was used to determine the density of algae, and all the sampling locations that were chosen showed a more than 90% coverage. In randomly placed 5×5 cm quadrats, algae were scraped off using a hammer and chisel as part of a quantitative algae sample procedure. Each location was described with six replicate quadrats, with 54 samples gathered for examination altogether. Sampling was performed at low tide, when the algae were completely exposed, to reduce the loss of polychaetes. In the lab, samples of algae were washed using a 500 μ m mesh sieve, and every branch was examined using a stereomicroscope. Using a variety of accessible keys, polychaetes were sorted, counted, and determined to the lowest possible taxonomic rank following the relevant literature [41–48]. The species names were verified in accordance with the latest nomenclature provided by the World Register of Marine Species [49].

2.3. Assessing Anthropogenic Pressure

The Land Uses Simplified Index (LUSI) was calculated as described by Buršić et al. [50] to quantify anthropogenic pressure in coastal areas. To prevent inaccuracies in assigning anthropogenic pressure levels to sampling locations, the land use data were temporally aligned with the field sampling. The LUSI evaluates land (urban, agricultural, industrial) and freshwater (salinity) pressure, adjusted by a coastline correction factor based on coastal morphology—concave coasts retain pressure longer, while convex coasts dilute them faster. Values range from 0.75 (minimal pressure) to 8.75 (strong pressure). A low LUSI value means coastal waters face minimal or diluted continental pressures, while a high LUSI value signifies strong, undiluted continental pressure [51].

The LUSI is calculated as follows:

$$\text{LUSI} = (\text{urban score} + \text{agricultural score} + \text{industrial score} + \text{riverine score}) \times \text{coastline correction factor.} \quad (1)$$

For this study, 2×2 km grids were used, derived from the CORINE Land Cover database (<https://land.copernicus.eu/pan-european/corine-land-cover>, accessed on 15 April 2024).

2.4. Species Composition Analysis

Based on relative abundance and frequency, taxonomic groups were classified into different categories. Dominance was assessed using relative abundance with the formula $A = (n_i/N) \times 100$, where n_i is the count of individuals per species i , and N is the total count. Following Tischler [52] and Travizi [53], five categories were used: eudominant ($>10\%$), dominant ($5\text{--}10\%$), subdominant ($2\text{--}5\%$), recedent ($1\text{--}2\%$), and subrecedent ($<1\%$). Frequency of occurrence was calculated as $F_a = N_a/N \times 100$, where N_a is the number of samples containing the species a , and N is the total number of samples. Species were classified as very frequent ($75\text{--}100\%$), frequent ($50\text{--}75\%$), widespread ($25\text{--}50\%$), and rare ($0\text{--}25\%$) [53,54]. To evaluate whether the distribution of species across categories differed significantly between areas with and without anthropogenic pressure, we used a chi-square test of independence. In cases where the expected frequencies in any cell were below 5, Fisher's exact test was applied to ensure accuracy.

To evaluate the structure and composition of polychaete assemblages within different areas, the following univariate indices were applied: species richness (S), Hill index ($N1$), relative abundance (N), Shannon and Wiener diversity index (H'), and Pielou index of equitability (J'). The species richness index (S) is a measure of the total number of recorded species, while the Hill index (in the case of this research, $N1$) is an indicator of the number of abundant species in the sample and a measure of the effective number of species. It was calculated as the exponential value of the Shannon index H' ($N1 = e^{H'}$); the Shannon index H' is calculated as

$$H' = -\sum_i p_i \ln(p_i) \quad (2)$$

and the Pielou index is calculated as the ratio between the Shannon index and Shannon maximum value [55]:

$$J' = H'/H'_{\max} \quad (3)$$

To assess the differences in polychaete relative abundance, the S and Hill index, as well as H' and J' indices, between areas under A.P. and without A.P. data were analyzed using the Wilcoxon rank-sum test. Prior to statistical testing, the data were checked for normality using the Shapiro-Wilk test.

Multivariate analyses were conducted to test for differences in polychaete assemblages at sampling locations with varying degrees of anthropogenic impact. To assess the contribution of individual species to the dissimilarity between high-impact and low-impact stations, we performed a SIMPER analysis using the vegan package in R version 4.4.2 [56]. Polychaete abundance data were obtained from 54 sampling stations, each classified according to their LUSI score, which indicates the level of anthropogenic pressure. The species abundance data were organized into a species-by-station matrix, and the LUSI score for each station served as the grouping variable for the analysis. The SIMPER function was applied to determine the average contribution of each species to the dissimilarity between the two groups (high-impact vs low-impact). The output from the SIMPER analysis included the contribution percentage of each species, their average abundance within each group (high and low impact), and the ratio of their contribution relative to their standard

deviation, which reflects the consistency of their contribution across the groups. A Principal Coordinates Analysis (PCoA) was performed to investigate the relationships between polychaete communities and environmental variables. The LUSI score, a composite measure of anthropogenic stress, was used as a categorical explanatory variable. The vegan package in R was utilized to perform the PCoA and visualize the results. The PCoA plot was generated using the ggplot2 package, with stations labeled and colored according to their respective LUSI scores.

A PERMANOVA analysis was performed to test the significance of the differences of polychaete assemblages based on two factors (fixed): pressure (two levels: “Under A.P.” and “Without A.P.”) and areas (4 levels: “NP Brijuni”, “Pula”, “Banjole”, and “Premantura”). The significance level (probability) was obtained using the Monte Carlo permutation method (P(MC)). A pairwise PERMANOVA test was also performed to assess differences between individual sampling locations. To test the homogeneity of multivariate dispersions, a distance-based test (PERMDISP) was performed. A non-metric MDS graph was created based on the Bray–Curtis similarity of square root-transformed data. PERMANOVA and PERMDISP analyses were conducted using the software package PRIMER v.6 [57,58].

3. Results

3.1. Categorization of Anthropogenic Pressure

LUSI values ranged from 0.75 to 5.00, with urban settlements being under the greatest pressure. Locations like Saccorgiana, Cintinera, Stupice, and Verige scored higher, indicating anthropogenic pressure. In Brijuni National Park, away from urban and agricultural areas, LUSI values were lower overall, but Verige stood out with the highest score, classifying it as under pressure. On the other hand, the sampling locations Verudela, Bumbište, Kamenjak, Javorika, and Dražice scored a lower LUSI value, indicating lower anthropogenic pressure compared to other locations. These location-specific LUSI values are available in a previously documented published paper by Buršić et al. [50], as this research is part of the same study conducted at these sites.

3.2. Polychaete Diversity Within *Corallina officinalis* Assemblages

The sampling results revealed that polychaetes were indeed rich in both species richness and abundance. A total of 62 polychaete taxa, including 54 identified to the species level, were recorded across 13 families, with 1479 individual specimens collected.

The relative abundance and frequency of occurrence of polychaetes varied based on the sampling location, specifically whether the area was under significant anthropogenic pressure or not. In stations without A.P., the relative abundance of polychaetes reached up to 19.26%, and their frequency of occurrence was as high as 80%. In contrast, in stations under A.P., the relative abundance peaked at 12.90%, with a maximum frequency of occurrence of 75% (Table 1).

Table 1. Species list of polychaetes, with relative abundance and frequency of occurrence (without A.P.—locations without anthropogenic impact; under A.P.—locations under anthropogenic impact).

Family	Species	Relative Abundance (%)			Frequency of Occurrence (%)		
		Without A.P.	Under A.P.	All Locations	Without A.P.	Under A.P.	All Locations
Cirratulidae	<i>Tharyx killariensis</i>	0.26	0.14	0.20	6.67	4.17	5.56
	<i>Timarete</i> sp.	0.53	0.55	0.54	10.00	16.67	12.96
	Cirratulidae indet.	1.58	0.83	1.22	3.33	8.33	5.56
Dorvilleidae	<i>Dorvillea rubrovittata</i>	0.13	0.00	0.07	3.33	0.00	1.85

Table 1. Cont.

Family	Species	Relative Abundance (%)			Frequency of Occurrence (%)		
		Without A.P.	Under A.P.	All Locations	Without A.P.	Under A.P.	All Locations
Eunicidae	<i>Lysidice collaris</i>	0.00	0.14	0.07	0.00	4.17	1.85
	<i>Lysidice ninetta</i>	0.26	0.14	0.20	3.33	4.17	3.70
	<i>Lysidice unicornis</i>	0.66	0.69	0.68	13.33	20.83	16.67
Lumbrinereidae	<i>Scoletoma funchalensis</i>	0.00	0.28	0.14	0.00	8.33	3.70
	<i>Scoletoma laurentiana</i>	0.13	0.00	0.07	3.33	0.00	1.85
Maldanidae	Nicomachinae indet.	0.13	0.00	0.07	3.33	0.00	1.85
Nereididae	<i>Eunereis longissima</i>	0.13	0.00	0.07	3.33	0.00	1.85
	<i>Neanthes</i> sp.	0.00	0.28	0.14	0.00	8.33	3.70
	<i>Nereis pelagica</i>	0.40	0.97	0.68	10.00	20.83	14.81
	<i>Nereis</i> cf. <i>pelagica</i>	9.37	6.38	7.91	63.33	70.83	66.67
	<i>Nereis perivisceralis</i>	0.53	0.14	0.34	13.33	4.17	9.26
	<i>Nereis rava</i>	1.85	1.11	1.49	26.67	16.67	22.22
	<i>Nereis</i> sp.	0.13	1.39	0.74	3.33	12.50	7.41
	<i>Perinereis cultrifera</i>	0.00	0.83	0.41	0.00	20.83	9.26
	<i>Perinereis macropus</i>	0.00	0.28	0.14	0.00	4.17	1.85
	<i>Platynereis dumerilii</i>	8.84	3.05	6.02	60.00	37.50	50.00
	<i>Platynereis nadie</i>	0.13	0.00	0.07	3.33	0.00	1.85
	Nereididae indet.	1.58	2.08	1.83	33.33	37.50	35.19
Oenoidae	<i>Arabella iricolor</i>	1.06	1.39	1.22	20.00	29.17	24.07
Ophellidae	Ophellidae indet. juv.	0.00	0.14	0.07	0.00	4.17	1.85
	<i>Nainereis</i> cf. <i>quadricuspida</i>	0.00	0.28	0.14	0.00	8.33	3.70
Orbinidae	<i>Eteone foliosa</i>	0.13	0.00	0.07	3.33	0.00	1.85
Phyllodocidae	Phyllodocidae indet.	0.13	0.00	0.07	3.33	0.00	1.85
Polynoidae	<i>Harmothoe imbricata</i>	0.13	0.00	0.07	3.33	0.00	1.85
	<i>Lepidonotus clava</i>	0.13	0.28	0.20	3.33	8.33	5.56
	<i>Lepidonotus</i> sp.	0.53	0.97	0.74	13.33	16.67	14.81
	Polynoidae indet.	0.13	0.00	0.07	3.33	0.00	1.85
Sabellidae	Sabellidae indet.	1.19	0.83	1.01	23.33	8.33	16.67
Syllidae/Syllinae	<i>Branchiosyllis exilis</i>	0.13	0.00	0.07	3.33	0.00	1.85
	<i>Haplosyllis spongicola</i>	0.00	0.42	0.20	0.00	8.33	3.70
	<i>Syllis alternata</i>	0.79	1.25	1.01	16.67	12.50	14.81
	<i>Syllis armillaris</i>	1.32	0.97	1.15	16.67	8.33	12.96
	<i>Syllis beneliahua</i>	1.06	1.53	1.28	20.00	25.00	22.22
	<i>Syllis columbretensis</i>	1.19	0.28	0.74	20.00	8.33	14.81
	<i>Syllis corallicola</i>	0.00	0.14	0.07	0.00	4.17	1.85
	<i>Syllis gerlachi</i>	1.98	3.47	2.70	33.33	37.50	35.19
	<i>Syllis gerundensis</i>	2.51	10.40	6.36	43.33	75.00	57.41
	<i>Syllis garciai</i>	0.13	0.28	0.20	3.33	4.17	3.70
	<i>Syllis gracilis</i>	1.58	1.39	1.49	36.67	16.67	27.78
	<i>Syllis krohnii</i>	0.66	0.55	0.61	16.67	12.50	14.81
	<i>Syllis rosea</i>	19.26	8.60	14.06	80.00	75.00	77.78
	<i>Syllis prolifera</i>	11.74	9.43	10.62	53.33	54.17	53.70
	<i>Syllis pulvinata</i>	0.00	0.28	0.14	0.00	8.33	3.70
	<i>Syllis</i> sp.	0.53	3.33	1.89	13.33	20.83	16.67
	<i>Syllis variegata</i>	1.72	1.80	1.76	26.67	41.67	33.33
	<i>Trypanosyllis coeliaca</i>	0.13	0.00	0.07	3.33	0.00	1.85

Table 1. Cont.

Family	Species	Relative Abundance (%)			Frequency of Occurrence (%)		
		Without A.P.	Under A.P.	All Locations	Without A.P.	Under A.P.	All Locations
Syllidae/ Exogoninae	<i>Brania arminii</i>	1.45	0.00	0.74	23.33	0.00	12.96
	<i>Brania pusilla</i>	0.79	1.80	1.28	10.00	41.67	24.07
	<i>Exogone (Exogone) dispar</i>	3.30	3.05	3.18	43.33	50.00	46.30
	<i>Exogone (Exogone) verugera</i>	0.40	0.28	0.34	10.00	8.33	9.26
	<i>Salvatoria clavata</i>	1.06	0.55	0.81	20.00	12.50	16.67
	<i>Salvatoria limbata</i>	2.37	1.80	2.10	23.33	29.17	25.93
	<i>Salvatoria yraidae</i>	1.06	0.55	0.81	20.00	12.50	16.67
	<i>Sphaerosyllis austriaca</i>	3.69	6.93	5.27	46.67	54.17	50.00
	<i>Sphaerosyllis pirifera</i>	7.78	12.90	10.28	53.33	58.33	55.56
Syllidae/ Eusyllinae	<i>Paraehlersia ferrugina</i>	3.03	4.58	3.79	43.33	45.83	44.44
	<i>Pyonosyllis dionisi</i>	0.00	0.28	0.14	0.00	4.17	1.85
Syllidae/ Autolitinae	Autolitinae indet.	0.26	0.00	0.14	6.67	0.00	3.70

At the family level of classification, a total of 13 distinct polychaete families were identified during the study. Among these, the Syllidae family emerged as the most dominant, exhibiting the highest diversity in terms of species richness, as well as the greatest abundance in the number of individual specimens collected (Figure 2).

The calculation of the relative abundance of species across different sampling locations, specifically in areas with a different intensity of anthropogenic pressure, showed that the subrecedent species were the most represented. This category included 33 out of 55 species in locations without A.P. and 34 out of 54 species in locations under A.P. (Table 2). In terms of frequency of occurrence, the rare species category was the most dominant, with 76% of the recorded species in areas without A.P. and 74% in areas under A.P. belonging to this category (Table 3). A comparison of relative abundance categories across locations under and without A.P. showed no statistically significant difference in distribution ($\chi^2 = 0.31$, $df = 4$, $p = 0.99$). The distribution of species by frequency of occurrence also did not significantly differ between locations under anthropogenic pressure and those without (Fisher's exact test, $p = 0.95$).

Table 2. Relative abundance of polychaete species in locations under and without anthropogenic pressure (A.P.).

Relative Abundance	Without A.P.		Under A.P.	
	Number of Species	%	Number of Species	%
eudominant (>10%)	2	3.70	2	3.70
dominant (5–10%)	3	5.56	4	7.41
subdominant (2–5%)	5	9.26	5	9.26
recedent (1–2%)	11	20.37	9	16.67
subrecedent (<1%)	33	61.11	34	62.96
TOTAL	54	100.00	54	100.00

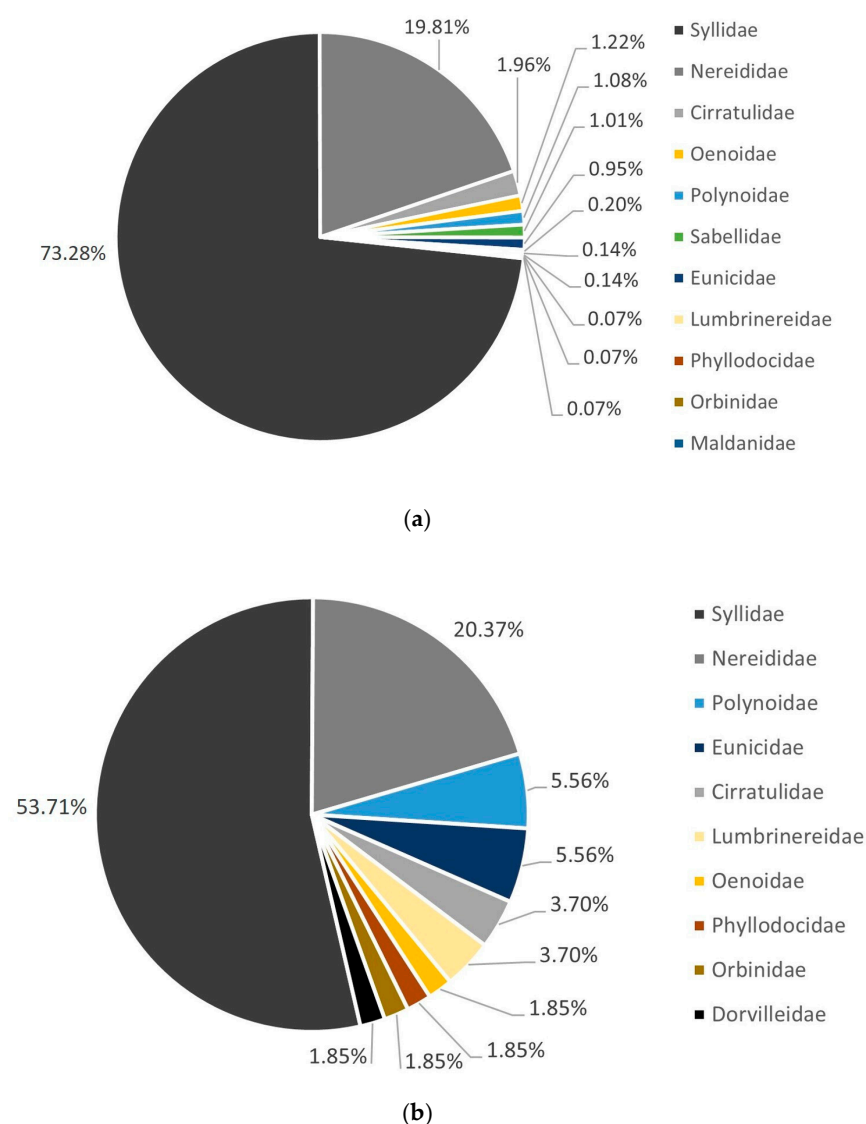


Figure 2. Relative abundance of polychaetes (a) and species richness (b) per each family recorded on all sampling locations.

Table 3. Frequency of occurrence of polychaete species in locations under and without anthropogenic pressure (A.P.).

Frequency of Occurrence	Without A.P.		Under A.P.	
	Number of Species	%	Number of Species	%
very frequent (75–100%)	1	1.85	0	0.00
frequent (50–75%)	4	7.41	6	11.11
widespread (25–50%)	8	14.81	8	14.81
rare (0–25%)	41	75.93	40	74.07
TOTAL	54	100.00	54	100.00

The Hill's diversity index N1 indicated a high level of diversity within the polychaete community, reflecting the effective number of equally abundant species and suggesting that the community is both species-rich and relatively evenly distributed. For locations without A.P., the N1 was 19.91, while in areas under A.P., it was slightly higher at 20.37, indicating a similar level of diversity across various conditions (Wilcoxon rank-sum test, $p > 0.05$). Species richness showed no variation (Wilcoxon rank-sum test, $p > 0.05$), with 45 species recorded in both areas, under and without A.P. (Figure 3). These results un-

underscore the ecological complexity of the area, with no single species dominating the assemblage and diversity being maintained under A.P.

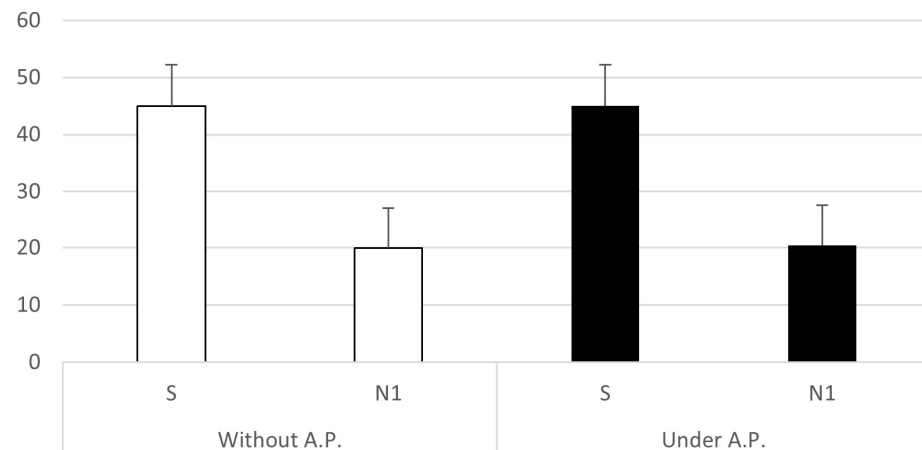


Figure 3. Mean values $\pm$ standard deviation of species richness (S) and Hill index (N1) for polychaete sampling locations without and under anthropogenic pressure (A.P.).

Polychaete relative abundances at sampling locations under and without A.P. reveal no significant differences (Wilcoxon rank-sum test, $p > 0.05$) and show a similar distribution (Figure 4). This suggests that, despite varying levels of anthropogenic pressure, there were no substantial shifts in the distribution of polychaete relative abundance across the studied stations. The boxplot in Figure 4 visually confirms this lack of difference, with overlapping distributions between the two groups.

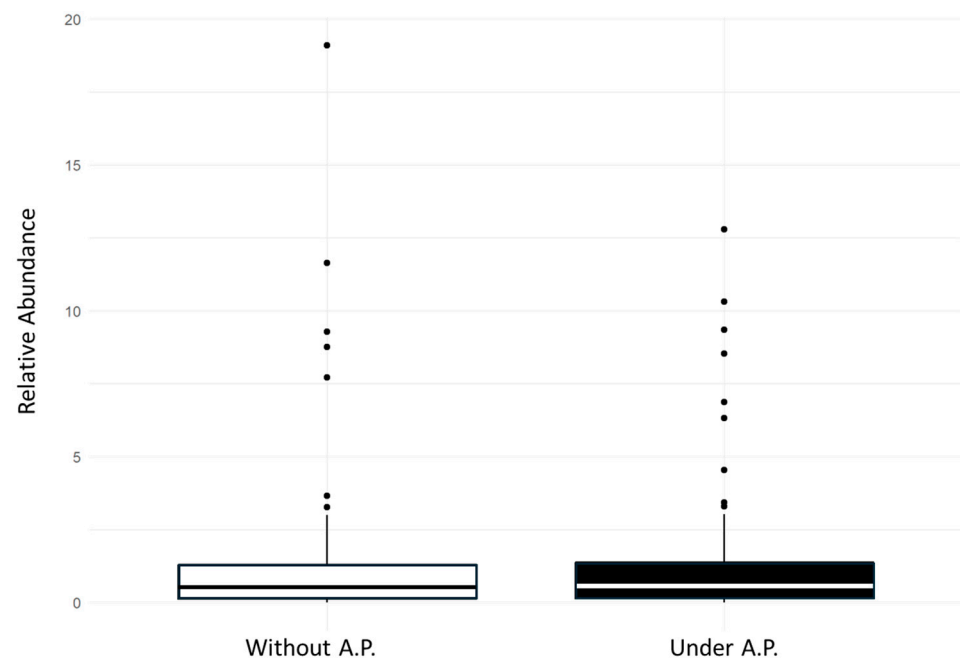


Figure 4. Polychaete relative abundance comparison between sampling locations under and without anthropogenic pressure.

Diversity indices (H' , J') did not differ significantly between areas under and without A.P. (Wilcoxon rank-sum test, $p > 0.05$) (Figure 5).

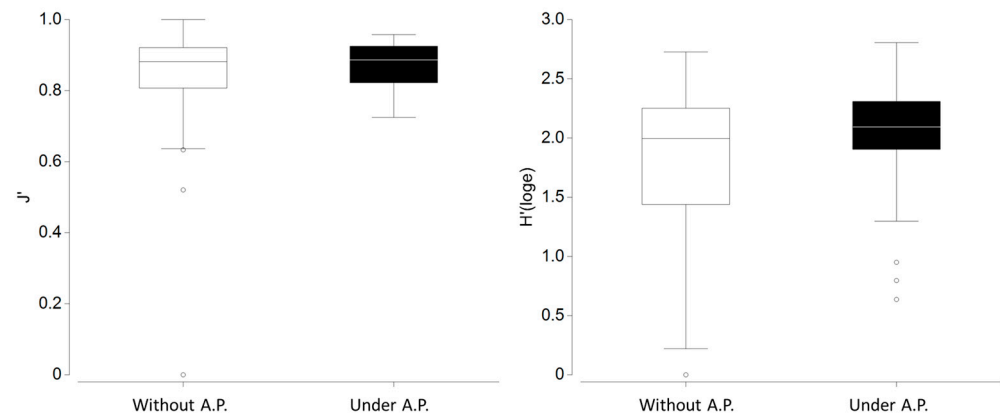


Figure 5. Boxplot of indices J' (Pielou index) and H' (Shannon–Wiener index) across sampling locations with a different anthropogenic pressure.

3.3. Polychaete Species Composition Analysis

Species with the highest contributions to the overall dissimilarity across the two site types were identified, providing an insight into which species were most affected by anthropogenic pressure. In the SIMPER analysis, *Sphaerosyllis pirifera* contributed most to the dissimilarity between the high-impact (under A.P.) and low-impact (without A.P.) stations, with an average contribution of 0.063. Other species showing significant contributions include *Syllis rosea* (contribution of 0.061), *Syllis prolifera* (0.054), and *Syllis gerundensis* (0.049), among others. Table 4 presents a full list of species, along with their average contributions, mean abundances in both high- and low-impact groups, and their ratio values, which reflect the consistency of each species' contribution.

Table 4. Species contributions to dissimilarity between stations under A.P. (high) and without A.P. (low) based on the SIMPER analysis.

Species	Contribution	SD	Ratio	Mean High	Mean Low
<i>Sphaerosyllis pirifera</i>	0.0628	0.0520	1.2075	23.25	11.80
<i>Syllis rosea</i>	0.0615	0.0565	1.0891	15.50	29.20
<i>Syllis prolifera</i>	0.0541	0.0382	1.4172	17.00	17.80
<i>Syllis gerundensis</i>	0.0488	0.0409	1.1948	18.75	3.80
<i>Platynereis dumerilii</i>	0.0361	0.0320	1.1257	5.50	13.40
<i>Sphaerosyllis austriaca</i>	0.0320	0.0464	0.6890	12.50	5.60
<i>Nereis cf. pelagica</i>	0.0292	0.0218	1.3390	11.50	14.20
<i>Paraehlersia ferrugina</i>	0.0217	0.0223	0.9754	8.25	4.60
<i>Syllis sp.</i>	0.0198	0.0193	1.0273	6.00	0.80
<i>Syllis gerlachi</i>	0.0176	0.0188	0.9345	6.25	3.00
<i>Exogone dispar</i>	0.0130	0.0099	1.3134	5.50	5.00
<i>Salvatoria limbata</i>	0.0109	0.0086	1.2663	3.25	3.60
<i>Brania pusilla</i>	0.0107	0.0095	1.1254	3.25	1.20
<i>Syllis gracilis</i>	0.0092	0.0061	1.4943	2.50	2.40
<i>Syllis armillaris</i>	0.0085	0.0097	0.8784	1.75	2.00
<i>Syllis variegata</i>	0.0080	0.0062	1.2922	3.25	2.60
<i>Nereis rava</i>	0.0074	0.0050	1.4831	2.00	2.80
<i>Brania arminii</i>	0.0072	0.0053	1.3599	0.00	2.20
<i>Arabella iricolor</i>	0.0072	0.0084	0.8601	2.50	1.60
<i>Nereis sp.</i>	0.0071	0.0120	0.5892	2.50	0.20
<i>Syllis alternata</i>	0.0065	0.0051	1.2800	2.25	1.20
<i>Syllis beneliahua</i>	0.0063	0.0088	0.7153	2.75	1.60
<i>Lepidonotus sp.</i>	0.0061	0.0073	0.8355	1.75	0.80
<i>Salvatoria clavata</i>	0.0054	0.0049	1.1065	1.00	1.60
<i>Syllis columbretenensis</i>	0.0054	0.0053	1.0188	0.50	1.80

Table 4. Cont.

Species	Contribution	SD	Ratio	Mean High	Mean Low
<i>Nereis pelagica</i>	0.0050	0.0039	1.2872	1.75	0.60
<i>Perinereis cultrifera</i>	0.0046	0.0042	1.0917	1.50	0.00
<i>Lysidice unicornis</i>	0.0038	0.0028	1.3515	1.25	1.00
<i>Timarete</i> sp.	0.0038	0.0040	0.9448	1.00	0.80
<i>Salvatoria yraidaea</i>	0.0036	0.0030	1.2065	1.00	1.60
<i>Syllis krohnii</i>	0.0033	0.0035	0.9650	1.00	1.00
<i>Nereis perivisceralis</i>	0.0024	0.0023	1.0331	0.25	0.80
<i>Exogone verugera</i>	0.0024	0.0022	1.0839	0.50	0.60
<i>Haplosyllis spongicola</i>	0.0023	0.0025	0.9121	0.75	0.00
<i>Syllis garciai</i>	0.0020	0.0027	0.7428	0.50	0.20
<i>Neanthes</i> sp.	0.0019	0.0020	0.9279	0.50	0.00
<i>Tharyx killariensis</i>	0.0019	0.0025	0.7426	0.25	0.40
<i>Pyonossyllis dionisi</i>	0.0019	0.0034	0.5446	0.50	0.00
<i>Lysidice ninetta</i>	0.0017	0.0025	0.6811	0.25	0.40
<i>Lepidonotus clava</i>	0.0016	0.0018	0.9166	0.50	0.20
<i>Nainereis</i> cf. <i>quadricuspida</i>	0.0016	0.0029	0.5491	0.50	0.00
<i>Syllis pulvinata</i>	0.0015	0.0016	0.9385	0.50	0.00
<i>Perinereis macropus</i>	0.0014	0.0025	0.5524	0.50	0.00
<i>Scoletoma funchalensis</i>	0.0014	0.0025	0.5524	0.50	0.00
<i>Branchiosyllis exilis</i>	0.0009	0.0018	0.4801	0.00	0.20
<i>Eunereis longissima</i>	0.0009	0.0018	0.4801	0.00	0.20
<i>Platynereis nadio</i>	0.0009	0.0018	0.4801	0.00	0.20
<i>Syllis corallicola</i>	0.0007	0.0012	0.5524	0.25	0.00
<i>Lysidice collaris</i>	0.0007	0.0012	0.5524	0.25	0.00
<i>Dorvillea rubrovittata</i>	0.0006	0.0013	0.4833	0.00	0.20
<i>Harmothoe imbricata</i>	0.0006	0.0013	0.4833	0.00	0.20
<i>Scoletoma laurentiana</i>	0.0006	0.0012	0.4839	0.00	0.20
<i>Trypanosyllis coeliaca</i>	0.0005	0.0010	0.4849	0.00	0.20
<i>Eteone foliosa</i>	0.0005	0.0010	0.4849	0.00	0.20

Species with the highest contributions to the overall dissimilarity were among the most abundant species recorded across all locations. The species with the highest recorded number of individuals were *Syllis rosea*, *S. prolifera*, and *Sphaerosyllis pirifera*. Among the ten most abundant species, eight belonged to the family Syllidae, while two were from the family Nereididae (Figure 6). Among the remaining 44 species, 28 had 10 or fewer individuals recorded, and 16 had up to 31 individuals. Looking at the relative abundance of polychaetes across sampling locations without and under A.P., some differences were noted in the top ten most abundant species. *Syllis rosea* was recorded as the most abundant species sampled in locations without A.P., while *Sphaerosyllis pirifera* was the most abundant in locations under A.P. (Figure 6).

The PERMANOVA analysis revealed that both the *Pressure* and *Area* factors had a significant effect on polychaete species composition, indicating a strong spatial variation. The interaction between *Pressure* and *Area* was not significant, indicating no strong combined effect of these factors on the polychaete community structure (Table 5). A pairwise comparison showed significant differences ($p < 0.05$) among all the four areas.

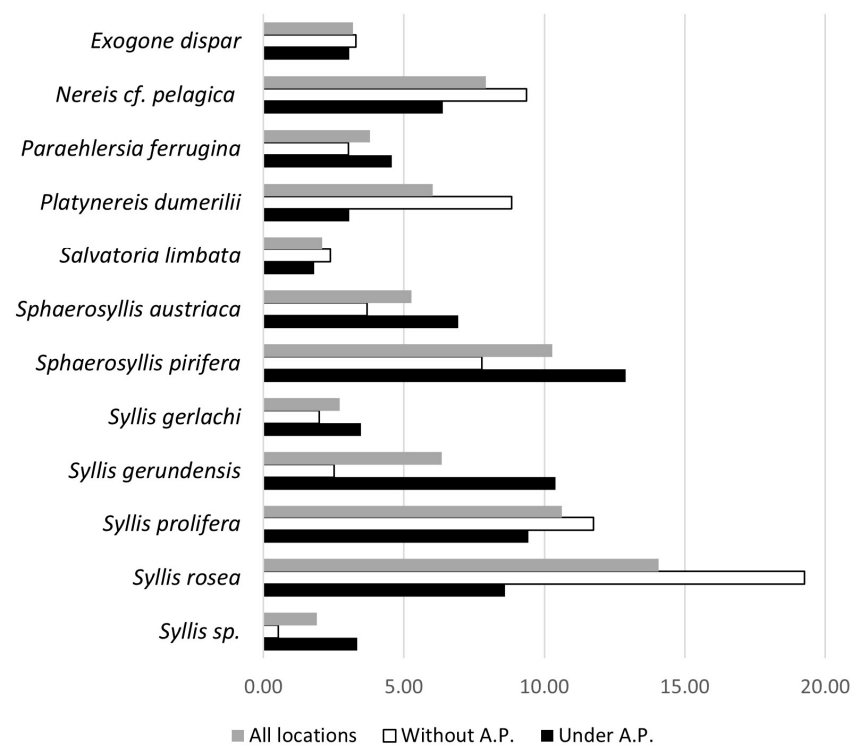


Figure 6. Relative abundance of the dominant polychaete species across all sampling locations (gray color) and in locations without (white color) and under (black color) anthropogenic pressure (A.P.).

Table 5. Results of PERMANOVA testing effects of *Pressure* and *Area* on polychaete community composition.

Source	Df	SS	MS	Pseudo-F	P(perm)
Pressure = Pr	1	4093.2	4093.2	1.7515	0.028
Area = Ar	3	22,863	7620.9	3.2611	0.0001
Pr × Ar	3	9322.5	3107.5	1.3297	0.0601
Res	46	107,500	2336.9		
Total	53	143,320			

Df = Degree of Freedom, SS = Sum of Squares, MS = Mean Square.

The PERMDISP analysis indicated a significant difference in the dispersion of polychaete communities among the sampling areas ($F = 4.138$, $P(\text{perm}) = 0.024$), suggesting heterogeneity in community variability, but not between sites under and without A.P. ($F = 0.227$, $P(\text{perm}) = 0.676$). Pairwise comparisons showed that dispersion in Pula was significantly different from Banjole ($t = 4.7771$, $P(\text{perm}) = 0.0002$) and Premantura ($t = 3.1137$, $P(\text{perm}) = 0.0075$) but not from NP Brijuni ($t = 1.9315$, $P(\text{perm}) = 0.1002$). No significant differences in dispersion were observed between other locations. The mean dispersion values showed that Pula had the highest variability (54.056 ± 2.3316), followed by NP Brijuni (45.953 ± 3.0415), Premantura (44.086 ± 2.195), and Banjole (41.165 ± 1.3587). These results suggest that polychaete community variability is most pronounced in Pula, while Banjole exhibits the least dispersion, as displayed in the MDS graph (Figure 7).

The Principal Coordinates Analysis (PCoA) plot reveals some extent of separation between polychaete communities based on LUSI scores, with Axis 1 and Axis 2 explaining 13.62% and 32.24% of the variation, respectively (Figure 8). While the separation is not entirely distinct, there is a noticeable trend that suggests polychaete communities from stations with different LUSI scores may exhibit some variation in their composition. This indicates that LUSI scores could influence the community structure to some extent, though other environmental factors might also be contributing to the observed patterns.

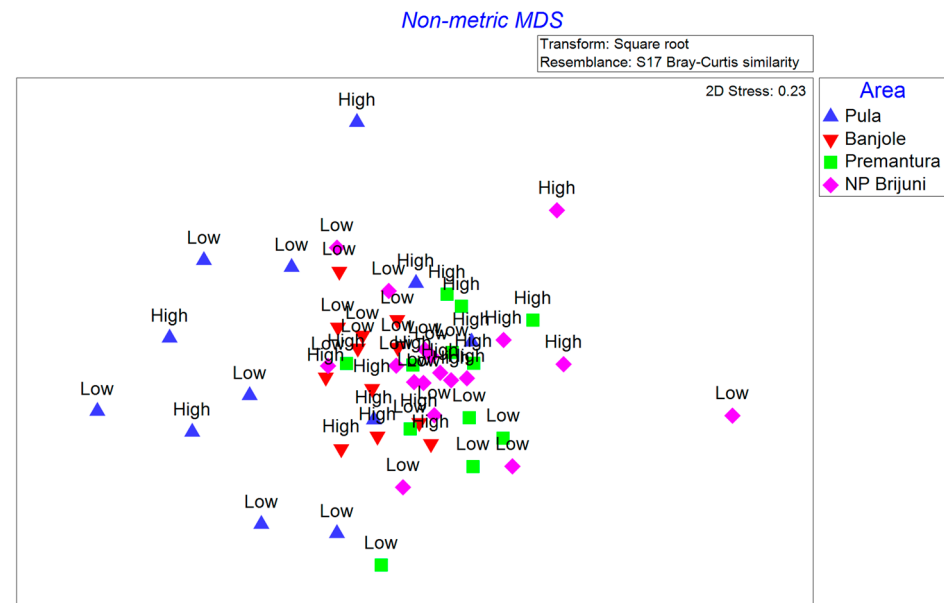


Figure 7. Non-metric multidimensional scaling (nMDS) ordination of polychaete community composition across sampling locations (low = locations without anthropogenic pressure, high = locations under anthropogenic pressure).

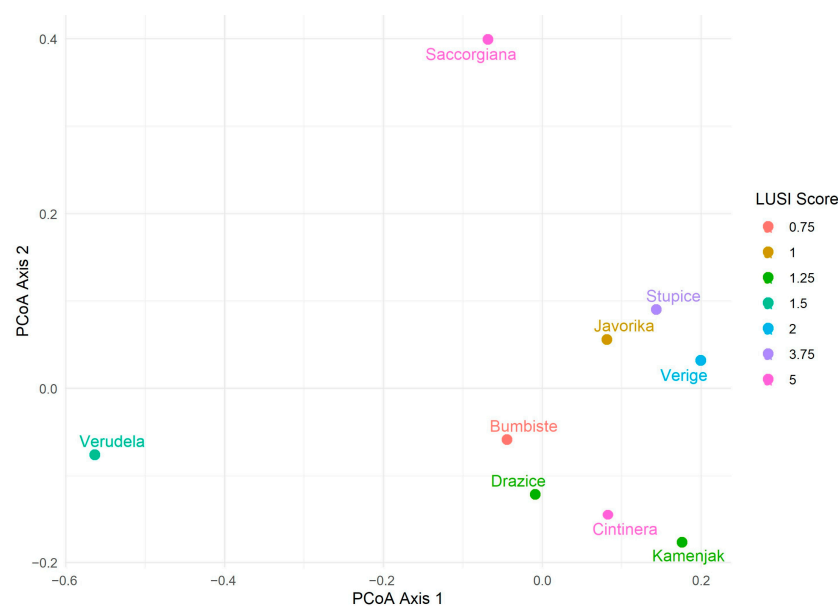


Figure 8. Principal Coordinates Analysis (PCoA) of polychaete communities based on LUSI scores.

4. Discussion

Our research is among the few studies on hard-bottom polychaetes in the Northern Adriatic Sea [17,32,59–67] and the first one to focus on *Corallina officinalis* assemblages in the intertidal zone. As emphasized by Mikac et al. [17], who conducted a recent comprehensive study on the diversity and distribution patterns of hard-bottom polychaetes in the area, further investigations are essential to better understand the diversity and spatiotemporal dynamics of these assemblages in the Northern Adriatic. Our study contributes valuable insights to this field. The most abundant and diverse families identified, particularly Syllidae, along with Nereididae and Eunicidae, are well recognized as key components of Mediterranean hard-bottom polychaete communities [17,68–72]. The high variability in these shallow habitats, as recorded in our study, likely stems from environmental fluctuations, including changes in temperature, salinity, hydrodynamics, and light intensity [73].

Our results suggest that Syllidae family members are widely distributed and highly adaptable to the environmental conditions in the study area. Their dominance is likely driven by ecological factors such as habitat preference, reproductive strategies, and resource availability, which favor their proliferation over other families. Syllids are abundant and diverse in various habitats, including sponges, seaweed, seagrass, corals, hydrozoans, and soft bottoms, due to their generalist feeding strategy and active lifestyle in interstitial spaces or crevices [74]. They have a recognized role as environmental indicators. Syllids thrive in diverse habitats such as coarse sediments, hard bottoms, kelp holdfasts, and interstitial spaces created by gravel, sand particles, or coralline structures, which provide shelter, mobility, and feeding opportunities [74–76].

The findings of this study align with those reported by Martins et al. [74], which highlight the dominance of Syllidae in kelp holdfast habitats and their primary association with hard-bottom substrates such as seagrass, kelp rhizomes, photophilic algae, corals, and sponges. Similarly, the present study identified Syllidae as the dominant family, exhibiting the highest diversity in species richness and abundance. While Martins et al. [74] specifically identified *Brania pusilla*, *Myrianida brachycephala*, *Syllis krohni*, and *S. variegata* as characteristic of kelp holdfasts with a cosmopolitan or Atlantic–Mediterranean distribution, this study recorded *Syllis rosea*, *S. prolifera*, and *Sphaerosyllis pirifera* as the most abundant species. Additionally, the predominance of Syllidae among the most abundant species in both studies further emphasizes the ecological importance of this family in benthic environments.

Notably, the subrecent species were the most abundant in both categories of locations, under and without anthropogenic pressure, with a slightly higher representation in areas under human pressure. This may indicate that certain species within this group are more resilient or adaptable to environmental changes. Giangrande et al. [16] emphasized that polychaetes have a more nuanced ecological role and can serve as valuable bioindicators in diverse environments. Additionally, they pointed out that certain families, such as Syllidae, are particularly sensitive to anthropogenic pressures, often decreasing in abundance or disappearing entirely under negative environmental conditions. A few hard-bottom taxa, such as *Syllis* cf. *hyalina*, *S. gracilis*, and *S. prolifera*, have shown opportunistic features and the ability to persist or even thrive under certain stress conditions [16]. Furthermore, the high proportion of rare species recorded in our study suggests a diverse but sparsely distributed species pool. While anthropogenic pressures had only a modest effect on the frequency of rare species, these patterns may reflect the broader resilience of marine ecosystems.

Species richness and Hill's diversity index (N1) remained largely consistent between sites exposed to A.P. and those without it, suggesting that these pressures do not significantly alter the overall species diversity or evenness within polychaete communities. The minor differences observed in these metrics may reflect shifts in community structure rather than a substantial loss of biodiversity. These results emphasize the resilience of the community in maintaining both richness and diversity.

The SIMPER analysis further highlights the role of A.P. in shaping species composition. Notably, *Sphaerosyllis pirifera* exhibited the highest contribution to dissimilarity, indicating its significant role in differentiating areas under and without A.P. Other species, such as *Syllis rosea*, *S. prolifera*, *S. gerundensis* and *Platynereis dumerilii*, also contributed notably. These findings are partially consistent with those of Mikac et al. [17], who found *S. rosea*, *P. dumerilii*, and *S. pirifera* among the top contributors to dissimilarities at different depths of hard-bottom polychaete assemblages, along with additional species such as *Nereis usticensis*, *S. prolifera*, and *Amphiglena mediterranea*. The combined findings on depth-related and human pressure-related factors emphasize the importance of a holistic conservation

strategy that integrates individual species dynamics with broader community interactions to preserve and restore ecosystem health.

The PERMANOVA analysis in this study revealed that spatial variation, represented by the factor *Area*, significantly influenced polychaete species composition, together with *Pressure*, suggesting that anthropogenic influences might contribute to community differences, though not conclusively. The absence of a significant interaction between *Area* and *Pressure* indicates that these factors do not combine to strongly affect the polychaete community structure. An additional analysis further highlighted significant heterogeneity in community dispersion between sampling areas, with Pula, the largest town in the region, showing a notably higher variability compared to rural locations such as Banjole, Premantura, and NP Brijuni. This difference may stem from Pula's greater environmental complexity or anthropogenic disturbance, compared to the more homogeneous conditions in less-impacted rural locations. As a larger urban center, Pula experiences more pronounced anthropogenic pressure, including urban, agricultural, and industrial impacts. Diversity indices (H' , J') supported this pattern, with a higher diversity generally observed in areas of lower anthropogenic pressure, consistent with broader ecological trends. For example, Giangrande et al. [16] found that polychaete diversity in Mediterranean ecosystems often declines under anthropogenic stress.

Regarding the potential effects of anthropogenic pressures on the biomass and coverage area of the red algae *C. officinalis* in each research area, we acknowledge that such factors may play a crucial role in shaping the availability of habitats and food for polychaetes. In areas with intense human activities, the introduction of pollutants and excess nutrients could lead to changes in algae biomass, which in turn could impact the polychaete assemblages that rely on this algae for shelter and food [77,78]. Eutrophication, for example, is known to typically increase algae biomass, but the excessive discharge of harmful pollutants could have varying effects on algae growth [79]. While our study did not directly measure these parameters, exploring these interactions could enhance our understanding of the ecological dynamics in these ecosystems.

No significant differences in polychaete relative abundance between stations under A.P. and those without it were recorded, suggesting that anthropogenic stressors may not have a strong immediate impact on polychaete abundance within the sampled locations. In contrast, Dafforn et al. [80] reported increased polychaete richness and abundance in heavily modified estuaries, which they attributed to nutrient enrichment rather than direct contamination effects, highlighting complex interactions between environmental factors. Their findings also underscore the complexity of interpreting diversity patterns, as multiple covarying factors, such as nutrient inputs and local species diversity, may strongly influence ecological responses beyond the effects of contamination alone. Further research incorporating other environmental factors and additional sampling locations may help elucidate whether more subtle ecological changes are occurring. Additionally, a longer-term study could shed light on potential delayed effects of anthropogenic disturbances on polychaete populations. Buršić et al. [27] examined the link between *C. officinalis* density and the abundance of various taxonomic groups, including polychaetes, in their broader study of the invertebrates associated with this algae. While the correlation between polychaete abundance and algae dry weight was not significant, they noted that environmental factors and habitat characteristics are likely to influence the structure and abundance of polychaete assemblages. As part of the same study of fauna associated with *C. officinalis*, but focusing on mollusks, Buršić et al. [50] found that anthropogenic pressure did not significantly affect the overall abundance of gastropods in the Southern Istrian coastal region. However, a decline in the average number of individuals of the most abundant gastropod and bivalve

species was noted at stations under anthropogenic pressure, particularly during summer periods, when our polychaete sampling also took place.

Noteworthy, our study also documented the presence of the non-indigenous polychaete *Lysidice collaris* [29], further supporting the evidence of its establishment in the Adriatic Sea. This finding aligns with recent research by Žunec et al. [81], which recorded the species in the central Adriatic, as well as previous reports from the Northern Adriatic [1,82,83]. Originally described from the Red Sea, *L. collaris* is widely distributed across tropical and temperate regions and has been recorded in various Mediterranean locations, typically inhabiting calcareous algae and coralligenous habitats. Its occurrence at multiple Adriatic sites suggests a well-established population, emphasizing the need for continued monitoring to assess its distribution and potential ecological impact. Regarding other species recorded in our study, the presence of *Pyonosyllis dionisi* was doubtful in the Northern Adriatic [1]. Moreover, other polychaetes are likely potentially newly recorded for the Adriatic Sea. This is the case for *Timarete* sp., which differs from *Timarete filigera*, the only species of the genus previously recorded in the Adriatic [1], as well as from the two non-indigenous species—*T. caribous* and *T. punctata*—documented in the Mediterranean [84,85]. The distinguishing features of *Timarete* sp. include one or two large, sigmoid dark brown hooks in the middle, posterior neuropodia, and the absence of black spots. This family is still poorly known and needs further revision [86]. There are still taxonomic uncertainties also within the genus *Nereis*. With *Nereis* sp., we indicated species with the homogomph falciger notochetae of *N. jacksoni* and *N. persica* (paragnaths were not visible). With *Nereis* cf. *pelagica*, we indicated animals with intermediate features between *N. pelagica* and *N. zonata*, both in terms of the shape of the homogomph falcigers notochetae and paragnath, when visible, in areas VI and VIII-VI-VIII [47].

5. Conclusions

In conclusion, this study highlights the resilience and adaptability of the polychaete assemblages associated with *C. officinalis* in the Northeastern Adriatic Sea, even in the presence of anthropogenic pressures. Subtle shifts in species composition and abundance suggest that anthropogenic stress may favor certain species in respect to others, particularly within the Syllidae family. Key species such as *Sphaerosyllis pirifera*, *Syllis rosea*, *Syllis gerundensis*, and *Platynereis dumerilii* were identified as significant contributors to the dissimilarity between locations without and under A.P., underscoring their potential as indicators of environmental disturbances. These findings emphasize the importance of periodic monitoring and conservation efforts, focusing not only on individual species but also on the broader community dynamics that define ecosystem health. Further research is needed to explore the long-term impacts of A.P. on marine biodiversity and the functioning of the ecosystem.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d17050302/s1>, Table S1: Geographic coordinates of sampling locations.

Author Contributions: Conceptualization, V.P., M.B. and L.I.; data curation, M.B., V.P., I.K. and T.V.Z.; formal analysis, V.P., M.B., N.I. and A.Ž.; funding acquisition, M.B., E.P. and B.M.; investigation, V.P., M.B., A.Ž., N.I., P.B. and L.I.; methodology, V.P., M.B. and I.K.; resources, M.B., P.B. and E.P.; visualization, V.P., M.B. and T.V.Z.; supervision, V.P., M.B. and B.M.; writing—original draft preparation, V.P. and M.B.; writing—review and editing, M.B., V.P., A.Ž., P.B., N.I., I.K., E.P., L.I., T.V.Z. and B.M. All authors have read and agreed to the published version of the manuscript.

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