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Changes in Composition of Mollusks within *Corallina officinalis* Turfs in South Istria, Adriatic Sea, as a Response to Anthropogenic Impact

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Abstract: A very common intertidal alga, *Corallina officinalis*, serves as a refuge for numerous invertebrates within its settlements. The composition and structure of invertebrates may differ in relation to different natural or human-induced stress, and this study examined the effects of anthropogenic impact on the abundance and diversity of mollusks residing within *C. officinalis* settlements. Sampling was conducted during two seasons (Season 1 = algae's maximum vegetation growth and Season 2 = algae's minimum vegetation growth). Gastropods and bivalves made up 50% of all invertebrates identified, with a total of 47 species of gastropods, 25 species of bivalves, and one polyplacophoran species recorded. Considering the overall count of individuals, 4562 gastropods, 21,738 bivalves, and 260 polyplacophorans were collected from all available *Corallina* samples. The results indicated that locations under human impact showed a reduced number of the most abundant gastropod and bivalve species and a reduced average number of individuals.

Keywords: mollusks; gastropods; bivalves; *Corallina officinalis*; anthropogenic impact; North Adriatic; Istrian coast

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1. Introduction

The Adriatic Sea, as part of the Mediterranean, is heavily influenced by various stressors, such as fisheries, temperature rise, acidification, maritime transport, tourism development, and pollution from land, resulting in biodiversity loss and ecosystem degradation [1]. Hard bottom benthic marine communities of coastal areas are heavily influenced by anthropogenic activities, especially in locations close to urban settlements and industrial zones [2–5]. Encrusting algae, such as *Corallina officinalis* Linneaus, the subject of this research, are common in coastal area because their structure makes it easier to withstand harsh conditions created by turbulent seawater movements and the destructive action of sea waves [6]. As it is obvious that every year there is increasing pressure on the coastal area, especially due to intensified urbanization, it is very important to know what kind of habitats and species are present in such areas and what their role is in the ecosystem [7,8]. Based on this knowledge, appropriate decisions can be made in the field of coastal zone management, which includes settlements of this red algae as well.

Due to their sedentary lifestyle and long-term exposure to nutrients and anthropogenic influence, macroalgae are a good indicator of changes in the marine environment. As a result of environmental stress, frequently attributed to human activities, algae may respond by reducing their population or even causing the disappearance of the most vulnerable species of algae. Subsequently, the vulnerable species, such as canopy-forming macroalgae belonging to the order Fucales, may be substituted by highly resistant and opportunistic species [9–13]. In many habitats, macroalgae and other flora determine the physical structure of the environment and influence the composition of organisms and their mutual interaction [14]. Various benthic groups, mostly macrofauna, have been used as indicators of different stressors or pollution in the environment [15–17].

Invertebrates are considered very suitable organisms as indicators of natural and anthropogenic changes, and for the Mediterranean Sea, there are various indices that use the ratio of sensitive and opportunistic groups and species in assessing the quality of coastal waters [14]. The ratio of opportunistic polychaetes and amphipod crustaceans (the BOPA index—the benthic opportunistic polychaetes amphipods index) was proposed as a measure for determining the ecological status of coastal waters based on pollution gradient from unpolluted to extremely polluted sites [18]. The BOPA index revised the previously proposed polychaete/amphipod ratio by reducing the effort in identifying individual taxa, which reduces the time and cost of assessing the water quality condition. The significance of biological indicators in evaluating the condition of marine ecosystems is highlighted by European laws such as the Water Framework Directive 2000/60/EC and the Marine Strategy Framework Directive 2008/56/EC. To determine the ecological health of European coastlines and estuaries, two indices were devised, namely, AMBI and multivariate-AMBI (M-AMBI), which can be computed using the AMBI software [19]. As *C. officinalis* settlements are abundant and have a high diversity of different invertebrate species [20–26], they are suitable for the implementation of the aforementioned indices.

The purpose of this study was to compare the composition of invertebrates, particularly mollusks, in areas affected by human activities and those unaffected. We expect to find more opportunistic and resilient species in human-impacted areas and higher biodiversity in non-impacted areas. Moreover, the seasonal dynamics of sampling, which coincided with the maximum and minimum growth phases of algae, were also taken into consideration during further analysis. This consideration was based on the expectation that the biomass of algae would have an influence on the abundance and diversity of mollusks.

2. Materials and Methods

2.1. Sampling Design

The research was conducted in the Northern Adriatic region, specifically in the coastal areas of Southern Istria and Brijuni National Park in Croatia. Considering different spatial scales, from larger to smaller, analyses were performed at the level of areas, locations, and sites. Four areas and nine locations were chosen based on prior knowledge of *C. officinalis* distribution, coast conditions (such as algae coverage, slope, and wind exposure), and anthropogenic impact. Each area had at least one location under and one location outside of anthropogenic impact. Within each location, two sites at least 100 m apart were selected, and within each site, three subsamples of algae were randomly collected within 5×5 cm replicate quadrates (Figure 1). This resulted in a total of 108 samples, 54 per sampling season. Season 1 was during the algae's maximum growth (sampling from November 2017 to April 2018), and Season 2 was during the algae's minimum growth (sampling from June to August 2018). Seasonal samples were taken into consideration when statistical multivariate analyses were performed.

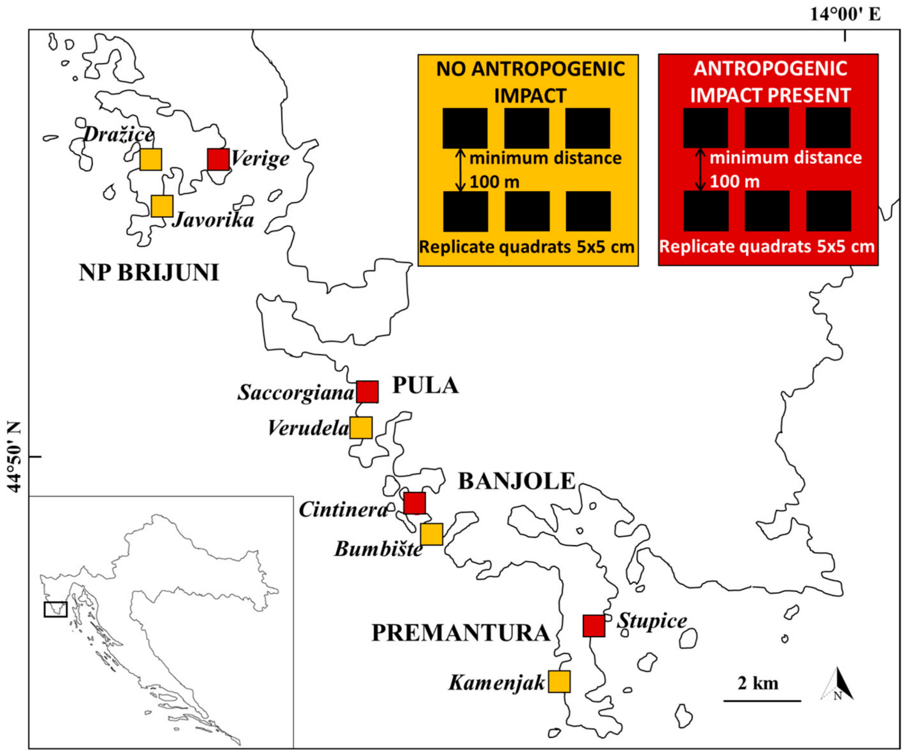

Figure 1. Locations under (red squares) and outside of anthropogenic influence (yellow squares) with a display of the sampling design. Six replicate quadrats 5 × 5 cm in size are displayed as black squares. Four sampling areas are Pula, Banjole, Premantura, and Brijuni National Park, and 9 locations are listed in italics.

2.2. Sampling Methods

Random quadrats were sampled in areas with nearly 100% algal coverage. Samples of algae with all associated invertebrates were taken with a hammer and chisel, stored in plastic containers, and fixed with alcohol. Sampling took place during low tide when the *C. officinalis* settlements were exposed to air to efficiently collect all organisms and minimize the loss of mobile invertebrates. In the laboratory, the samples were washed through a 0.5 mm mesh sieve, and all invertebrates were separated and identified under a microscope to the lowest possible taxonomic level. Given the objectives of this research, whenever possible, mollusks were identified at the species level using various available keys [27–35]. Following the isolation of all invertebrates, the algae were dried at 80 °C for a duration of 24 h, after which their weight was measured. Algal biomass values determined as dry weight are more reliable than values determined as wet weight since wet algal samples may contain variable amounts of seawater.

2.3. Quantifying Anthropogenic Impact

In order to quantify the anthropogenic impact, the Land Uses Simplified Index (LUSI) [36] was calculated. This index is based on collecting information that describes anthropogenic impact, the impact of freshwater from inhabited, industrial, and agricultural areas and rivers, and information about the morphology of the coast. The categories of impact are placed into two groups, land impact and freshwater impact. For the land impact, the score depends on the percentage of coverage of urban settlements, agricultural areas, or commercial and industrial areas, while for freshwater impact, the score depends on salinity. Each category is assigned a score. In addition to the score, a correction factor is applied depending on the type of coast, whether it is concave, convex, or straight (Table 1). This factor is the greatest for the concave type of coast that relates to bays and inlets, as any land impact is retained for longer in such habitats as opposed to the convex coast, where there is a large dilution capacity of seawater. The LUSI index values provide a quantitative estimation of the land impact on the investigated area and have a range of values from 0.75 to 8.75. Lower values indicate areas where there is no anthropogenic impact or that impact is minimal, and as the value increases, the impact is stronger. The LUSI index is calculated by the following formula: LUSI = (urban score + agricultural score + industrial score + riverine score) x coastline correction factor [36].

Table 1. Scores for calculating the LUSI index. Adapted from Flo et al. 2019 [36].

Land Impact			**Freshwater Impact**	**Pressure Score**
Urban Area	**Agricultural Area**	**Industrial Area**	**Salinity**	
	≤10%	≤10%	≥37.5	**0**
≤33%	10–40%	>10%	34.5–37.5	**1**
33–66%	>40%		<34.5	**2**
>66%				**3**
Correction factor for coastline morphology			concave	×**1.25**
			convex	×**0.75**
			straight	×**1.00**

Given that the maximum aerial distance between stations in Southern Istria was about 9 km in the present research, the calculation of this index was adapted, and for each research area, a 2 × 2 km square was used in the CORINE (COoRdination of INformation on the Environment) display. CORINE is a digital database that shows land use, i.e., on a map, one can see if a certain area is, for example, forest land, urban environment, or agricultural land (https://land.copernicus.eu/pan-european/corine-land-cover, accessed on 15 April 2021).

The mollusk fauna was analyzed with regard to the tolerance and sensitivity of certain species to the gradient of environmental stress. According to five proposed ecological groups (EG I-V) [19], present species were classified into one of the existing groups. Group I consists of species that are very sensitive to organic pollution and are present in conditions where there is no pollution. Group II consists of species that are indifferent to pollution and are always present, but to a lesser extent, with lower abundance and without significant variations over time. Group III consists of species that tolerate organic pollution, Group IV consists of secondary opportunistic species, and Group V consists of primary opportunistic species. The regularly updated list is available as part of the AZTI application at the web link https://ambi.azti.es, accessed on 6 July 2023, and includes over 11,000 taxa, providing information on their classification within specific groups. The software computes the percentage of each ecological group in each sample (%EGI, %EGII, etc.) and provides a classification of pollution or disturbance in the sample [37]. Based on the contribution of individual ecological groups, the value of the AZTI's Marine Biotic Index (AMBI) can be calculated, which ranges from 1 to 6, with lower values corresponding to unpolluted areas and higher to polluted areas. The formula for calculation is as follows: AMBI = $[(0 \times \%\ EGI) + (1.5 \times \%\ EGII) + (3 \times \%\ EGIII) + (4.5 \times \%\ EGIV) + (6 \times \%\ EGIV)]/100$ [19].

2.4. Statistical Data Processing

Multivariate methods were used to process the collected data using PRIMER v.6 [38]. For the statistical analysis of fluctuations in the abundance of gastropods and bivalves, a four-factor permutation analysis of variance (PERMANOVA) was used with the dry weight of the calcareous alga *C. officinalis* as a covariable. Four factors descriptions were as follows: (1) the Season factor is included in the analysis as a fixed factor with two levels (Season 1—November 2017–April 2018 and Season 2—June 2018–August 2018); (2) the factor Anthropogenic impact is fixed and has two levels (under anthropogenic impact and outside anthropogenic impact); the levels of this factor are set based on the LUSI index; (3) the Area factor is a random factor with 4 levels (Pula, Banjole, Premantura, NP Brijuni); and (4) the Location factor is a random factor and is nested in the interaction An × Ar with two levels (two randomly selected Locations for each combination of An and Ar factor levels). For factors and interactions in which statistically significant results were determined, additional permutation comparison tests were analyzed.

3. Results

3.1. Categorization of Research Locations Depending on Anthropogenic Impact

The LUSI index values ranged from 0.75 to 5.00, and the percentage of cover of urban settlements had the greatest impact on the overall scoring. When examining individual locations within each of the four study areas, the calculation confirms that the locations of Saccorgiana, Cintinera, Stupice, and Verige can be classified as locations under anthropogenic influence because the index values are higher than other locations without such influence (Table 2). Since Brijuni National Park is located outside of urban areas without significant agricultural influence, the LUSI index values are generally lower. However, when comparing all three locations within the NP Brijuni area, the Verige location has the highest index value and is, therefore, classified as a location under anthropogenic influence in that area.

Table 2. LUSI indices for research areas of Pula, Banjole, Premantura, and NP Brijuni (* denotes locations under anthropogenic impact). Individual values are expressed as follows: urban area; agricultural area; industrial area; and freshwater impact, as explained in Table 1).

Area	Location	Individual Values					Score
PULA	Verudela	1	0	1	0	0.75	**1.50**
	Saccorgiana *	2	1	1	0	1.25	**5.00**
BANJOLE	Bumbište	1	0	0	0	0.75	**0.75**
	Cintinera *	2	1	1	0	1.25	**5.00**
PREMANTURA	Kamenjak	1	0	0	0	1.25	**1.25**
	Stupice *	2	1	0	0	1.25	**3.75**
NP BRIJUNI	Javorika	1	0	0	0	1.00	**1.00**
	Dražice	1	0	0	0	1.25	**1.25**
	Verige *	1	0	1	0	1.00	**2.00**

3.2. Diversity of Mollusks within Corallina Officinalis Settlements

Results of the combined sampling carried out during both seasons showed that gastropods and bivalves made up 50% of all isolated invertebrates. In total, 47 species of gastropods and 25 species of bivalves were recorded. In terms of the total number of individuals, 4562 gastropods and 21,738 bivalves were isolated.

Another group of mollusks, the polyplacophorans, were present with just one species, *Acanthochitona fascicularis* (Linnaeus, 1767), and 260 individuals, but since the diversity of gastropods and bivalves dominated, a detailed overview of these two groups is presented further in this paper. Species were classified into corresponding ecological groups (EG), from EG I to EG V (Table 3). The share of each mollusk species in the total number of individuals was calculated separately for sampling locations with and without anthropogenic impact using the following formula: S = (Na/N) × 100. Here, Na represents the number of individuals of species "a", and N represents the total number of all individuals within the specific category of interest, either outside anthropogenic impact or under anthropogenic impact. These values are necessary for calculating the AMBI index.

Table 3. Species list of gastropods and bivalves (EG—ecological group assigned based on the updated list of AZTI application; outside A.P.—locations outside anthropogenic impact; under A.P.—locations under anthropogenic impact).

Family	**Species**	**EG**	**Share in the Total Number of Mollusks (%)**	
			Outside A.P.	**Under A.P.**
GASTROPODA				
Aplysiidae	*Aplysia* sp.juv. (Linnaeus, 1767)	I		0.01
Cerithiidae	*Bittium reticulatum* (da Costa, 1778)	I	7.21	2.55
Cerithiidae	*Cerithium vulgatum* (Bruguière, 1792)	II	0.01	
Cerithiopsidae	*Cerithiopsis tubercularis* (Montagu, 1803)	I		0.01
Cingulopsidae	*Eatonina cossurae* (Calcara, 1841)		3.72	3.82
Cingulopsidae	*Eatonina* sp. (Thiele, 1912)		0.04	0.01
Columbellidae	*Columbella rustica* (Linnaeus, 1758)	I	0.02	
Dorididae	Dorididae indet.juv. (Rafinesque, 1815)	I	0.01	
Eulimidae	*Vitreolina antiflexa* (Monterosato, 1884)	I	0.02	0.02
Fissurellidae	Fissurellidae indet.juv (J. Fleming, 1822)		0.01	0.02
Mitridae	*Episcomitra cornicula* (Linnaeus, 1758)			0.01
Muricidae	*Hexaplex trunculus* (Linnaeus, 1758)	I		0.01
Muricidae	*Muricopsis cristata* (Brocchi, 1814)		0.01	
Muricidae	*Ocenebra* cf. *edwardsii* (Payraudeau, 1826)		0.04	0.05
Omalogyridae	*Ammonicera fischeriana* (Monterosato, 1869)		0.00	0.05
Patellidae	*Patella caerulea* (Linnaeus, 1758)		0.12	0.05
Phasianellidae	*Tricolia pullus* (Linnaeus, 1758)	I	0.01	
Pyramidellidae	*Brachystomia eulimoides* (Hanley, 1844)		0.03	0.06
Pyramidellidae	*Megastomia winfriedi* (Peñas and Rolán, 1999)		0.22	0.16
Pyramidellidae	*Odostomia plicata* (Montagu, 1803)	II	0.01	0.01
Pyramidellidae	*Parthenina emaciata* (Brusina, 1866)	I	0.03	0.01
Pyramidellidae	*Spiralina alpinoligustica* (Sacco, 1892)	I	0.02	0.03
Rissoellidae	*Rissoella* sp. (Gray, 1847)	I	1.38	0.16
Rissoidae	*Alvania poucheti* (Dautzenberg, 1889)	I	0.17	0.03
Rissoidae	*Alvania rudis*(Philippi, 1844)	I		0.01
Rissoidae	*Alvania* sp. 1 (Risso, 1826)	I		0.03
Rissoidae	*Alvania* sp. 2 (Risso, 1826)	I	0.01	
Rissoidae	*Alvania* cf. *carinata juv.* (da Costa, 1778)			0.02
Rissoidae	*Cingula trifasciata* (J. Adams, 1800)	I	0.03	0.01
Rissoidae	*Crisilla beniamina* (Monterosato, 1884)		0.09	0.01
Rissoidae	*Crisilla innominata* (R. B. Watson, 1897)		3.60	0.68
Rissoidae	*Crisilla iunoniae* (Palazzi, 1988)		0.13	0.03
Rissoidae	*Crisilla* cf. *maculata* (Monterosato, 1869)		2.13	0.95
Rissoidae	*Pusillina philippi* (Aradas and Maggiore, 1844)		0.31	0.06
Rissoidae	*Rissoa splendida* (Eichwald, 1830)		0.07	0.02
Rissoidae	*Setia* sp. (H. Adams and A. Adams, 1852)		0.01	
Scissurellidae	*Scissurella costata* (d'Orbigny, 1824)		1.62	1.79

Table 3. *Cont.*

Family	Species	EG	Share in the Total Number of Mollusks (%)	
			Outside A.P.	Under A.P.
Scissurellidae	*Sinezona cingulata* (O. G. Costa, 1861)		0.19	0.06
Siphonariidae	*Siphonaria* cf. *pectinata juv.* (Linnaeus, 1758)	I	0.05	0.11
Triphoridae	*Monophorus perversus* (Linnaeus, 1758)	I	0.01	0.00
Tritoniidae	*Duvaucelia manicata* (Deshayes, 1853)	I	0.01	0.03
Trochidae	*Clanculus* sp.juv. (Montfort, 1810)		0.01	
Trochidae	*Gibbula* sp.juv. (Risso, 1826)	I		0.01
Trochidae	*Gibbula* cf. *turbinoides* (Deshayes, 1835)	I	0.04	0.03
Trochidae	*Phorcus turbinatus* (Born, 1778)		0.27	0.16
Trochidae	*Steromphala adriatica* (Philippi, 1844)	I	0.00	0.01
Gastrpoda indet.juv.			0.25	0.02
BIVALVIA Arcidae	*Arca noae* (Linnaeus, 1758)	I	0.01	0.02
Arcidae	*Arca* sp. (Linnaeus, 1758)	I	0.05	0.05
Carditidae	*Cardita calyculata* (Linnaeus, 1758)	I	1.11	1.63
Chamidae	*Chama gryphoides* (Linnaeus, 1758)		0.01	
Hiatellidae	*Hiatella rugosa* (Linnaeus, 1767)	I	5.46	3.04
Lasaeidae	*Lasaea* cf. *rubra* (Gmelin, 1791)	II	0.09	0.61
Limidae	*Lima lima* (Linnaeus, 1758)	I	0.02	0.01
Lucinidae	*Lucinella* sp. (Monterosato, 1884)	I	0.01	
Mytilidae	*Gregariella semigranata* (Reeve, 1858)	I		0.02
Mytilidae	*Lithophaga lithophaga* (Linnaeus, 1758)			0.01
Mytilidae	*Modiolus barbatus* (Linnaeus, 1758)	I		0.01
Mytilidae	*Musculus* cf. *costulatu* (Risso, 1826)	I	7.95	5.05
Mytilidae	*Musculus* sp. (Röding, 1798)	I	0.01	0.02
Mytilidae	*Mytilus galloprovincialis* (Lamarck, 1819)	III	62.19	77.32
Noetiidae	*Striarca lactea* (Linnaeus, 1758)	I	0.03	0.02
Pectinidae	*Flexopecten glaber* (Linnaeus, 1758)	I		0.01
Spondylidae	*Spondylus* sp. Linnaeus, 1758			0.01
Veneridae	*Clausinella* sp. (Gray, 1851)	I	0.01	
Veneridae	*Irus irus* (Linnaeus, 1758)	I	0.01	0.13
Veneridae	Veneridae indet. 1	I	0.01	0.02
Veneridae	Veneridae indet. 2	I		0.06
Veneridae	Veneridae indet. 3	I	0.05	0.08
Veneridae	Veneridae indet. 4	I		0.01
Veneridae	Veneridae indet. 5	I		0.01
Veneridae	Veneridae indet. 6	I		0.01
POLYPLACOPHORA Acanthochitonidae	*Acanthochitona fascicularis* (Linnaeus, 1767)	I	1.11	0.81

By comparing the AMBI index values based on the composition of recorded mollusk species, it can be observed that locations under anthropogenic impact have higher values than those outside of the impact. The largest contribution to the value of the AMBI index was made by the species *Mytilus galloprovincialis*, which is classified in ecological group III, and its increase in dominance in locations with anthropogenic impact caused the increase in the value of the index (Table 4).

Table 4. AMBI index and the share of individual ecological groups in the total abundance of mollusks with regard to the sampling location.

Ecological Group	Share in the Total Number of Mollusks	
	Without Anthropogenic Impact	**With Anthropogenic Impact**
I	24.83%	14.02%
II	0.10%	0.62%
III	62.19%	77.32%
AMBI indexs	**1.87**	**2.33**

Considering all sampling locations and both sampling seasons, the most abundant gastropod species were *Bittium reticulatum*, *Crisilla* cf. *maculata*, *Eatonina cossurae*, and *Scissurella costata*, while bivalves were numerically most represented by species *Mytilus galloprovincialis*, *Musculus costulatus*, *Hiatella rugosa*, and *Cardita calyculata* (Figure 2).

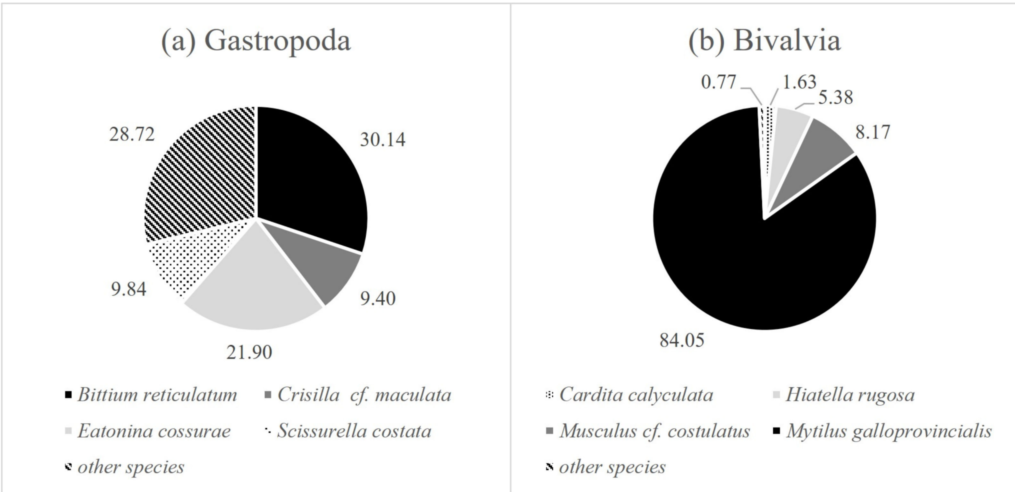

Figure 2. Abundance of gastropod (**a**) and bivalve (**b**) species recorded within *C. officinalis* settlements in all sampling locations and during both seasons.

3.3. Multivariate Analysis of Gastropods

PERMANOVA analysis was applied to determine statistically significant differences in the number of individual gastropod and bivalve species with regard to sampling location and time and with regard to the presence or absence of anthropogenic influence. The PERMANOVA model consisted of four factors (*Season*, *Anthropogenic Impact*, *Sampling Area*, and *Sampling Location*) which are described in detail in Section 2. Besides testing the influence of these main factors, the impact of their interaction was also examined using permutation comparison tests.

The dry weight of *C. officinalis* significantly influenced the number of individuals of gastropod species found (Table 5). Variations on all spatial scales were statistically significant. It was also established that there is a difference in the number of individuals according to the factor *Season* and *Sampling Area*, which, together with the statistically significant interaction *Anthropogenic Impact* × *Area*, was investigated in detail with comparative tests. The statistical significance of the comparison of gastropods' species composition between seasons was recorded (t = 3.7183, p = 0.0003). Permutational comparison tests showed no statistical significance within levels for the *Anthropogenic Impact* × *Area interaction*.

Table 5. PERMANOVA for the total species composition present within gastropods with four factors. Dry weight of *C. officinalis* is a covariable. The effects of the four factors (*Season*, *Anthropogenic Impact*, *Sampling Area*, and *Sampling Location*) on the number of individuals of recorded gastropod species were tested.

			Gastropoda		
Source	*Df*	*SS*	*MS*	*Pseudo-F*	*P(MC)*
Corallina	1	7458.7	7458.7	3.9186	**0.0022**
Season = Se	1	47,363	47,363	12.821	**0.0002**
Anthropogenic impact = An	1	4520.4	4520.4	0.85593	0.5389
Area = Ar	3	20,338	6779.3	2.784	**0.0037**
Se × An	1	4455.5	4455.5	2.3209	0.0822
Se × Ar	3	11,077	3692.2	1.3807	0.1898
An × Ar	3	15,143	5047.5	2.1675	**0.0257**
Location (Se × Ar)	8	19,251	2406.4	2.1989	**0.0002**
Se × An × Ar	3	5543.9	1848	0.71144	0.7675
Se × Location (An × Ar)	8	20,816	2602.1	2.3777	**0.0001**
Error	63	68,946	1094.4		

The sampling area (quadrat) was 5 × 5 cm in size. The description of the factors and other details are explained in Section 2. *Df* = Degree of Freedom; *SS* = Sum of Squares; *MS* = Mean Square; *P(MC)* = significance level (probability) was obtained by Monte Carlo permutation method. Total number of measurements N = 96; number of measurements in each combination of factors n = 3.

Gastropod species were analyzed using the statistical method of Principal Coordinate Analysis (PCO) in relation to the research locations, sampling period, and anthropogenic impact. The two-dimensional projection shows the separation of locations with regard to the sampling period and anthropogenic influence. Locations sampled during Season 2 are clearly separated from locations sampled during Season 1, which is visible on the first axis (PCO1) that represents 32.2% of the total variability (Figure 3). For locations under and outside of anthropogenic impact, the separation is a little less pronounced but still present.

The species *Rissoella* sp. contributes the most to distinguishing the Kamenjak and Verudela locations, which are outside of anthropogenic impact. During Season 2, the species *Eatonina cossurae* contributed the most to distinguishing the locations of Saccorgiana and Cintinera, which are under anthropogenic influence.

3.4. Multivariate Analysis of Bivalves

The number of individual mollusk species was found to depend on the biomass of *C. officinalis* algae, but the significance of this relationship was slightly elevated compared to the 5% threshold (*P(MC)* = 0.078). A statistical difference in the number of individuals based on the factor *Season* was determined and is presented with other results in Table 6. Statistical significance was found at the one-meter, ten-meter, and kilometer spatial scales. Fluctuations in the number of individuals were analyzed using comparative tests based on the factors of *Season* and *Area*. A significant difference was observed in species composition between winter and summer for bivalve species (t = 2.0103, p = 0.0367), which was obtained using permutation comparison tests.

Principal coordinates analysis (PCO) was also made for the bivalve species present in relation to research location, season, and anthropogenic impact (Figure 4). On the two-dimensional projection, there is no clear separation of locations with regard to the season and anthropogenic impact.

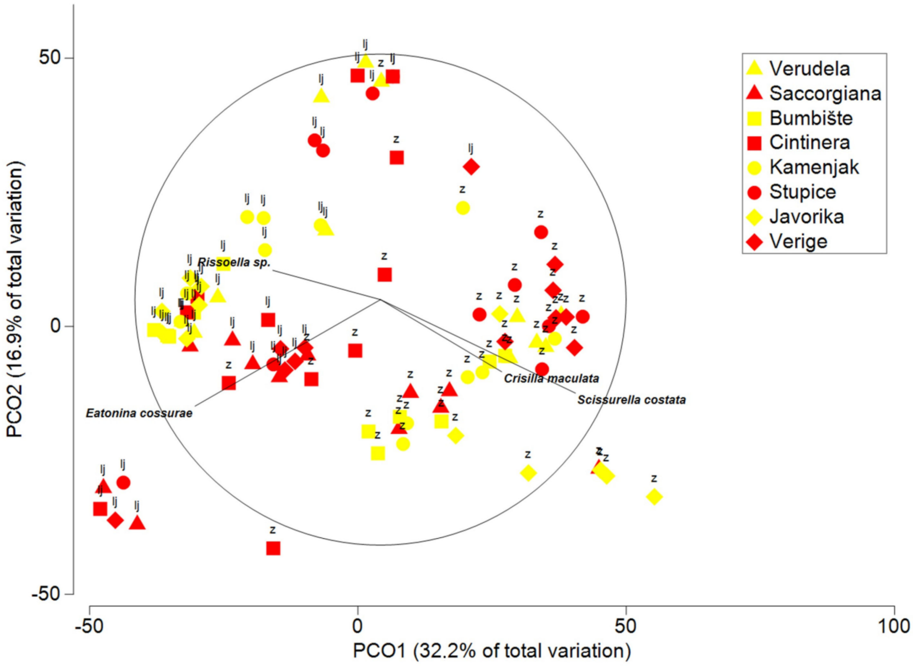

Figure 3. PCO ordination of gastropod species. The factors were as follows: *Sampling area* (Pula, Banjole, Premantura, Brijuni National Park), *Anthropogenic impact* (locations outside anthropogenic impact are marked in yellow; locations under anthropogenic impact are marked in red), *Season* (z-Season 1; lj-Season 2) and *Sampling location* (listed in the legend).

Table 6. PERMANOVA for the total species composition present within bivalves with four factors. Dry weight of *C. officinalis* is a covariable. The effects of the four factors (*Season*, *Anthropogenic Impact*, *Sampling Area*, and *Sampling Location*) on the number of individuals of recorded bivalve species were tested.

			Bivalvia		
Source	*Df*	*SS*	*MS*	*Pseudo-F*	*P(MC)*
Corallina	1	1451.7	1451.7	2.2751	0.078
Season = Se	1	8411.5	8411.5	4.656	**0.0263**
Anthropogenic = An	1	859.8	859.8	0.89527	0.511
Area = Ar	3	8668.5	2889.5	3.7172	**0.0031**
Se × An	1	1673.3	1673.3	1.4399	0.2881
Se × Ar	3	5473.5	1824.5	1.8771	0.0809
An × Ar	3	2775	924.99	1.2457	0.3078
Location (An × Ar)	8	6141.8	767.73	2.2408	**0.0009**
Se × An × Ar	3	3350.9	1117	1.1873	0.343
Se × Location (An × Ar)	8	7540.9	942.61	2.7512	**0.0001**
Error	63	21585	342.62		

The sampling area (quadrat) was 5 × 5 cm in size. The description of the factors and other details are explained in Section 2. *Df* = Degree of Freedom, *SS* = Sum of Squares, *MS* = Mean Square, *P(MC)* = significance level (probability) was obtained by Monte Carlo permutation method. Total number of measurements N = 96; number of measurements in each combination of factors n = 3.

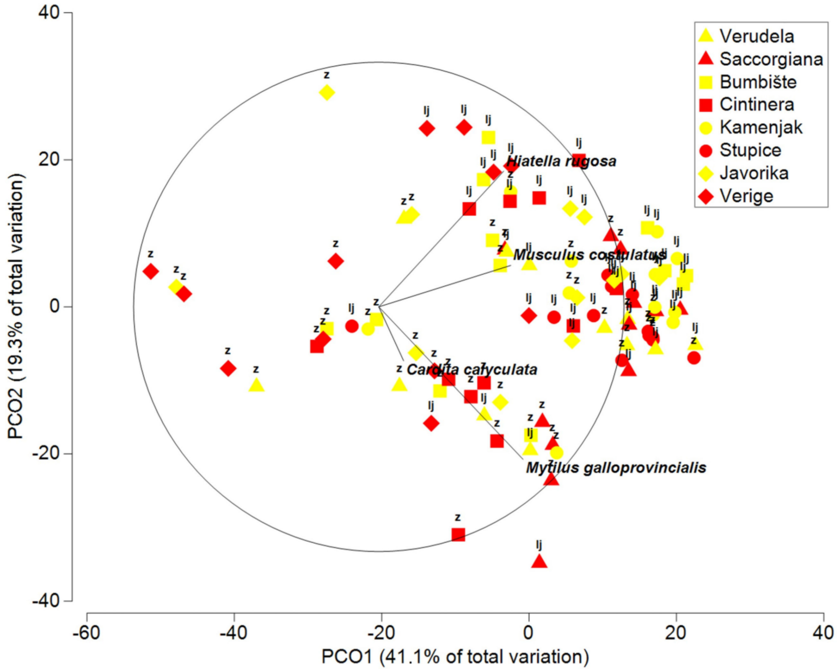

Figure 4. PCO ordination of bivalve species. The factors were as follows: *Sampling area* (Pula, Banjole, Premantura, Brijuni National Park); *Anthropogenic impact* (locations outside anthropogenic impact are marked in yellow, locations under anthropogenic impact are marked in red); *Season* (z-Season 1; lj-Season 2); and *Sampling location* (listed in the legend).

The first axis (PCO1) represents 41.1% of the total variability, while for the second axis (PCO2), this value is 19.3%. Since there is no clear separation, it can only be concluded that the species *Mytilus galloprovincialis*, *Hiatella rugosa*, *Cardita calyculata*, and *Musculus* cf. *costulatus* dominated in both sampling seasons.

4. Discussion

In monitoring activities, invertebrates are frequently employed as indicators due to their ability to react to a wide range of natural and human-induced changes. As mentioned earlier, macrofaunal species are divided into five ecological groups [19], and there is an up-to-date list available at https://ambi.azti.es, comprising more than 11,000 taxa. Our study focused on mollusks and found that they constituted 50% of all the identified invertebrates, bringing attention to this particular taxonomic group. Out of the 73 mollusk species documented in our research, 46 were included in the aforementioned list, with 42 belonging to Group I, three to Group II, and only one to Group III. As most of the species belong to Group I, it could be concluded that the mollusk fauna of hard bottom substrates in the Southern Istrian region of Croatia is dominated by sensitive species. However, the species *Mytilus galloprovincialis* was extremely dominant in terms of its abundance during both seasons. It was made up of 90% of all specimens sampled during Season 1 and 80% of all specimens sampled during Season 2. Therefore, it could still be concluded that *C. officinalis* settlements tolerate organic pollution to some extent. Despite being designed for soft bottoms, the AMBI index was used in this research due to its high sensitivity to environmental variations on hard bottoms [39].

An overview of fluctuations in the number of individuals of the most abundant species of gastropods and bivalves confirms that there are differences in all spatial scales considering *C. officinalis* distribution and that certain species fluctuate depending on anthropogenic impact and the sampling season. The gastropod species *Bittium reticulatum* showed variability in its numbers between different areas and within locations. The variations were influenced by different factors, such as sampling period and anthropogenic impact, particularly in the Banjole area. It was found to be more abundant in areas with less human impact and during Season 1 (winter) when its dominance was at the maximum level. In a study carried out in the western Mediterranean, it was discovered that *B. reticulatum* dominates in the fall and causes a decrease in the diversity of other species; thus, it could have been expected that the dominance would be more pronounced in the winter period [40]. This species usually lives in large groups and feeds on algae and organic debris. It prefers sheltered coastal areas with solid substrate and various algae as its habitat. Research has shown that the proximity of a sewage outlet influences mollusk composition, with higher abundance observed in locations close to the outlet [41]. This could be due to increased sedimentation since *B. reticulatum* feeds on deposited organic matter (it consumes sediment and organic matter for nutrition). *B. reticulatum* has been found in areas with low-quality seawater and poor water movement, higher sedimentation rates, and organic matter levels [42], indicating that it has a wide distribution and can survive in challenging conditions.

No significant variation was found in the abundance of *Crisilla maculata* at any spatial scale or in relation to the sampling period or human impact. This species' dominance is common, as many species from the Rissoidae family are present in intertidal macroalgal communities [43]. *Crisilla maculata* thrives in this habitat because, similar to some other Rissoidae species, it feeds on diatoms, epiphytic algae, and food in the sediment of the algae and between branches of the dense thallus [44,45].

The abundance of *Eatonina cossurae* did not vary between research areas but fluctuated significantly based on human impact and the sampling period. During Season 2, the dominance of *E. cossurae* species increased several times. Research of the anthropogenic impact (influence of sewage outlet) on mollusk composition has shown a slightly higher abundance of the *Eatonina* genus near the sewage outlet [41], which is consistent with our research, where a slight increase in individuals was observed in locations under anthropogenic impact.

A significant fluctuation in the abundance of *Scissurella costata* was observed based on human impact. This species was previously found to be abundant in *Cystoseira compressa* and *Carpodesmia crinita* settlements due to its diet of food trapped in sediment in the algal thallus [46]; thus, its abundance in *C. officinalis*, with favorable conditions and food availability, was expected.

A statistically significant difference was found in *Hiatella rugosa* abundance between locations, regardless of the sampling period. This genus has been recorded in previous studies in *C. officinalis* algal communities, though never in large numbers, with individuals reaching a maximum size of 10 mm and attached to the lower parts of the alga by bissus threads [47], as observed in this study. *Hiatella* species are typically filter feeders that can inhabit cracks, holes in rocks, be partially buried in sediment, or attached to invertebrates or algae by filaments. They are tolerant to varying depths, temperatures, and salinities, making them well-suited to life in intertidal zones where these parameters fluctuate greatly. Some species of *Hiatella* can be found even in areas with lower water quality, meaning that they have lower survival requirements [42].

The species *Musculus costulatus* showed statistically significant fluctuations between locations based on the sampling period. This species is also known to tolerate lower water quality conditions [42]. Research showed that its dominance could lead to reduced species diversity within algal branches during spring [40]. In the present study, the dominance of *M. costulatus* doubled during the summer sampling period (Season 2) when the number of total recorded species declined, indicating a similar effect within *C. officinalis* settlements.

The variability of *Cardita calyculata* was determined based on its occurrence period and human impact at the largest geographical scale (between areas). During Season 1, a significantly higher number of individuals was counted, with a slightly greater presence in areas affected by human activities compared to pristine locations. Previous studies have shown that this species mostly thrives in environments with low pollution levels and regular water renewal [48], suggesting that the difference in individual numbers caused by the human impact will not be significantly different.

Mytilus galloprovincialis showed statistically significant spatial variations at all geographical scales, being the dominant bivalve species and leading to differences between sampling areas. Juvenile forms of *M. galloprovincialis* often dominate algal settlements on coastal rocky habitats, as recorded in a study in Southern Italy, where they constituted 96.6% of the total abundance in three *Cystoseira* algal species [46]. The adult forms were found in close proximity, attached to the rocky substrate, competing for space with algae. High densities and biomasses of *M. galloprovincialis* have been observed to increase quickly if they find a suitable habitat, and the shell structure can provide refuge for other species [49]. This research also recorded its high abundances of 90% (Season 1) and 80% (Season 2), indicating the suitability of the habitat for *M. galloprovincialis*. In habitats with high algae density, macrofaunal organisms have been shown to have a lower density, explained by the high presence of juvenile bivalves from the Mytilidae family [50]. A high presence of Mytilidae was also recorded (*Gregariella semigranata*, *Lithophaga lithophaga*, *Modiolus barbatus*, *Musculus costulatus*, *Musculus* sp., and *Mytilus galloprovincialis*), making up 95% of all bivalves during Season 1 and 90% during Season 2. Samples with lower mytilid abundances showed a higher diversity of species. Juvenile Mytilidae are attached to filamentous algae branches before settling on the substrate as adults, and the species *C. officinalis* serves as a temporary substrate. Mussels are attached to seaweed using byssal threads, dominating and occupying potential attachment surfaces for other species, leading to a significant impact on the abundance and density of other mollusk species. The filtration activity of mussels, as they feed by filtering suspended particles from the water, can have indirect effects on larval populations of other species. Through their filtration process, mussels remove a considerable number of suspended larvae from the water column, reducing the chances of successful larval settlement and recruitment for other species [51,52].

Overall, the combined effects of competition for space and larval elimination through filtration by mussels contribute to the observed impacts on the abundance and density of other mollusk species within the ecosystem. These interactions further highlight the complexity of ecological dynamics and the influence of species' interactions on community structure and composition.

5. Conclusions

The anthropogenic impact has not greatly affected the overall abundance of gastropods inhabiting hard bottom substrates of the Southern Istrian coastal region in Croatian parts of the Northern Adriatic Sea. However, if the number of individuals of the most abundant species of gastropods and bivalves is considered, some differences can be observed, i.e., a decrease in average abundance at locations under human influence. The average number of individuals of the most abundant species of gastropods is lower at locations sampled under anthropogenic impact during both sampling seasons. For bivalves sampled during algae's minimum growth (Season 2), a decrease in the average number of individuals was observed at locations with human impact compared to locations without that impact. The dominance of the species *Mytilus galloprovincialis* and other mussels from the family Mytilidae greatly affects the composition of other invertebrates.

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