ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 60 (2): 127 - 136, 2019	ORIGINAL SCIENTIFIC PAPER

A preliminary study on the diversity of invertebrates associated with *Corallina officinalis* Linnaeus in southern Istrian peninsula

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Red coralligenous algae Corallina officinalis *provides shelter to many invertebrate species from wave actions, predation and desiccation stress in the intertidal area. Physical structure and complexity of the habitat have a major influence on biodiversity of this community. The aim of this preliminary study was to examine the diversity of invertebrate assemblages inside the red algae* C. officinalis *turf. Three sampling locations were chosen where algal cover range was above 90%. On each location sampling was done quantitatively by scraping off within 3 replicate quadrats 20 x 20 cm in size. A total of 30,518 specimens were isolated from all sampling locations. The prevalent groups were amphipod crustaceans, polychaetes, bivalves and gastropods that made a total of 86% of all macrofaunal groups associated with algal turfs. The most abundant group were amphipods that made 42% of the total separated individual invertebrates. Our study demonstrated that* C. officinalis *is a very important habitat with high abundance and diversity of invertebrate assemblages.*

Key words: *invertebrates,* Corallina officinalis, *mapping, biodiversity, Istrian coast*

INTRODUCTION

In many marine habitats macroalgae and other flora determine the physical structure of the environment and affect the composition of organisms and their interaction (MATIAS *et al*., 2007). This is also the case with the red incrusting algae *Corallina officinalis* Linneaus (GUIRY & GUIRY, 2019) which modifies the environment with its characteristic structure. Thus, it provides shelter to many macrofaunal species from wave action, predation and dehydration in the intertidal zone (LIUZZI & GAPPA, 2008). Many infaunal and epifaunal species seek shelter here during periods of unfavorable weather conditions (BERTNESS *et al*., 2006).

Physical structure and complexity of habitats have a major impact on the biodiversity of this community (KELAHER, 2002) and previous research around the world recorded up to 329,000 invertebrates per one square meter (BROWN & TAYLOR, 1999; KELAHER *et al*., 2001; BUSSELL *et al*., 2007; LIUZZI & GAPPA, 2008) which confirms that this algal turf is a very important habitat for preserving coastal biodiversity. Changes in structure and composition of macrofauna associated with coralline algae have been explored worldwide (KELAHER, 2003; KELAHER *et al*., 2004; MATIAS *et al*., 2007; LIUZZI & GAPPA, 2008; MATIAS *et al*., 2015; LAVENDER, *et al*., 2017). In the southwest Atlantic, changes in the structure of macrofaunal communities within the coralline algae settlements have been observed at local and regional scale since this type of habitat can occur along thousands of kilometers of shoreline. This broad distribution makes it suitable to analyze changes in benthic biodiversity and taxonomic turnover at different spatial scales. The study conducted by LIUZZI & GAPPA (2008) concluded that changes in assemblage structure at the local scale were of a much smaller magnitude than those observed at regional scale and may be attributed to small differences in habitat topography, intertidal elevation, wave exposure, and distance to harbors or different sources of pollution. Invertebrates are considered very suitable organisms as indicators of natural and anthropogenic changes and there are various indices that use the ratio of sensitive and opportunistic species and groups in the assessment of the quality of coastal waters (DAUVIN & RUELLET, 2007; BORJA *et al.,* 2009; CABANA *et al*., 2013).

In the Mediterranean Sea *Corallina officinalis* is most commonly found in the intertidal zone, but it is also present at higher depths in subtidal area (BABBINI & BRESSAN, 1997). The mapping carried out in the eastern Adriatic has shown that settlements of this coralline algae were registered across 13% of the surveyed coastline (NIKOLIĆ *et al*., 2013). Investigations in the area of the western coast of Istria recorded algae from the genus *Corallina* (*C. officinalis* and *C. elongata*) at almost all research sites, with *C. officinalis* dominating in areas without anthropogenic influence (IVEŠA *et al*., 2009).

The lack of data about the abundance and composition of macrofauna associated with such widespread settlements of *C. officinalis* in Adriatic Sea determined the aim of this study, which was to examine the diversity of invertebrate assemblages inside the coralline turf and to acquire qualitative and quantitative data about invertebrates within its settlements in the northern Adriatic.

MATERIAL AND METHODS

Study area

The study area included the southern part of the Istrian peninsula (Adriatic Sea, Croatia) which is made of karstic coast and predominantly limestone in the intertidal area. Samples were taken where coastal slope was horizontal on sampling locations named Saccorgiana (44°50’31” N, 13°50’02” E), Banjole (44°49’10” N, 13°51’20” E) and Kamenjak (44°46’38” N, 13°54’20” E) (Fig. 1). All locations are semiexposed, particularly to southwest wind direction.

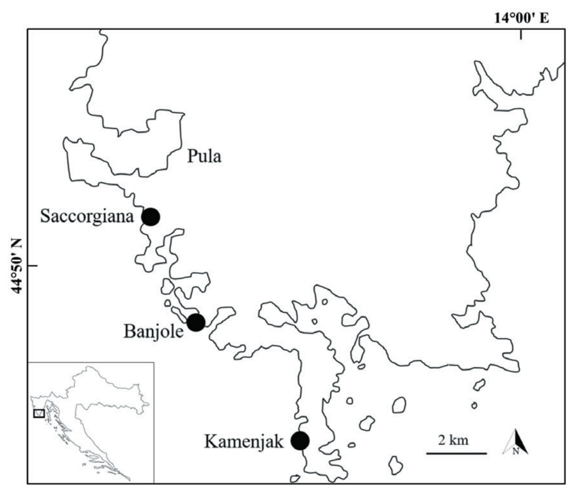

Fig. 1. Sampling locations at southern Istrian peninsula

Sampling

Sampling was performed from January till May 2016 at previously mentioned sampling locations. Sampling was done where the range of algal turf coverage was between 75% and 100% in a continuous belt about 50 cm wide, where the coastal slope was horizontal and where the exposure to wind and waves was quite high. On each location sampling was done quantitatively by scraping off within 3 replicate quadrats 20 x 20 cm in size. Samples were taken with hammer and chisel and fixed in 4% formalin. Sampling was done during low tide to minimize the loss of mobile invertebrates. In the laboratory samples, including *C. officinalis*, were rinsed through a sieve with 0.5 mm mesh size and stored in 70% ethanol for further processing. Macrofaunal organisms were sorted and counted under a binocular microscope and were identified to the lowest possible taxonomic level. After separation of all macrofaunal organisms, wet and dry weight of *C. officinalis* was also recorded. Algae were dried at 80°C for 24 hours. Mollusks were identified to the species level, whenever possible. Species were determined according to NORDSIEC, 1968, 1969; PARENZAN, 1970, 1974; SABELLI *et al.*, 1990; POPPE & GOTO, 1993; GIANUZZI-SAVELLI *et al.*, 1996 and GOFAS *et al.*, 2011a,b. For each taxa total number of individuals was recorded. Descriptive statistics and graphical representation of results was done using MS Excel considering all sampling locations as one. The nomenclature of the species was checked according to the World Register of Marine Species (WORMS, 2019).

RESULTS

Altogether 30,518 invertebrates were isolated from *Corallina* samples belonging to 24 different taxonomic groups whether is Phylum, Class, Order or Family. Average density value was 84,775 individuals per square meter (ind m^{-2}). The highest value was recorded for Kamenjak with 116,250 ind m^{-2}, while the average *Corallina* dry weight was lowest compared to other sampling locations (Table 1).

Table 1. Maximum density of individuals (ind m-^{2}) and average dry weight (dw) of C. officinalis

	max ind m^{-2}	mean dw (g)
Banjole	81,625	197.92
Saccorgiana	99,500	162.29
Kamenjak	116,250	129.99

The prevalent invertebrates were amphipod crustaceans, polychaetes, bivalves and gastropods that made a total of 86% of all individuals associated with algal turfs at all research locations. The most abundant group were amphipods with 42% of all separated invertebrates (Fig. 2) and they were by far the most dominant taxonomic group with more than 4,200 recorded individuals (Figure 3). Some taxonomic groups

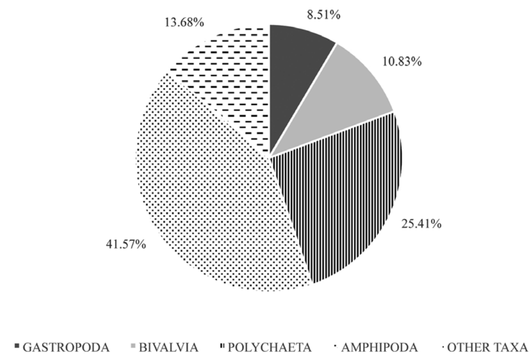

Fig 2. The most abundant taxonomic groups in C. officinalis *assemblages in studied area.*

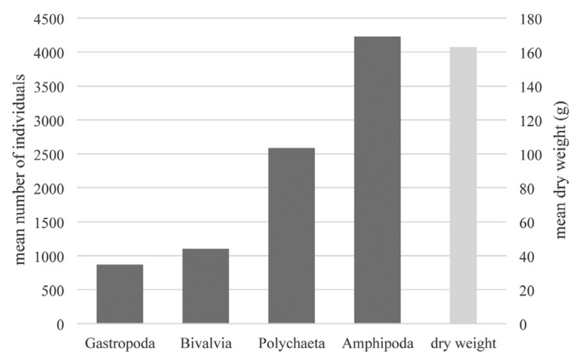

Fig. 3. Average number of individuals per each of the most abundant taxonomic groups in studied area

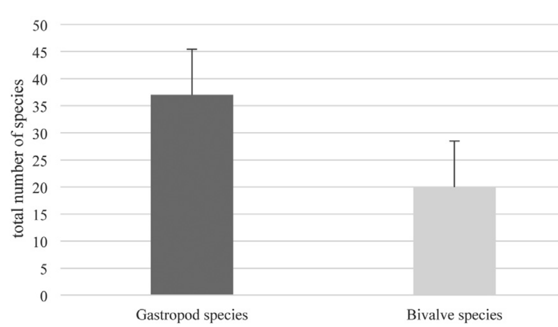

Fig. 4. Cumulative total value of gastropod and bivalve species in studied area

were represented with just a few individuals and had a total percentage of occurrence less than 0.1% (Table 2). These groups were Plathyhelminthes, Aplacopohora, Cumacea, Diptera, Anthozoa, Porifera and Echinoidea. A total of 37 gastropod and 20 bivalve species were recorded, but not all species were present at all sampling locations (Table 3 & 4; Fig. 4). Twelve of 37

Table 2. Qualitative and quantitative composition of invertebrates

Taxonomic group	Banjole	Saccorgiana	Kamenjak	TOT	%	mean	SD
Platyhelminthes	2	1	1	4	0.01	1.3	0.6
Nemertea	49	15	8	72	0.24	24.0	21.9
Nematoda	222	296	759	1277	4.18	425.7	291.0
Sipuncula	15	16	44	75	0.25	25.0	16.5
Gastropoda	358	1683	555	2596	8.51	865.3	714.9
Bivalvia	324	1589	1393	3306	10.83	1102.0	680.9
Polyplacophora	11	36	49	96	0.31	32.0	19.3
Aplacophora	0	1	0	1	0.00	0.3	0.6
Polychaeta	2407	3065	2282	7754	25.41	2584.7	420.6
Decapoda	14	23	34	71	0.23	23.7	10.0
Cumacea	13	4	1	18	0.06	6.0	6.2
Tanaidacea	391	148	172	711	2.33	237.0	133.9
Isopoda	150	313	15	478	1.57	159.3	149.2
Amphipoda	4606	2515	5564	12685	41.57	4228.3	1559.2
Caprellidae	4	5	345	354	1.16	118.0	196.6
Copepoda	0	12	64	76	0.25	25.3	34.0
Crustacea varia	3	0	0	3	0.01	1.0	1.7
Pantopoda	234	199	11	444	1.45	148.0	119.9
Acari	35	198	15	248	0.81	82.7	100.4
Diptera	0	0	14	14	0.05	4.7	8.1
Anthozoa	1	0	0	1	0.00	0.3	0.6
Porifera	1	0	0	1	0.00	0.3	0.6
Echinoidea	1	2	0	3	0.01	1.0	1.0
Ophiuroidea	66	64	100	230	0.75	76.7	20.2

Table 3. Qualitative and quantitative composition of gastropods

Species	Banjole	Saccorgiana	Kamenjak	TOT	%	mean	SD
Alvania discors (T. Allan, 1818)	0	5	1	6	0.23	2.0	2.6
Alvania parvula (Jeffreys, 1884)	2	0	0	2	0.08	0.7	1.2
Alvania scabra (Philippi, 1844)	0	4	0	4	0.15	1.3	2.3
Alvania sp. *juv.*	0	0	2	2	0.08	0.7	1.2
Bittium reticulatum (da Costa, 1778)	151	1209	329	1689	65.06	563.0	566.5
Brachystomia eulimoides (Hanley, 1844)	3	0	0	3	0.12	1.0	1.7
Cerithium vulgatum Bruguière, 1792	0	2	0	2	0.08	0.7	1.2
Cerithiopsis tubercularis (Montagu, 1803)	1	0	0	1	0.04	0.3	0.6
Cingula trifasciata (J. Adams, 1800)	1	3	0	4	0.15	1.3	1.5
Clanculus cruciatus (Linnaeus, 1758)	0	1	1	2	0.08	0.7	0.6
Crisilla innominata (R. B. Watson, 1897)	8	75	15	98	3.78	32.7	36.8
Columbella rustica (Linnaeus, 1758)	1	1	10	12	0.46	4.0	5.2
Eatonina cossurae (Calcara, 1841)	9	136	0	145	5.59	48.3	76.1
Epitonium clathratulum (Kanmacher, 1798)	2	0	0	2	0.08	0.7	1.2
Fissurellidae indet. *juv*	1	2	3	6	0.23	2.0	1.0

Gibbula sp. *juv.*	6	0	0	6	0.23	2.0	3.5
Hexaplex trunculus (Linnaeus, 1758)	0	1	2	3	0.12	1.0	1.0
Jujubinus striatus (Linnaeus, 1758)	2	1	0	3	0.12	1.0	1.0
Megastomia winfriedi Peñas & Rolán, 1999	0	14	0	14	0.54	4.7	8.1
Melarhaphe neritoides (Linnaeus, 1758)	0	2	0	2	0.08	0.7	1.2
Monophorus perversus (Linnaeus, 1758)	3	2	0	5	0.19	1.7	1.5
Muricopsis cristata (Brocchi, 1814)	0	5	4	9	0.35	3.0	2.6
Ocenebra edwardsii (Payraudeau, 1826)	1	0	0	1	0.04	0.3	0.6
Patella cf. caerulea juv. Linnaeus, 1758	2	0	0	2	0.08	0.7	1.2
Pusia ebenus (Lamarck, 1811)	0	1	1	2	0.08	0.7	0.6
Pusia tricolor (Gmelin, 1791)	0	1	0	1	0.04	0.3	0.6
Pusilllina philippi (Aradas & Maggiore, 1844)	3	0	0	3	0.12	1.0	1.7
Scissurella costata d'Orbigny, 1824	84	103	65	252	9.71	84.0	19.0
Setia fusca (Philippi, 1841)	0	6	0	6	0.23	2.0	3.5
Setia maculata (Monterosato, 1869)	68	90	113	271	10.44	90.3	22.5
Siphonaria cf. pectinata (Linnaeus, 1758) juv.	3	1	2	6	0.23	2.0	1.0
Spiralinella incerta (Milaschewich, 1916)	1	8	2	11	0.42	3.7	3.8
Steromphala adriatica (Philippi, 1844) juv.	1	2	1	4	0.15	1.3	0.6
Tritia elongata (Bucquoy, Dautzenberg & Dollfus, 1882)	0	1	0	1	0.04	0.3	0.6
Tritia incrassata (Strøm, 1768)	2	0	0	2	0.08	0.7	1.2
Tritonia manicata Deshayes, 1853	2	5	2	9	0.35	3.0	1.7
Vitreolina antiflexa (Monterosato, 1884)	1	2	2	5	0.19	1.7	0.6

Table 4. Qualitative and quantitative composition of bivalves

Species	Banjole	Saccorgiana	Kamenjak	**TOT**	**%**	**mean**	**SD**
Aequipecten opercularis (Linnaeus, 1758)	1	1	0	2	0.06	0.7	0.6
Arca noae Linnaeus, 1758	7	8	2	17	0.51	5.7	3.2
Arca tetragona Poli, 1795	2	8	0	10	0.30	3.3	4.2
Arca sp.	3	4	0	7	0.21	2.3	2.1
Cardiadae indet.	27	131	52	210	6.35	70.0	54.3
Cardita calyculata (Linnaeus, 1758)	0	1	0	1	0.03	0.3	0.6
Ctena decussata (O. G. Costa, 1829)	1	0	0	1	0.03	0.3	0.6
Flexopecten glaber (Linnaeus, 1758)	0	1	0	1	0.03	0.3	0.6
Hiatella sp.	0	15	55	70	2.12	23.3	28.4
Irus irus (Linnaeus, 1758)	0	1	0	1	0.03	0.3	0.6
Lasaea sp.	0	1	0	1	0.03	0.3	0.6
Lima lima (Linnaeus, 1758)	2	0	0	2	0.06	0.7	1.2
Lima sp.	0	2	0	2	0.06	0.7	1.2
Mimachlamys varia (Linnaeus, 1758)	3	0	0	3	0.09	1.0	1.7
Modiolus barbatus (Linnaeus, 1758)	16	31	10	57	1.72	19.0	10.8
Musculus costulatus (Risso, 1826)	73	161	323	557	16.85	185.7	126.8
Mytilus galloprovincialis Lamarck, 1819	0	0	5	5	0.15	1.7	2.9
Mytilaster sp.	187	1218	942	2347	70.99	782.3	533.7
Pododesmus patelliformis (Linnaeus, 1761)	2	0	0	2	0.06	0.7	1.2
Veneridae indet.	0	6	4	10	0.30	3.3	3.1

(32%) gastropod species were present at all sampling locations, while for bivalves 5 of 20 (25%) species were common for all locations. The structure of the total bivalve fauna was determined by the distinctive dominance of the family Mytilidae (genus *Mytilaster* and *Musculus costulatus*), whereas the total gastropod fauna was determined by *Bittium reticulatum, Scissurella costata* and *Setia maculata* as the most abundant species. The lowest abundance of bivalves and gastropods were recorded for Banjole, while Kamenjak, as the location with higher abundance of invertebrates, had the lowest number of bivalve and gastropod species recorded.

DISCUSSION

High numbers of invertebrates were recorded in many worldwide studies of macrofauna associated with *Corallina officinalis* turfs. In the United Kingdom, it was estimated that up to 329,000 individual invertebrates can be found per one square meter (BUSSELL *et al*., 2007). Research in New Zealand has reached 80,000 ind m^{-2} (BERTHELSEN *et al.,* 2015b), in South America up to 304,000 ind m^{-2} (LIUZZI & GAPPA, 2008), and in Australia up to 250,000 ind m^{-2} (KELAHER *et al*., 2001). In this preliminary study the average number of invertebrates per m^2 was close to 85,000, ranging from approximately 64,000 to more than 116,000 ind m^{-2} (Table 5).

Our study recorded 59 molluscan taxa which is the second highest number of recorded molluscan taxa in similar prior studies, comparing to the highest number of 82 taxa recorded in a study conducted in Australia by KELAHER *et al.* (2001) (Table 5). If this comparison is done only for recorded bivalve taxa, our study shows the highest number of 20 recorded bivalve taxa followed by 16 taxa recorded in Argentina by KELAHER *et al.* (2007a). Comparing the species list of mollusks there are 11 genera that are common for present and previous studies: *Cingula* (DOMMASNES, 1969), *Eatonina* (KELAHER, 2003), *Fisurella, Hiatella, Siphonaria,* (KELAHER et al., 2007a; KELAHER et al., 2007b), *Lasaea* (AKIOKA *et al*.,1999; KELAHER et al., 2007a; KELAHER et al., 2007b), *Modiolus* (AKIOKA *et al*.,1999), *Muricopsis* and *Pusilina* (BERTHELSEN *et al*., 2015b), *Musculus* (KELAHER *et al.*, 2007a) and *Mytilus* (AKIOKA *et al*., 1999; KELAHER *et al*., 2007a). These are all studies conducted in different regions than the Adriatic Sea, in Australia, New Zealand, Chile, Argentina, Japan and Norway, so a difference in species composition is to be expected.

LIUZZI & GAPPA (2008) pointed out in their study a lower diversity of macrofaunal organisms in habitats where the algae density is higher, which is explained by the extremely high abundance of juvenile mussels from the Mytilidae family. Species belonging to Mytilidae family (*Modiolus barbatus, Musculus costulatus, Mytilus galloprovincialis* and *Mytilaster* sp.) showed high abundance even in our study and made 90% of the total number of isolated bivalves. Looking at each sampling location separately, Kamenjak had the highest percentage of species belonging to Mytilidae family and the lowest number of bivalve and gastropod species recorded which corresponds to LIUZZI & GAPPA (2008) conclusions.

Prior analyses of the turf forming algal biomass effect on the invertebrate species richness showed a positive relationship between the number of species of invertebrates and the biomass of algae (MATIAS *et al*., 2015). Another positive effect on the overall diversity of macrofaunal assemblages of this habitat was recorded, which is the fact that turf forming algae retain a larger amount of sediment that serves as a secondary habitat for many macrofaunal species and meiofauna (AIROLDI & CINELLI, 1997).

The effects of changes in the habitat structure on the number and distribution of species within were also previously studied. MATIAS *et al.* (2011) concluded that reducing the diversity of habitats would have adverse effects on the diversity of taxonomic groups, as the habitat structure itself greatly affects the spatial distribution of species. Therefore, given the complexity of the algae's habitat, the composition of macrofaunal species varies. Within our preliminary study samples were taken during the period when algal density was at its peak, as the focus was to determine possible maximum diversity of invertebrates

Table 5. Comparison of the number of taxa in coralline turf on different locations throughout the world.

Location	No. of molluscan taxa	No. of gastropod taxa	No. of bivalve taxa	No. of all taxa	Max. density (ind m^{-2})	References
Croatia	59	37	20	79	116,250	Present study
Norway	26	18	8	70	/	DOMMASNES, 1969
Japan	22	15	6	91	/	AKIOKA *et al*., 1999
Australia (South-Eastern)	82	65	11	147	250,000	KELAHER *et al*., 2001
Ireland (West Coast)	24	17	6	24	/	KELAHER *et al*., 2004
Chile (Central)	27	15	7	27	/	
Australia (East Coast)	58	46	6	58	/	
Chile (Northern)	35	23	8	94	/	KELAHER & CASTILLA, 2005
Argentina (Southern)	38	18	16	38	77,000	KELAHER *et al*. 2007a
Chile (Central)	30	19	8	30	/	KELAHER *et al*., 2007b
Wales	27	/	/	123	329,000	BUSSELL *et al*., 2007
Argentina (North & Central)	21	/	/	118	304,400	LIUZZI & GAPPA, 2008
New Zealand	46	38	2	118	53,643	BERTHELSEN *et al*., 2015a
New Zealand	/	48	/	129	80,000	BERTHELSEN *et al*., 2015b

within this alga, so a seasonal dynamic should be investigated to have a more precise conclusion about the abundance trends and the effect of algal structure on the macrofaunal composition.

CONCLUSIONS

These preliminary results showed that the diversity of invertebrates associated with *Corallina officinalis* in northern Adriatic Sea is high and comparable to the numbers recorded in other studies that were conducted throughout the world. *Corallina* as a habitat has great potential for investigating different anthropogenic impacts as many indicator invertebrate species are commonly found within its settlements. Along with the seasonal dynamic of macrofaunal composition, future studies will examine how the anthropogenic influence is affecting macrobenthic communities associated with *C. officinalis,* an important coastal habitat with abundant and diverse macrofaunal assemblages.

ACKNOWLEDGEMENTS

The authors are grateful to the anonymous reviewers for their suggestions and comments, which allowed significant improvement of the manuscript.

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Received:10 March 2019
Accepted:30 October 2019

Preliminarno istraživanje raznolikosti bezkralježnjaka unutar naselja alge *Corallina officinalis* na području južne Istre

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SAŽETAK

Crvena inkrustirajuća alga *Corallina officinalis* pruža zaklon mnogim beskralješnjacima u području mediolitorala od djelovanja valova, predacije i isušivanja. Karakteristična struktura i složenost staništa imaju veliki utjecaj na bioraznolikost ove zajednice. Cilj ovog rada bio je istražiti raznolikost faune beskralješnjaka unutar naselja crvene alge *C. officinalis*. Tri lokacije uzorkovanja odabrane su na području gdje je pokrivenost algom bila veća od 90%. Na svakoj lokaciji uzorkovanje je provedeno kvantitativno u 3 replikata, struganjem unutar kvadrata veličine 20 x 20 cm. Ukupno je izolirano 30.518 jedinki, a dominantne skupine bile su amfipodni rakovi, mnogočetinaši, školjkaši i puževi.

Ovi taksoni su činili ukupno 86% svih beskralješnjaka pronađenih unutar staništa alge. Najbrojnija skupina su amfipodni rakovi koji su činili 42% ukupnih izoliranih beskralježnjaka. Naše je istraživanje pokazalo da je naselje alge *C. officinalis* vrlo važno stanište s visokom brojnošću i raznolikošću beskralješnjaka.

Ključne riječi: beskralješnjaci, *Corallina officinalis*, kartiranje, bioraznolikost, obala Istre