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**SREDOZEMSKA KAMENA KORALA (*CLADOCORA CAESPITOSA*)  
KOT BIOGRADNIK ŽIVLJENJSKEGA PROSTORA V TRŽAŠKEM  
ZALIVU**

DOKTORSKA DISERTACIJA

**MEDITERRANEAN STONY CORAL (*CLADOCORA CAESPITOSA*) AS  
A HABITAT BUILDER IN THE GULF OF TRIESTE**

DOCTORAL DISSERTATION

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This doctoral dissertation work is a completion of Interdisciplinary Doctoral Programme in Biosciences (Biotechnical Faculty, University of Ljubljana). The work was conducted at the Marine Biology Station in Piran, which is part of the National Institute of Biology (Ljubljana) and was financed by the National Agency for Research of the Republic of Slovenia. On the basis of the Statute of University of Ljubljana, and by decision of Senate of Biotechnical Faculty of the University of Ljubljana, dated from 5<sup>th</sup> July 2014, the continuation to doctoral postgraduate studies of interdisciplinary Doctoral Programme in Bioscience, field: Biology, was approved and prof. dr. Lovrenc Lipej as the supervisor was confirmed.

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### KEY WORDS DOCUMENTATION (KWD)

ND	Dd
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LA	En
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AB	The Mediterranean stony coral <i>Cladocora caespitosa</i> (Linnaeus, 1767) is a shallow-water coral, capable to host a diversified faunal assemblage still poorly known. Species-area relationship (SAR) describes the pattern in which the species richness increases with area and is considered a good tool to estimate biodiversity. The aim of this work was to apply SAR in areas where <i>C. caespitosa</i> was abundant. Sampling surveys were performed from 2012 to 2014 with SCUBA diving at 5 sampling sites between 4 m and 9 m of depth. Investigations were performed with a combination of traditional methods (colony collection, sorting and determination) and non-destructive SCUBA diving methods (quadrat analysis and linear transect) at 3 levels: “microscale”, biodiversity within <i>C. caespitosa</i> colonies (scale of cm <sup>2</sup> ), “mesoscale”, biodiversity among colonies (scale of m <sup>2</sup> ) and “macroscale”, fish assemblages associated with the area dominated by <i>C. caespitosa</i> (scale of ten or so m <sup>2</sup> ). About 300 different taxa among invertebrates and fish were found, some of them rare and poorly known. There was an important component of juveniles. The importance of <i>C. caespitosa</i> as a habitat builder and the suitability of SAR to estimate of species richness were proved.

## KLJUČNA DOKUMENTACIJSKA INFORMACIJA (KDI)

ŠD	Dd
DK	UDK 574:592 (043.3) = 111
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AV	PITACCO, Valentina, M.Sc. Morska Biologija
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IN	SREDOZEMSKA KAMENA KORALA <i>CLADOCORA CAESPITOSA</i> KOT BIOGRADNIK ŽIVLJENJSKEGA PROSTORA V TRŽAŠKEM ZALIVU
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OP	XIV, 124 str., 14 pregl., 44 sl., 6 pril., 185 vir.
IJ	En
JI	en/sl
AI	Sredozemska kamena korala <i>Cladocora caespitosa</i> (Linnaeus, 1767) je kolonijska korala, ki živi v plitvih vodah. Zaradi svoje oblike in velikosti lahko gosti zelo raznoliko živalsko skupnost. Odnos med površino in vrstno pestrostjo (Species-Area Relationship - SAR) opisuje večanje vrstne pestrosti z naraščanjem površine in je uporabno orodje za ocenjevanje biotske raznovrstnosti. Namen disertacije je aplikacija modela SAR na območjih, kjer je bila <i>C. caespitosa</i> pogosta. Vzorčevanja smo izvedli med leti 2012 in 2015 z uporabo avtonomne potapljaške opreme na 5 vzorčevalnih postajah med 4 in 9 m globine. Preiskave so bile opravljene s kombinacijo standardnih metod (zbiranje kolonij, sortiranje in določevanje vrst v laboratoriju) in nedestruktivnih potapljaških metod (analiza kvadratov in linearnih transektov) na 3 nivojih: "mikroskala", biodiverziteta v kolonijah (cm <sup>2</sup> ), "mezoskala", biodiverziteta med kolonijami (m <sup>2</sup> ) in "makroskala", ribje vrste v okolju s prevladujočo kameno koralo (10 m <sup>2</sup> ). Popisali smo 290 različnih taksonov nevretenčarjev in rib, med katerimi so bili nekateri redki ali slabo poznani. V kolonijah je bilo veliko mladostnih primerkov. Rezultati potrjujejo pomembno vlogo sredozemske kamene korale kot biogradnika in primernost uporabe SAR za ugotavljanje vrstne pestrosti.

## TABLE OF CONTENTS

<b>KEY WORDS DOCUMENTATION (KWD)</b> .....	<b>III</b>
<b>KLJUČNA DOKUMENTACIJSKA INFORMACIJA (KDI)</b> .....	<b>IV</b>
<b>INDEX OF TABLES</b> .....	<b>VIII</b>
<b>INDEX OF FIGURES</b> .....	<b>IX</b>
<b>INDEX OF ANNEXES</b> .....	<b>XIII</b>
<b>ABBREVIATIONS AND SYMBOLS</b> .....	<b>XIV</b>
<b>PUBLICATIONS</b> .....	<b>XVII</b>
<b>1 INTRODUCTION</b> .....	<b>1</b>
1.1 THE MEDITERRANEAN STONY CORAL .....	3
1.2 THE DISTRIBUTION OF THE MEDITERRANEAN STONY CORAL IN THE STUDY AREA .....	4
1.3. PREVIOUS RESEARCH ON <i>CLADOCORA CAESPITOSA</i> IN SLOVENIA .....	5
1.4 THREATS AND CONSERVATION PERSPECTIVES .....	6
<b>2 AIM OF THE STUDY</b> .....	<b>7</b>
<b>3 MATERIAL AND METHODS</b> .....	<b>8</b>
3.1 STUDY AREA .....	8
3.2 CHARACTERISTICS OF SAMPLING SITES .....	8
3.3 FIELD WORK AND LABORATORY WORK .....	11
<b>3.2.1 Microscale</b> .....	<b>13</b>
<b>3.2.2 Mesoscale</b> .....	<b>15</b>
<b>3.2.3 Macroscale</b> .....	<b>17</b>
3.3 DATA ANALYSIS .....	18
<b>3.3.1 Microscale</b> .....	<b>18</b>
3.3.1.1 Biometric analysis of colonies .....	18
3.3.1.2 Macroinvertebrate assemblages .....	19

3.3.1.3 Species-area relationship.....	21
<b>3.3.2 Mesoscale.....</b>	<b>22</b>
<b>3.3.3 Macroscale .....</b>	<b>24</b>
<b>4 RESULTS .....</b>	<b>26</b>
4.1 MICROSCALE .....	26
<b>4.1.1 Characteristics of <i>C. caespitosa</i> colonies .....</b>	<b>26</b>
<b>4.1.2 SAR.....</b>	<b>29</b>
4.1.2.1 Mollusc assemblages.....	30
4.1.2.2 Polychaete assemblages .....	33
4.1.3.1 Crustacean assemblages .....	44
4.2 MESOSCALE.....	60
<b>4.2.2 SAR.....</b>	<b>65</b>
<b>4.2.3 Megabenthic assemblages.....</b>	<b>66</b>
4.3 MACROSCALE.....	73
<b>4.3.1 Fish assemblages.....</b>	<b>73</b>
4.4 BIODIVERSITY ASSOCIATED WITH BEDS OF <i>C. CAESPITOSA</i> .....	82
<b>5 DISCUSSION.....</b>	<b>85</b>
5.1 DISCUSSION ON THE METHODS.....	85
<b>5.1.1 Sampling effort .....</b>	<b>85</b>
<b>5.1.2 Taxonomical accuracy .....</b>	<b>87</b>
<b>5.2.3 Method limitations .....</b>	<b>88</b>
<b>5.2.4 Micro and macroenvironmental parameters.....</b>	<b>89</b>
5.2 MICROSCALE .....	91
<b>5.2.1 SAR.....</b>	<b>91</b>
<b>5.2.2 Mechanisms underlying SAR.....</b>	<b>93</b>

<b>5.2.3 Associated macrofaunal community</b> .....	<b>95</b>
<b>5.2.4 Mollusc assemblages inhabiting colonies of <i>C. caespitosa</i></b> .....	<b>97</b>
<b>5.2.5 Polychaetes assemblages inhabiting colonies of <i>C. caespitosa</i></b> .....	<b>99</b>
<b>5.2.5 Crustacean assemblages inhabiting colonies of <i>C. caespitosa</i></b> .....	<b>99</b>
5.3. MESOSCALE .....	100
<b>5.3.1 SAR</b> .....	<b>100</b>
<b>5.3.2 Megabenthic assemblages</b> .....	<b>101</b>
5.4 MACROSCALE.....	102
<b>5.4.1. SAR</b> .....	<b>102</b>
<b>5.4.2 Fish assemblages</b> .....	<b>103</b>
5.5 BIODIVERSITY ASSOCIATED WITH <i>C. CAESPITOSA</i> .....	104
<b>5.5.1 Nature conservation implication</b> .....	<b>105</b>
<b>6 CONCLUSION</b> .....	<b>109</b>
<b>7 SUMMARY (POVZETEK)</b> .....	<b>111</b>
<b>8 REFERENCES</b> .....	<b>118</b>
<b>ANNEXES</b> .....	<b>1</b>

## INDEX OF TABLES

Table 1: Sampling sites with coordinates, depth range and date of sampling.....	12
Table 2: Maximum (D1) and minimum (D2) axes, height (H), <i>Is</i> index, weight (W), net ( $V_{net}$ ) total ( $V_{tot}$ ) and interstitial volume ( $V_{int}$ ) and covered surface (A) of studied colonies at the five studied sites.....	26
Table 3: Macroinvertebrate species determined to the species level. ....	38
Table 4: List of megabenthic taxa found with the two methods. ....	64
Table 5: Estimates of species richness for each site and for the whole area using different functions (underwater counting). ....	66
Table 6: Estimates of species richness for each site and for the whole area using different functions (photographic technique).....	66
Table 7: Checklist and frequency of occurrence of recorded invertebrate taxa using underwater visual count at the four studied sites (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Cape Ronek – RR and Strunjanček- STR).....	68
Table 8: Checklist and frequency of occurrence of recorded fish species at the four studied sites (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Cape Ronek – RR and Strunjanček- STR) .....	74
Table 9: Number of coastal fish species recorded at each sampling sites.....	75
Table 10: Estimates of species richness for each site and for the whole area using different functions.....	77
Table 11: Total number of taxa, species and individuals counted in the present work. For colonial organisms a colony was counted as one individual. * = colonial organisms excluded.....	83
Table 12: Comparison between sites using Bray-Curtis distance applied to most frequent (>25 % of frequency) species recorded at leveles of micro, meso and macroscale. ....	83
Table 13: Comparison between sites using Bray-Curtis distance applied to all species recorded at leveles of micro, meso and macroscale. ....	83
Table 14: Comparison of methods applied at different scales. <sup>1</sup> = hours spent underwater; <sup>2</sup> = dimension of sampling units (m <sup>2</sup> ); <sup>3</sup> = total surface analyzed (m <sup>2</sup> ); <sup>4</sup> = % of taxa determined till the level of species. X = few hours, xx = few days, xxx = many days, xxxxx = many months.....	89



## INDEX OF FIGURES

Figure 1: Sampling sites where <i>C. caespitosa</i> is abundant (Debeli Rtič - DR, Pacug - PA, Piranček - PR, Cape Ronck - RR and Strunjanček- STR). .....	9
Figure 2: Dotcharts showing sampling depth, number of colonies of <i>C. caespitosa</i> (CC), average colony length (av_D1), ratio between the length of the biggest and the smallest colony and percentage of living polyp per each quadrat on horizontal axes and sampling sites on vertical axes. Right above is the dotchart with temperature oscillations in different sampling months. Dots = quadrats. ....	10
Figure 3: Research design with the three levels of investigations.....	11
Figure 4: Sampling design for the micro, meso and macroscale.....	12
Figure 5: Sampling (a) of colonies of <i>C. caespitosa</i> (Photo: L. Lipej, 2012) and measurement (b) in laboratory (Photo: V. Pitacco, 2012).....	14
Figure 6: Metal frame used for underwater visual estimation of macrofauna (mesoscale). ....	16
Figure 7: Species counting with pictures analysis using PhotoQuad software. ....	17
Figure 8: Sampling scheme for macroscale .....	18
Figure 9: Boxplots showing how sampling depth, percentage of living polyps, interstitial volume, algal coverage, coverage of sponges and mud accumulation for each colony varied among sites. .	28
Figure 10: Linear regressions between the total number of non colonial invertebrates and the area covered by colonies of <i>C. caespitosa</i> in cm <sup>2</sup> . All axes are log-transformed. Dots = sampled colonies. ....	30
Figure 11: Linear regressions between the area covered by colonies in cm <sup>2</sup> and the total number of taxa of molluscs (a), the number of taxa of bivalves (b) and gastropods (c). All axes are log-transformed. Dots = sampled colonies. ....	32
Figure 12: Linear regressions between the area covered by colonies of <i>C. caespitosa</i> in cm <sup>2</sup> and the total number of taxa of polychaetes (a), total number of taxa of mobile polychaetes (b) and of sessile polychaetes (c). Dots = sampled colonies.....	34
Figure 13: Linear regressions between the area covered by colonies of <i>C. caespitosa</i> in cm <sup>2</sup> and the total richness of crustaceans (a), the richness of decapods (b) and the richness of amphipods (c). Dots = sampled colonies. ....	36
Figure 14: Average percentage of richness (a) and abundances (b) of phyla of non-colonial organisms. ....	37

Figure 15: Variation of abundances (a), richness (b), Margalef (c), Shannon (d), Pielou (e) and Simpson (f) indices according to the total colony volume (cm <sup>3</sup> ). .....	41
Figure 16: Average richness (a) and abundances (b) of the different functional guilds for mollusc (mol), polychaetes (poly), crustaceans (crust), tunicates, echinoderms and sipunculids (other) and for the total assemblages (tot). .....	42
Figure 17: Average richness (a) and abundances (b) of the different feeding guilds for mollusc (moll), polychaetes (poly), crustaceans (crust), tunicates, echinoderms and sipunculids (other) and for the total assemblages (tot). .....	43
Figure 18: Cluster analysis for log-transformed abundances of non colonial invertebrate taxa in each colony at the different sampling sites.....	44
Figure 19: Diversity indices of mollusc assemblages in each <i>C. caespitosa</i> colony. Colonies are ordered by size (total volume). Margalef index of richness (d), Shannon diversity index (H'), Pielou index of equitability (J') and Simpson index of dominance (D <sub>λ</sub> ) calculated for each colony (=dots). .....	45
Figure 20: Taxa richness (a) and taxa abundance (b) of functional groups of molluscs for each colony of <i>C. caespitosa</i> . Colonies are ordered by size (total volume). FL = free living, motile species, EP = epilithic species, living their entire lives attached to a substrate, EN = endolithic species, living in holes bored in hard substrates, SB = soft bottom dwelling species, ND = no data available. ....	47
Figure 21: Taxa richness (a) and abundance (b) of feeding guilds of molluscs for each colony of <i>C. caespitosa</i> . P = motile predators, Pa = parasites, O = omnivores, SF = suspension feeders capturing seston particles with their gills or with mucous strings, MG = micrograzers feeding on algae, cyanobacteria or detritus attached to algal fronds. ....	49
Figure 22: Cluster analysis for log-transformed abundances of molluscs in each colony at the different sampling sites. ....	50
Figure 23: Diversity indices of polychaete assemblages in each <i>C. caespitosa</i> colony. Colonies are ordered by size (total volume). Margalef index of richness (d), Shannon and Wiener diversity index (H'), Pielou index of equitability (J') and Simpson index of dominance (D <sub>λ</sub> ) calculated for each colony.....	51
Figure 24: Richness (a) and abundances (b) of functional groups of polychaetes for each colony of <i>C. caespitosa</i> . Colonies are ordered by size (total volume). FL = free living, motile species, EP = epilithic species, living their entire lives attached to a substrate, EN = endolithic species, living in holes bored in hard substrates, SB = soft bottom dwelling species, DM = discretely motile species; ND = no data available.....	53
Figure 25: Taxa richness (a) and abundance (b) of feeding guilds of polychaetes for each colony of <i>C. caespitosa</i> . C = motile predators, DF= deposit feeders, O = omnivores, SF = suspension feeders	

capturing seston particles with their gills or with mucous strings, MG = micrograzers feeding on algae, cyanobacteria or detritus attached to algal fronds.....	55
Figure 26: Cluster analysis for log-transformed abundances of polychaetes in each colony at the different sampling sites. Each colony is numbered according to size.....	56
Figure 27: Diversity indices of crustacean assemblages in each <i>C. caespitosa</i> colony. Colonies are ordered by size (total volume). Margalef index of richness (d), Shannon and Wiener diversity index ( $H'$ ), Pielou index of equitability ( $J'$ ) and Simpson index of dominance ( $D_r$ ) calculated for each colony.....	58
Figure 28: Taxa richness (a) and abundance (b) of feeding guilds of crustaceans for each colony of <i>C. caespitosa</i> . C= motile predators, DF= deposit feeders, O = omnivores, SF = suspension feeders capturing seston particles with their gills or with mucous strings, Pa = ectoparasites, ND = no data found. ....	59
Figure 29: Cluster analysis for log-transformed abundances of crustaceans in each colony at the different sampling sites. ....	60
Figure 30: Cumulative curves according to the original sequence of recording (a,b) and by calculating the mean of species-area curve and its standard deviation from random order of quadrats, sampled without replacement (c,d), for the entire dataset (a,c) and for each site separately (b,d). Vertical lines = SD. Data were recorded in situ with underwater visual counting. ....	62
Figure 31: Cumulative species-area curves for megabenthic invertebrates according to the original sequence of recording (a,b) and by calculating the mean of species-area curve and its standard deviation from random order of quadrats, sampled without replacement (c,d), for the entire dataset (a,c) at sites Pacug, Piranček, Ronek and Strunjanček (b,d). Data were obtained from photographs. ....	63
Figure 32: Average percentage of richness (a) and abundance (b) of different phyla detected with underwater counting. For colonial organisms colonies were counted. ....	67
Figure 33: Variation of diversity indices among different sampling sites. ....	70
Figure 34: Rarefaction curves for megabenthic species found at each site with (a) and without (b) the most abundant species ( <i>R. Dubia</i> and <i>P. triqueter</i> ), with average species richness for random subsamples of individuals. Vertical lines = SD.....	71
Figure 35: Cluster for log-trasformed abundance data of megabenthic invetebrates obtained with underwater visual method. ....	72
Figure 36: Species accumulation curves for fish species found at the first and second passages and combined data with transects in order of occurrence (a) and in random order (b). Each transect is weighted according to its length. Vertical lines = SD.....	75

Figure 37: Species accumulation curves for fish species found at all sites (a,c) and at each site separately (b,d), with transects in order of occurrence (a,b) and in random order (c,d). Each transect is weighed according to its length. Vertical lines= SD. ....	76
Figure 38: Rarefaction curves for fish species found at each site with (a) and without (b) gregarious species ( <i>S. salpa</i> and <i>C. chromis</i> ), with average species richness for random subsamples of individuals. Vertical lines = SD. ....	78
Figure 39: Dotcharts showing sampling depth (upper graph) and number of colonies of <i>C. caespitosa</i> (lower graph) per each transect on horizontal axes and sampling sites on vertical axes. Dots = transects. ....	79
Figure 40: Boxplot showing depth, colony density, fish abundances and richness in relation with different sampling sites. ....	80
Figure 41: Cluster analysis for presence-absence of fish in each transect at the different sampling sites (without species with less than 25 % of frequency). ....	81
Figure 42: Boxplots showing values of different diversity indices among sampling sites. Margalef index of richness (d), Shannon diversity index ( $H'$ ), Pielou index of equitability ( $J'$ ) and Simpson index of dominance ( $D_\lambda$ ) calculated considering normalised species richness and abundances ( $/100m^2$ ) for each transect. ....	82
Figure 43: Average of species richness, Shannon and Simpson indices from different sampling sites, calculated at different scales. ....	84
Figure 44: Cumulative curve of all taxa (a), taxa of mollusc, polychetes, crustaceans and others (b) for cumulative sampling volume ( $cm^3$ ). ....	86

## INDEX OF ANNEXES

Annex A: Differences between sampled sites for <i>C. caespitosa</i> tested with Kruskal-Wallis chi-squared for biometrical characteristics.....	1
Annex B: Average dimensions of corallites of <i>C. caespitosa</i> for each sampling site. D1 = calyces maximum axis, D2 = calyces minimum axis, H = corallite length, A = calyces surface ( $D1 * D2 * \pi$ ). .....	4
Annex C: Correlation results.....	5
Annex D: AIC values.....	7
Annex E: Trellis graph showing relationship between richness of non-colonial invertebrates and area covered by colonies in each of the sampled site.....	8
Annex F: Trellis graph showing relationship between number of taxa of molluscs and area (cm <sup>2</sup> ) covered by colonies in each of the sampled site. ....	9
Annex G: Trellis graph showing relationship between number of taxa of polychaetes (tot_poly) and area covered (cm <sup>2</sup> ) by colonies (A) in each of the sampled site. ....	10
Annex H: Trellis graph showing relationship between number of taxa of crustaceans (tot_crust) and area (cm <sup>2</sup> ) covered by colonies (A) in each of the sampled site. ....	11
Annex I: Trellis graph showing relationship between richness of non-colonial invertebrates and area (cm <sup>2</sup> ) covered by colonies for each of the sampling month. A = Area covered by colonies (in cm <sup>2</sup> ), S_tot = non-colonial invertebrate richness (number of taxa). ....	12
Annex L: Trellis graph showing relationship between richness of non-colonial invertebrates and area (cm <sup>2</sup> ) covered by colonies for each of the sampling depth (5 to 9 m). ....	13

## ABBREVIATIONS AND SYMBOLS

A	Area
C	Carnivores
Chao	Chao bias-corrected function
boot	bootstrap
Crust	Crustaceans
d	Margalef index of richness
$D\lambda$	Simpson index of dominance
Deca	Decapods
DF	Deposit feeders
DM	discretely mobile
EN	Endolithic
EP	Epilithic
FL	Free living
G	Grazers
H'	Shannon diversity index
KW	Kruskal Wallis chi-squared test
J'	Peilou index of equitability
Jack1	first order jackknife function
Jack2	second order jakknife function
LP	Percentage of living polyps
MG	Micrograzers
Mol	Molluscs
n	number of samples
N	Taxa abundance
ND	No data available
O	Omnivores
se	Standard error

S	Taxa richness
SAR	Species Area Relationship
SB	Soft bottom species
SF	Suspension feeders
P	Motile predator
Pa	(ecto)parasite
Poly	Polychaetes
WMW	Wilcoxon test

## PUBLICATIONS

PITACCO, Valentina, ORLANDO-BONACA, Martina, MAVRIČ, Borut (author, photographer), LIPEJ, Lovrenc. Macrofauna associated with a bank of *Cladocora caespitosa* (Anthozoa, Scleractinia) in the Gulf of Trieste (North Adriatic). *Annales, Series historia naturalis*, ISSN 1408-533X, 2014, vol. 24, n. 1, pp. 1-14, illustr. <http://zdjp.si/it/docs/Annales/naturalis/n24-1/pitacco-bonaca-mavric-lipej.pdf>. [COBISS.SI-ID [3179855](#)]

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## 1 INTRODUCTION

Species-area relationships (SARs) are among the best known and most studied patterns in ecology (Rosenzweig, 1995). They describe the pattern in which the species richness (S) increases with area (A). Nowadays SAR is recognized as one of the few true laws of ecology (Gotelli, 2001).

Models based on SAR have been proposed in conservation biology to project the expected loss of species richness from a region undergoing specified levels of habitat degradation (e.g. Connor and McCoy, 2001; Ulrich, 2005) and to estimate local species richness for hotspot identification (e.g. Veech, 2000).

The species area curve is central for the theory of island biogeography (MacArthur and Wilson, 1963). The pattern holds not only for geographic islands, that is a pieces of land surrounded by water (MacArthur and Wilson, 1963; Holt et al., 1999), but also for 'island' system where similar habitat types are separated in space by relatively unfavourable habitats islands (MacArthur, 1972). SAR applications have been widely used for terrestrial ecosystems, applied to plants (Arrhenius, 1921; Gleason, 1922) and birds (MacArthur and Wilson, 1963; Preston, 1960), conversely there are only few similar estimates for the marine realm (Neigel, 2003).

The Arrhenius equation (Arrhenius, 1921) is generally considered the first quantitative formulation of SAR and is still one of the most frequently applied (Dengler, 2009). This relationship is typically nonlinear and has the general form of a power function:  $S = c A^z$ , where S is species richness, A is area, c and z are constants that are fitted to data. To date, many other functions have being used to describe the SARs, some of them were used to better fit real data, others were based on merely theoretical assumptions (Dengler, 2009). Attempts have been made to review these functions, in order to solve terminological confusion and evaluate the different methods (Tjørve, 2009; Dengler, 2009). But despite the large number of articles published, there is still no general agreement either on the shape of the species-area curve or on its biological interpretation (Connor and McCoy, 2001; Scheiner, 2009; Tjørve, 2009; Williams et al., 2009). To a large degree, this is because SAR models and their explanations depend on different factors such as scale

(Palmer and White, 1994; Crawley and Harral, 2001; Turner and Tjørve, 2005), methodology (Diekmann, 2007; Dengler, 2009) and time (Rosenzweig, 1995; Carey et al., 2006).

For the largest scale, the biological province, diversity depends on the rate of speciation, for islands depends on the rate between colonization and extinction and for provincial patches depends on the habitat heterogeneity and rate of replacement of individuals in sink populations (Rosenzweig, 1999).

From the methodological point of view sampled area itself is a complex variable and could be decomposed in several components such as grain (minimum scale sampled), extent (maximum scale sampled), number of sample units, shape of the unit and geometry of placement (Palmer and White, 1994).

Pattern in species richness have been studied at two main levels (Romdal and Grytnes, 2007): regional level, deriving data mainly from literature and scientific collections, and local level, focusing on field inventories in a specific area. The first approach has been largely employed and proved to be effective at the scale of kilometers. Instances are a study of SAR for benthic invertebrates in coastal lagoons (Guilhaumon et al., 2012) and SAR for fish related with MPA (Levin et al., 2009). Less investigations were performed at local scale and very few works tried to apply the SAR to habitat at the scale of  $m^2$  or  $cm^2$ , such as coral heads (Abele and Patton, 1976), boulders (McGuinness, 1984), rock walls (Smith and Witman, 1999), mussel beds (Witman, 1985) and artificial settling plates (Osman, 1978; Anderson, 1998).

For the purpose of the present work the following distinction of types of SAR will be considered:

- (1) total species richness vs. total area, among habitats differing in area (*e.g.*, islands in an archipelago; a “type-2” relationship *sensu* Rosenzweig, 1995)
- (2) local species richness in a sample of defined size, among habitats differing in area (a “type-3” relationship *sensu* Rosenzweig, 1995).

The choice was made due to practical reasons. The different sampling scheme implies different nature of SAR and different underlying factors, requiring also different statistical approaches. In the first case, areas of different size are analysed, therefore plotting species richness against area, the curve obtained is made of independent points and regression can be performed. In the second case, random samples of defined size are collected. The curve obtained is cumulative and each value of richness is dependent on the previous ones. Therefore other methods of extrapolation have to be used, based on frequencies, number of rare species or permutations.

### 1.1 THE MEDITERRANEAN STONY CORAL

The Mediterranean stony coral *Cladocora caespitosa* (Linnaeus, 1767) is the only native colonial and obligate zooxanthellate coral in the Mediterranean Sea (Zibrowius, 1980). The symbiosis with the zooxanthellae is centered on nutritional exchange; and enables the corals to deposit their carbonate skeletons. Zooxanthellae living in symbiosis with *C. caespitosa* are dinoflagellates of *Symbiodinium* clade A Temperate (Visram et al., 2006).

*C. caespitosa* is endemic to the Mediterranean, with a few records in the neighbouring eastern Atlantic, along the southern Portuguese and Moroccan coasts (Zibrowius, 1980). It is a phaceloid coral, characterized by distinct corallites which develop vertically, each having its own wall independent of the others. Colonies are hemispherical bush-like with shape and sphericity changing with hydrodinamism of the area (Kružić and Benković, 2008).

It has adapted to live in different environments, from a shallow photophilic algal community to deeper circalittoral assemblages (Zibrowius, 1980; Schiller, 1993). It is one of the few examples of long-lived species with an important structural role in Mediterranean infralittoral belt (Zibrowius, 1980). Usually it is considered as a part of the Biocoenosis of Photophilic Algae (AP) (Balduzzi et al., 1994; Aguilar et al., 2006; Voultsiadou et al., 2011).

Colonies may live solitarily, in a form of 'beds', that is numerous colonies living more or less close to each other, or form 'banks' made of colonies connected together in a large

formation more than 1 m high and covering several square metres (Zibrowius, 1980; Schiller, 1993; Morri et al., 1994).

This coral, physiologically and morphologically similar to the typical tropical reef-building relatives (Peirano et al. 1994; Kružić and Požar-Domac, 2003), is able to host a diversified faunal assemblage. Coral associates are defined as sessile invertebrates that live on or within the coral skeleton (Risk et al., 2001). Even if this is well-known for tropical species, cold-water corals also provide essential habitat for thousands other species, in particular animals like sponges, polychaetes, crustaceans, molluscs, bryozoans and fish (Freiwald et al., 2004).

Only few studies are available on the macrofauna associated with the mediterranean stony coral. Species associated with *C. caespitosa* were reported from different sites in Adriatic (Sciscioli and Nuzzaci, 1970; Zavodnik, 1976; Schiller, 1993), Ionian Sea (Lumare, 1965) and Aegean Sea (Arvanitidis and Koukouras, 1994; Koukouras et al, 1998; Antoniadou and Chintiroglou, 2010). Most of these works focused on a single taxonomic group, namely polychaetes (Sciscioli and Nuzzaci, 1970; Arvanitidis and Koukouras, 1994) and echinoderms (Zavodnik, 1976). One unique available paper considered all taxa of associated invertebrates (Koukouras et al., 1998).

Coral associated species represent an important food source for other invertebrates and benthic fishes, therefore areas with massive presence of *C. caespitosa* could attract them. Fish assemblages associated with other habitat types, such as macroalgae and seagrasses have been intensively investigated (e.g. Pais et al., 2007, Cheminée et al., 2015), in particularly in the Gulf of Trieste (Lipej et al., 2003; 2005; Orlando Bonaca and Lipej, 2005, 2007; Orlando Bonaca et al., 2008; Lipej et al., 2009). However, the role of Mediterranean stony coral for benthic fishes has not being subject of investigation yet.

## 1.2 THE DISTRIBUTION OF THE MEDITERRANEAN STONY CORAL IN THE STUDY AREA

Although common in the whole Mediterranean *C. caespitosa* is abundant only locally (Peirano et al., 1994). In the northern Adriatic *C. caespitosa* is more frequent and abundant along the eastern coast, e.g. Prvić, Pag and Mljet Islands (Kružić and Benković, 2008).

On the north-western part of the Gulf of Trieste only single living colonies were recorded at different sites, e.g. inside the Marine Reserve of Miramare (Ciriaco et al., 1998; Montagna et al., 2007), on rocky outcrops locally called *tegnùe*, *trezze*, *presùre* or *grèbeni* (Casellato and Stefanon, 2008). A subfossil bank made mostly of debris from corallites of *C. caespitosa*, without living colonies, was studied at a site (Dosso di Santa Croce) closed to the Laboratorio di Biologia Marina - now OGS, Trieste (Orel et al., 2006).

On the south-eastern part of the Gulf, along the Slovenian coast, *C. caespitosa* is very abundant and forms 'beds' from 3 m to 10 m of depth at 7 different sites (Schiller, 1993; Zunino, 2013). Only at Cape Ronek it extends also in deeper water forming a singular biogenic formation between 12.4 and 21 m depth (Lipej et al., 2006). This biogenic formation is made of a detritic layer of dead corallites and, to a lesser extent, of coralline algae, on the top of which living colonies of *C. caespitosa* grow. To date in the entire Mediterranean Sea only four comparable formations (in Sicily, Sardinia, Corsica and the Aegean Sea) have been reported (Peirano et al., 1994; Koukouras et al., 1998).

### 1.3. PREVIOUS RESEARCH ON *CLADOCORA CAESPITOSA* IN SLOVENIA

First investigations concerning the Mediterranean stony coral in the Gulf of Trieste were performed in 1984 and 1989 (Schiller, 1993). Colonies of *C. caespitosa* at two sites in the bay of Piran were investigated at level of biometry, biology, ecology and distribution, and the associated macrofauna was sorted to higher taxonomic levels, as well (Schiller, 1993). Other investigations followed only recently, focusing mainly on the effects of global changes (Lipej et al., 2013; Kružić et al., 2014). *C. caespitosa* distribution, growth rate, occurrence of tissue necrosis, coral bleaching and mass mortality events have been monitored, in particular in the Strunjan Nature Reserve (Lipej et al., 2013) and at Cape Madona marine natural monument (Kružić et al., 2014). An investigation on distribution and biometric characteristics of *C. caespitosa* was performed in 2012 at 7 sites along the Slovenian coast (Zunino, 2013) and data were compared with historical data (Schiller, 1993).

This singular biogenic formation at Cape Ronek was object of a preliminary study in November 2010 (Pitacco et al., 2014a), aiming at describing the macrofauna living inside

colonies of *C. caespitosa* and invertebrates found on the detritic bottom mainly made of dead corallites. Our results suggested that macrofaunal communities associated with *C. caespitosa* at the study area deserved special investigation, since it was rich and diversified.

#### 1.4 THREATS AND CONSERVATION PERSPECTIVES

*C. caespitosa* is undergoing a rapid decrease in both size and spatial distribution (Morri et al., 2000; Rodolfo-Metalpa et al., 2005). Among major threats there are changes in global climate. Global warming and the related acidification of the ocean pose a serious threat for this species and the associated macrofauna (Rodolfo-Metalpa et al., 2005, 2011, Kersting et al., 2013). Laboratory experiments demonstrated that prolonged higher than average temperatures may induce necrosis in polyps, which have been observed also *in situ* (Rodolfo-Metalpa et al., 2005). Moreover, even if this coral was able to calcify also in undersaturated conditions, having large parts of its skeleton exposed, it showed evident marks of dissolution at low pH, and its calcification ability was disrupted when acidification was combined with elevated temperatures (Rodolfo-Metalpa et al., 2011). Mass-mortality events of *C. caespitosa* such as those recorded in the NW Mediterranean Sea (Perez et al., 2000; Rodolfo-Metalpa et al., 2005; Garrabou et al., 2009), were in fact related with thermal anomalies (Rodolfo-Metalpa et al., 2005; Kersting et al., 2013).

Coral bleaching in *C. caespitosa*, a disruption of symbioses between coral hosts and photosynthetic microalgal endosymbionts, by the loss of algal pigments or elimination of algal cells (Brown et al., 1997), have been documented, as well (Schiller, 1993; Lipej et al., 2013; Kružić et al., 2014). The loss of the pigmented zooxanthellae causes the coral to lose colour and eventually die, since a major portion of the coral's nutrition comes from the photosynthetic products of the algae. Bleaching is probably related to increasing seawater temperatures, as well (Lipej et al., 2013; Kružić et al., 2014).

Anthropogenic activities such as industrial and urban sewage discharges and coastal works are also threatening *C. caespitosa* (Kružić and Požar-Domac, 2007).

## 2 AIM OF THE STUDY

Biogenic constructions are very important in supporting biodiversity (Cocito, 2004). Being a long-lived and large-size organism, *C. caespitosa* fulfils the requisites for the formation of well-structured bioconstructions (Jackson, 1977; Wood, 1999), thus we expected associated communities to be very rich in terms of species richness and areas with high density of coral colonies to be important for biodiversity. We also expect a correlation between the size of the sampling area and species richness.

The main goals of the work were:

1. to test the hypothesis that the number of associated macroinvertebrate species increases in relation with the colony size, following Arrhenius power-equation model
2. to test if SAR models can be effectively applied at the levels of micro, meso and macroscale for the estimation of species richness
3. to test the importance of the Mediterranean stony coral as habitat builder for biodiversity, checking whether an increase in spatial heterogeneity is related to an increase in species richness.

### 3 MATERIAL AND METHODS

#### 3.1 STUDY AREA

The Gulf of Trieste is a shallow semi-enclosed embayment located in the northernmost part of the Adriatic Sea (Mediterranean Sea). It extends from Cape Savudrija (Croatia) to Grado (Italy) and includes the entire Slovenian coast. The maximum depth (approximately 33 m) is found in waters off Piran. The area is characterized by the lowest winter temperatures in the Mediterranean Sea which can fall below 10° C (Boicourt et al., 1999). Salinity is about 37 on average, but it is influenced near the coast by fresh water inputs (Mozetič et al., 1998). During the summer, a typical thermal stratification of the water column develops, due to surface heating and fresh water inflow (Boicourt et al., 1999). In winter months, the water column is characterized by considerable vertical homogeneity due to autumnal cooling processes and wind mixing (Mozetič et al., 1998). The embayed situation of the Gulf of Trieste, together with dominant winds blowing in an offshore direction (from the North-East) and very shallow waters create a quite sheltered condition (Boicourt et al., 1999).

The coastal morphology of the study area varies from steep rocky cliffs to gradual sloping beaches consisting of gravel and pebbles (Ogorelec et al., 1991). The rocky substratum of the Slovenian coast consists mainly of Eocene flysch layers, with alternating solid sandstone and soft marl (Ogorelec et al., 1997).

In past decades the coastal sea suffered from many anthropogenic impacts such as intensive fishing, sewage outfalls and mariculture (Francé and Mozetič, 2006; Mozetič et al., 1998; Grego et al., 2013). Nowadays almost 80 % of the natural shoreline of the Slovenian coast has been destroyed (Turk, 1999).

#### 3.2 CHARACTERISTICS OF SAMPLING SITES

Five sampling sites, which were previously assessed as rich with *C. caespitosa* colonies, were selected along the Slovenian coast (Figure 1): Debeli Rtič - DR, Pacug - PA, Piranček - PI, Cape Ronek – RR and Strunjanček- STR. Three of them (DR, STR and RR) are



located in marine protected areas, and sampling was performed with the permission of the Marine Reserve Authorities.

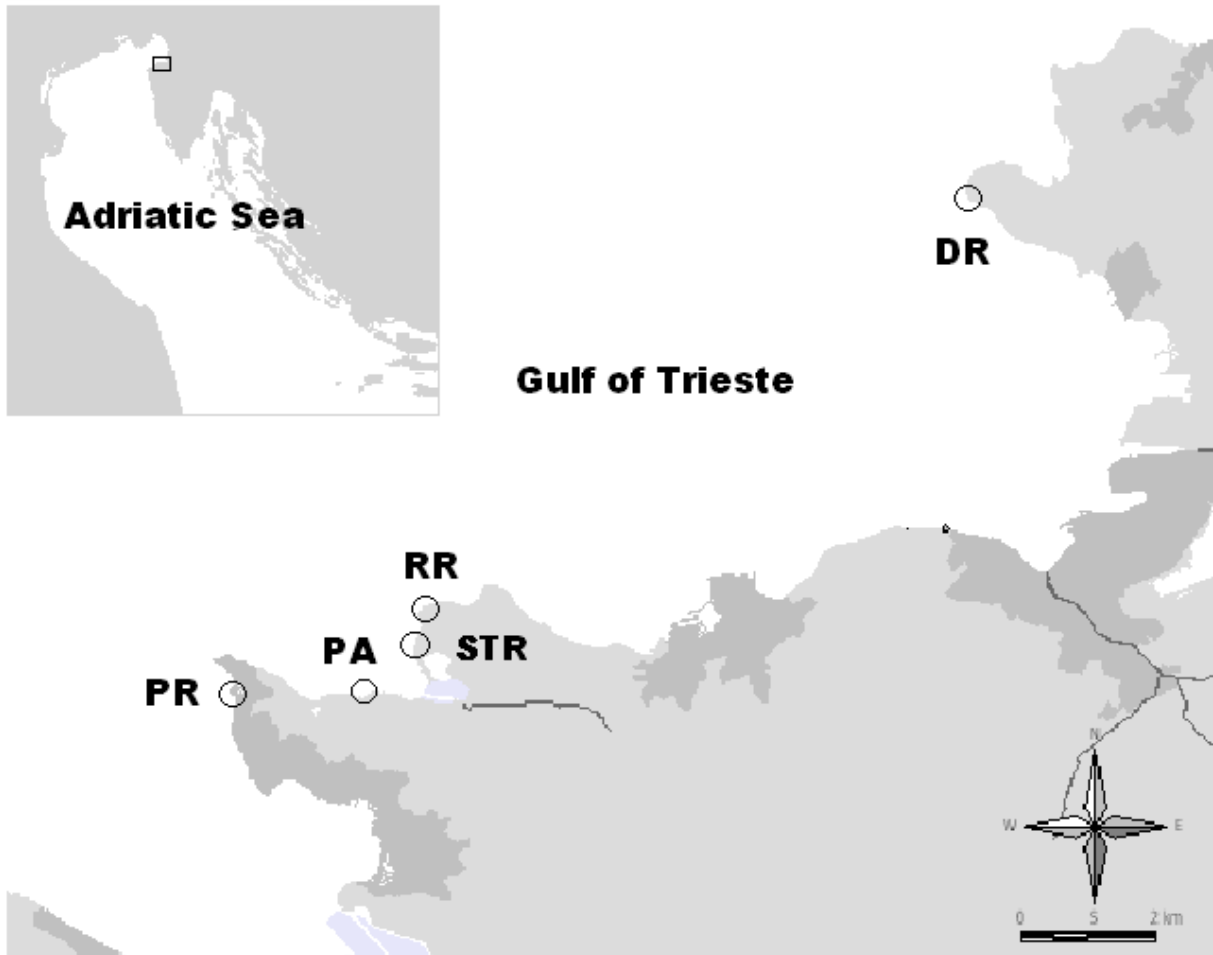


Figure 1: Sampling sites (Debeli Rtič - DR, Pacug - PA, Piranček - PR, Cape Ronek – RR and Strunjanček-STR).

Slika 1: Vzorčevalne postaje (Debeli Rtič - DR, Pacug - PA, Piranček - PR, Rt Ronek – RR in Strunjanček-STR).

Sampling surveys were performed from 2012 to 2015 with SCUBA diving between 4 m and 9 m of depth (Table 1).

Sampling depth varied according to the abundance of colonies of *C. caespitosa*. The deepest sampling sites were RR and PR, while STR was the shallowest (Figure 6). The number of colonies (KW chi-squared = 11.32,  $df = 4$ ,  $p$ -value = 0.02319) and average colony size (KW chi-squared = 11.846,  $df = 4$ ,  $p$ -value = 0.01853) changed slightly among

few sites. The biggest colonies were found at site PR, where the density per square meter was also high. At site PA and DR colony density was high but colonies were smaller. At site STR colony density was low and colonies small (Figure 2). No differences in colony density or colony size were found between samples collected in 2013 and 2014 ( $p$ -value > 0.05). Temperature changed according to seasonality, with higher values in July and August (Figure 2). There was a moderate positive correlation between depth and number of colonies per square meter ( $r_s = 0.530$ ;  $p$ -value = 0.0001).

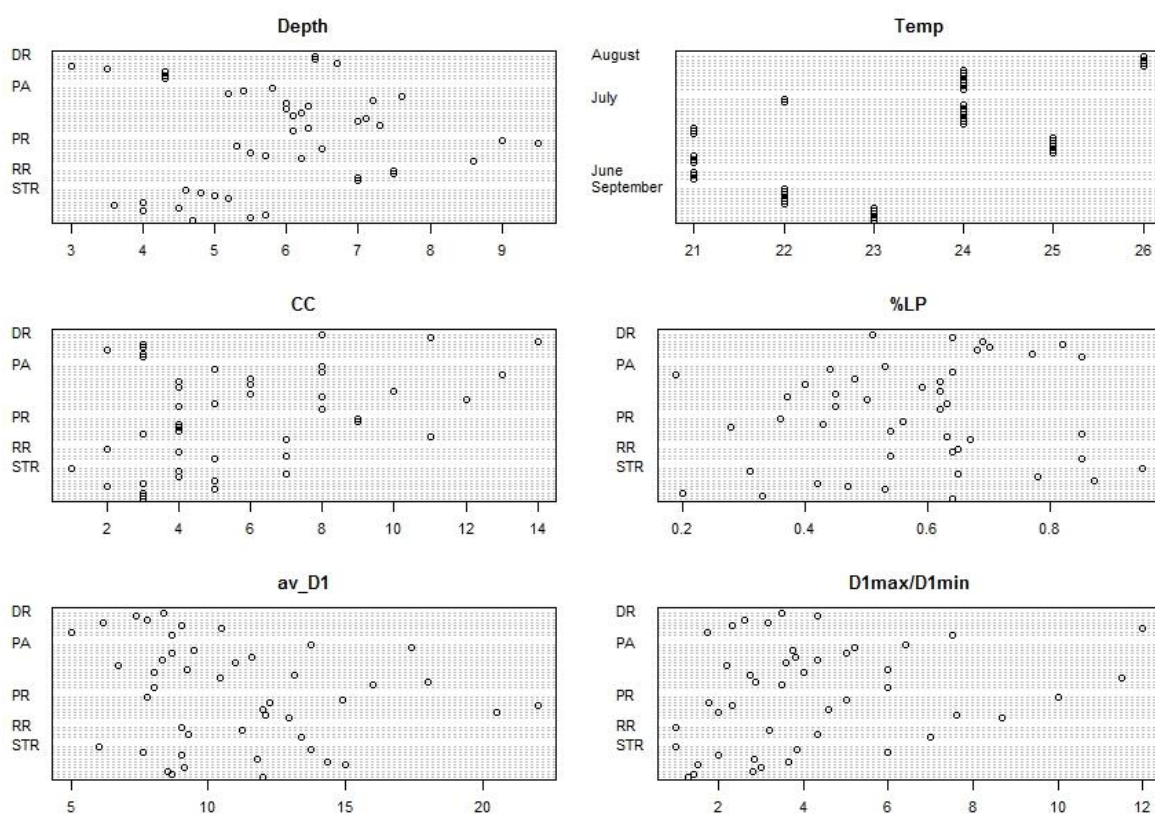


Figure 2: Dot charts showing sampling depth, number of colonies of *C. caespitosa* (CC), average colony length (av\_D1), ratio between the length of the biggest and the smallest colony and percentage of living polyp per each quadrat on horizontal axes and sampling sites on vertical axes. Right above is the dotchart with temperature oscillations in different sampling months. Dots = quadrats.

Slika 2: Dotcharts, ki ponazarjajo globino vzorčevanja, število kolonij *C. caespitosa* (CC), povprečne dolžine kolonij (av\_D1), razmerje med dolžino najmanjših in največjih kolonij in delež živih polipov na vsakem kvadratu na vodoravnih oseh in vzorčevalne postaje na navpičnih oseh. Desno zgoraj je dotchart, ki prikazuje gibanje temperature po posameznih mesecih. Pike = vzorčevalni kvadrati.

### 3.3 FIELD WORK AND LABORATORY WORK

In order to assess faunistic diversity associated with *C. caespitosa* a combination of standard techniques and non-destructive methods was used.

Research was carried out at three different levels (Figure 3):

- “microscale”: biodiversity inside *C. caespitosa* colonies, scale of square centimeters;
- “mesoscale”: biodiversity within colonies, scale of square meters;
- “macroscale”: biodiversity within the area dominated by *C. caespitosa* colonies, scale of ten or so square meters.

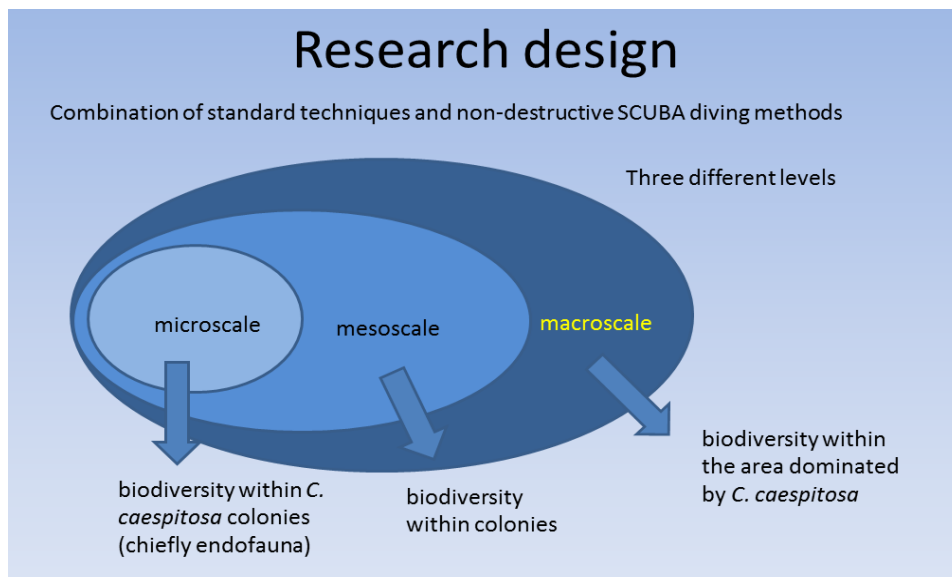


Figure 3: Research design with the three levels of investigations.

Slika 3: Vzorčevalni načrt s tremi nivoji raziskav.

In order to test the Species Area Relationship three different sampling designs were used (Figure 4):

- Sampling physically separate areas, consisting in single colonies of *C. caespitosa*, for the microscale
- Sampling quadrats, each one subdivided in 4 adjacent subquadrats for the mesoscale

- Sampling linear transects subdivided in two for the macroscale

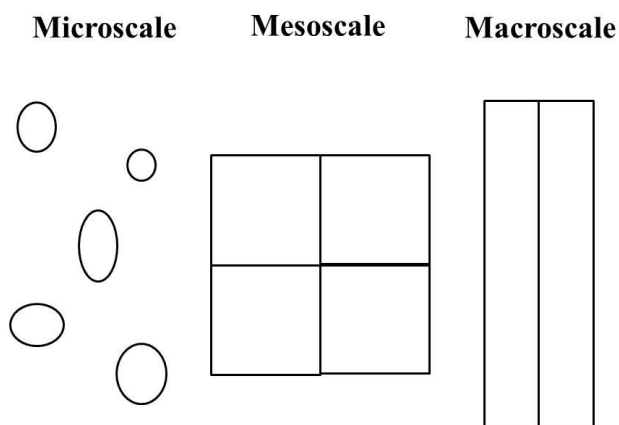


Figure 4: Sampling design for the micro, meso and macroscale.

Slika 4: Vzorčevalni načrt za mikro, mezo in makroskalo.

Table 1: Sampling sites with coordinates, depth range and date of sampling.

Preglednica 1: Vzorčevalne postaje s koordinatami, globinami in datumi vzorčevanja.

Code	Site	Latitude (N)	Longitude (E)	Sampling dates	Depth range
<b>RR</b>	<b>Rtič Ronek</b>	45°32'25"	13°36'56"	9.7.2012 19.7.2013 26.9.2014	6-10 m
<b>PR</b>	<b>Piranček</b>	45°31'38"	13°34'30"	24.7.2012 19.6.2013 31.7.2013 24.7.2014	5-10 m
<b>STR</b>	<b>Strunjanček</b>	45°32'5"	13°36'10"	22.8.2012 1.8.2013 7.8.2014	3-6 m
<b>PA</b>	<b>Pacug</b>	45°31'34"	13°35'24"	10.8.2012 18.7.2013 25.7.2014 17.9.2014	5-8 m

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<b>DR</b>	<b>Debeli rtič</b>	45°35'28"	13°42'88"	19.10.2012 3.7.2013 9.9.2014	5-7m
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### 3.2.1 Microscale

The microscale level consists in the study of benthic organisms living inside colonies. At this level animals are strictly associated with corals, so a destructive traditional method was necessary, being the unique proper technique for determination of cryptic animals such as polychaetes. In order to reduce the impact of sampling procedure on *C. caespitosa* population only two large-sized colonies were collected. In this way we can check if our predictions for small colonies could be applied also for the bigger ones.

In summer 2012 5 colonies of *C. caespitosa* of different dimensions were collected at each site for the characterization of invertebrate assemblages. At size STR one additional recruit (5 cm of length) was collected. Colonies fixed to small rocks and detritus, which could be easily detached from the substrate without hammer and chisel, were collected and immediately put in plastic buckets. Collected colonies were put in buckets full of seawater and brought to laboratory (Figure 5). This method proved to be efficient for macroinvertebrates living in tropical corals (Abele and Patton, 1976). Since associated faunal community could vary between living and dead colonies (Cantera et al., 2003). The precise percentages of living polyps within colonies were estimated later in laboratory, together with the percentage of the colony covered by algae, sponges and the amount of mud trapped inside.

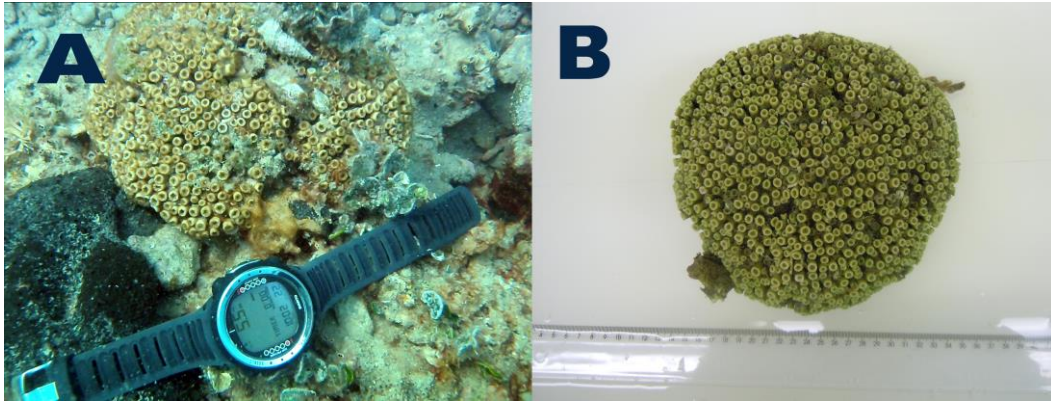


Figure 5: Sampling (a) of colonies of *C. caespitosa* (Photo: L. Lipej, 2012) and measurement (b) in laboratory (Photo: V. Pitacco, 2012).

Slika 5: Vzorčevanje (a) kolonij *C. caespitosa* (Foto: L. Lipej, 2012) in merjenje (b) v laboratoriju (Foto: V. Pitacco, 2012).

In laboratory minimum axis (width,  $D_2$ ), maximum axis (length,  $D_1$ ) and height ( $H$ ) of each colony were measured with a ruler. Each colony was weighted with a scale ( $d = 0.01$  g) for colonies smaller than 1500 g and hanging scale ( $d = 5$  g) for bigger colonies. For each colony the volume was measured thorough water displacement, before (net volume,  $V_{net}$ ) and after (total volume,  $V_{tot}$ ) covering them with plastic foils (Schiller, 1993). Interstitial volume ( $V_i$ ) was calculated as follows:  $V_i = V_{tot} - V_{net}$ . The area covered by each colony (A) was calculated using the classical formula for the calculation of the area of an ellipse:  $A = (D_1/2) (D_2/2) \pi$ .

The Index of sphericity (Riegl, 1995) was calculated for each colony:

$$Is\text{-index} = \text{Maximal Height/Maximal Diameter} \quad \dots (1)$$

The *Is*-index expresses a degree of sphericity, *i.e.*, how close is the shape of the colony to a hemisphere. In the case of hemispheric shape the index is 0.5.

For each colony 30 corallites among the longest were chosen and for each of them maximum (length) and minimum diameters (width) and length were measured with caliper ( $d=0.01$  cm).

Samples were preserved in 70 % ethanol and eventually all colonies were broken down for macrobenthic invertebrates sorting and determination. In order to remove mud trapped among corallites, samples were sieved through a 0.5 mm mesh.

Organisms that were alive at the moment of sampling were determined to the lowest possible taxonomic level according to relevant literature and their abundance was estimated. Colonial species were also determined and their coverage on a surface of 20 × 20 cm was calculated, but they were excluded from indices calculation. For determination a stereomicroscope and a microscope were used for details (morphological characteristics). Main literature references were: Tebble (1966), Ghisotti and Sabelli (1970), Parenzan (1970-1976), Torelli (1982), Cossignani and Ardovini (2011), and Scaperotta et al., (2009-2013) for molluscs; Carrera-Parra (2006), Fauvel (1923,1927), Fauchald (1977), Bianchi (1981) Barnich and Fiege (2003), San Martin (2003), Viéitez (2004) for polychaetes; Naylor (1972), Ruffo (1982-1998), Harrison and Ellis (1991), Falciai and Minervini (1992), and Hayward and Ryland (1995) for crustaceans, Occhipinti Ambrogi (1981) for bryozoans; Tursi (1980) for tunicates, Tortonese (1965) for echinoderms and Sarà (1972) for sponges; Pancucci-Papadopoulos et al. (1999) for sipunculids and additional relevant articles. The nomenclature follows World Register of Marine Species (WoRMS, 2015). For molluscs and echinoderms individuals with less than half the known adult size of the species were considered juveniles and were counted separately.

### **3.2.2 Mesoscale**

The mesoscale level consists in the study of invertebrates inhabiting bottom areas within *C. caespitosa* colonies. Sampling was performed during warm months in 2013 and 2014. Data were collected *in situ* using quadrat sampling method, a non-destructive diving visual census methodology, used for benthic fish and invertebrate assemblages investigations (Nieder et al., 2000; Lipej et al., 2008). In order to assess the proper sample size a preliminary test was carried out, comparing different quadrats of 0.5 x 0.5, 1 x 1 and 2 x 2 meters size. Given time limitation of SCUBA-diving activities, 1 x 1 meter size resulted to be the most appropriate quadrat size. A metal frame of 1 x 1 meter separated in 4 subquadrats (Figure 6) was placed in areas where *C. caespitosa* was more abundant, with a minimum of 2 colonies per quadrat.

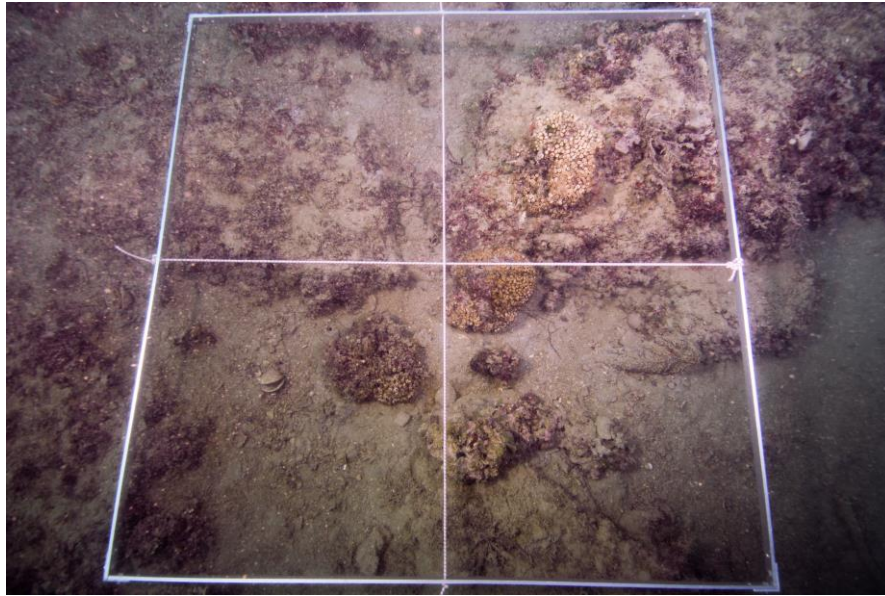


Figure 6: Metal frame used for underwater visual estimation of macrofauna (mesoscale).

Slike 6: Kovinski okvir za podvodno vizuelno oceno makrofavnice (mezoskala).

A total of 18 quadrats were analysed in 2013 and 27 in 2014 with a minimum of 3 replicates per site. Each quadrat was photographed and data on benthic assemblages were collected *in situ*. Species were determined to the lowest possible taxonomic level and organisms were counted and marked during diving on a diver slate. For colonial organisms colonies were counted. Sampling time was dependent on the heterogeneity of substrate inside the quadrats. The number and dimensions of *C. caespitosa*'s colonies, the percentage of dead corallites and the rugosity of the substrate for each replicate were calculated later from pictures.

In order to check if the sampling effort was enough accurate to get a picture of biodiversity at the studied sites, in 2014 and 2015 additional photograph sets of 25 quadrats were taken at 4 studied sites (Ronek, Pacug, Strunjanček and Piranček). For each quadrat at least 4 photographs were taken, one for each subquadrat of 0.5 x 0.5 m, for a total of 400 photographs. All photographs were analysed using PhotoQuad software (Trygonis and Sini, 2012), in order to count the number of megabenthic species present (Fig. 3.5).

“Megabenthos” is operatively defined as the benthic fraction with organisms large enough to be identified in seafloor images (Gage and Tyler, 1992). Since the determination of certain species of invertebrates and macroalgae requires sample collection and a detailed



analysis in laboratory, some of them were left to the genus or family level and the following operational definitions were employed. The term 'turf' was used to describe small cushion-shaped algal communities (less than 1 cm height) with dominant species of the genera such as *Ceramium*, *Laurencia*, *Polysiphonia* and *Dictyota*, and mucus produced by Cyanobacteria and other bacteria or diatoms (Steneck and Dethier, 1994).

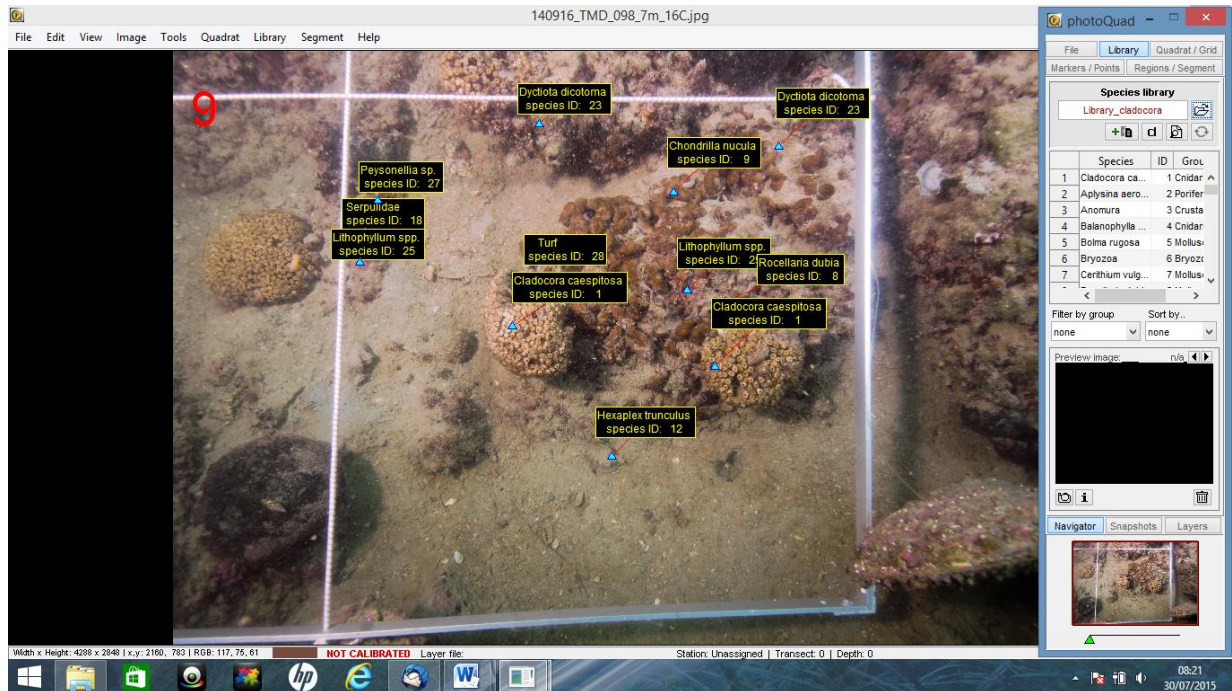


Figure 7: Species counting with pictures analysis using PhotoQuad software.

Slika 7: Preštevanje vrst z analizo fotografij s programom PhotoQuad software.

Species accumulation curves were calculated using vegan package for R (Oksanen et al., 2015) with two methods: the first adds sites in the same order they first occurred in the data, the second finds the mean number of species per area and its standard deviation from random subsampling without replacement (Gotelli and Colwell, 2001).

### 3.2.3 Macroscale

The macroscale level consists in the study of the associated benthic ichthyofauna. The fieldwork was carried out using SCUBA diving from June to September of 2013, 2014 and 2015, when the fish species were most active. Data were collected *in situ* using the visual transect technique (Harmelin, 1987), a non-destructive diving census methodology. Horizontal transects (MacPherson, 1994; Orlando Bonaca and Lipej, 2005; Lipej and

Orlando Bonaca, 2006) from 30 to 50 m in length were laid out at different depths, depending on the presence of colonies of *C. caespitosa*. Transects lengths were chosen in order to include homogeneous habitat. Depth was constant over the whole length of the transect. For each range, a fixed transect was established on the bottom with meter-marks. Fish were counted mostly within 2 m, 1 m to the left and 1 m to the right of the line. When possible 2 consecutive passages on the same transect were performed, to test the accuracy of the method. During the surveys, a constant swimming speed was maintained. A number from 2 to 6 transects were performed at each site each year, for a total of 51 transects distributed over five locations (Fig. 8). Species names and abundances were marked during diving on a diver slate.

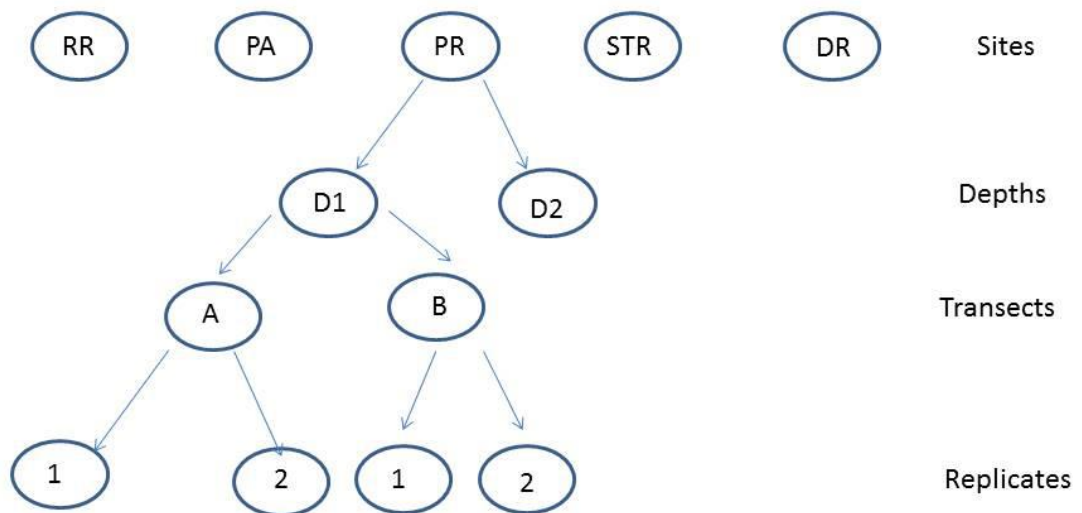


Figure 8: Sampling scheme for macroscale

Slika 8: Vzorčevalni načrt za makroskalo

### 3.3 DATA ANALYSIS

#### 3.3.1 Microscale

##### 3.3.1.1 Biometric analysis of colonies

To test if colony minimum and maximum axis, height, weight, surface covered, net volume, interstitial volume and total volume, *Is*-index and percentage of living polyps varied among sites a chi square test applied to Kruskal-Wallis ranks (Kruskal and Wallis, 1952) was run. The same test and a *post-hoc* Tukey test were run to check for differences in maximum and minimum calyces diameters among sites.

Correlation between colonies axes, covered surface, total volume and percentage of living polyps in each colony was analysed with Spearman's coefficients for non-parametric distributions (Spearman, 1907).

### 3.3.1.2 Macroinvertebrate assemblages

Data exploration techniques were used to check the presence of outliers, influential points, collinearity between variables and normality. Log transformation was applied when needed. Data exploration procedures followed Zuur et al. (2007) and were performed with R version 2.4.0.

Cumulative curves for taxa richness with increasing sample volume were created to check whether the sample size was representative for the sampled area. Curves were also created for the dominant taxonomic groups separately.

Trellis graphs showing the relationship between colony size (surface covered) and number of total taxa, taxa of molluscs, polychaetes and crustaceans for each sampling site were used to check whether this pattern is site independent or not. The same graphs were used to check for variability among sampling depth and month. Graphs were created using Lattice package for R (Sarkar, 2008).

Invertebrate richness and abundances for each colony were normalised for 1000 cm<sup>3</sup> and a chi square tests applied to Kruskal-Wallis ranks (Kruskal and Wallis, 1952) was run to test if there was variability among sites. The same test was performed to check if taxa richness and abundance normalised for 1000 cm<sup>3</sup> of each functional group, feeding guild and taxonomical group (mollusc, polychaetes and crustaceans) varied among sites.

Non-parametric Spearman's correlation was used to test how normalized taxa richness and abundance changed with colony size.

The number of taxa (S) and the number of individuals (N) were counted for non colonial organisms and the Margalef index of richness (d), the Shannon and Wiener diversity index ( $H'$ ), the Pielou index of equitability ( $J'$ ), and the Simpson index of dominance ( $D'$ ) (Clarke and Warwick, 2001) were calculated for each colony. Indices were also calculated for the three main groups: molluscs, polychaetes and crustaceans. When it was not possible to determine organisms to the level of species, a conservative approach was applied in order to determine the number of taxa present in one sample: organisms were counted as different taxa only if they showed evident differences from the ones determined to the species level. Polychaetes belonging to families Spirorbidae and Terebellidae were omitted from indices calculation. There was a huge number of individuals of the family Spirorbidae compared with low richness (about 3 species) and their habit of living inside tiny calcareous tubes, makes their correct sorting and determination time consuming. Members of the family Terebellidae instead live inside corallites without hard protection structures. Consequently it was difficult to extract them in condition good enough for correct species determination (the entire animal is needed). Percentage of dominance (% D) and frequency (% F) was calculated for each taxon. Bionomical affinity was defined mainly following relevant literature: Pérès and Picard (1964), Pérès (1967), Vio and De Min (1996), Zenetos (1996) and Solis-Weiss et al. (2004) for molluscs, Simboura and Nicolaidou (2001), San Martin (2003), Viéitez (2004) for polychaetes.

Each species was assigned to one of the following trophic group: predators feeding on other invertebrates (C), (ecto)parasites and specialized carnivores (Pa), omnivores with more than one feeding mode (O), suspension feeders capturing seston particles with their gills or with mucous strings (SF), deposit feeders, feeding on organic debris, and occasionally algae and bacteria, present in the sediment (DF), micrograzers feeding on algae, cyanobacteria or detritus attached to algal fronds (MG) and grazers, feeding on macroalgae (G). Feeding guilds were assessed according to Solis-Weiss et al. (2004), Rueda et al., (2009) and Cossignani and Ardochini (2011) for molluscs, Fauchald and Jumars (1979), Solis-Weiss et al. (2004) for polychaetes, Tortonese (1965) for echinoderms.

Moreover, each species was assigned to one of four functional groups, combining the classification of Reed and Mikkelsen (1987), Hrs-Brenko and Legac (2006) and Fauchald and Jumars (1979): free living, motile species (FL), sessile epilithic species, living the whole life attached to a substrate (EP), endolithic species, living in holes and crevices bored in hard substrates (EN), soft bottom dwelling species (SB) and discretely mobile species (DM), which were known to live attached to the substrate and move freely at different growth stages (Hrs-Brenko and Legac, 2006) or tubicolous species, capable of moving also out of the tube for some period (Fauchald and Jumars, 1979).

### 3.3.1.3 Species-area relationship

Relationships between colony size (covered area – A as colony size descriptor) and total taxa, mollusc, polychaete and crustacean taxa richness and abundance were tested with Spearman's coefficients for non-parametric distributions (Spearman, 1907).

Regression lines were calculated to describe these relationships between taxa richness and colony size. For molluscs regression lines were also calculated to describe the relationship between colony size and taxa of bivalves and gastropods, separately. For polychaetes regression lines were calculated to describe the relationship between colony size and mobile (FL, DM and SB) and sessile taxa (EP and EN), separately.

Regression lines were calculated to describe the relationship between colony size and richness of total taxa, mollusc, polychaete and crustacean taxa using total colony's volume ( $V_{tot}$ ) as colony size descriptor, as well.

Multiple regressions were used to test if factors not related with colony size, such as depth, percentage of living polyps, sediment trapped inside the colony, sponge and algal coverage, have significant influence on SAR model. Non-linear regression models were also used to choose the best model among the most frequently used to fit SAR data: Arrhenius, Gleason, Gitay, Lomolino and Michaelis-Menten models (Dengler, 2009). Adjusted R-squared and AIC (Akaike Information Criterion) were used to choose the best model. They are defined by:

$$AIC = n(\log SS_{residual}) + 2(K+1) - n \log(n) \quad \dots (2)$$

$$\text{Adjusted } R^2 = 1 - (\text{SS}_{\text{residuals}} / (n - K)) / (\text{SS}_{\text{total}} / (n - 1)) \quad \dots (3)$$

Where  $n$  = sample size,  $\text{SS}_{\text{residual}}$  = sum of squared deviations of observed values from fitted values,  $\text{SS}_{\text{total}}$  = sum of squared deviations of observed data from the mean,  $K$  = number of parameters. With a lower AIC, the model is considered better in explaining the data.

Analyses on residuals were performed to verify the assumption of normality, homogeneity, independence and absence of pattern in the residuals for model validation.

Based on chosen regression models the expected values were estimated and compared with observed values using a Wilcoxon-Mann-Whitney (WMW) statistic (Mann and Whitney, 1947). Calculations were performed using vegan package (Oksanen et al., 2015) for R.

Spearman's coefficients (Spearman, 1907) were used also to test the relation between colony size and taxa richness of the main groups of macroinvertebrates (polychaetes, molluscs and crustaceans) separately. For each of these three groups the same relationship was tested for different functional groups, feeding guilds and taxonomic subunits (bivalves and gastropods for molluscs, dominant families for polychaetes).

Cluster using abundance data with log transformation, Bray-Curtis distance and Ward linkage was performed for polychaete assemblages. Simper analysis was later carried out with vegan package (Oksanen et al., 2015), in order to check which species have more weight in producing the observed pattern.

All analyses were performed using R version 2.4.0.

### 3.3.2 Mesoscale

Data collected with underwater counting techniques were analysed first. Dotcharts were created to check for differences among sites according to sampling depth, density of colonies of *C. caespitosa*, average colony length (maximum axis), ratio between the length of the biggest and the smallest colony and average percentage of living polyps per each quadrat. A dot chart was also created to show variation of temperature with sampling month. Non-parametric Spearman's correlation (Spearman, 1907) was used to test if colony density was related with sampling depth and chi square tests applied to Kruskal-

Wallis ranks (Kruskal and Wallis, 1952) was used to check if differences among sites in colony density and dimension were significant.

To check the representativeness of our samples in relation to the sampling effort, species accumulation curves were performed for the total data set and for each site separately using vegan package (Oksanen et al., 2015) for R. Cumulative curves were built with two methods: according to the original sequence of recording and by calculating the mean of species-area curve and its standard deviation from random order of quadrats, sampled without replacement.

In order to estimate the number of unseen species and add them to the observed species richness incidence-based estimates using the frequencies of species were used (Colwell and Coddington, 1994). The functions are the following: Chao bias-corrected (4), first order jackknife (5), second order jackknife (6), bootstrap (7).

$$S_P = S_0 + (a_1(a_1 - 1) / 2(a_2 + 1)) (N - 1) / N \quad \dots (4)$$

$$S_P = S_0 + a_1((N - 1) / N) \quad \dots (5)$$

$$S_P = S_0 + (a_1(2N - 3) / N) - a_2 (N - 2)^2 / N(N - 1) \quad \dots (6)$$

$$S_P = S_0 + \sum_{i=1}^{S_0} (1 - p_i)^N \quad \dots (7)$$

where  $S_P$  is the extrapolated richness in a pool,  $S_0$  is the observed number of species in the collection,  $a_1$  and  $a_2$  are the number of species occurring only in one or only in two sites in the collection,  $p_i$  is the frequency of species  $i$ , and  $N$  is the number of sites in the collection.

Rarefaction curves to check how richness of benthic invertebrates increases with increasing number of individuals were performed at each site separately. Curves were created calculating average species richness for random subsamples of individuals.

In order to test which is the minimum sampling effort for the study sites, data obtained from photographs analysis were used to create cumulative curves.

Frequency of occurrence (% F) was calculated for each taxon and for each sampling site. Species present only at 1, 2, 3, 4 or all sites were also counted.

The number of taxa (S) and the number of individuals (N) were counted for non colonial organisms and the Margalef index of richness (d), the Shannon and Wiener diversity index ( $H'$ ), the Pielou index of equitability ( $J'$ ), and the Simpson index of dominance ( $D\lambda$ ) (Clarke and Warwick, 2001) were calculated for each sampling site.

Cluster using presence-absence data, Jaccard distance and Ward linkage were performed for fish assemblage. Jaccard distance was chosen because it does not consider double zeros to calculate similarity.

### 3.3.3 Macroscale

To check if successive passages on the same transect influenced our results species accumulation curves were calculated for the first passage, the second and for the two parallels combined using vegan package (Oksanen et al., 2015) for R. Cumulative curves were built with the same methods used for the mesoscale, but each transect was weighted according to its length. Methods for estimating species pool were also the same. Wilcoxon-Mann-Whitney (WMW) statistic was used to compare both counts and estimated species pool for the three groups (Mann and Whitney, 1947).

To check the representativeness of our samples in relation to the sampling effort, species accumulation curves were performed for all transects together and for each site separately. Also in this case cumulative curves were built with the same methods used for the mesoscale, but each transect was weighted according to its length. Wilcoxon-Mann-Whitney (WMW) statistic was used to compare estimated species pool for each sampling site (Mann and Whitney, 1947).

Dot charts were created to check for differences in sampling depth and density of colonies of *C. caespitosa* among sites. Non-parametric Spearman's correlation (Spearman, 1907) was used to test if colony density was related with sampling depth and if fish richness and density were related with colony density. The same test was also used to check if fish richness increase with increasing abundances.



Chi square tests applied to Kruskal-Wallis ranks (Kruskal and Wallis, 1952) was used to check if fish richness and density per 100 m<sup>2</sup> differed among sampling sites, months or years.

Rarefaction curves to check how fish richness increases with increasing number of individuals were performed at each site separately. Curves were created calculating average species richness for random subsamples of individuals.

Frequency of occurrence (% F) was calculated for each species for each site. Species exclusive of each site were also counted.

The number of taxa (S) and the number of individuals (N) were counted and the Margalef index of richness (d), the Shannon and Wiener diversity index (H'), the Pielou index of equitability (J'), and the Simpson index of dominance (D<sub>λ</sub>) (Clarke and Warwick, 2001) were calculated for each transect using normalised data (100 m<sup>2</sup>).

Cluster using presence-absence data, Jaccard distance and Ward linkage were performed for fish assemblages. Jaccard distance was chosen because it does not consider double zeros to calculate similarity.

## 4 RESULTS

### 4.1 MICROSCALE

#### 4.1.1 Characteristics of *C. caespitosa* colonies

Mediterranean stony coral colony size varied from the smallest at site STR 5.5 x 5 x 5 cm to the biggest at site PR 38 x 31.4 x 17.8 cm. Most of them had a maximum axis smaller than 23 cm. Only at PR bigger colonies were present (Table 2).

Table 2: Maximum (D1) and minimum (D2) axes, height (H), *Is* index, weight (W), net ( $V_{net}$ ) total ( $V_{tot}$ ) and interstitial volume ( $V_{int}$ ) and covered surface (A) of studied colonies at the five studied sites.

Preglednica 2: Največja (D1) in najmanjša (D2) dolžina, višina (H), *Is* indeks, masa (W), neto prostornina ( $V_{net}$ ), skupna prostornina ( $V_{tot}$ ) in intersticijska prostornina ( $V_{int}$ ), ter pokrovnost (A) kolonij na petih vzorčevalnih postajah.

		D1	D2	H	<i>Is_ind</i>	W	$V_{net}$	$V_{tot}$	$V_{int}$	A (cm <sup>2</sup> )
<b>RR</b>	Av	17.18	14.64	9.41	0.55	1116.82	609	968	359	210.53
	Sd	4.55	4.65	2.66	0.08	829.22	505	626	226	107.70
	Max	22.30	18.60	13.30	0.63	2060.00	1325	1590	680	324.02
	Min	11.00	8.00	6.90	0.43	176.75	110	195	85	69.12
<b>PR</b>	Av	22.54	19.12	13.84	0.63	3213.65	1934	2654	720	399.73
	Sd	10.72	9.27	5.56	0.12	3231.32	1872	2409	645	351.96
	Max	38.00	31.40	20.30	0.75	7760.00	4560	5550	1715	937.14
	Min	10.50	9.00	6.00	0.47	226.01	105	245	140	74.22
<b>STR</b>	Av	17.40	14.44	11.44	0.65	1166.78	796	1138	342	207.01
	Sd	3.66	4.38	3.66	0.13	799.75	550	717	179	103.58
	Max	22.70	19.80	15.50	0.85	2390.00	1630	2165	535	353.01
	Min	13.70	9.80	7.20	0.50	474.13	280	430	150	110.07
<b>PA</b>	Av	17.84	13.16	9.16	0.53	1039.30	513	978	465	198.97
	Sd	6.10	4.27	2.74	0.10	615.67	339	539	255	102.12
	Max	23.30	17.60	12.00	0.67	1380.00	830	1505	820	298.58
	Min	8.20	6.20	5.50	0.41	117.21	40	150	110	39.93
<b>DR</b>	Av	14.80	12.84	9.64	0.66	874.22	506	837	331	159.10
	Sd	4.04	4.08	2.72	0.07	941.18	565	911	349	99.67
	Max	21.30	19.80	14.50	0.71	2540.00	1505	2445	940	331.23
	Min	11.40	9.20	8.10	0.53	238.00	120	260	100	82.37

Colonies were mostly rounded and spherical ( $Is$ -index =  $0.62 \pm 0.12$  SD). Coral morphology ( $Is$ -index) and other biometrical parameters, such as colony minimum and maximum axis, height, weight, surface covered, colony surface, net volume, interstitial volume and total volume did not varied significantly among sites (

**Annex A**). All those characteristics (except  $Is$ -index) were positively correlated between each others (

**Annex C**). Dimensions of calyces (

**Annex B**) instead were not constant among sites (

**Annex A**).

The percentage of living polyps, algal and sponge cover, percentage of interstitial volume and mud content varied among colonies at some sites (Figure 9) but no significant differences were found among the five sampling sites (

**Annex A**). There was a weak decrease in the percentage of interstitial volume with increasing colony volume ( $r_s = -0.412$ , p-value = 0.037), and weak decrease of the percentage of living polyps with increasing percentage of algae ( $r_s = -0.491$ , p-value = 0.011) and mud ( $r_s = -0.440$ , p-value = 0.024).

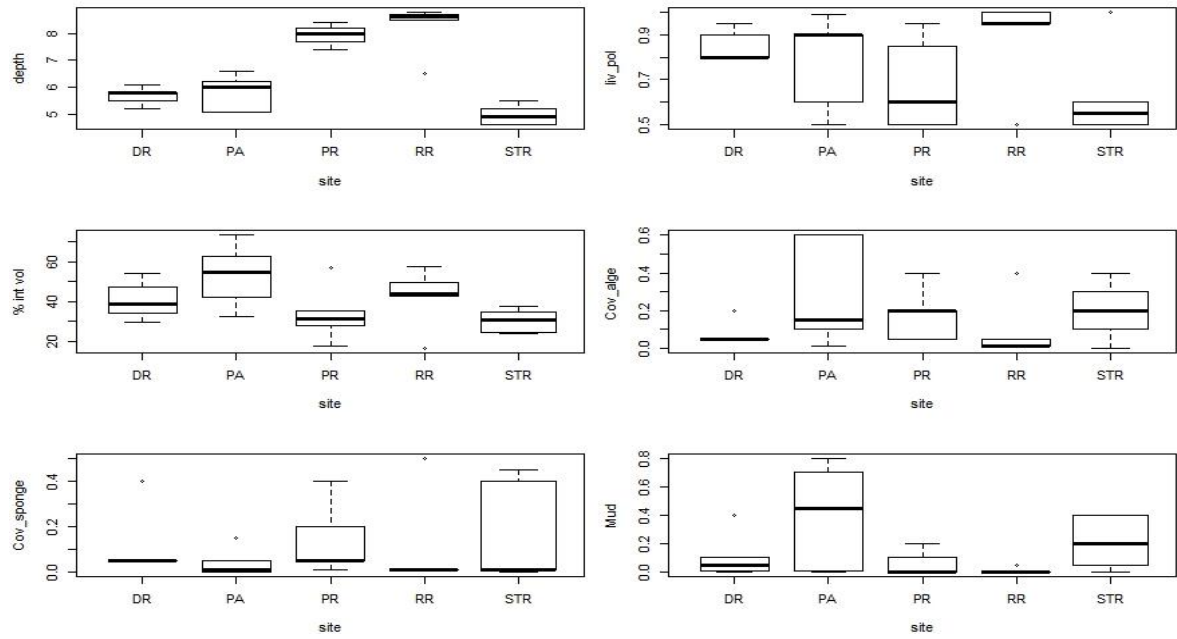


Figure 9: Boxplots showing how sampling depth, percentage of living polyps, interstitial volume, algal coverage, coverage of sponges and mud accumulation for each colony varied among sites.

Slika 9: Boxplots, ki prikazujejo, kako se razlikujejo vzorčevalna globina, delež živih polipov, intersticijska prostornina, pokrovnost alg, pokrovnost spužev in kopičenje mulja na kolonijah posameznih postaj.

#### 4.1.2 SAR

The number of taxa of non colonial invertebrates increased with increasing colony size and the relationship between taxa richness and the surface covered by colonies was strong ( $r_s = 0.813$ ;  $p\text{-value} < 0.001$ ).

The general pattern of this relationship between total number of taxa and colony size was consistent in all sampled site (Trellis graph, Annex E) and also considering taxa richness of molluscs (Annex F), polychaetes (Annex G) and crustaceans separately (Annex H). The same pattern was constant among sampling months (Annex I) and depths (Annex L), as well.

The best model describing how taxa richness increased with colony size (Figure 10) was a linear regression on log-transformed variables (see AIC values for a comparison with non-linear regression models - Arrhenius, Gleason, Gitay, Lomolino and Michaelis-Menten - Annex D).

Colony size was the factor exerting a major influence on invertebrate richness (regression in Figure 10 explains about 70 % of the relation between richness and colony size). The amount of mud inside colonies had a moderate relation with total taxa richness (Annex C). Other factors, such as depth, percentage of algae and sponges coverage were not related with taxa richness (Annex C) and did not had a significant influence on the relation between richness and colony size. Nevertheless, the best model explaining the relationship between invertebrate richness and colony size (78 % of the relation explained), was a multiple linear regression considering also depth and percentage of mud in each colony (comparison of AIC values, see Annex D, and analysis of residuals).

Predictions of species richness slightly improved also using colony volume instead of area covered as colony size descriptor (WMW test:  $V = 169$ ,  $p\text{-value} = 1$  for volume and  $V = 321$ ,  $p\text{-value} < 0.001$  for covered area, AIC values see Annex D).

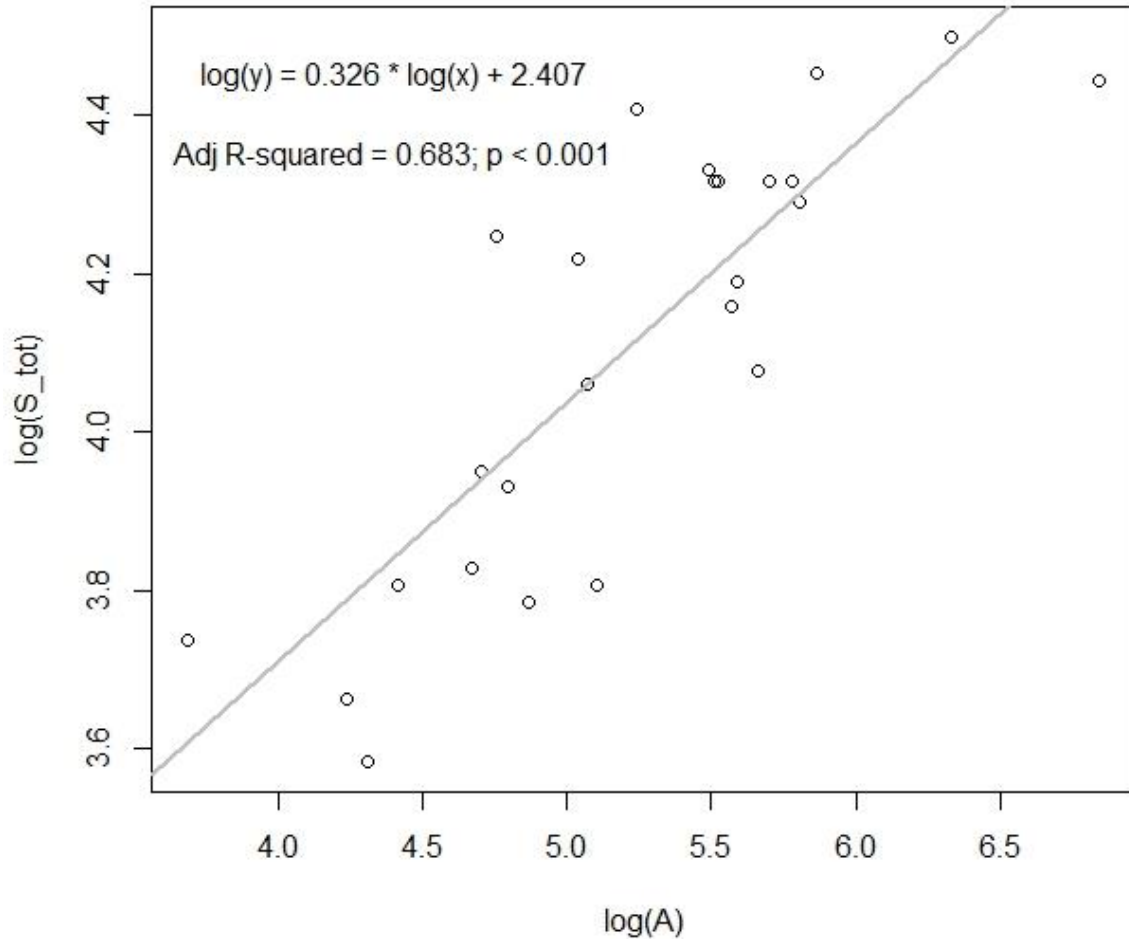


Figure 10: Linear regressions between the total number of non colonial invertebrates and the area covered by colonies of *C. caespitosa* in  $\text{cm}^2$ . All axes are log-transformed. Dots = sampled colonies.

Slika 10: Linearna regresija med skupnim številom nekolonialnih nevretenčarjev in površino, ki jo pokriva posamezna kolonija *C. caespitosa* ( $\text{cm}^2$ ). Osi so log-preoblikovane. Dots = vzorčene kolonije.

#### 4.1.2.1 Mollusc assemblages

The number of mollusc taxa increased with increasing *C. caespitosa* colony size (area covered by colonies - A) and this relation was strong ( $r_s = 0.720$ ;  $p$ -value  $< 0.001$ ). Colony size was the best predictor for mollusc richness (regression in Figure 11a explains 74 % of the relation between mollusc richness and colony size). The model explaining mollusc richness only with colony size showed the best fit. Other factors such as depth, percentage

of living polyps, percentage of algae or sponges coverage and mud content were not significantly related with mollusc richness (AIC values in Annex D and analysis of residuals).

The same was observed considering gastropods ( $r_s = 0.695$ ;  $p\text{-value} < 0.001$ ) and bivalves ( $r_s = 0.641$ ;  $p\text{-value} < 0.001$ ) separately (regression lines in Figure 11b, c).

In the cases of bivalves and gastropods expected values did not differ significantly from observed values (WMW test  $V = 152$ ,  $p\text{-value} = 0.791$  for bivalves and  $V = 112$ ,  $p\text{-value} = 0.919$  for gastropods), whereas for mollusc total taxa richness there was a significant difference (WMW test  $V = 270$ ,  $p\text{-value} = 0.003$ ) between expected and observed values. Prediction of mollusc total richness improved considering total colony volume as colony size descriptor (WMW test  $V = 160$ ,  $p\text{-value} = 0.958$ , AIC values in Annex D).

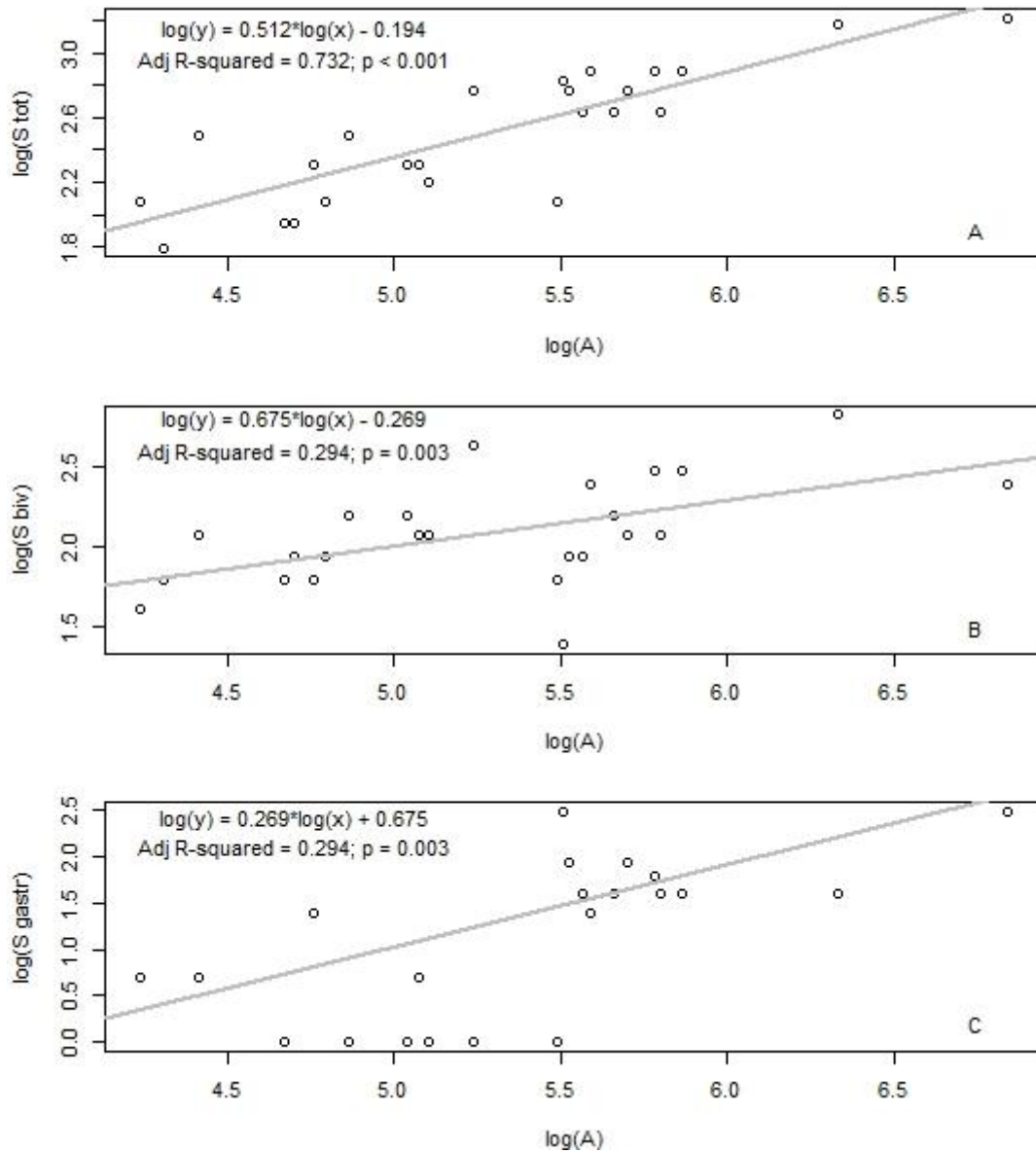


Figure 11: Linear regressions between the area covered by colonies in  $\text{cm}^2$  and the total number of taxa of molluscs (A), the number of taxa of bivalves (B) and gastropods (C). All axes are log-transformed. Dots = sampled colonies.

Slika 11: Linearna regresija med površino, ki jo pokriva kolonija (v  $\text{cm}^2$ ) in skupnim številom taksonov mehkužcev (A), število taksonov školjk (B) in polžev (C). Osi so log-preoblikovane. Dots = vzorčene kolonije.



#### 4.1.2.2 Polychaetes

Polychaetes taxa richness increased with increasing colony size (Figure 12) and this relation was strong (Annex C). Nevertheless, richness of polychaetes was also moderately related with the amount of mud present inside the colonies, and it slightly decreased with increasing depth (Annex C). Colony size alone explained only partially the variability of polychaete richness (bivariate linear regression explained 58 % of the relation). The amount of mud present inside the colonies exerted a significant influence on taxa richness, as well (multiple regression model explained 75 % of the relationship).

The richness of motile polychaetes increased with colony size, as well ( $r_s = 0.7514$ ; p-value  $< 0.001$  for A) (Figure 12). Conversely, richness of sessile polychaetes was not related with the dimension of colonies (Figure 12).

For what polychaete assemblages were concerned, area covered by colonies was a good predictor of taxa richness (Wilcoxon test  $V = 161$ , p-value = 0.9789 for total polychaete taxa and  $V = 170$ , p-value = 0.8532 for only motile taxa).

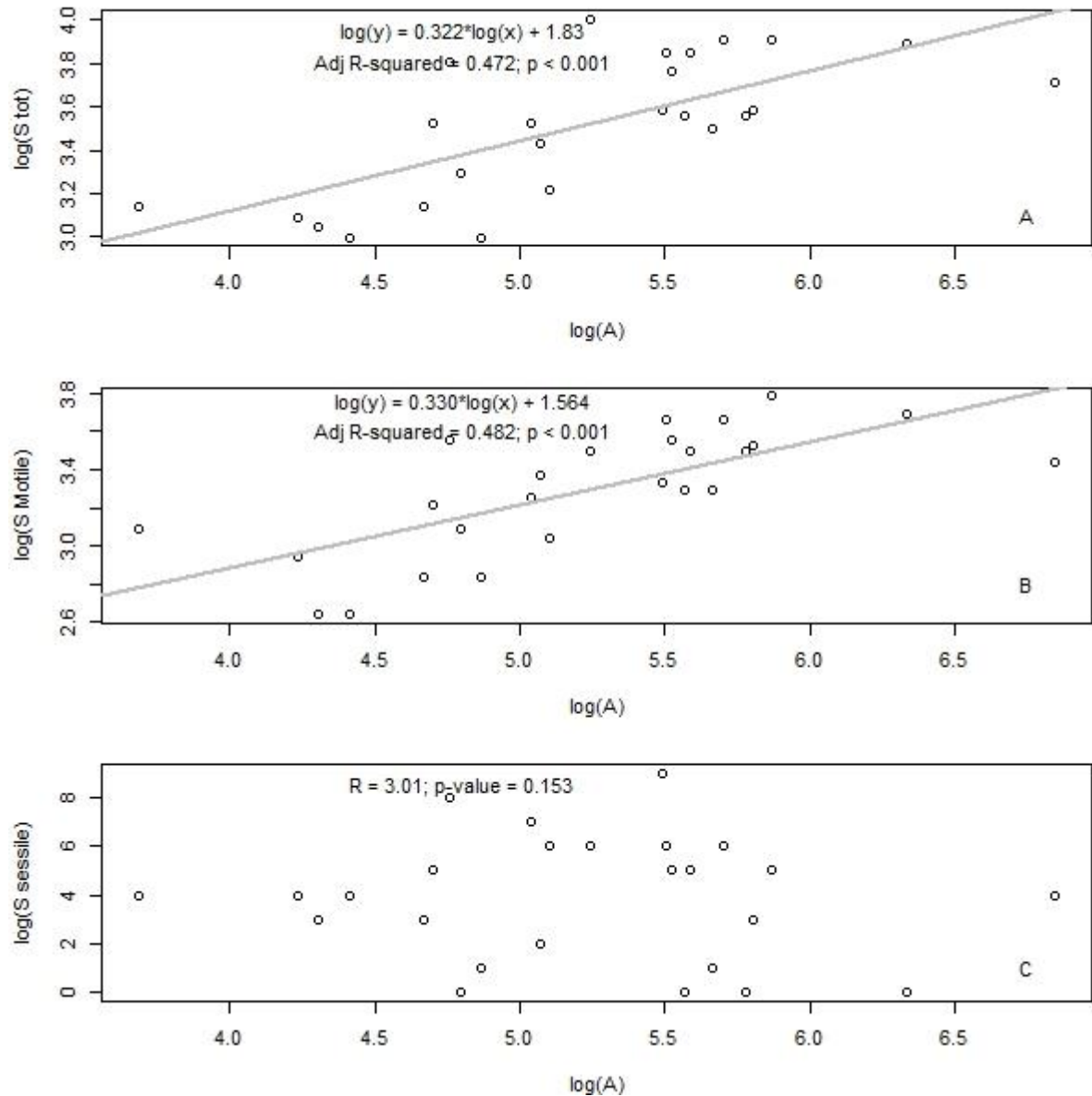


Figure 12: Linear regressions between the area covered by colonies of *C. caespitosa* in  $\text{cm}^2$  and the total number of taxa of polychaetes (A), total number of taxa of mobile polychaetes (B) and of sessile polychaetes (C). Dots = sampled colonies.

Slika 12: Linearna regresija med površino, ki jo pokriva kolonija v  $\text{cm}^2$  in skupnim številom taksonov mnogoščetincev (A), številom taksonov mobilnih mnogoščetincev (B) in sedentarnih mnogoščetincev (C). Osi so log-preoblikovane. Dots = vzorčene kolonije.

#### 4.1.2.4 Crustaceans

Crustacean taxa richness increased with increasing colony size (Figure 13) but the relation was not strong ( $r_s = 0.519$ ;  $p\text{-value} = 0.0078$ ) and colony size was not a good predictor for

taxa richness (linear regression in Figure 13 explains only 23 % of the relation between richness of crustaceans and area covered by colonies).

Crustacean richness was not significantly related with any other factors such as depth, percentage of living polyps, percentage of algae or sponges coverage and mud content (Correlation values in Annex C, AIC values in Annex D and analysis of residuals). Even if sampling depth and months seemed to influence the relationship between crustacean richness and colony size (AIC values in Annex D and pattern of residuals), the model considering also these factors (multiple linear regression) explained only 43 % of the relationship).

Considering only decapods, there was a moderate relationship between richness and colony size ( $r_s = 0.553$ ;  $p\text{-value} = 0.004$ ), as well. Conversely amphipods richness was weakly related with colony size ( $r_s = 0.458$ ;  $p\text{-value} = 0.024$ ).

Richness of decapods showed no relation with the percentage of living polyps, muds or depth (Annex C). Even if the percentage of living polyps seemed to affect the relationship between species and area (multiple linear regression  $p\text{-values}$  in Annex C and AIC values in Annex D), the percentage of the relationship explained by the model was still too low (38 %) to enable a good prediction.

Considering regression between total crustacean richness and area expected values did not differ significantly from observed values ( $p\text{-value} = 0.979$ ) and covered area resulted as a better predictive factor compared with total volume (AIC values in Annex D). The same was observed considering only decapods (Wilcoxon test  $V = 143$ ,  $p\text{-value} = 0.615$  and AIC values in Annex D).

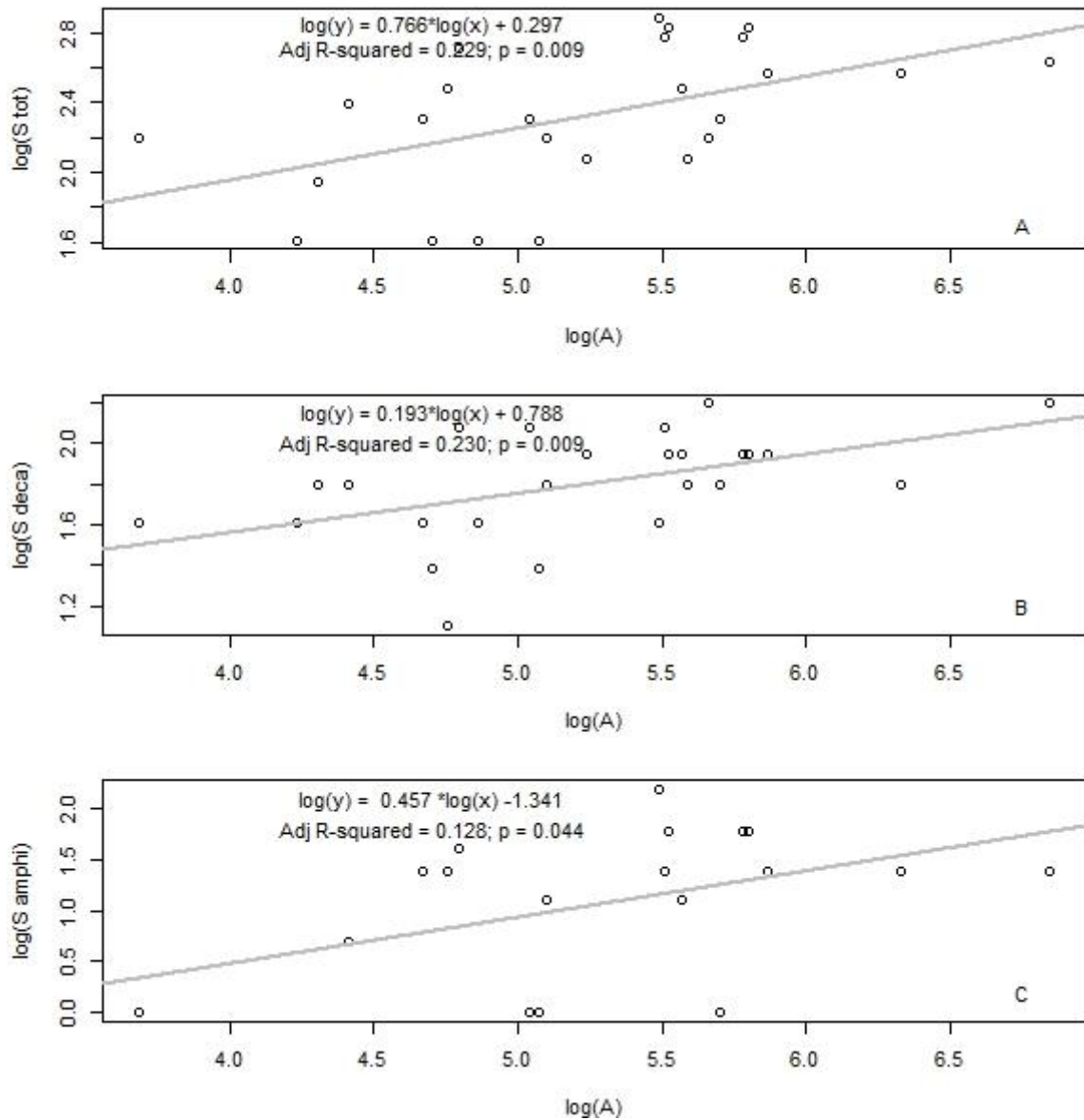


Figure 13: Linear regressions between the area covered by colonies of *C. caespitosa* in  $\text{cm}^2$  and the total richness of crustaceans (a), the richness of decapods (b) and the richness of amphipods (c). Dots = sampled colonies.

Slika 13: Linearna regresija med površino, ki jo pokriva kolonija (v  $\text{cm}^2$ ) in skupnim številom taksonov rakov (a), številom taksonov rakov deseteronožcev (b) in postranic (c). Osi so log-preoblikovane. Dots = vzorčene kolonije.

### 4.1.3 Invertebrate community

A total of 222 different taxa were found: 95 polychaetes, 64 molluscs, 43 crustaceans, 5 tunicates, 5 bryozoans, 3 sponges, 4 echinoderms, 1 cnidarian and 1 sipunculid. Among non-colonial organisms 11561 invertebrates were counted and 177 taxa were determined to the species level (Table 3). Polychaetes were the most abundant (46 %), followed by molluscs (26 %) and crustaceans (18 %) (Figure 14).

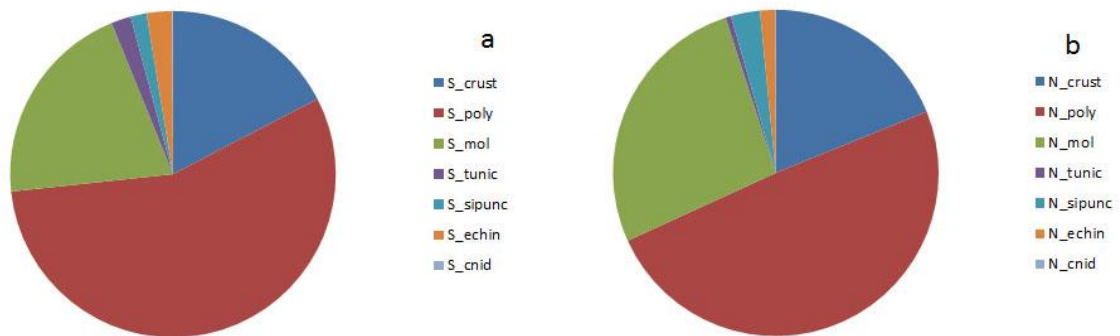


Figure 14: Average percentage of richness (a) and abundances (b) of phyla of non-colonial organisms per colony.

Slika 14: Povprečni delež števila vrst (a) in abudanc (b) nekolonijskih debel nevretenčarjev na kolonijo.

The most frequent and abundant species (present in every colony) were: *Rocellaria dubia*, *Hiatella arctica*, *Athanas nitescens*, *Lysidice ninetta*, *Eunice vittata* and sipunculids, followed by *Syllis variegata*, *Pilumnus hirtellus*, *Alpheus dentipes* and *Arca noae* (> 90 %).

Among molluscs more than half (58 %) of counted individuals were juveniles and 16 taxa were found only as juvenile forms (*Lepidopleurus cajetanus*, *Jujubinus* sp., *Cerithium vulgatum*, *Hexaplex trunculus*, *Vexillum* sp., *Barbatia barbata*, *Lithophaga lithophaga*, Ostreidae, *Irus irus*, *Petricola lithophaga*, *Kellia suborbicularis*, *Kurtiella bidentata*, *Coralliophila lithophagella*, *Gouldia minima*). This proportion of juvenile molluscs was highly variable among colonies and a slight variability was observed also among sites (Kruskal-Wallis chi-squared = 9.8437, df = 4, p-value = 0.043). The highest proportion of juveniles was observed in samples collected in September (75 % at site PA) and July (72 % at site RR and 64 % at site PR), the lowest was observed in August (53 % at site STR) and October (51 % at site DR).

Among echinoderms, *Ophiotrix* spp. was found only in juvenile form while *Amphipholis squamata* was found both in adult and juvenile forms.

Also among polychaetes some individuals with less than half the average adult size were observed (e.g. *S. vermicularis* and *E. torquata*). Stolons of some species of genera *Syllis* and *Trypanosyllis* were observed and some individuals of *Pileolaria* spp. incubated eggs in the operculum.

Among crustaceans females of different species such as *Alpheus dentipes*, *Apseudes acutifrons*, *Athanas nitescens*, *Gnathia vorax*, *Lysianassa longicornis*, *Parapseudes latifrons*, *Pilumnus hirtellus*, *Synalpheus gambarelloides* and *Thoralus cranchii* were observed carrying eggs. At site RR one pranzia larvae of the isopod *Gnathia vorax*, together with one adult male and a female carrying eggs, were found in the same colony.

Table 3: Macroinvertebrate taxa determined to the species level.

Preglednica 3: Vrste ugotovljenih makronevretenčarjev.

<b>Taxonomic list</b>	
<b>Mollusca</b>	Phyllodoce madeirensis
<i>Lepidopleurus cajetanus</i>	<i>Pterocirrus macroceros</i>
<i>Chiton olivaceus</i>	<i>Glycera alba</i>
<i>Acanthochitona fascicularis</i>	<i>Glycera tessellata</i>
<i>Diodora gibberula</i>	<i>Parasabella tommasi</i>
<i>Diodora graeca</i>	<i>Parasabella langerhansi</i>
<i>Emarginella huzardii</i>	<i>Flabelliderma cinari</i>
<i>Clanculus cruciatus</i>	<i>Euphrosyne foliosa</i>
<i>Bittium latreilli</i>	<i>Paleanotus chrysolepis</i>
<i>Bittium reticulatum</i>	<i>Leocrates claparedii</i>
<i>Cerithium vulgatum</i>	<i>Dodecaceria concharum</i>
<i>Marshallora adversa</i>	<i>Aphelochaeta filiformis</i>
<i>Monophorus thiriota</i>	<i>Cirriformia tentaculata</i>
<i>Cerithiopsis tubercularis</i>	<i>Cirratulus cirratulus</i>
<i>Cerithiopsis nana</i>	<i>Caulleriella viridis</i>
<i>Alvania cimex</i>	<i>Amphitrite cirrata</i>
<i>Alvania geryonia</i>	<i>Neoamphitrite affinis</i>
<i>Rissoina bruguieri</i>	<i>Nicolea venustula</i>
<i>Manzania crassa</i>	<i>Branchiosyllis exilis</i>
<i>Serpuloides arenaria</i>	<i>Brania pusilla</i>
<i>Muricopsis cristata</i>	<i>Eurysyllis tuberculata</i>

continued

Continuation of Table 3: Macroinvertebrate taxa determined to the species level.

<b>Taxonomic list</b>	
<i>Ocinebrina edwardsii</i>	<i>Exogone naidina</i>
<i>Hexaplex trunculus</i>	<i>Haplosyllis spongicola</i>
<i>Polliia dorbignyi</i>	<i>Paraehelersia ferruginea</i>
<i>Mangelia stossiciana</i>	<i>Salvatoria clavata</i>
<i>Arca noae</i>	<i>Sphaerosyllis pirifera</i>
<i>Barbatia barbata</i>	<i>Syllides fulvus</i>
<i>Striarca lactea</i>	<i>Syllis alternata</i>
<i>Lithophaga lithophaga</i>	<i>Syllis armillaris</i>
<i>Modiolus barbatus</i>	<i>Syllis beneliahuae</i>
<i>Modiolula phaseolina</i>	<i>Syllis corallicola</i>
<i>Modiolarca subpicta</i>	<i>Syllis columbretensis</i>
<i>Brachidontes pharaonis</i>	<i>Syllis ferrani</i>
<i>Lima hians</i>	<i>Syllis gerlachi</i>
<i>Chlamys varia</i>	<i>Syllis gerudensis</i>
<i>Chlamys multistriata</i>	<i>Syllis gracilis</i>
<i>Anomia ephippium</i>	<i>Syllis krohnii</i>
<i>Galeomma turtoni</i>	<i>Syllis prolifera</i>
<i>Chama gryphoides</i>	<i>Syllis variegata</i>
<i>Pseudochama gryphina</i>	<i>Trypanosyllis aeolis</i>
<i>Irus irus</i>	<i>Trypanosyllis zebra</i>
<i>Rocellaria dubia</i>	<i>Trypanosyllis coeliaca</i>
<i>Hiatella artica</i>	<i>Xenosyllis scabra</i>
<i>Hiatella rugosa</i>	<b>Arthropoda</b>
<i>Ctena decussata</i>	<i>Achaeus cranchii</i>
<i>Petricola lithophaga</i>	<i>Alpheus dentipes</i>
<i>Kellia suborbicularis</i>	<i>Athanas nitescens</i>
<i>Kurtiella bidentata</i>	<i>Galathea intermedia</i>
<i>Coralliophila lithophagella</i>	<i>Gouretia denticulata</i>
<i>Gouldia minima</i>	<i>Pagurus anachoretus</i>
<i>Diplodonta rotundata</i>	<i>Pilumnus hirtellus</i>
<b>Polychaeta</b>	<i>Pilumnus spinifer</i>
<i>Vermiliopsis striaticeps</i>	<i>Pisidia blutelli</i>
<i>Vermiliopsis infundibulum</i>	<i>Pisidia longimana</i>
<i>Pomatocerus triqueter</i>	<i>Synalpheus gambarelloides</i>
<i>Serpula concharum</i>	<i>Eualus (Thoralus) cranchii</i>
<i>Serpula vermicularis</i>	<i>Xantho pilipes</i>
<i>Hydroides pseudouncinata pseudouncinata</i>	<i>Eisothistos macrurus</i>
<i>Simplaria pseudomilitaris</i>	<i>Gnathia vorax</i>
<i>Pileolaria militaris</i>	<i>Janira maculosa</i>
<i>Eunice torquata</i>	<i>Apseudes acutifrons</i>

continued

Continuation of Table 3: Macroinvertebrate taxa determined to the species level.

<b>Taxonomic list</b>	
<i>Lysidice ninetta</i>	<i>Leptochelia savignyi</i>
<i>Lysidice collaris</i>	<i>Parapseudes latifrons</i>
<i>Nematonereis unicornis</i>	<i>Balanus trigonus</i>
<i>Eunice vittata</i>	<i>Caprella acanthifera</i>
<i>Eunice siciliensis</i>	<i>Corophium acutum</i>
<i>Eunice schizobranchia</i>	<i>Dexamine spinosa</i>
<i>Marphysa fallax</i>	<i>Leptocheirus guttatus</i>
<i>Lumbrinereis coccinea</i>	<i>Leucothoe euryonyx</i>
<i>Lumbrinereis latreilli</i>	<i>Leucothoe richiardii</i>
<i>Scoletoma impatiens</i>	<i>Leucothoe spinicarpa</i>
<i>Scoletoma fragilis</i>	<i>Liljeborgia dellavallei</i>
<i>Lumbrinereis gracilis</i>	<i>Lysianassa costae</i>
<i>Scoletoma funchalensis</i>	<i>Lysianassa longicornis</i>
<i>Arabella geniculata</i>	<i>Lysianassa pilicornis</i>
<i>Perinereis cultrifera</i>	<i>Maera grossimana</i>
<i>Ceratonereis costae</i>	<i>Maera inaequipes</i>
<i>Nereis rava</i>	<i>Metaphoxus simplex</i>
<i>Harmothoe areolata</i>	<i>Orchomene humilis</i>
<i>Harmothoe extenuata</i>	<i>Phtisica marina</i>
<i>Harmothoe fragilis</i>	<i>Stenothoe monoculoides</i>
<i>Harmothoe gilchristi</i>	<b>Echinodermata</b>
<i>Harmothoe impar</i>	<i>Amphipholis squamata</i>
<i>Harmothoe spinifera</i>	<i>Ophioderma longicaudum</i>
<i>Lepidonotus clava</i>	<i>Paracentrotus lividus</i>
<i>Polynoe scolopendrina</i>	<b>Bryozoa</b>
<i>Notomastus latericeus</i>	<i>Schizoporella errata</i>
<i>Pseudoleiocapitella fauvelii</i>	<i>Schizoporella unicornis</i>
<i>Leiochrides australis</i>	<b>Porifera</b>
<i>Dasybranchus caducus</i>	<i>Aplysina aerophoba</i>
<i>Dasybranchus galjole</i>	<i>Chondrilla nucula</i>

Many of the parameters used to describe invertebrate assemblages (richness and abundance of each functional group and of each feeding guild) were related with colony size (Annex C).

Taxa abundances increased with increasing colony size (Annex C), as well. Conversely density of taxa and individuals (per 1000 cm<sup>3</sup>) decrease with increasing colony size



(Annex C). The same was observed considering only polychaetes and crustaceans (Annex C), whereas density of molluscs did not show this pattern (Annex C).

Values of Margalef and Shannon indices increased with colony size (Figure 15). The Margalef index varied from 6.37 (at site PR) to 12.48 (at site STR), Shannon index varied from 2.93 (at site PR) to 3.69 (at site PA). Conversely Simpson and Pielou indices had no relation with colony size (Figure 15). The Simpson index varied from 0.91 (at site PA) to 0.96 (at site PR) and the Pielou index from 0.74 (at site PR) to 0.92 (at site PA).

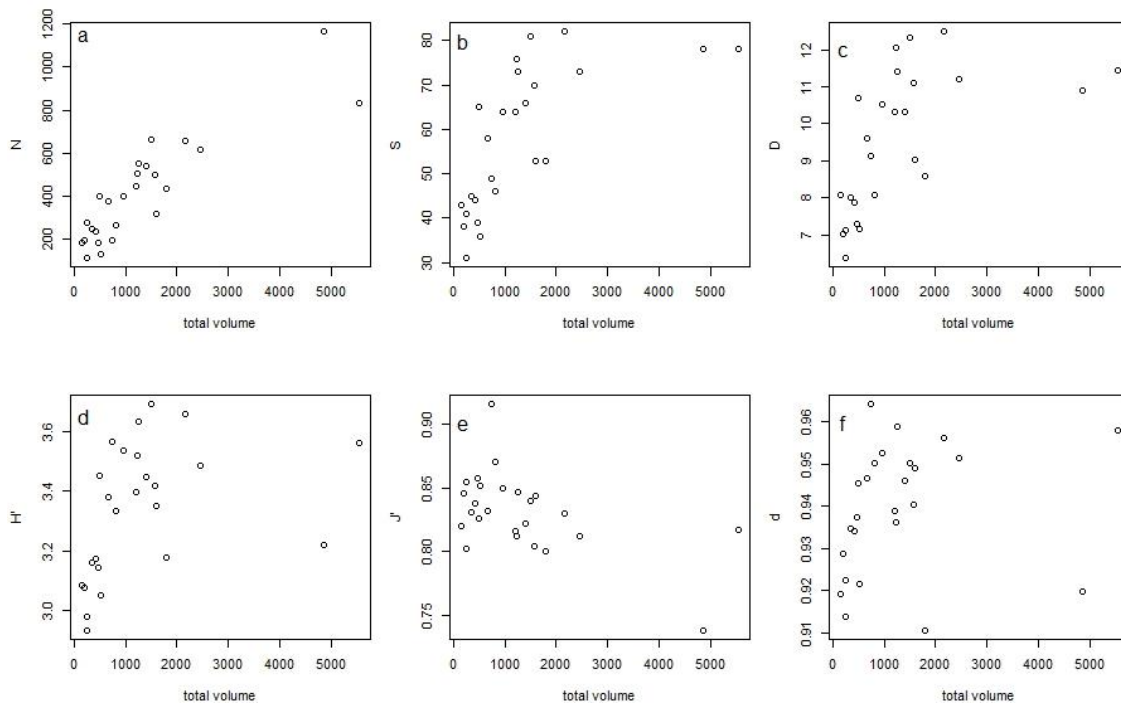


Figure 15: Variation of abundances (a), richness (b), Margalef (c), Shannon (d), Pielou (e) and Simpson (f) indices according to the total colony volume ( $\text{cm}^3$ ).

Slike 15: Nihanje števila osebkov (a), števila vrst (b), Margalefov (c), Shannonov (d), Pieloujev (e) in Simpsonov (f) indeks glede na prosdornino celotne kolonije ( $\text{cm}^3$ ).

The community of non-colonial invertebrates was dominated by free living (for richness) and discretely mobile (for abundances) organisms, followed by epilithic and endolithic organisms (Figure 16). Free living invertebrates were mainly crustaceans, polychaetes, echinoderms and molluscs, discretely mobile were mainly polychaetes and molluscs. Molluscs and polychaetes were differentiated in different functional groups, while

crustaceans, echinoderms, tunicates and sipunculids were only found in a few functional groups (Figure 16).

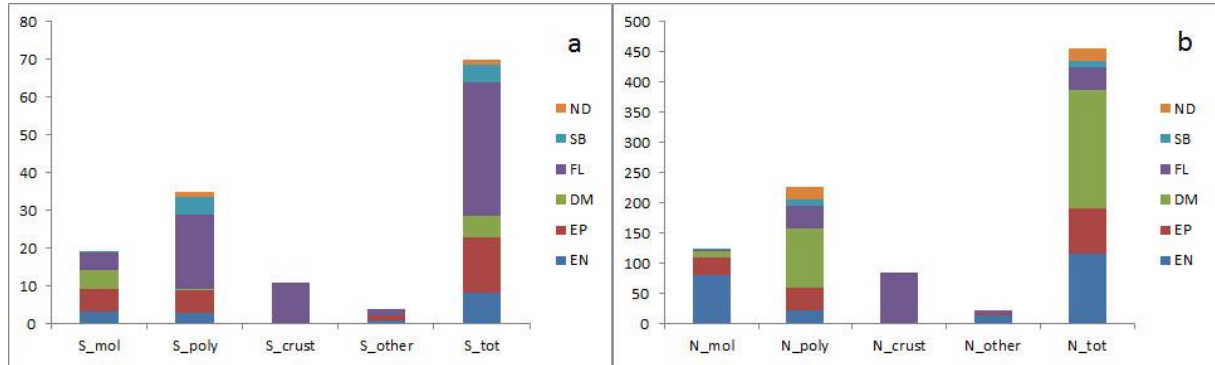


Figure 16: Average richness (a) and abundances (b) of the different functional guilds for mollusc (mol), polychaetes (poly), crustaceans (crust), tunicates, echinoderms and sipunculids (other) and for the total assemblages (tot).

Slike 16: Povprečna vrstna pestrost (a) in abudanca (b) različnih funkcionalnih skupin mehkužcev (mol), mnogoščetincev (poli), rakov (crust), plaščarjev, iglokožcev in pršivcev (other) in celotno združbo (tot).

Richness and abundance of invertebrates of different functional groups (epilithic, endolithic, discretely mobile, soft bottom and free living) significantly increased with colony size, with the exception of abundance of epilithic invertebrates (Annex C). Soft bottom and free living invertebrates were also related with an increasing amount of mud inside the colonies (Annex C). There was instead a negative relation between the percentage of living polyps and groups such as discretely mobile (for richness and abundance), endolithic (for abundance), epilithic and soft bottom invertebrates (for richness) (Annex C).

Richness and abundances of invertebrates of different functional groups did not vary significantly among sites, with the exception of richness of discretely mobile invertebrates. The same parameters normalized for 1000 cm<sup>3</sup> did not show differences, as well (

**Annex A).**

The community of non-colonial invertebrates was dominated by carnivores (for richness), followed by deposit feeders and suspension feeders considering both richness and abundances (Figure 17). Carnivores were mainly polychaetes and molluscs, deposit feeders

were mainly polychaetes, crustaceans, echinoderms and sipunculids, suspension feeders were mainly molluscs, polychaetes and crustaceans.

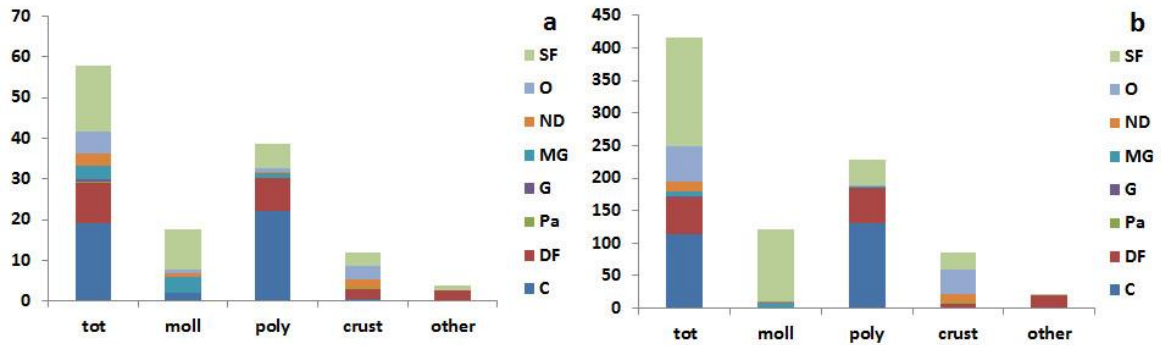


Figure 17: Average richness (a) and abundances (b) of the different feeding guilds for mollusc (mol), polychaetes (poly), crustaceans (crust), tunicates, echinoderms and sipunculids (other) and for the total assemblages (tot).

Slike 17: Povprečna vrstna pestrost (a) in abundanca (b) različnih prehranjevalnih skupin mehkužcev (mol), mnogoščetincev (poly), rakov (crust), plaščarjev, iglokožcev in pršivcev (other) in celotno združbo (tot).

Richness and abundance of invertebrates of different feeding groups significantly increased with colony size, as well (Annex C), with the exception of spongivores, ectoparasitic invertebrates and macrograzers, present with only few individuals. There was a negative relation between the percentage of living polyps and abundance of suspension feeders, carnivores and omnivores (Annex C).

The composition of invertebrate assemblages (based on presence-absence data) in each colony showed a higher similarity among colonies with similar size than among colonies at the same sampling site. Only colonies from the site STR are grouped in the same cluster. Invertebrate assemblages can be clearly divided into two groups (anosim = 0.309; p-value = 0.001), one with assemblages inhabiting colonies smaller than 1.2 dm<sup>3</sup> and another found in bigger colonies. Exceptions are samples from sites STR2 and STR4, grouped with smaller colonies and sites such as PA1 and DR2 grouped with bigger colonies. The same results (Figure 18) were obtained with log-transformed abundance data (anosim = 0.666; p-value = 0.001). This separation in groups is due mainly to the bivalves *Rocellaria dubia*

and *Hiatella arctica*, crustaceans *Athanas nitescens* and *Pisidia longimana*, polychaetes *Notomastus latericeus*, *Lysidice ninetta*, *Serpula concharum*, *Ceratonereis costae*, *Eunice vittata*, *Nereis rava*, *Syllis variegata* and sipunculids, accounting cumulatively for 50 % of dissimilarity. All these species are very frequent and abundant.

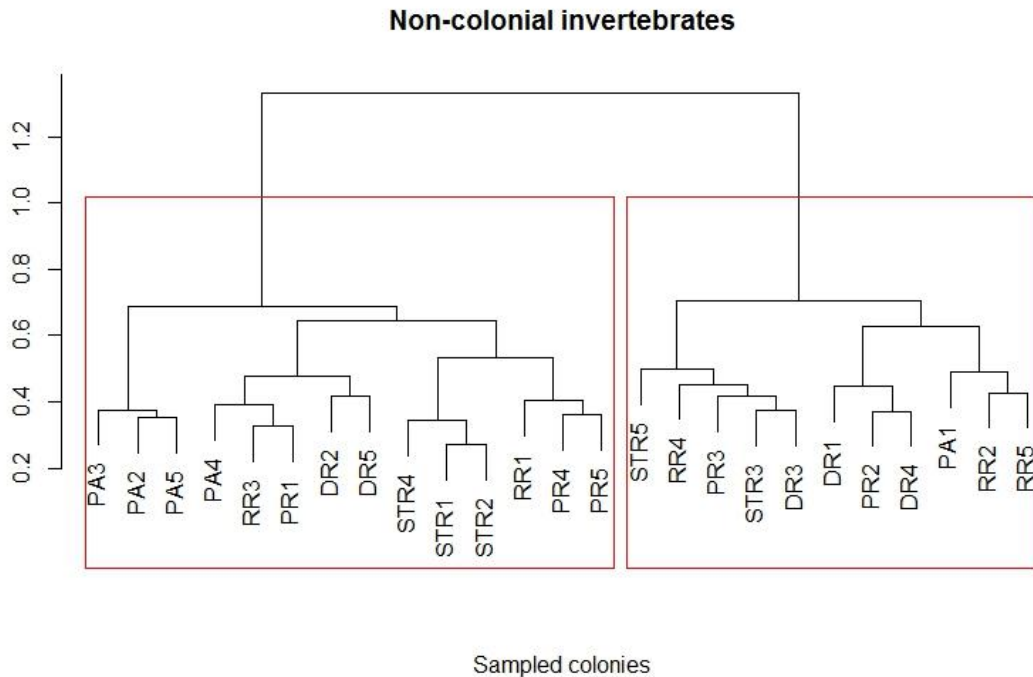


Figure 18: Cluster analysis for log-transformed abundances of non colonial invertebrate taxa in each colony at the different sampling sites.

Slika 18: Klusterska analiza za logaritmsko preoblikovane vrednosti abundance nekolonialnih nevretenčarskih taksonov v vsaki koloniji na različnih vzorčevalnih postajah.

#### 4.1.3.1 Mollusc assemblages

Altogether, 3034 molluscs belonging to 64 different taxa (3 Polyplacophora, 31 Gastropoda and 30 Bivalvia) were counted in the colonies of *C. caespitosa*. Bivalves were the dominant group with 2767 individuals (91 %), followed by gastropods with 199 (7 %) and polyplacophorans with 68 (2 %). The most frequent and abundant species were bivalves *Rocellaria dubia* and *Hiatella arctica* present in all studied colonies with 34 % and 28 % of dominance, respectively. Very frequent (>75 % of frequency of occurrence) were also bivalves *Arca noae*, *Striarca lactea*, *Modiolus barbatus* and *Chama gryphoides*. All these species were present both in adult and juvenile forms.

Margalef and Shannon diversity indices increased with colony size. The Margalef index varied from 1.34 to 4.32 at site PR, the Shannon index varied from 1.08 to 2.53 at site PA, the Simpson index from 0.51 to 0.90 at site PA. The Pielou index varied from 0.51 at site PR to 0.90 at site PA, without any relation with colony size (Figure 19).

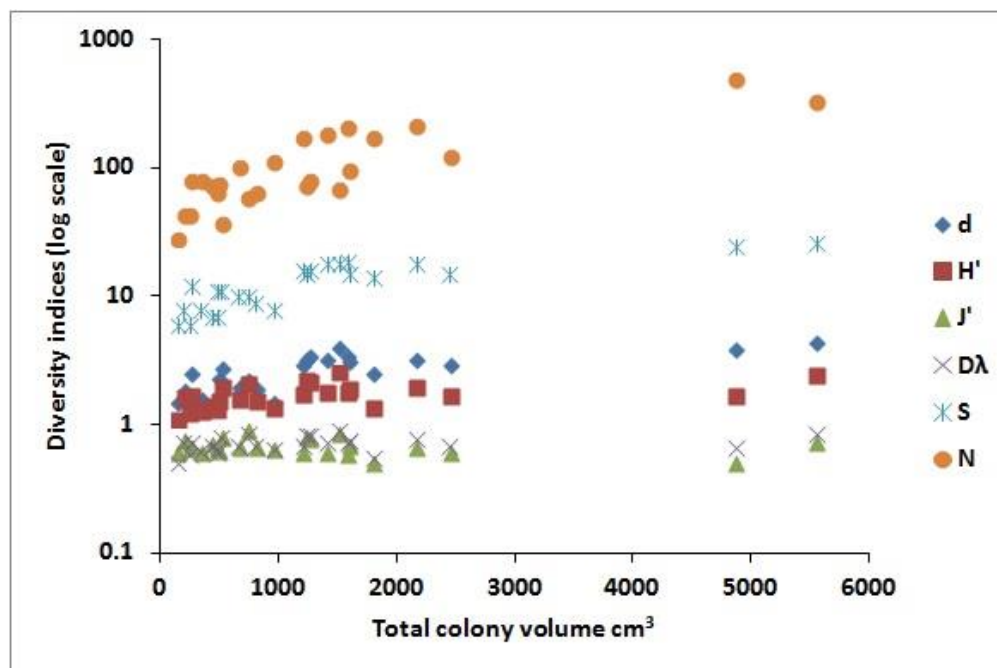


Figure 19: Diversity indices of mollusc assemblages in each *C. caespitosa* colony. Colonies are ordered by size (total volume). Margalef index of richness (d), Shannon diversity index ( $H'$ ), Pielou index of equitability ( $J'$ ) and Simpson index of dominance ( $D\lambda$ ) calculated for each colony (=dots).

Slika 19: Diverzitetni indeksi združb mehkužcev v posamezni koloniji kamene korale. Kolonije so razvrščene po velikosti (prostornina). Margalefov indeks (d), Shannonov indeks ( $H'$ ), Pieloujev indeks ( $J'$ ) in Simpsonov indeks ( $D\lambda$ ) izračunan na vsako kolonijo (= pike).

About 72 % of taxa, which were identified to the species level, belonged to the biocoenosis of photophilic algae (AP), while a low percentage of species belonged to the biocoenosis of the coastal detritic bottoms (DC), the muddy detritic bottoms (DE), terrigenous mud (VTC) and superficial muddy sands in sheltered areas (SVMC). No characteristic species of any biocoenosis were found.

Considering taxa richness, free living animals were dominant (51 %), followed by epilithic (33 %) and endolithic taxa (7 %). Conversely, considering animal abundance (Figure 20),

endolithic animals were dominant (67 %), followed by epilithic (24 %) and free living molluscs (9 %). The most frequent and abundant endolithic species were *Rocellaria dubia* and *Hiatella arctica*, epilithic were *Arca noae*, *Striarca lactea*, *Modiolus barbatus*, *Chama gryphoides* and free living were *Chiton olivaceus* and *Clanculus cruciatus*.

Taxa richness of epilithic, endolithic and free living mollusc increased with colony size (Annex C). Their abundances also increased with colony size: strong positive correlations were observed between total volume and abundance of free living, epilithic and endolithic molluscs (Annex C). Other groups showed no significant correlation with colony size (Annex C) and were not present in every colony.

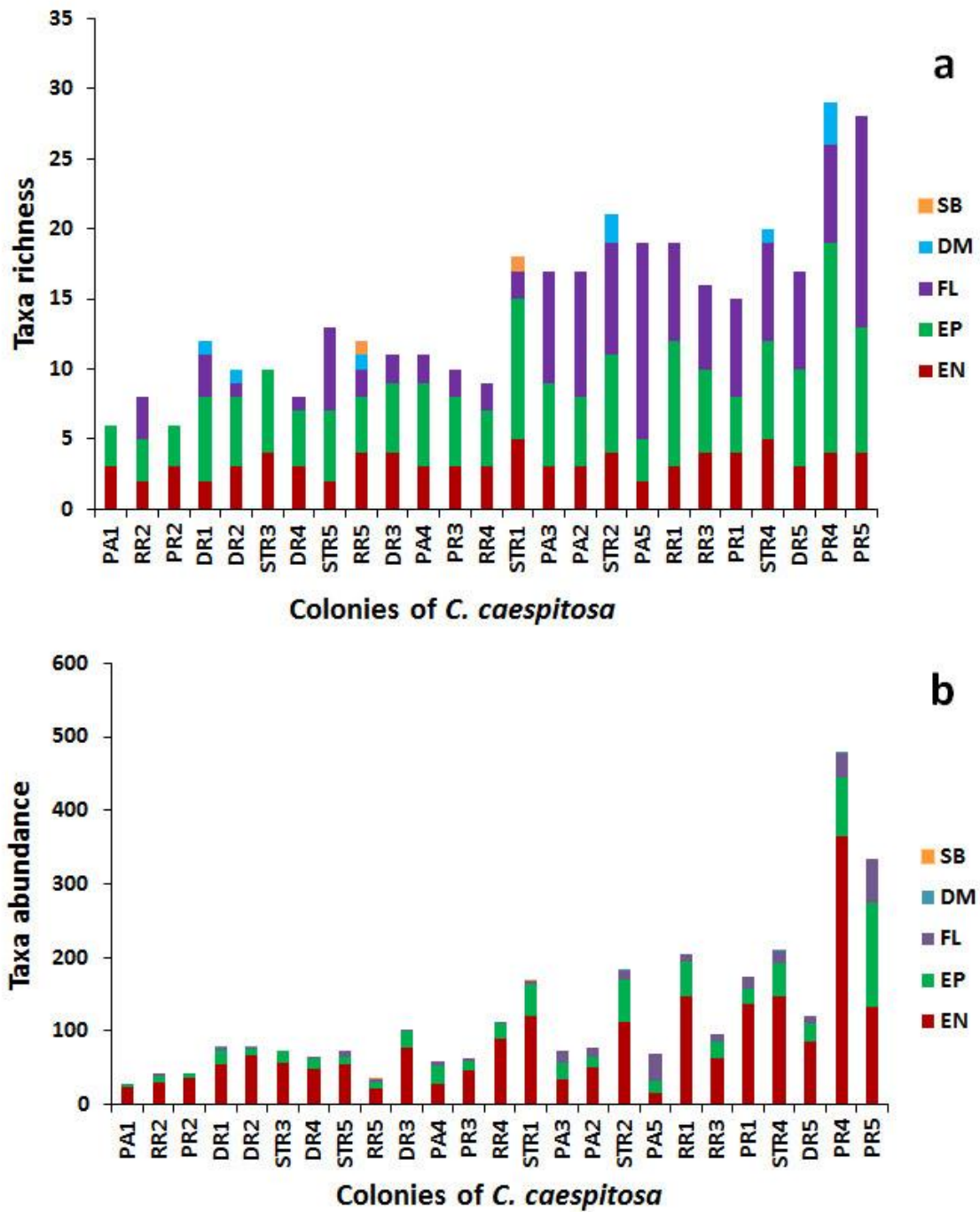


Figure 20: Taxa richness (a) and taxa abundance (b) of functional groups of molluscs for each colony of *C. caespitosa*. Colonies are ordered by size (total volume). FL = free living, motile species, EP = epilithic species, living their entire lives attached to a substrate, EN = endolithic species, living in holes bored in hard substrates, SB = soft bottom dwelling species, ND = no data available.

Slika 20: Število taksonov (a) in abudanca taksonov (b) funkcionalnih skupin mehkužcev za posamezno kolonijo *C. caespitosa*. Kolonije so razvrščene po velikosti (prostornina). FL = prosto živeče, premikajoče se vrste, EP = epilitske vrste, ki živijo vse življenje pritrjene na podlago, EN = endolitske vrste, ki živijo v luknjah na trdih podlagah, SB = vrste mehkega dna, ND = ni razpoložljivih podatkov.

Filter feeders were dominant (Figure 21) considering both abundance (50 %) and taxa richness (91 %), followed by micrograzers (25 % of abundances and 33 % taxa richness). The most frequent and abundant filter feeders were *R. dubia* and *H. arctica*, present in all colonies. The most frequent and abundant micrograzers were *C. olivaceus* and *C. cruciatus*.



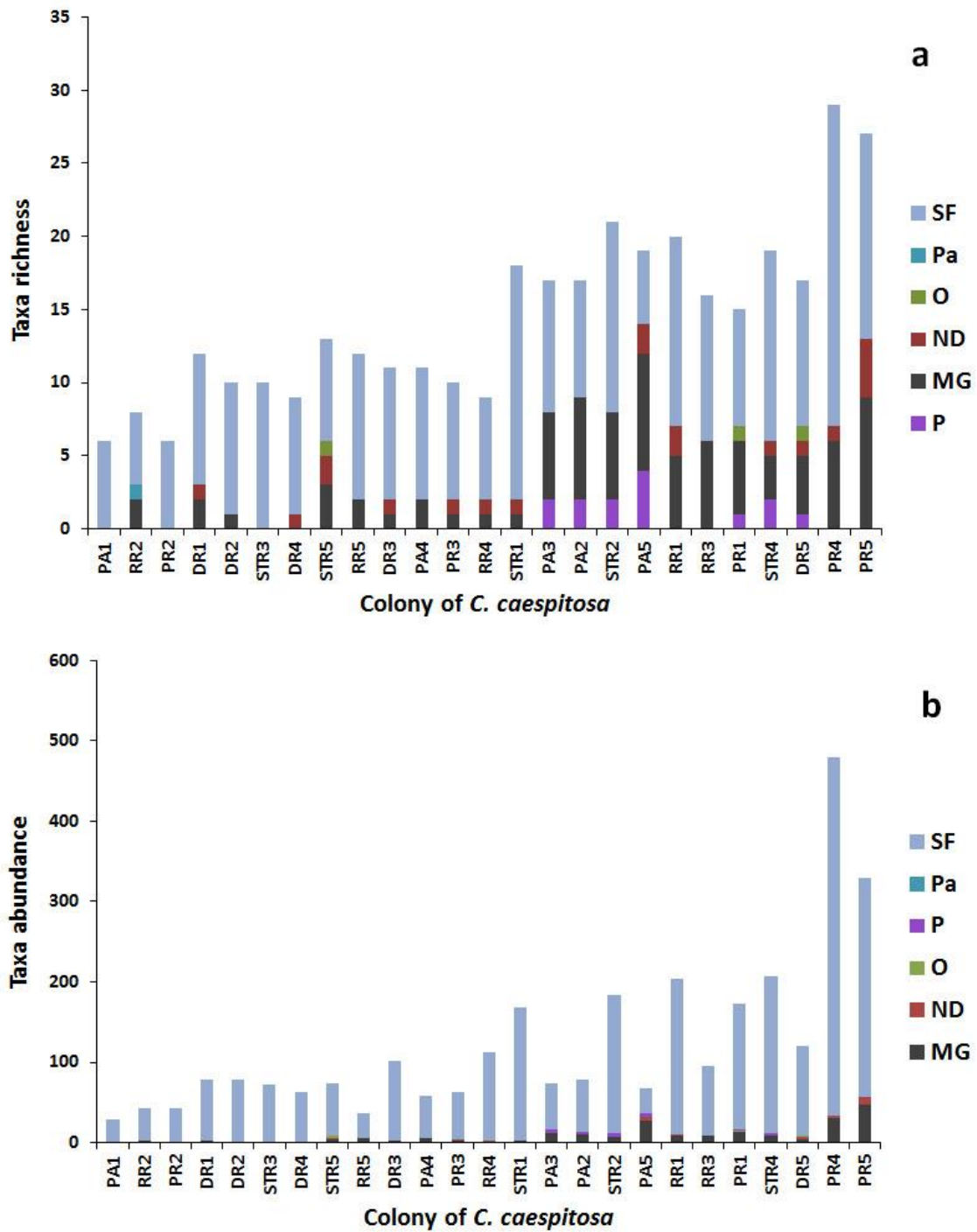


Figure 21: Taxa richness (a) and abundance (b) of feeding guilds of molluscs for each colony of *C. caespitosa*. P = motile predators, Pa = parasites, O = omnivores, SF = suspension feeders capturing seston particles with their gills or with mucous strings, MG = micrograzers feeding on algae, cyanobacteria or detritus attached to algal fronds.

Slika 21: Število taksonov (a) in abundanca (b) taksonov mehkužcev za posamezno kolonijo *C. caespitosa*. P = premikajoči plenilci, Pa = paraziti, O = vsejedi, SF = suspenzijofagi, MG = mikropašni rastlinojedci.

Mollusc assemblages are divided into two groups (Anosim= 0.418, p-value = 0.001), but these groups are not clearly related neither with colony size nor sampling sites (Figure 22).

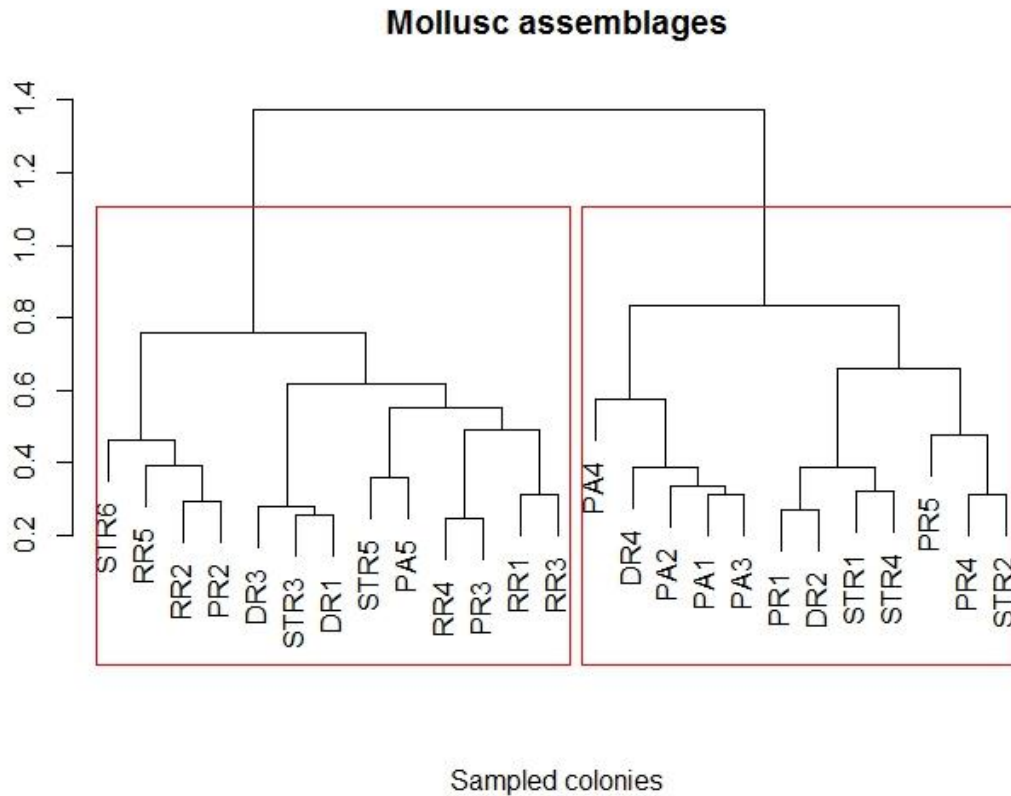


Figure 22: Cluster analysis for log-transformed abundances of molluscs in each colony at the different sampling sites.

Slika 22: Klusterska analiza za-logaritemsko preoblikovano abudanco mehkužcev v posamezni koloniji na različnih vzorčevalnih postajah.

#### 4.1.3.2 Polychaete assemblages

Altogether, 5827 polychaetes belonging to 92 different taxa were counted in the colonies of *C. caespitosa* and 83 of them were determined to the species level.

Three additional taxa belonging to the family Spirorbidae (*Pileolaria militaris*, *Simplaria pseudomilitaris* and *Janua* sp.) were present but they were excluded from calculation. The dominant families were Serpulidae, Eunicidae, Syllidae and Nereididae, accounting altogether for 62 % of polychaetes counted. These families, together with Cirratulidae, Terebellidae and Polynoidae were present in all studied colonies.

The most frequent (>75 % of frequency of occurrence) and abundant (>5 % of dominance) species were *Vermiliopsis striaticeps*, *Pomatoceros triqueter*, *Serpula concharum*, *Eunice torquata*, *Lysidice ninetta*, *L. unicornis*, *Harmothoe areolata*, *Notomastus latericeus*, *Dodecaceria concharum*, *Syllis variegata*. Species of the family Terebellidae were also frequent and abundant.

The Margalef index varied from 3.56 to 7.91, the diversity (Shannon) index varied from 2.03 to 3.36, the Pielou index from 0.70 to 0.91 and the Simpson index from 0.78 to 0.95 (Figure 23).

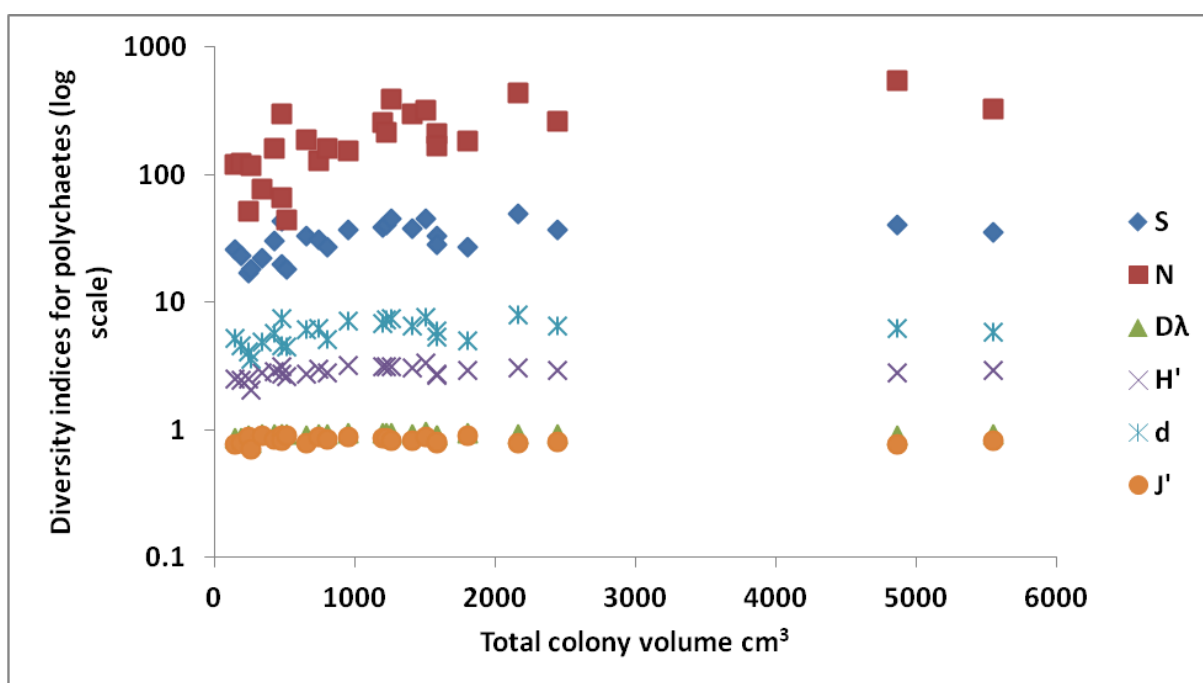


Figure 23: Diversity indices of polychaete assemblages in each *C. caespitosa* colony. Colonies are ordered by size (total volume). Taxa richness (S), taxa abundance (N), Margalef index of richness (d), Shannon and Wiener diversity index (H'), Pielou index of equitability (J') and Simpson index of dominance ( $D_{\lambda}$ ) calculated for each colony.

Slika 23: Diverzitetni indeksi združb mnogoščetincev v posamezni koloniji *C. caespitosa*. Kolonije so razvrščene po velikosti (prostornina). Število taksonov (S), število osebkov (N), Margalefov indeks (d), Shannonov in Wienerjev indeks (H), Pieloujev indeks (J) in Simpsonov indeks ( $D_{\lambda}$ ), izračunani za vsako kolonijo.

Free living animals were dominant (Figure 24) for both richness (56 %) and abundance (48 %), followed by epilithic (15 % of richness and 18 % of abundance) and soft bottom species for richness (15 %) and discretely motile for abundances (18 %). Among free

living species the most frequent and abundant species were *Ceratonereis costae*, *Nereis rava* and *Syllis variegata*, among epilithic were *Serpula concharum*, *Pomatoceros triqueter* and *Vermiliopsis striaticeps*, among soft bottom dwellers were *Cirriformia tentaculata*, *Lumbrineris latreilli* and *Scoletoma impatiens*, and among discretely motile were *Eunice torquata*, *E. vittata* and *Notomastus latericeus*.

Free living and discretely motile organisms are related to colony size for both richness and abundance (Annex C). Abundance of soft bottom species is related with colony size, but their richness is better related with the presence of mud trapped in the colony (Annex C).

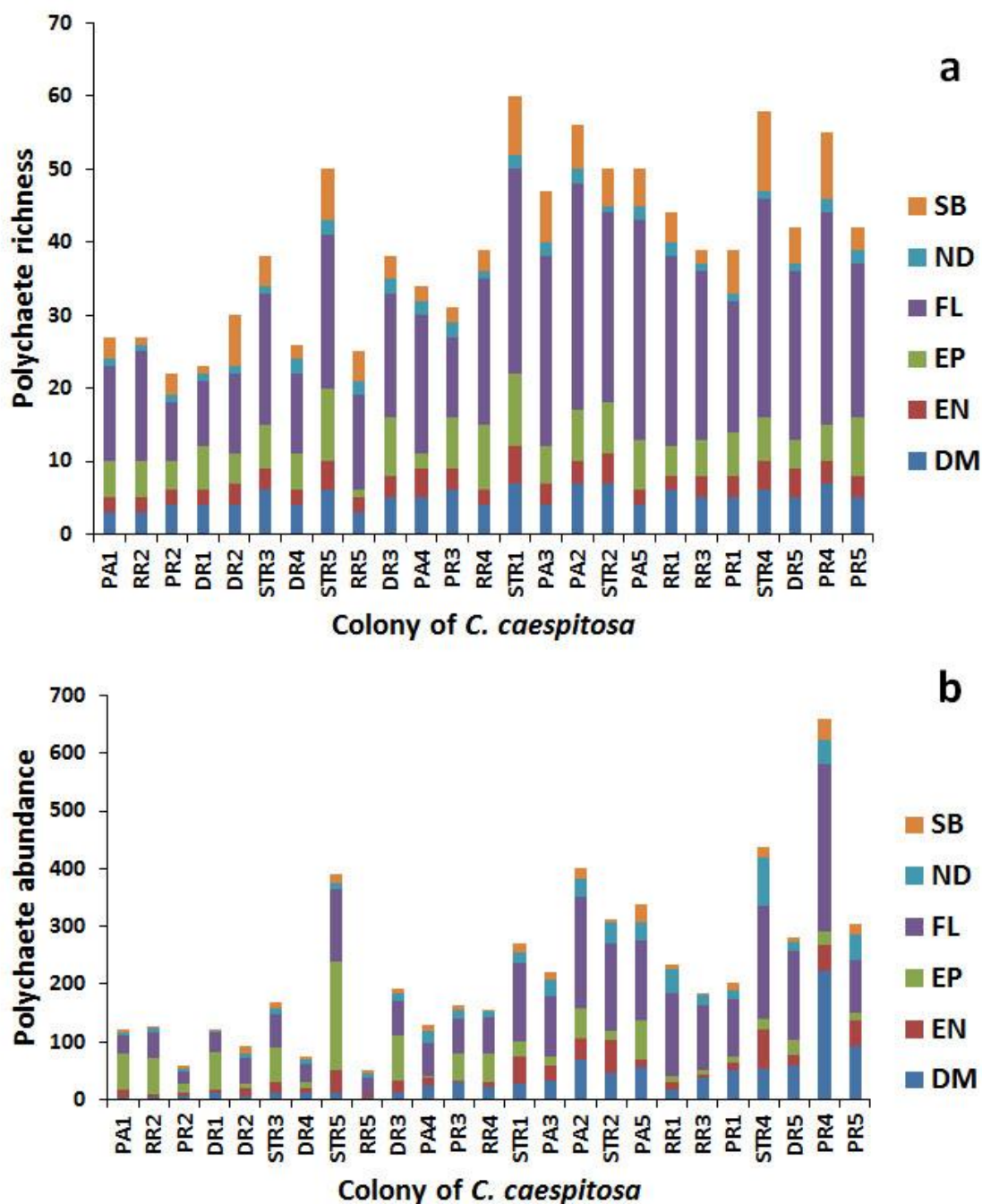


Figure 24: Richness (a) and abundances (b) of functional groups of polychaetes for each colony of *C. caespitosa*. Colonies are ordered by size (total volume). FL = free living, motile species, EP = epilithic species, living their entire lives attached to a substrate, EN = endolithic species, living in holes bored in hard substrates, SB = soft bottom dwelling species, DM = discretely motile species; ND = no data available

Slika 24: Število taksonov (a) in abundanca (b) taksonov funkcionalnih skupin mnogoščetincev za posamezno kolonijo *C. caespitosa*. Kolonije so razvrščene po velikosti (volumen). FL = prosto živeče, premikajoče se vrste, EP = epilitske vrste, ki živijo vse življenje pritrjene na podlago, EN = endolitske vrste, ki živijo v luknjah na trdih podlagah, SB = vrste mehkega dna, ND = ni razpoložljivih podatkov.

Carnivores were dominant (Figure 25) considering both abundance (58 %) and taxa richness (56 %), followed by deposit feeders (23 % of abundance and 24 % of taxa richness) and filter feeders (16 % of abundance and 11 % taxa richness). The most frequent and abundant carnivores were *E. vittata*, *C. costae*, *N. rava* and *S. variegata*. The most frequent and abundant deposit feeders were *N. latericeus* and terebellids, while the most frequent and abundant filter feeders were *S. concharum*, *P. triqueter* and *V. striaticeps*.

Abundance and taxa of carnivores and deposit feeders were positively correlated with colony size (Annex C).

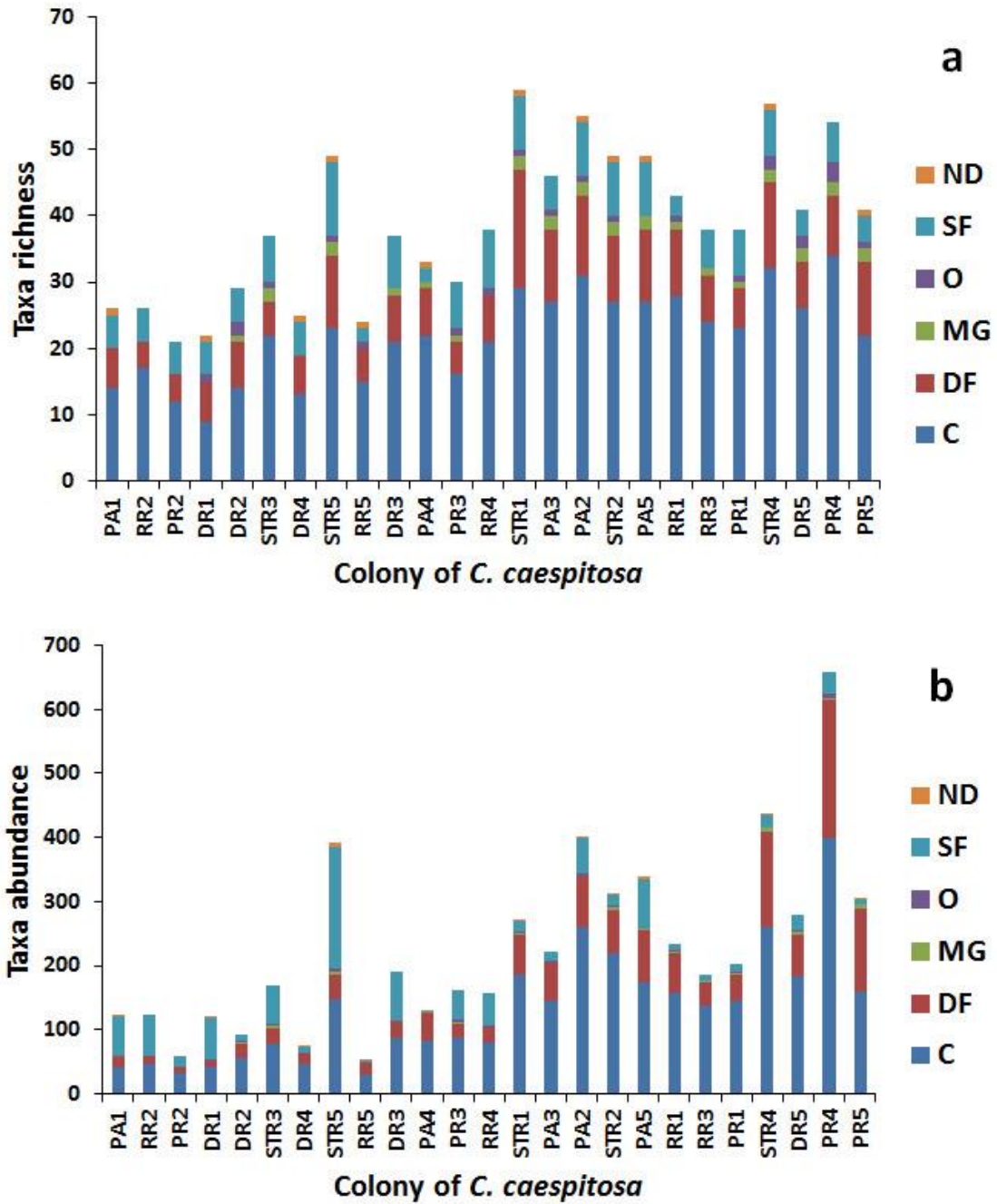


Figure 25: Taxa richness (a) and abundance (b) of feeding guilds of polychaetes for each colony of *C. caespitosa*. C = motile predators, DF= deposit feeders, O = omnivores, SF = suspension feeders capturing seston particles with their gills or with mucous strings, MG = micrograzers feeding on algae, cyanobacteria or detritus attached to algal fronds, ND = no data available.

Slika 25: Število taksonov (a) in abundanca (b) taksonov mnogoščetincev za posamezno kolonijo *C. caespitosa*. C = premikajoči se plenilci, DF = detritivori, O = omnivori, SF = suspenzijofagi, MG = mikropašni rastlinojedci, ND = ni razpoložljivih podatkov.

Polychaetes assemblages are clearly divided into two groups, one with assemblages inhabiting colonies smaller than 1 dm<sup>3</sup> and another found in bigger colonies (Figure 26). Samples from sites STR5 and PA4 are exceptions. This separation in groups is due mainly to the following species: *Notomastus latericeus*, *Syllis ferrani*, *S. gerlachi*, *Serpula concharum*, *Nematoneis unicornis* and *Lysidice ninetta*. The differences are not only due to different abundances, but *N. latericeus*, *S. ferrani* and *S. gerlachi* are much more frequent (up to 100 %) in bigger colonies than in smaller ones (at around 50-60 %).

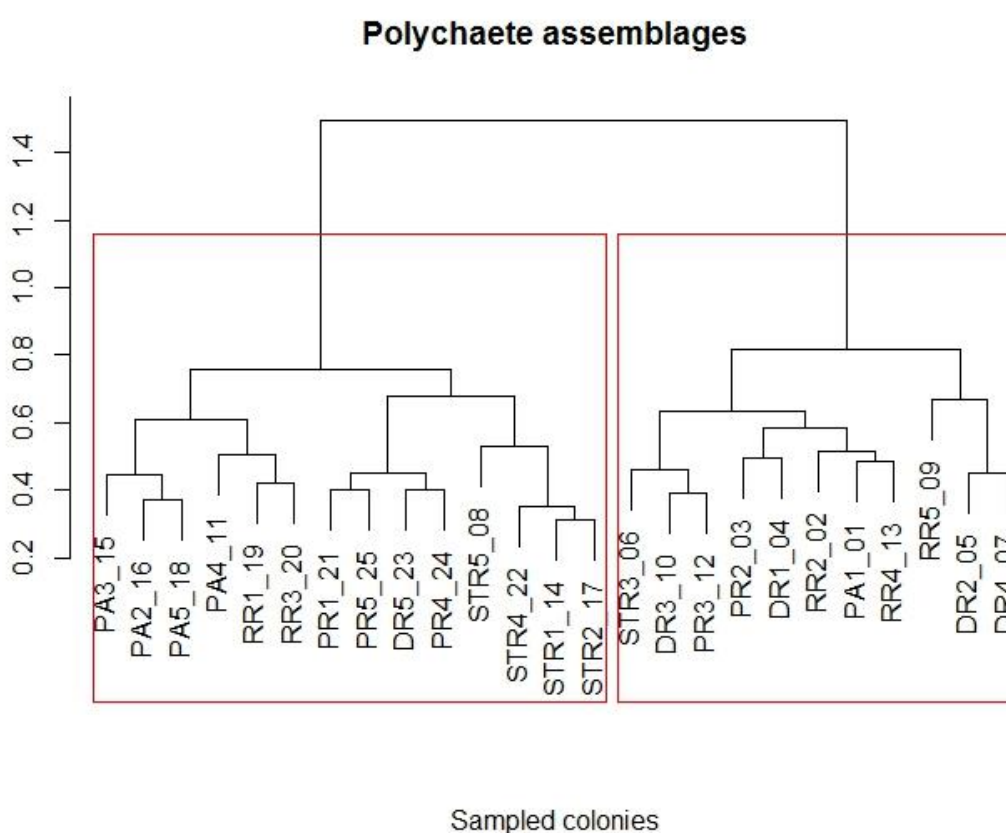


Figure 26: Cluster analysis for log-transformed abundances of polychaetes in each colony at the different sampling sites. Each colony is numbered according to size.

Slika 26: Klusterska analiza za-log preoblikovano abundanco mnogoščetincev v posamezni koloniji na različnih vzorčevalnih postajah. Vsaka kolonija je oštevilčena glede na velikost.



#### 4.1.3.3 Crustacean assemblages

Altogether, 2135 crustaceans belonging to 43 different taxa (20 amphipods, 15 decapods, 3 isopods, 3 tanaidaceans, 1 cirriped, 1 mysid and 1 cumacean) were counted in the colonies of *C. caespitosa* and 37 of them were determined to the species level.

Decapods and amphipods were dominant in term of abundance (1630 decapods, 193 amphipods) and richness (15 decapods, 19 amphipods), respectively. Altogether they accounted for 85 % of crustaceans counted and 83 % of taxa.

The most frequent (>75 % of frequency of occurrence) and abundant (>5 % of dominance) species were *Athanas nitescens*, *Pilumnus hirtellus*, *Alpheus dentipes*, *Eualus cranchii* and *Pisidia longimana*.

Richness (S) and abundance (N) increased with colony size (Figure 27). The same was observed considering decapods and amphipods separately (Figure 27).

The Margalef index varied from 1.03 to 3.43 from colonies collected at site RR. The diversity (Shannon) index varied from 1.16 to 2.35 at site RR, and the Simpson index from 0.58 at RR to 0.88 at site STR. The Pielou index varied from 0.66 to 0.95 at site STR, without relation with colony size (Figure 27).

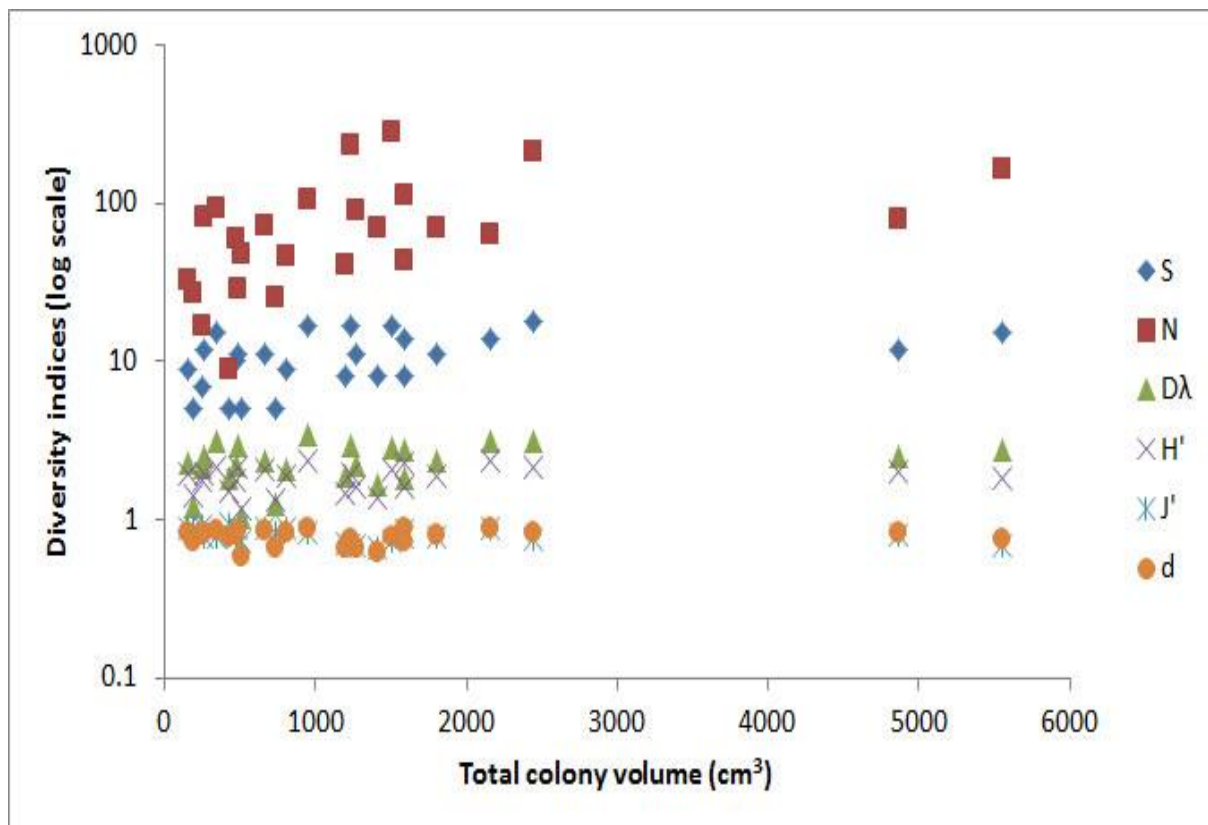


Figure 27: Diversity indices of crustacean assemblages in each *C. caespitosa* colony. Colonies are ordered by size (total volume). Margalef index of richness (d), Shannon and Wiener diversity index ( $H'$ ), Pielou index of equitability ( $J'$ ) and Simpson index of dominance ( $D_\lambda$ ) calculated for each colony.

Slika 27: Diverzitetni indeksi združb rakov v posamezni koloniji *C. caespitosa*. Kolonije so razvrščene po velikosti (prostornina). Margalefov indeks (d), Shannonov in Wienerjev indeks (H), Pieloujev indeks (J) in Simpsonov indeks ( $D_\lambda$ ), izračunani za vsako kolonijo.

All crustaceans were free living with the exception of the isopod *Gnathia vorax*, which is ectoparasitic and cirriped *Balanus trigonus*, which is sessile.

Suspension feeders and omnivores were dominant considering both abundance (43 % for omnivores, 31 % for suspension feeders) and taxa richness (28 % for suspension feeders and 26 % for omnivores). Taxa richness of deposit feeders was also consistent (22 % of taxa richness), in particular in some colonies (Figure 28). The most frequent and abundant omnivores were the decapods *Alpheus dentipes* and *Pilumnus hirtellus*. The most frequent and abundant deposit feeder was *Liljeborgia dellavallei*, the most frequent and abundant filter feeders were *Parapseudes latifrons*, *Pisidia* sp. and *Leptochelia savignyi*.

Taxa richness of omnivores, deposit feeders and suspension feeders were positively correlated with colony size (annex C).

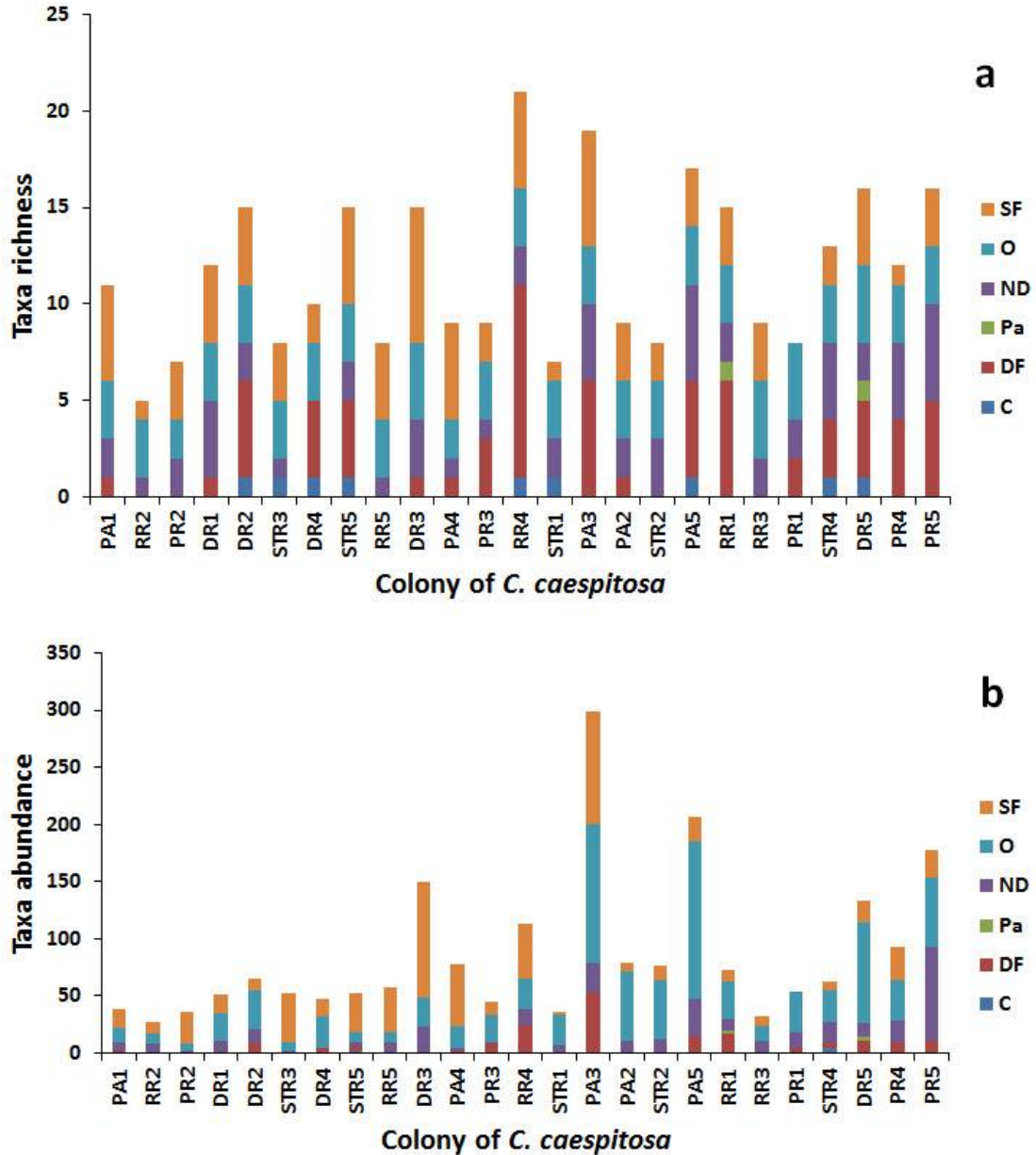


Figure 28: Taxa richness (a) and abundance (b) of feeding guilds of crustaceans for each colony of *C. caespitosa*. C = motile predators, DF = deposit feeders, O = omnivores, SF = suspension feeders capturing seston particles with their gills or with mucous strings, Pa = ectoparasites, ND = no data available.

Slika 28: Števila taksonov (a) in abundanca (b) prehranjevalnih skupin rakov za vsako kolonijo *C. caespitosa*. C = premikajoči se plenilci, DF = detritivori, Pa = paraziti, O = omnivori, SF =suspenzijofagi, MG = mikropašni rastlinojedci, ND = ni razpoložljivih podatkov.

Crustacean assemblages are divided into two groups (Anosim= 0.330, p-value=0.001), but these groups are not clearly related neither with colony size nor sampling sites (Figure 29).

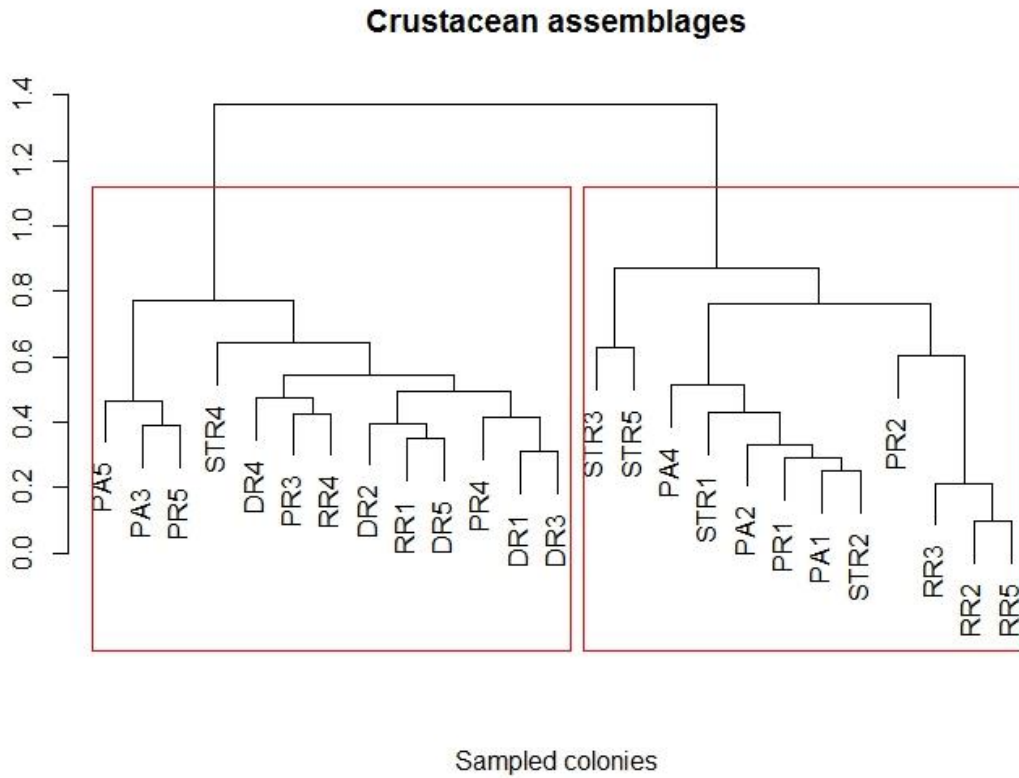


Figure 29: Cluster analysis for log-transformed abundances of crustaceans in each colony at the different sampling sites.

Slika 29: Klasterška analiza za-log preoblikovano abundanco rakov v psosomezni koloniji na različnih vzorčevalnih postajah.

## 4.2 MESOSCALE

### 4.2.1 Comparison of methods used

The comparison between the two techniques showed that despite the bigger number of samples analysed, the total number of taxa identified with the photographic technique was lower than the one obtained with the underwater method (46 against 61 taxa) and this difference increase considering only lower taxonomical levels, such genus or species (Table 4). Considering all species recorded for mesoscale 46 % of them were recorded with both methods, 48 % with underwater counting and 6 % only with photo analysis. For half of the species recorded only with underwater counting, determination was performed in laboratory and 40 % of them have been recorded also at level of microscale.

Species accumulation curves for megabenthic invertebrates recorded with underwater visual counting (Figure 30) tended to a horizontal asymptote when all collected samples were used, but not when a curve was drawn for each sampling sites.

Data recorded from a larger number of quadrats with photographic techniques (Figure 31) suggested that samples collected with underwater counting were not numerous enough to get a representative picture of each site, and thus enabling a comparison. In fact, even if cumulative curves seemed similar, some differences among sampling site could be masked by the limited number of sample collected. Cumulative curves obtained from pictures in fact were long enough to enable discrimination between sites: site RR showed the highest richness (> 40 taxa) compared with other sites (< 30 taxa).

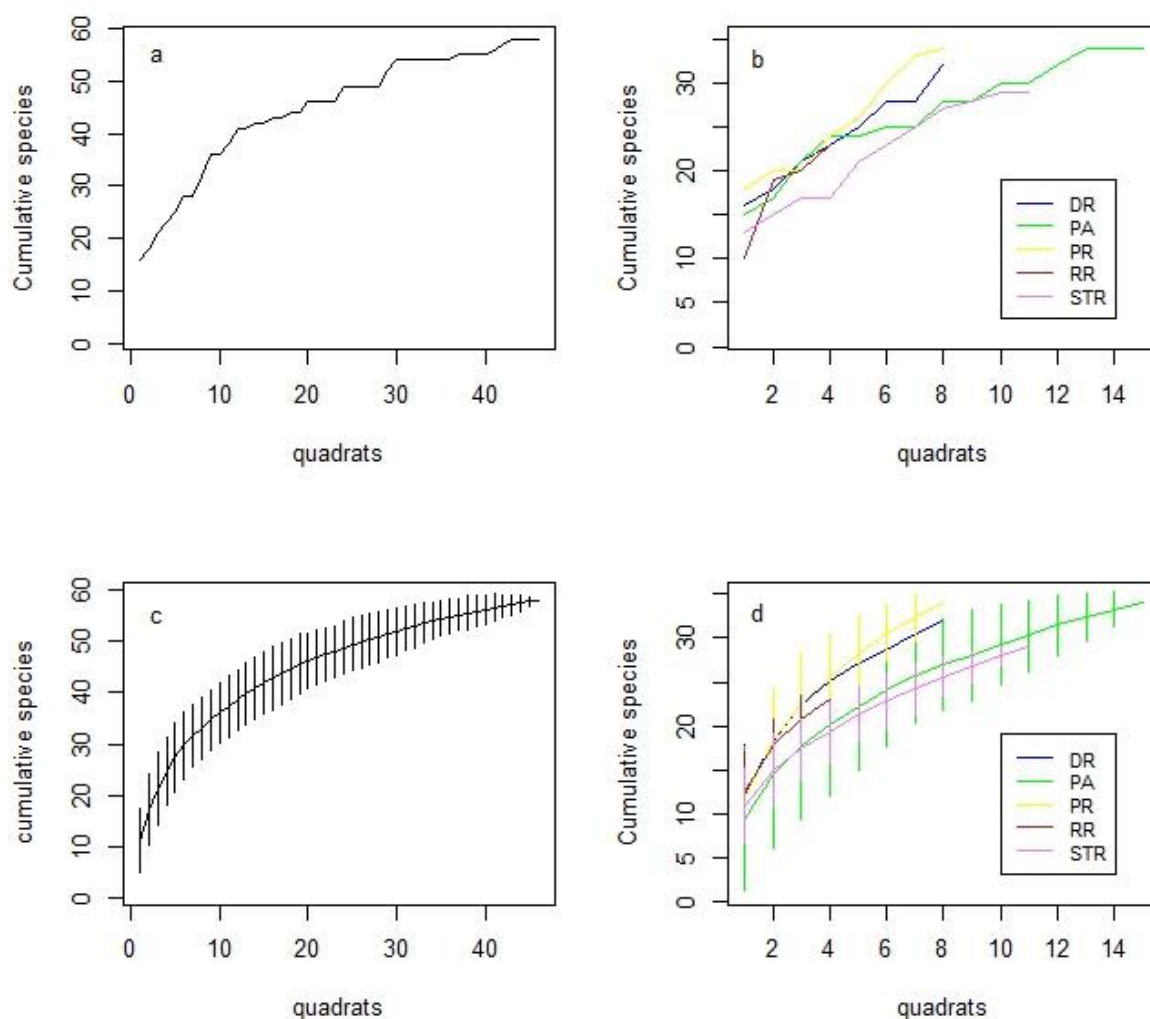


Figure 30: Cumulative curves according to the original sequence of recording (a, b) and from random order of quadrats, (c, d), for the entire dataset (a, c) and for each site separately (b, d). Vertical lines = SD. Data were recorded in situ with underwater visual counting.

Slika 30: Kumulativne krivulje v vrstnem redu pojavljanja (a, b) in v naključnem vrstnem redu (c, d), za celotno bazo podatkov (a, c) in za vsako postajo posebej (b, D). Navpične črte = standardni odkloni. Podatki so bili pridobljeni s podvodno opazovalno metodo.

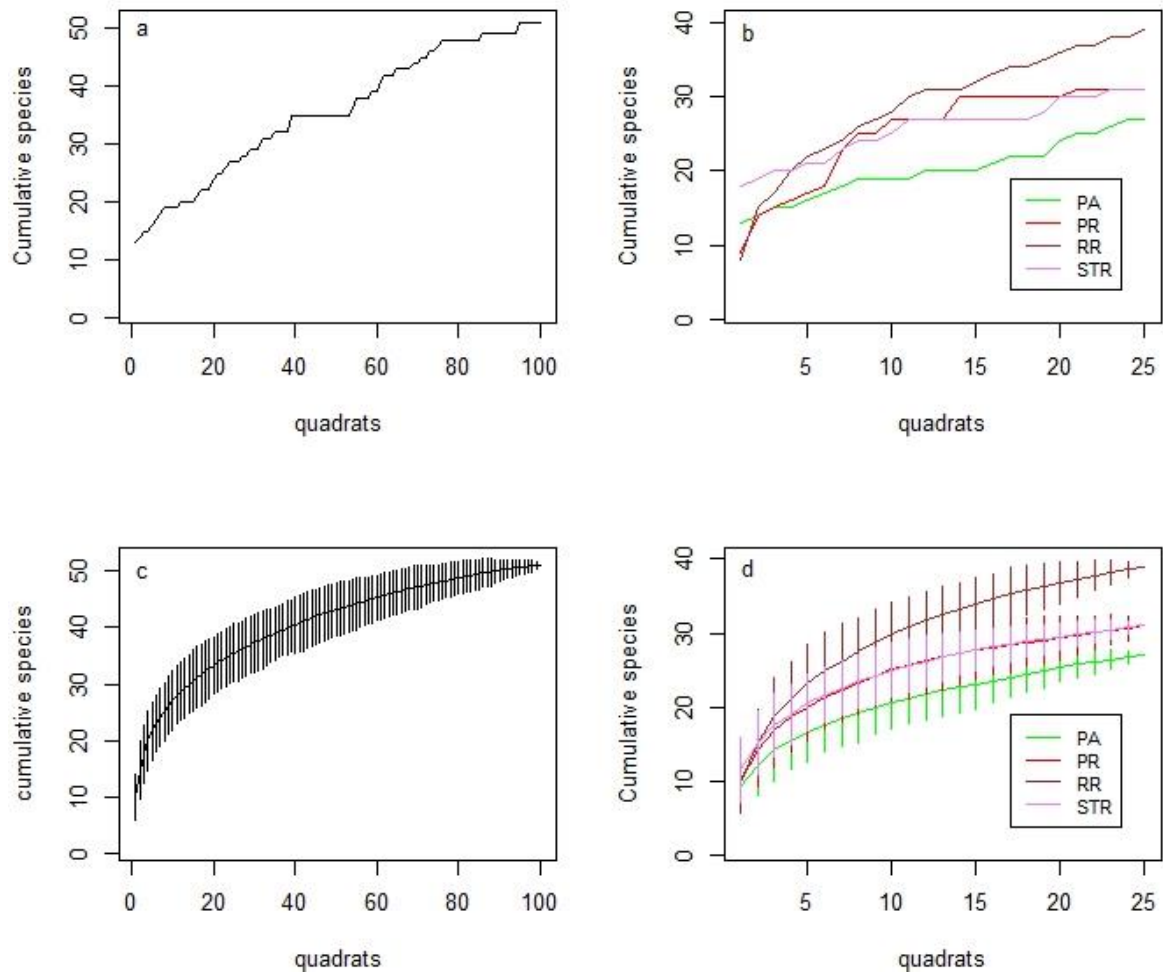


Figure 31: Cumulative curves for benthic invertebrates according to the original sequence of recording (a,b) and from random order of quadrats (c,d), for the entire dataset (a,c) and for each site separately (b,d). Data were obtained from photographs.

Slika 31: Kumulativne krivulje za število taksonov bentoških nevretenčarjev v vrstnem redu pojavljanja (a, b) in v naključnem redu (c,d), na vseh lokalitetah (a,c) in na posameznih lokalitetah (b,d). Podatki so bili pridobljeni s fotografsko metodo.

Table 4: List of megabenthic taxa found with the two methods.

Preglednica 4: Seznam pridnenih taksonov nevretenčarjev z uporabo dveh metod.

<b>Underwater counting</b>	<b>Photo analysis</b>
<i>Aiptasia mutabilis</i>	<i>Aiptasia mutabilis</i>
<i>Anomia ephippium</i>	Anemonia sp.
Anomura	Anomura
<i>Aplysina aerophoba</i>	<i>Aplysina aerophoba</i>
<i>Arcae noae</i>	<i>Arca noae</i>
<i>Balanophyllia italica</i>	Ascidia 1
<i>Barbatia barbata</i>	Ascidia 2 (colonial)
<i>Bittium reticulatum</i>	<i>Balanophyllia europaea</i>
Bivalvia	<i>Balanus</i> sp.
<i>Bolma rugosa</i>	<i>Bolma rugosa</i>
Bryozoa	Bryozoa
<i>Cacospongia scalaris</i>	<i>Cerithium</i> sp.
<i>Cerithium vulgatum</i>	<i>Cerithium vulgatum</i>
<i>Chiton olivaceus</i>	Dyctioceratida indet
<i>Chlamys glabra</i>	<i>Chondrilla nucula</i>
<i>Chlamys varia</i>	<i>Chondrosia reniformis</i>
<i>Chondrilla nucula</i>	<i>Cladocora caespitosa</i>
<i>Chondrosia reniformis</i>	<i>Cliona viridis</i>
<i>Cladocora caespitosa</i>	<i>Cliona celata</i>
<i>Clanculus cruciatus</i>	<i>Cliona schmidtii</i>
<i>Cliona celata</i>	Buccinoidea
<i>Cliona schmidtii</i>	<i>Conus mediterraneus</i>
<i>Columbella rustica</i>	<i>Diplosoma spongiforme</i>
<i>Conus mediterraneus</i>	<i>Eriphia spinifrons</i>
<i>Cucumaria planci</i>	<i>Hexaplex trunculus</i>
Decapoda	<i>Holothuria tubulosa</i>
Dyctioceratida indet	<i>Ircinia variabilis</i>
<i>Eudendrium</i> sp.	<i>Lithophaga lithophaga</i>
<i>Euthria (Buccinulum) cornea</i>	<i>Microcosmos</i> sp.
<i>Flabellina ischitana</i>	<i>Nassarius</i> sp.
Gastropod	<i>Ostra edulis</i>
<i>Hexaplex trunculus</i>	Natantia
<i>Holothuria tubulosa</i>	<i>Pinna nobilis</i>
<i>Ircinia variabilis</i>	<i>Pomatoceros triqueter</i>
<i>Jujubinus exasperatus</i>	Porifera indet1
<i>Lithophaga lithophaga</i>	Porifera indet2
<i>Maja crispata</i>	Porifera ident3
<i>Microcosmus sulcatus</i>	<i>Rocellaria dubia</i>

Continued



Continuation from Table 4. List of megabenthic taxa found with the two methods.

<b>Underwater counting</b>	<b>Photo analysis</b>	
<i>Modiolus barbatus</i>	Sabellidae	
<i>Muricopsis cristatus</i>	<i>Serpula vermicularis</i>	
<i>Nassarius incrassatus</i>	Serpulidae	
<i>Ophioderma longicauda</i>	<i>Serpulorbis arenaria</i>	
<i>Ophiothrix</i> spp.	<i>Sphaerechinus granularis</i>	
<i>Oscarella lobularis</i>	Spirorbidae	
<i>Ostrea edulis</i>	Venus verrucosa	
<i>Phallusia fumigata</i>		
<i>Pinna nobilis</i>		
<i>Pomatoceros triqueter</i>		
<i>Protula tubularia</i>		
<i>Rocellaria dubia</i>		
<i>Sarcotragus spinolosus</i>		
<i>Schizoporella sanguinea</i>		
<i>Serpula vermicularis</i>		
Serpulidae indet		
<i>Serpulorbis arenaria</i>		
Spirorbidae indet		
<i>Tethya aurantium</i>		
Trochidae indet		
Tunicata cf. Ascidia		
<i>Thuridilla hopei</i>		
<i>Venus verrucosa</i>		
<b>61</b>	<b>45</b>	<b>Total taxa</b>
<b>54</b>	<b>35</b>	<b>Family level</b>
<b>50</b>	<b>31</b>	<b>Genus level</b>
<b>48</b>	<b>26</b>	<b>Species level</b>

#### 4.2.2 SAR

The two methods gave different results in terms of estimation of species richness. Using data obtained with underwater counting with extrapolation techniques we obtained an expected total number of taxa ranging from 64 to 74, according to the different functions used (Table 5). Calculation based on photographic technique gave values about 8-10 taxa lower for total richness.

Extrapolation of taxa richness for each site applied to data obtained with underwater counting (Table 5) showed site RR as the poorest in terms of species richness, while using the photographic technique it resulted the richest (Table 6).

Table 5: Estimates of species richness for each site and for the whole area using different functions (underwater counting).

Preglednica 5: Ocene vrstne pestrosti za posamezno lokaliteto in za celotno območje z uporabo različnih funkcij (podvodna štetja).

Site	Species	Chao	chao.se	jack1	jack1.se	jack2	boot	boot.se	n
<b>DR</b>	32	53	17.75	43	5.17	50	37	2.63	8
<b>PA</b>	34	60	21.66	46	5.12	55	39	2.66	15
<b>PR</b>	34	48	10.33	46	5.63	53	39	2.95	8
<b>RR</b>	23	31	6.90	30	4.31	33	26	2.31	4
<b>STR</b>	29	62	30.42	40	3.92	48	34	1.92	11
<b>All</b>	58	66	6.01	71	4.04	74	64	2.44	46

Table 6: Estimates of species richness for each site and for the whole area using different functions (photographic technique). Abbreviation at p. XIV.

Preglednica 6: Ocene vrstne pestrosti za posamezno lokaliteto in za celotno območje z uporabo različnih funkcij (fotografska metoda). Okrajšave na str. XIV.

Site	Species	chao	chao.se	jack1	jack1.se	jack2	boot	boot.se	n
<b>PA</b>	27	37.24	9.880	34.68	2.715	39.395	30.35	1.571	25
<b>PR</b>	31	42.76	12.64	37.72	3.494	42.396	33.94	1.889	25
<b>RR</b>	39	44.55	4.865	47.64	3.195	49.748	43.30	1.910	25
<b>STR</b>	31	42.76	12.646	37.72	2.539	42.396	33.96	1.453	25
<b>All</b>	51	58.48	5.905	61.89	3.572	64.909	56.33	2.069	100

#### 4.2.3 Megabenthic assemblages

With underwater visual counting a total of 61 different taxa were found: 23 molluscs, 11 sponges, 4 echinoderms, 4 cnidarians, 3 polychaetes, 3 crustaceans, 3 tunicates, 2 bryozoans. A total of 6764 invertebrates and 1 fish were counted and 48 taxa were

determined to the species level. Molluscs were the most rich (47 %) and abundant (79 %) taxa, followed by sponges (19 % of taxa and 16 % of abundance) (Figure 32). With the photographic technique molluscs and sponges were confirmed as the richest groups. Additionally, three more species of invertebrates and four fish (*Serranus scriba*, *Serranus hepatus*, *Syngnathus acus* and *Parablennius rouxi*) were observed, for a total of 55 different species.

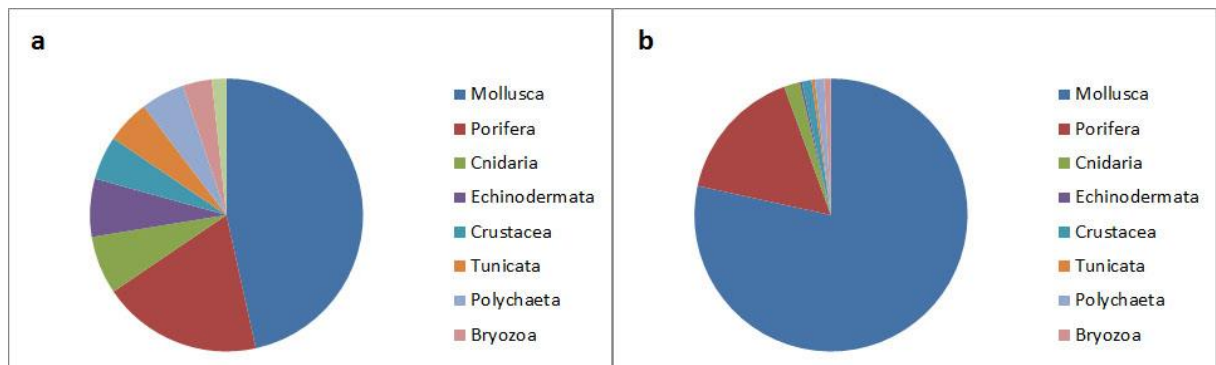


Figure 32: Average percentage of richness (a) and abundance (b) of different phyla detected with underwater counting. For colonial organisms colonies were counted.

Slika 32: Delež števila vrst (a) in abundance (b) različnih živalskih debel, ugotovljenih s podvodno metodo popisovanja. Pri kolonialnih organizmih smo šteli število kolonij.

The most frequent and abundant species were the bivalve *Rocellaria dubia* (98 % of frequency of occurrence) and the polychaete *Pomatoceros triqueter* (76 %), followed by the solitary coral *Balanophylla europea*, (70 %) the sponge *Aplysina aerophoba* (65 %), pagurids (67 %) and molluscs *Cerithium vulgatum* (65 %) and *Hexaplex trunculus* (50 %). All these species were found at all study sites (Table 7). The only species frequent at some sites (PR and PA) and almost absent at other (RR and STR), was the sponge *Chondrilla nucula*.

Table 7: Frequency of occurrence (%) of recorded invertebrate taxa using underwater visual count at the five studied sites (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Cape Ronsek – RR and Strunjanček- STR).

Preglednica 7: Frekvenca pojavljanja (%) nevretenčarskih taksonov z uporabo podvodnega popisa na petih vzorčevalnih lokacijah (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Rt Ronsek - RR in Strunjanček - STR).

<b>Taxa</b>	<b>PR</b>	<b>STR</b>	<b>DR</b>	<b>PA</b>	<b>RR</b>	<b>n. of sites</b>
<i>Anomura</i> indet.	63	55	88	67	75	5
<i>Aplysina aerophoba</i>	75	91	88	20	75	5
<i>Balanophyllia europea</i>	50	91	63	80	25	5
<i>Bolma rugosa</i>	25	45	50	33	75	5
<i>Cerithium vulgatum</i>	88	27	63	80	75	5
<i>Pomatoceros triqueter</i>	75	100	38	73	100	5
<i>Rocellaria dubia</i>	88	100	100	93	100	5
<i>Schizobrachiella sanguinea</i>	25	91	25	20	75	5
<i>Serpula vermicularis</i>	50	27	13	20	75	5
<i>Chiton olivaceus</i>	13	0	13	13	25	4
<i>Chondrosia reniformis</i>	75	9	63	20	0	4
<i>Cliona celata</i>	13	82	0	60	25	4
<i>Columbella rustica</i>	0	9	38	33	75	4
<i>Hexaplex trunculus</i>	63	73	50	40	0	4
<i>Holothuria tubulosa</i>	25	27	75	20	0	4
<i>Jujubinus exasperatus</i>	13	18	0	47	50	4
Serpulidae indet.	38	0	13	7	50	4
<i>Serpulorbis arenaria</i>	25	45	50	0	25	4
<i>Arca noae</i>	50	0	0	27	50	3
<i>Bittium</i> spp.	13	0	0	7	25	3
<i>Cacospongia scalaris</i>	25	27	0	0	100	3
<i>Chondrilla nucula</i>	88	0	25	40	0	3
Dyctioceratida indet.	0	9	0	7	25	3
<i>Phallusia fumigata</i>	13	9	50	0	0	3
<i>Protula</i> spp.	13	0	63	0	25	3
<i>Turridilla hopei</i>	0	9	13	7	0	3
<i>Venus verrucosa</i>	0	9	13	7	0	3
<i>Aiptasia mutabilis</i>	13	0	13	0	0	2
<i>Anomia ephippium</i>	0	0	13	7	0	2
Bivalvia indet.	0	9	0	7	0	2
Bryozoa indet.	13	9	0	0	0	2
<i>Chlamys varia</i>	13	9	0	0	0	2
<i>Clanculus cruciatus</i>	13	0	0	7	0	2
<i>Cliona schmidti</i>	13	0	0	13	0	2
<i>Conus mediterraneus</i>	0	0	0	33	50	2

Continued

Continuation of table 7: Frequency of occurrence (%) of recorded invertebrate taxa using underwater visual count at the five studied sites (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Cape Ronek – RR and Strunjanček- STR).

Nadaljevanje tabele 7

<b>Taxa</b>	<b>PR</b>	<b>STR</b>	<b>DR</b>	<b>PA</b>	<b>RR</b>	<b>n. of sites</b>
<i>Lithophaga lithophaga</i>	13	0	0	7	0	2
<i>Microcosmos</i> spp.	0	9	38	0	0	2
<i>Nassarius incrassatus</i>	25	0	0	7	0	2
<i>Oscarella lobularis</i>	0	0	0	7	25	2
Spirorbidae indet.	0	27	13	0	0	2
<i>Tethya aurantium</i>	0	0	25	13	0	2
Tunicata cf. <i>Ascidia</i>	13	9	0	0	0	2
<i>Barbatia barbata</i>	0	0	13	0	0	1
<i>Chlamys glabra</i>	0	0	13	0	0	1
<i>Cucumaria planci</i>	0	0	13	0	0	1
Decapoda indet.	0	9	0	0	0	1
<i>Eudendrium</i> sp.	13	0	0	0	0	1
<i>Euthria (Buccinulum) cornea</i>	13	0	0	0	0	1
<i>Flabellina ischitana</i>	13	0	0	0	0	1
Gastropoda indet.	13	0	0	0	0	1
<i>Ircinia variabilis</i>	0	45	0	0	0	1
<i>Maja verrucosa</i>	0	27	0	0	0	1
<i>Modiolus barbatus</i>	0	0	38	0	0	1
<i>Muricopsis cristatus</i>	0	0	0	7	0	1
<i>Ophioderma longicauda</i>	13	0	0	0	0	1
<i>Ophiothrix</i> spp.	0	0	0	7	0	1
<i>Ostrea edulis</i>	0	9	0	0	0	1
<i>Pinna nobilis</i>	13	0	0	0	0	1
<i>Sarcotragus spinolosus</i>	0	0	50	0	0	1
Trochidae indet.	0	0	13	0	0	1

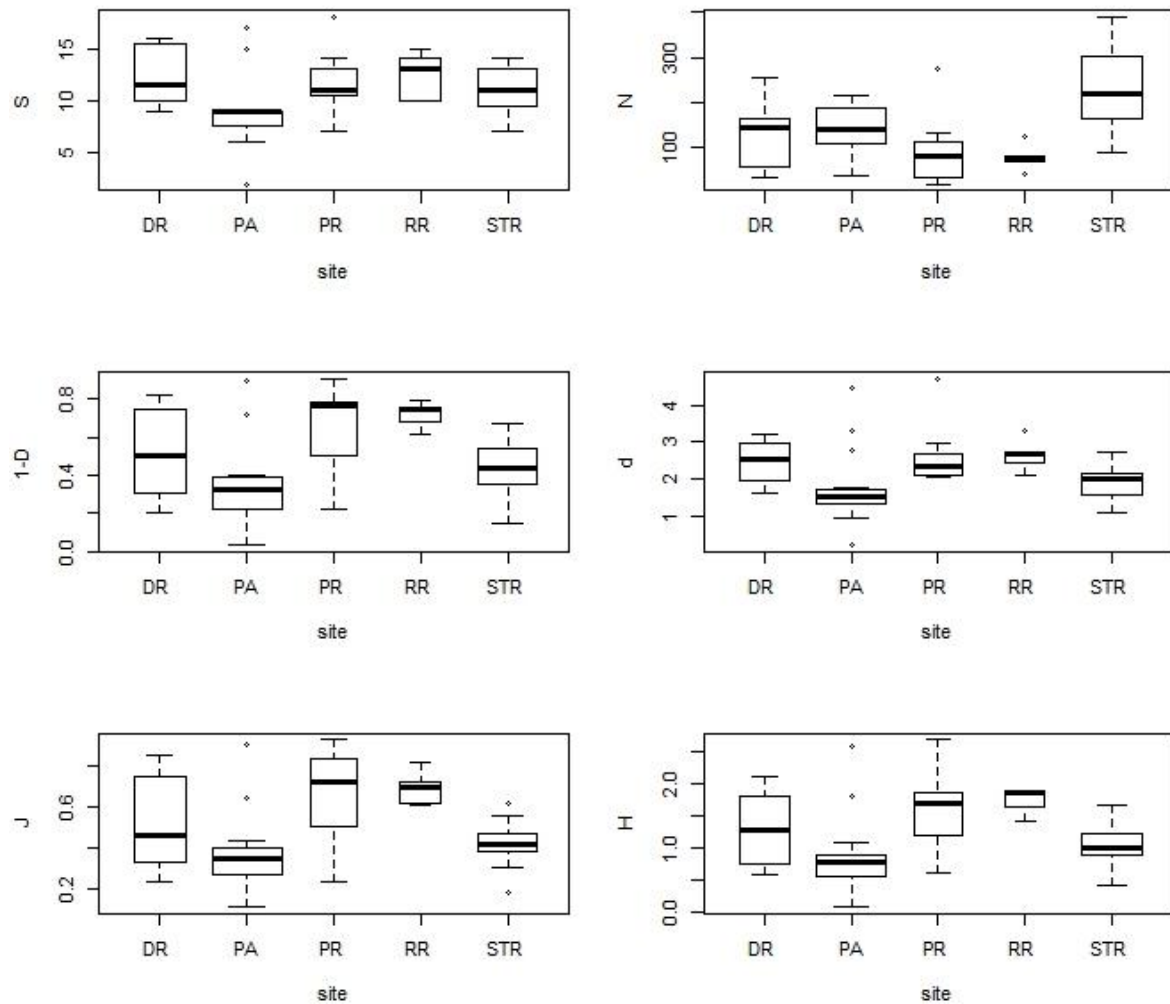


Figure 33: Variation of diversity indices among different sampling sites. Margalef index (d), Shannon diversity index ( $H'$ ), Pielou index ( $J'$ ) and Simpson index ( $D_\lambda$ ).

Slika 33: Spremenljivost različnih diverzitetnih indeksov na različnih postajah. Margalefov indeks (d), Shannonov indeks (H), Pieloujev indeks (J) in Simpsonov indeks ( $D_\lambda$ ).

Species richness did not varied significantly among sampling sites (KW chi-squared = 8.5698, df = 4, p-value = 0.073), but a variation was observed for abundances (KW chi-squared = 16.287, df = 4, p-value = 0.0026), and consequently in diversity indices (Figure 33). The absence of variability in species richness among sites was confirmed with photographic techniques. Rarefaction curves showed that most of the variation of abundance was due to two very abundant species *R. dubia* and *P. triqueter* (Figure 34).

Without these two species, number of species increase with number of individuals in the same way at the different site (with the exception of site STR).

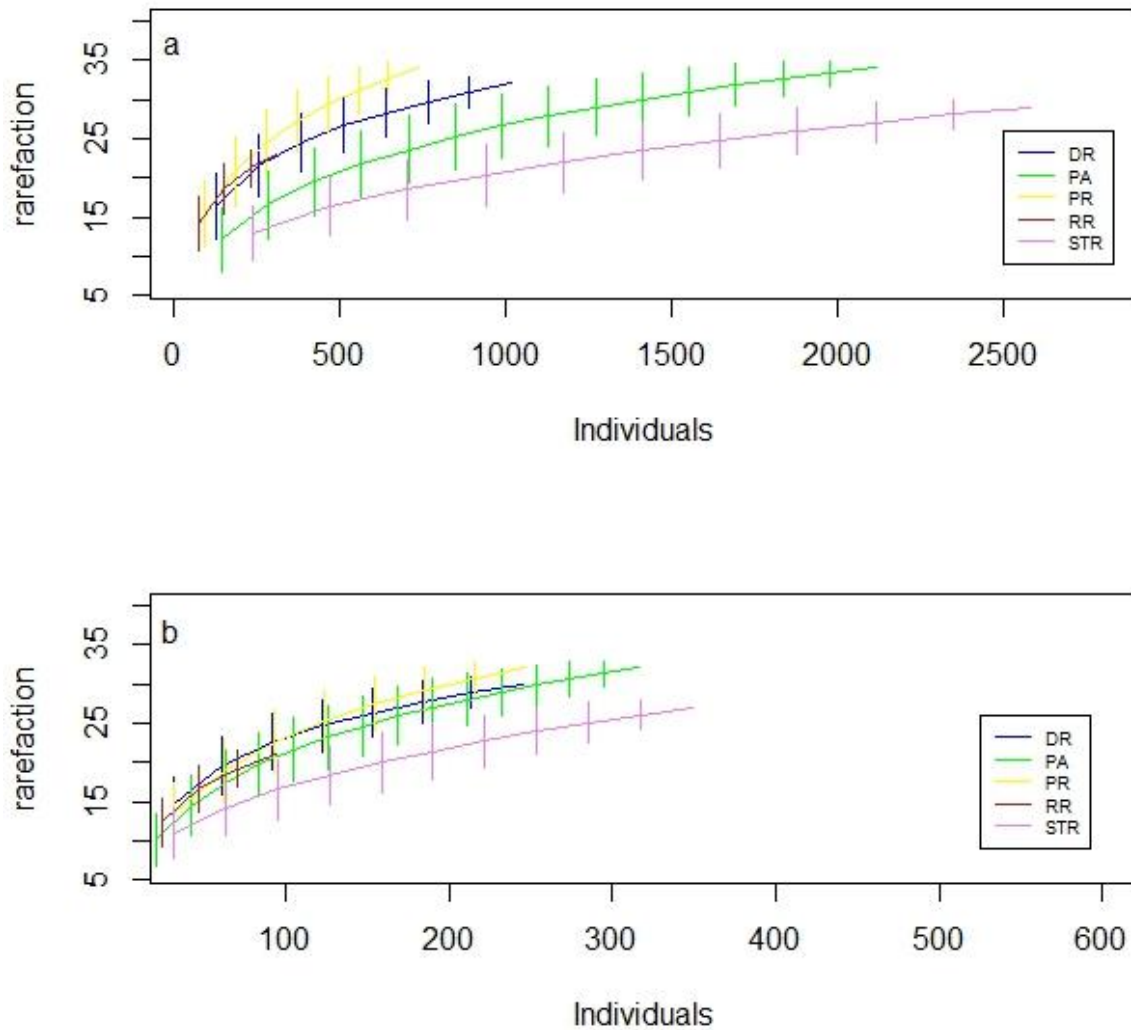


Figure 34: Rarefaction curves for benthic species found at each site with (a) and without (b) the most abundant species (*R. Dubia* and *P. triqueter*), with average species richness for random subsamples of individuals. Vertical lines = SD.

Slika 34: Graf odvisnosti števila vrst od njihove abundance za bentoške vrste na posameznih lokalitetah z najbolj številčnimi (a) in brez (b) najbolj številčnih vrst (*R. dubia* in *P. triqueter*), s povprečno pestrostjo vrst za naključne podvzorce osebkov. Navpične črte = SD.

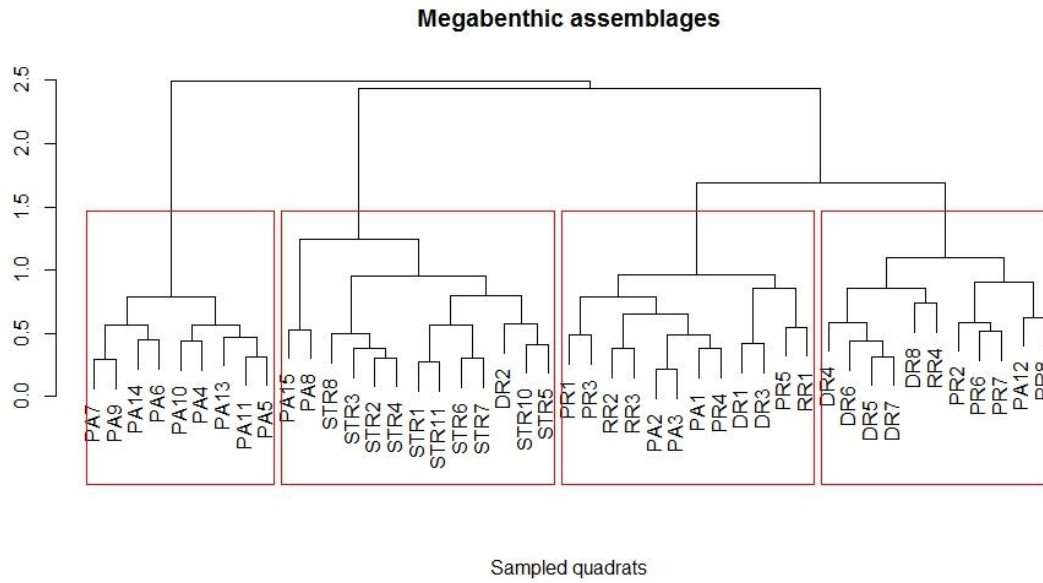


Figure 35: Cluster for log-transformed abundance data of megabenthic invertebrates obtained with underwater visual method.

Slika 35: Klusterska analiza logaritmsko preoblikovanih podatkov številčnosti megabentoških nevretenčarjev, pridobljenih s podvodno vizualno metodo.

Cluster analysis showed that sites STR and PA represent distinct groups, compared with other sites (Figure 35).



## 4.3 MACROSCALE

### 4.3.1 Fish assemblages

A total of 1383 fish were counted and 25 different species were identified (Table 8), among them only 8 were recorded at all sampling sites (Table 9). Species such as *Serranus scriba*, *Gobius cruentatus*, *Chromis chromis*, *Diplodus vulgaris*, *Serranus hepatus*, *Symphodus cinereus*, *Parablennius rouxi* and *Symphodus tinca*, were the most frequent.

Results of fish counting did not differ significantly between two successive passages on the same transect, nor between each passage and the mean value of the two (WMW test, p-value > 0.05). Species accumulation curve (Figure 36) showed that the total number of species counted with the second passage was lower than in the first, but calculated expected values were not significantly lower (t-test = 1.460, p-value = 1.832). Combining the two counts the expected value was not significantly higher than the first count (t-test = -0.158; p-value = 0.876). Therefore for calculation we assumed no differences between one and two replicates for the same sample.

Table 8: Frequency of occurrence (%) of recorded fish species at the five studied sites (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Cape Ronek – RR and Strunjanček- STR).

Preglednica 8: Frekvenca pojavljanja (%) zabeleženih vrst rib na petih vzočevalnih lokalitetah (Piranček - PR, Debeli Rtič - DR, Pacug - PA, Rt Ronek - RR in Strunjanček- STR).

Number	List of species	Percentage of frequency				
		PR	DR	PA	RR	STR
1	<i>Chromis chromis</i>	86	57	57	82	81
2	<i>Conger conger</i>	7	0	0	18	0
3	<i>Coris julis</i>	29	14	0	9	75
4	<i>Diplodus annularis</i>	7	0	14	45	25
5	<i>Diplodus puntazzo</i>	14	14	0	45	13
6	<i>Diplodus vulgaris</i>	64	71	57	91	100
7	<i>Gobius cruentatus</i>	100	57	100	100	81
8	<i>Gobius fallax</i>	36	0	43	82	13
9	<i>Labrus merula</i>	14	29	0	0	0
10	<i>Hippocampus hippocampus</i>	0	0	14	0	0
11	<i>Mullus surmuletus</i>	7	0	0	9	38
12	<i>Parablennius gattorugine</i>	36	14	14	0	13
13	<i>Parablennius rouxi</i>	57	29	29	64	63
14	<i>Parablennius tentacularis</i>	0	14	29	0	6
15	<i>Pomatoschistus bathi</i>	0	0	0	55	6
16	<i>Sarpa salpa</i>	0	14	14	55	25
17	<i>Serranus hepatus</i>	36	43	57	73	69
18	<i>Serranus scriba</i>	86	100	57	73	100
19	<i>Spicara flexuosa</i>	0	0	14	0	0
20	<i>Spondylliosoma cantharus</i>	14	14	29	0	13
21	<i>Symphodus cinereus</i>	93	57	86	91	19
22	<i>Symphodus ocellatus</i>	57	14	29	27	0
23	<i>Symphodus roissali</i>	21	0	0	0	0
24	<i>Symphodus tinca</i>	36	29	14	27	44
25	<i>Tripterygion delaisi</i>	7	0	14	0	13
<b>Total number of species</b>		<b>20</b>	<b>16</b>	<b>18</b>	<b>17</b>	<b>19</b>
<b>% of total number of species</b>		<b>80</b>	<b>64</b>	<b>72</b>	<b>68</b>	<b>76</b>

Table 9: Number of coastal fish species recorded at each sampling sites.

Preglednica 9: Število obrežnih ribjih vrst, zabeleženih na posameznih postajah.

species found at all sites	8
species found only in protected areas (RR, STR, DR)	1
species found only at one site	3
species found only at Ronek	1
species found only at Strunjanček	0
species found only at Debeli rtič	0
species found only at Pacug	2
species found only at Piranček	2
mean number of species per transect at Ronek	$9.45 \pm 2.70$
mean number of species per transect at Strunjanček	$7.94 \pm 1.98$
mean number of species per transect at Debeli rtič	$5.71 \pm 1.60$
mean number of species per transect at Pacug	$6.71 \pm 1.50$
mean number of species per transect at Piranček	$8.07 \pm 1.89$
	mean $\pm$ SD

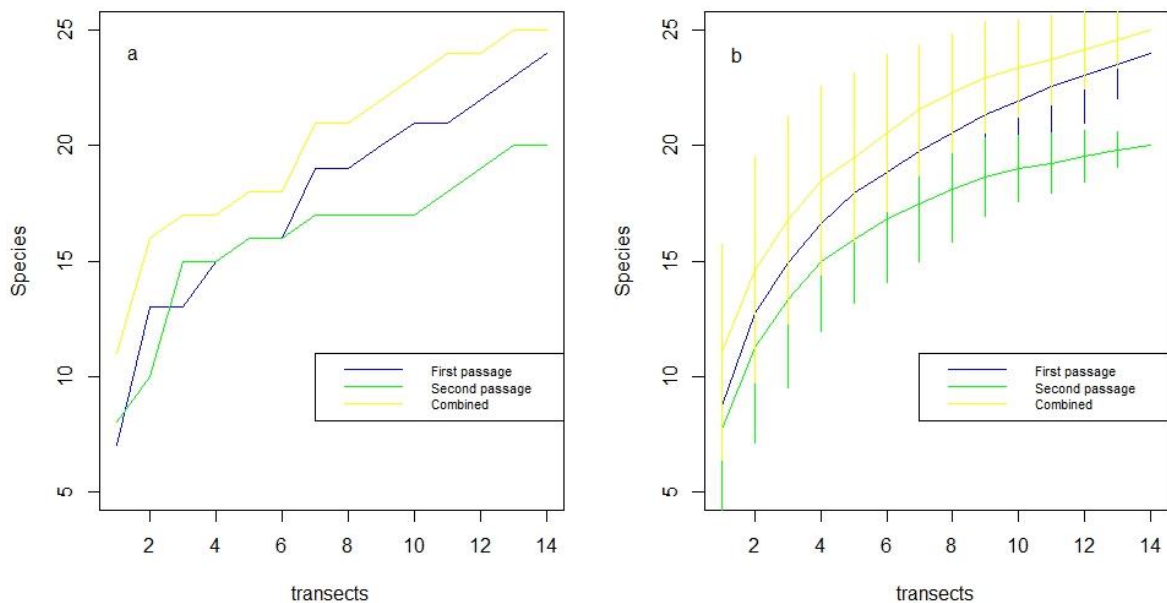


Figure 36: Species accumulation curves for fish species found at the first and second passages and combined data with transects in order of occurrence (a) and in random order (b). Each transect is weighted according to its length. Vertical lines = SD.

Slika 36: Kumulativna krivulja ribjih vrst na prvem in drugem linearnem popisu in kombinirani podatki iz transektov v vrstnem redu pojavljanja (a) in v naključnem vrstnem redu (b). Transekti so obteženi glede na njihovo dolžino. Navpične črte = SD.

Species accumulation curve showed that the total number of transects performed was enough to get a representative picture of fish richness associated with the study area (Figure 37a, c). Comparing species accumulation curves for each sampling site (Figure 37b,d), we saw that the equilibrium was reached at site STR and RR for a lower number of species, compared with site PR. At sites PA and DR the cumulative species curve did not reach an asymptote, but the shape of cumulative curves indicate that species richness at these sites should be higher than at STR. Site STR is the only with richness significantly lower than other sites. Estimations using different extrapolation functions gave the same results (Table 10). Only sites RR and STR differed significantly (

Annex A).

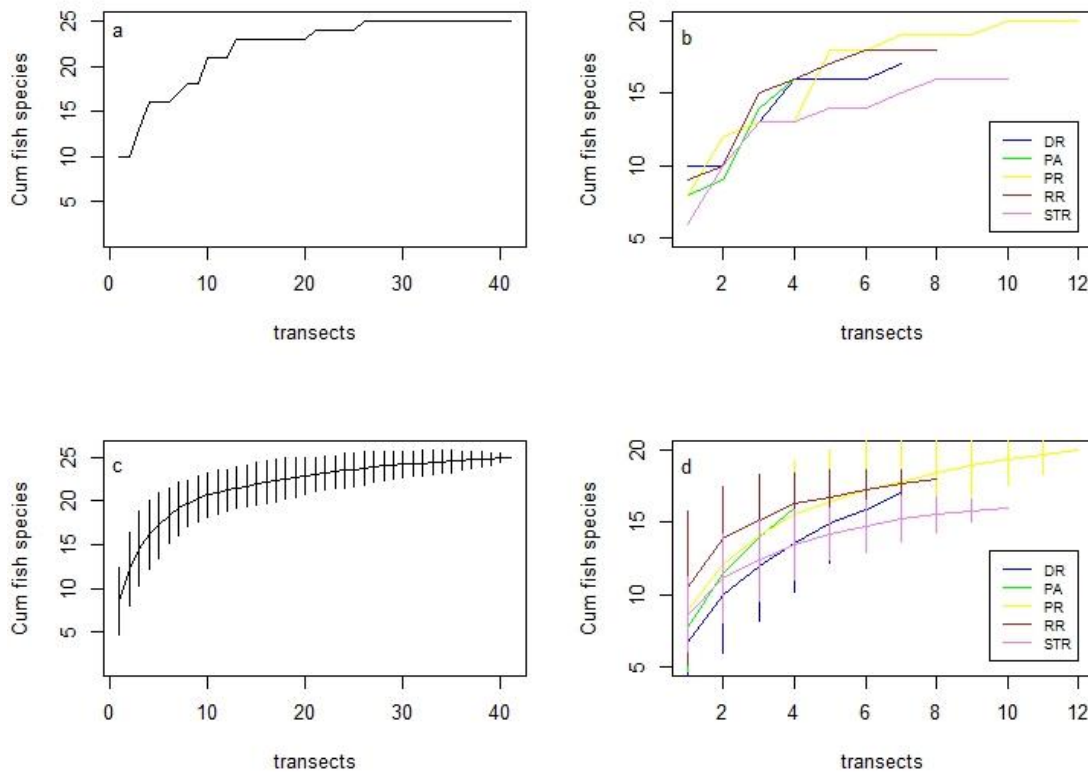


Figure 37: Species accumulation curves for fish species found at all sites (a,c) and at each site separately (b,d), with transects in order of occurrence (a,b) and in random order (c,d). Each transect is weighed according to its length. Vertical lines= SD.

Slika 37: Kumulativna krivulja ribjih vrst, popisanih na vseh lokalitetah (a, c) in na posameznih lokalitetah (b, d) iz transektov glede na frekvenco pojavljanja (a, b) in v naključnem redu. Vsak transekt je obtežen glede na njegovo dolžino. Navpične črte = SD.

Table 10: Estimates of species richness for each site and for the whole area using different functions.

Preglednica 10: Ocena vrstne pestrosti za vsako lokaliteto in za celotno območje z uporabo različnih funkcij.

Site	Species	chao	chao.se	jack1	jack1.se	jack2	boot	boot.se	n
<b>DR</b>	17	24.00000	7.164728	23.00000	3.464102	26.21429	19.69203	1.9149717	7
<b>PA</b>	16	22.00000	5.744563	22.00000	3.464102	24.66667	18.78516	1.9072063	4
<b>PR</b>	20	23.66667	4.881940	23.66667	2.279132	25.48485	21.72062	1.5066699	12
<b>RR</b>	18	21.93750	6.333505	20.62500	1.515544	22.23214	19.18651	0.9937674	8
<b>STR</b>	16	16.60000	1.200000	17.80000	1.272792	17.26667	17.05487	0.9875462	10
<b>All</b>	25	29.39024	7.025914	27.92683	1.689806	29.85305	26.29693	0.9270696	41

The number of colonies of *C. caespitosa* per transect varied among sampling sites (Figure 39). The highest density of colony was observed with in the deepest transect at site PR, while the lowest was observed in more shallow waters at sites DR, PA and STR. Nevertheless, the relation between density of colonies and sampling depth ( $r_s = 0.311$ , p-value = 0.020) was weak.

Fish richness and abundance were strongly related ( $r_s = 0.750$ , p-value < 0.001). Fish richness was not related with number of colonies ( $r_s = 0.188$ , p-value = 0.170), but there was a weak increase of fish abundances with density of colonies ( $r_s = 0.359$ , p-value = 0.007). Fishes richness increased with increasing fishing abundance at all sites, with the exception of PA, but only at site PR this relation was significative ( $r_s = 0.642$ , p-value < 0.024).

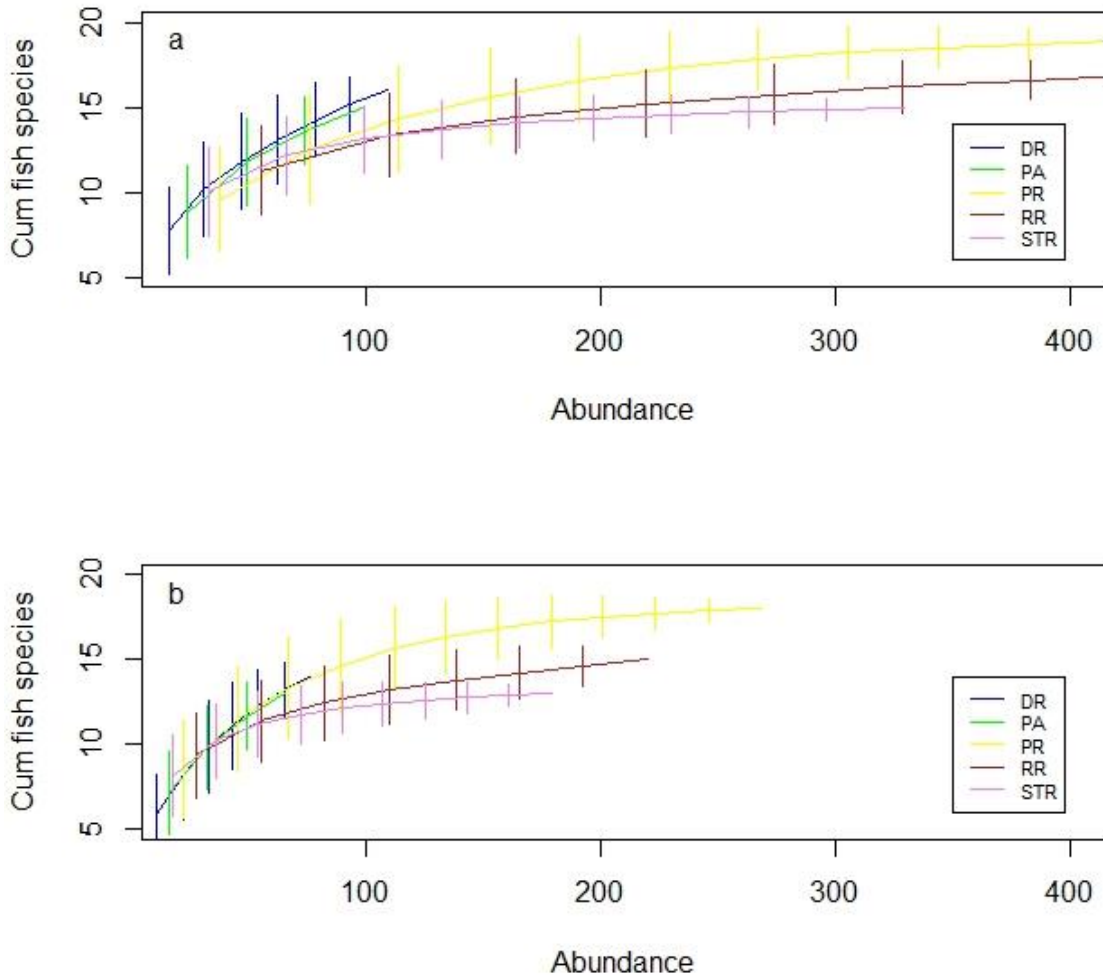


Figure 38: Rarefaction curves for fish species found at each site with (a) and without (b) gregarious species (*S. salpa* and *C. chromis*), with average species richness for random subsamples of individuals. Vertical lines = SD.

Slika 38: Graf odvisnosti števila vrst od njihove abundance prikazuje vrste rib na posameznih lokalitetah, z (a) in brez (b) jatnih vrst (*S. salpa* in *C. chromis*), s povprečno pestrostjo vrst za naključni podvzorec osebkov. Navpične črte = SD.

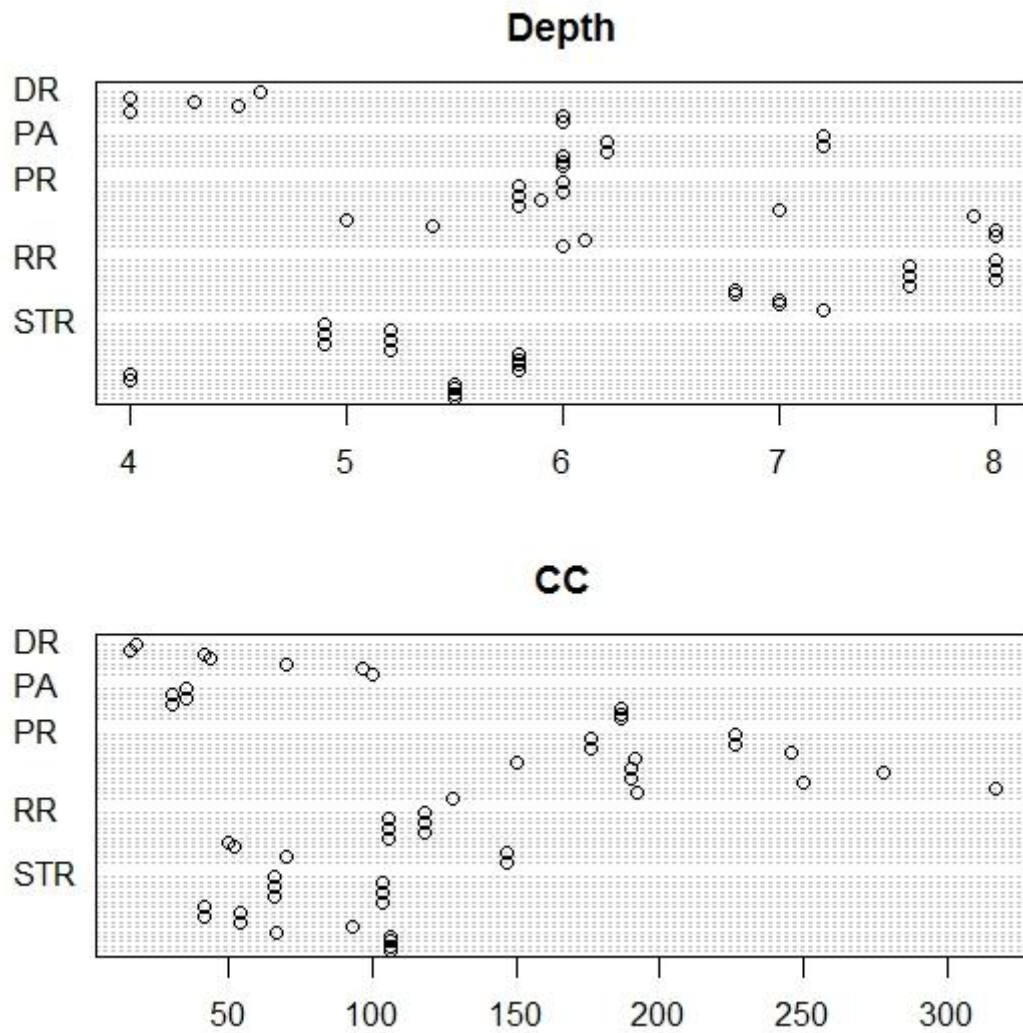


Figure 39: Dotcharts showing sampling depth (upper graph) and number of colonies of *C. caespitosa* (lower graph) per each transect on horizontal axes and sampling sites on vertical axes. Dots = transects.

Slika 39: Dotcharts prikazujejo globino vzorčenja (zgornji graf) in število kolonij *C. caespitosa* (spodnji graf) na vsakem transektu in lokaliteti. Pike = transekti.

Normalised fish richness (100 m<sup>2</sup>) did not differ among different sampling months (KW = 6.0183, *df* = 3, *p*-value = 0.111), but differed between sites (KW = 13.022, *df* = 4, *p*-value = 0.011) and years (KW = 13.824, *df* = 2, *p*-value = 0.0009). No differences were found between 2013 and 2014, so differences for 2015 could be a bias due to the lower number of sampling sites investigated. Given the big variation of species richness within sites (Figure 40), the main difference was given by lower values at site DR (Annex A).

Normalised abundances differed among sampling sites (KW chi-squared = 23.766,  $df = 4$ ,  $p$ -value < 0.001) as well, and the same was observed without considering gregarious species, namely *Salpa salpa* and *Chromis chromis* (Annex A). Normalised abundances did not differ among sampling month, but differed for year (Annex A). But without considering gregarious species also the difference among years was not significant (Annex A). At sites PR, STR and RR fish density per transect was higher than at sites DR and PA (Figure 40). Differences were significant only for site PA, and between sites DR and RR, but without considering gregarious species, most of these differences became not significant (Annex A).

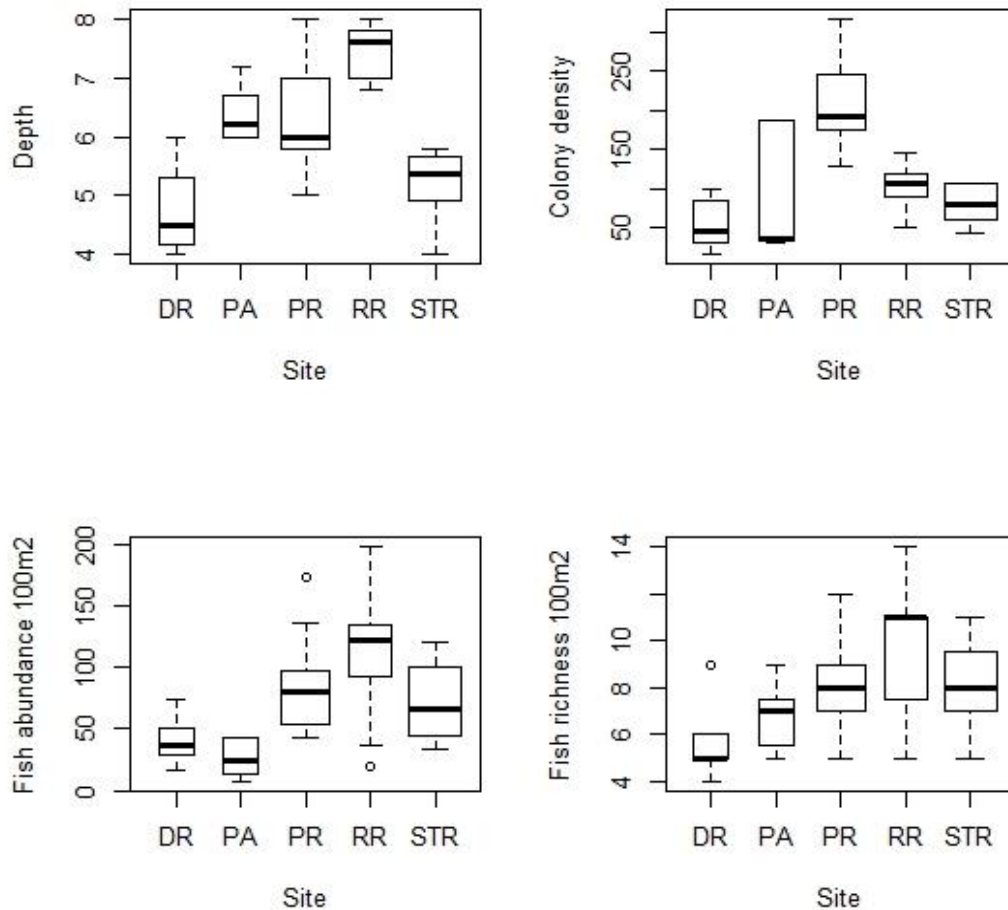


Figure 40: Boxplot showing depth, colony density, fish abundances and richness in relation with different sampling sites.

Slika 40: Boxplot graf prikazuje globino, gostoto kolonij, abundance rib in število vrst glede na različne lokalitete.



No clear pattern of variation of the composition of resident fish for each transect (considering presence absence data, excluding species with less than 25 % of frequency) was observed with multivariate analysis (Figure 41). Grouping of transects cannot be explained by sampling sites, month or depth.

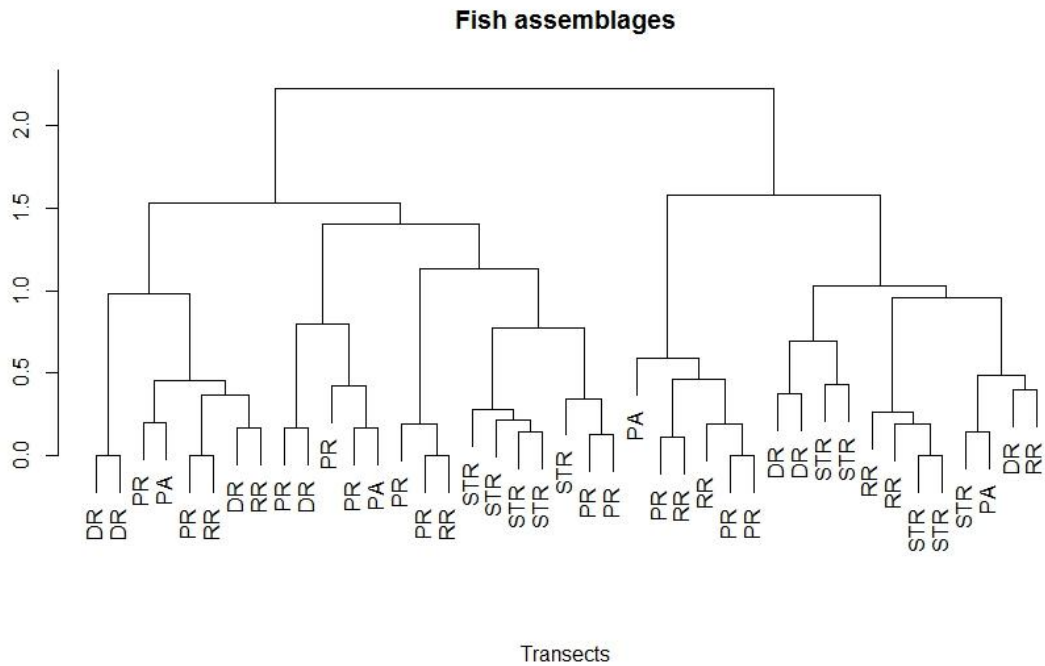


Figure 41: Cluster analysis for presence-absence of fish in each transect at the different sampling sites (without species with less than 25 % of frequency).

Slika 41: Klusterska analiza na podlagi prisotnosti-odsotnosti rib na posameznem transektu na različnih lokalitetah (brez vrst, ki se pojavljajo s frekvenco manjšo od 25 %).

Conversely diversity indices did not differ significantly among month with the exception of Pielou index, which is higher in August than in other months (Figure 42).

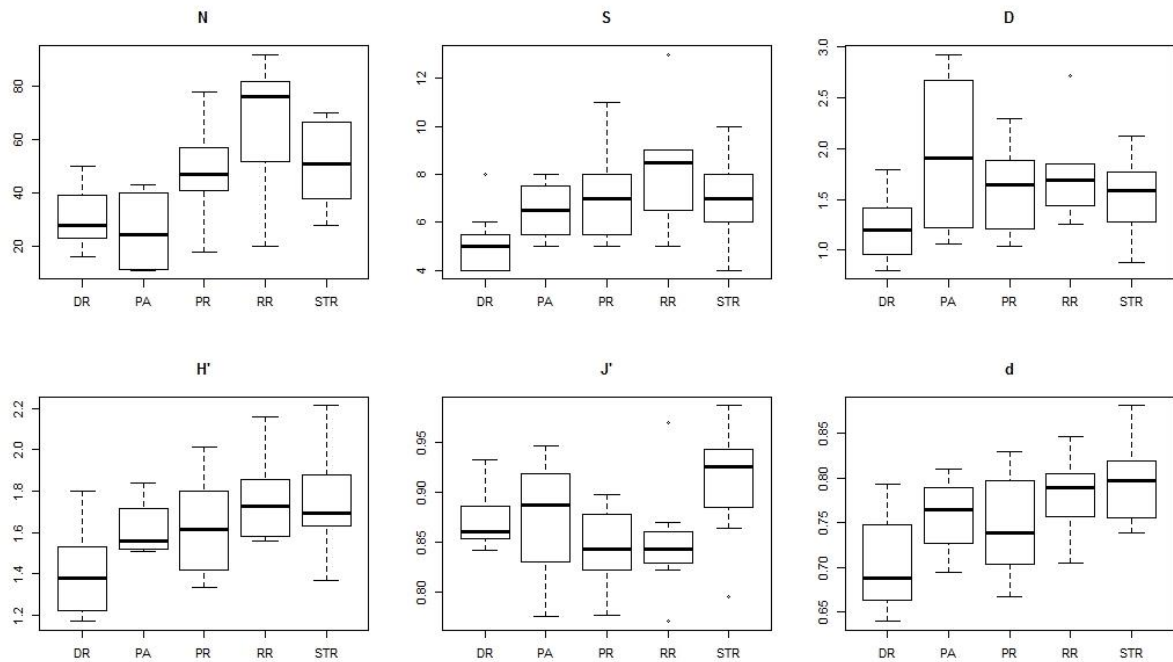


Figure 42: Boxplots showing values of different diversity indices among sampling sites. Margalef index of richness (d), Shannon diversity index ( $H'$ ), Pielou index of equitability ( $J'$ ) and Simpson index of dominance ( $D_\lambda$ ) calculated considering normalised species richness and abundances ( $/100m^2$ ) for each transect.

Slika 42: Boxploti prikazujejo vrednosti različnih diverzitetnih indeksov na različnih lokalitetah. Margalefov indeks (d), Shannonov indeks (H'), Pieloujev indeks (J') in Simpsonov indeks ( $D_\lambda$ ), izračunani glede na normalizirane vrednosti vrstne pestrosti in abundance ( $100m^2$ ) za posamezni transekt.

#### 4.4 BIODIVERSITY ASSOCIATED WITH BEDS OF *C. CAESPITOSA*.

Altogether 290 taxa were found associated with *C. caespitosa* beds, 234 were determined to the level of species (Table 11). Most of them were detected at the microscale levels (Figure 43), accounting for 77 % of total number of taxa, 76 % of total number of species and 59 % of total abundance. Animals recorded at mesoscale levels followed with 25 % of taxa, 22 % of species and 34 % of individuals (recorded only with visual method). Fish assemblages instead account for only 9 % of total species and 7 % of total abundance.

Table 11: Total number of taxa, species and individuals counted in the present work. For colonial organisms a colony was counted as one individual. \* = colonial organisms excluded.

Preglednica 11: Skupno število taksonov, vrst in osebkov, prešteti v tem delu. V primeru kolonijskih organizmov smo kolonijo upoštevali kot en osebek. \* = kolonijski organizmi izključeni.

	<b>Number of taxa</b>	<b>Number of species</b>	<b>Abundances</b>
<b>Microscale</b>	222	177	11561*
<b>Mesoscale photo</b>	46	27	/
<b>Mesoscale visual</b>	61	48	6765
<b>Total mesoscale</b>	71	52	/
<b>Macroscale</b>	25	25	1383
<b>Total</b>	290	234	19709

Assemblages at levels of micro, meso and macroscale together were quite homogeneous among sites, considering only the stable component (species with more than 25 % of frequency) (Table 12) or the whole assemblage (Table 13). For the microscale colonial organisms were not considered.

Table 12: Comparison between sites using Bray-Curtis distance applied to most frequent (>25 % of frequency) species recorded at levels of micro, meso and macroscale.

Preglednica 12: Primerjava med lokacijami z uporabo Bray-Curtis razdalje, za najbolj pogoste (> 25 % frekvenčnih) vrste, zabeležene na vseh treh ravneh (mikro, mezo in makro).

	<b>DR</b>	<b>PA</b>	<b>PR</b>	<b>RR</b>
<b>PA</b>	0.25			
<b>PR</b>	0.27	0.25		
<b>RR</b>	0.22	0.23	0.23	
<b>STR</b>	0.24	0.23	0.22	0.25

Table 13: Comparison between sites using Bray-Curtis distance applied to all species recorded at levels of micro, meso and macroscale.

Tabela 13: Primerjava med lokalitetami na podlagi Bray-Curtisove podobnosti za vrste, ugotovljene na vseh treh ravneh (mikro, mezo in makro).

	<b>DR</b>	<b>PA</b>	<b>PR</b>	<b>RR</b>
<b>PA</b>	0.34			
<b>PR</b>	0.32	0.31		
<b>RR</b>	0.30	0.31	0.29	
<b>STR</b>	0.34	0.34	0.31	0.32

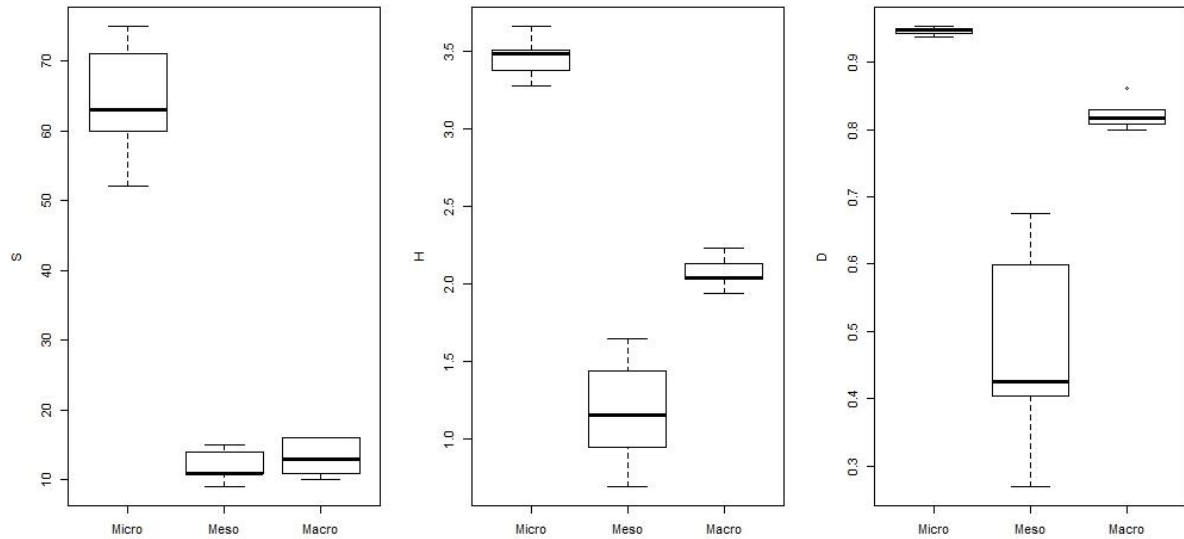


Figure 43: Average of species richness, Shannon and Simpson indices from different sampling sites, calculated at different scales.

Slika 43: Povprečje števila vrst ter Shannonov in Simpsonov indeks na različnih lokalitetah, preračunan za tri različne skale vzorčenja.

## 5 DISCUSSION

### 5.1 DISCUSSION ON THE METHODS

#### 5.1.1 Sampling effort

The methods used in our study revealed to be appropriate for achieving a representative picture of macrobenthic invertebrates living inside colonies (microscale), megabenthic invertebrate community (mesoscale) and of fish community (macroscale), as shown by cumulative curves in Figure 44, Figure 31 and Figure 38.

The sampling effort for the microscale (sampled volume) was appropriate in order to get a representative picture of the entire invertebrate community and of the three dominant phyla (molluscs, polychaetes and crustaceans) considered separately (see cumulative curves, Figure 44). Variation of species richness with sampling size differed among the different groups. For polychaetes and crustaceans the curve was very steep at the beginning and reaching fast the asymptote, while for molluscs there was a moderate increase of taxa at the beginning and the curve reached the asymptote slower.

The minimum effective sampling volume varied according to considered taxa. For molluscs a volume of 25.000 cm<sup>3</sup> is necessary to obtain a good estimate of the richness, whereas for polychaetes and crustaceans a volume of 15.000 cm<sup>3</sup> could be enough. This variability could be related to different biological traits of the groups and the order of colonization. Polychaetes are among the first colonizers of corals, finding a proper habitat among corallites of even small colonies, while only few endolithic molluscs are found in small recruits. The analysed small recruit (< 100 cm<sup>3</sup>) hosted just one species, the bivalve *Hiatella arctica*, which is one of the first invertebrates to colonize other bioconstructions (*Mesophyllum lichenoides* concretions, Sartoretto, 1998) and hard substrates (concrete blocks, Manoudis et al., 2005), as well. Conversely in the same colony 10 different taxa of polychaetes were found. Polychaetes are not only the first colonizers but they also account for the greatest diversity.

Moreover the structure of the community was also different. Mollusc and decapod assemblages were characterised by few dominant and frequent species, while the majority

of species were uncommon with random distribution. Polychaete assemblages instead showed a higher number of species and 30 % of them were frequent or very frequent.

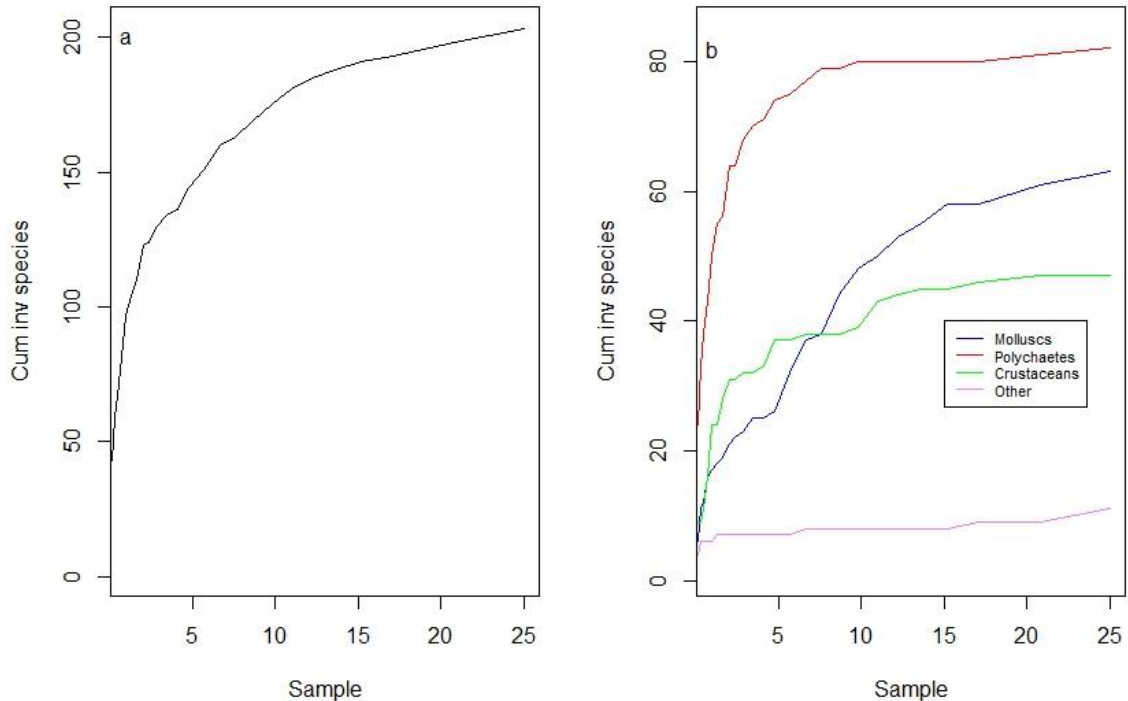


Figure 44: Cumulative curve of all taxa (a), taxa of mollusc, polychetes, crustaceans and others (b) for cumulative sampling volume ( $\text{cm}^3$ ).

Slika 44: Kumulativna krivulja vseh taksonov nevretenčarjev (a), taksoni mehkužcev, mnogoščetincev, rakov in drugih (v) glede na kumulativni obseg vzorčenja ( $\text{cm}^3$ ).

At the mesoscale level the sampling effort using both underwater counting and photographic technique seemed adequate to get a representative picture of macroinvertebrate community living in areas where the colonies of *C. caespitosa* are abundant (cumulative curves for the whole dataset). Nevertheless, richness estimates with the two techniques differed significantly. Richness estimates based on data collected with visual counting method were higher, because with this method, small organisms hiding among rocks whose detection would be impossible with simple observation, were collected and determined in laboratory.

Conversely, considering each sampling site separately, data collected with this method were inappropriate to compare the different sites. In fact, taxa cumulative curves for some sites, such as site RR, were far from reaching a clear asymptote. If one or more accumulation curves fail to reach an asymptote, the curves themselves may often be compared, after appropriate scaling, but when communities are far from reaching the asymptote, comparison is risky and easily subject to pitfalls (Gotelli and Colwell, 2001).

With the photographic technique, a higher number of samples can be collected with one SCUBA dive, since species counting is done later on at the computer. We used this technique to check how many samples we should collect to have a representative picture of megabenthic communities at the studied sites. With the photographic technique 25 samples of 1 m<sup>2</sup> resulted enough for a proper comparison between sites.

At the macroscale level sampling effort was adequate to get a representative picture of fish assemblages associated with beds of *C. caespitosa* (see cumulative curves). Nevertheless, considering each sampling site separately, samples collected per sites PA and DR were not enough, since the cumulative curves were far from reaching an asymptote. This was due to the lowest number of samples for these sites, compared with others, but also to the lowest fish density per transect. In fact, fish density at sites DR and PA was lower than at other sites, but species richness increased with number of individuals at the same rate at all sites (rarefaction curves). Therefore, at the site RR, STR and PR a smaller number of samples was necessary to get a representative estimate, compared with DR and PA.

### **5.1.2 Taxonomical accuracy**

The importance of taxonomical accuracy for ecological studies is still objective of scientific debate (Giangrande, 2003). Taxonomical uncertainty constitutes a limit for correct SAR estimation at micro and mesoscale levels. Some of the species found in the present work could not be determined to the species level for different reasons. One problem was related with the poor conditions of certain animals, so that the examination of diagnostic features was difficult. Specific taxonomic groups need different fixation and preservation techniques, so the only way to guarantee an optimal preservation of specimens is to focus the investigation on one or few taxonomic groups. For more general

ecological investigation a compromise have to be reached in the choice of preservation techniques. For the present work, the method was chosen in order to enable a good preservation for non-colonial organisms, known to account for the highest richness inside *C. caespitosa* colonies (Koukouras et al., 1998; Pitacco et al., 2014a) and therefore likely to exert a bigger influence on SAR. Another big problem was related with the extraction of soft and fragile animals, such as terebellid and cirratulid polychaetes from the inner part of corallites.

Another limit was due to the high number of juveniles, not always showing the characteristic features important for determination of the adult stage. Sometimes juveniles of some species could even resemble adults of other species, as was the case of some polychaetes of genus *Syllis* (San Martin, 2003).

Finally, the taxonomical position of some species is currently under debate and in need of revision. This is the case of mollusc species such as *Raphitoma laviae* and *R. bicolor*, species of the genus *Cerithium* (Gofas et al., 2011), the two species of *Hiatella* (*H. arctica* and *H. rugosa*) (Crocetta and Spanu, 2008), polychaetes like species of the genus *Serpula* and *Lysidice ninetta* and *L. collaris* (Giangrande and Gambi, *pers. com.*). All these problems can lead to an underestimation of total biodiversity.

### **5.2.3 Method limitations**

At the microscale level the methods used were extremely time consuming (Table 14). Time required to open a single colony, sort and determine associated macrofauna could vary from few days for smallest colonies, to various months for the biggest. More than 90 % of time spent for species determination was dedicated to polychaetes. Another drawback of the method is its destructivity that makes it unsuitable for more extensive research and monitoring. Moreover, this technique cannot be applied in areas where *C. caespitosa* is not so frequent and abundant like the study area.

At the mesoscale levels two different techniques were applied. The two methods gave consistent results in terms of description of the stable components of the community (most frequent and abundant species). The photographic method enables us to investigate a larger area with the same amount of time compared with underwater counting (Table 14). With



underwater counting more species were recorded and the taxonomic accuracy was bigger, due to the possibility of collecting animals whose determination was not possible underwater. Conversely analyses performed from photographs, despite the bigger sampling extent (Table 14) did not added significant additional information in terms of the total species richness.

A different consideration should be done if we consider each sampling site separately. From the analysis of cumulative curves it was clear that the sampling extent covered with the underwater methods proved to be insufficient for a comparison among different sites, so for this purpose the photographic technique was more appropriate. Extrapolation based on data collected with underwater counting, indicated site RR as the poorest in terms of species richness, but this results was biased by the low number of samples collected. Extrapolation based on data collected with the photographic technique instead indicated site RR as the richest. This technique enables also the repeatability of the analysis and their improvement, studying additional variables (*e.g.* substrate characteristics), also some time after sampling. Consequently, considering also that this technique is cheaper (time spent underwater is reduced), it could be more appropriate for monitoring purpose and estimation of richness.

Table 14: Comparison of methods applied at different scales. <sup>1</sup> = hours spent underwater; <sup>2</sup> = dimension of sampling units (m<sup>2</sup>); <sup>3</sup> = total surface analyzed (m<sup>2</sup>); <sup>4</sup> = % of taxa determined till the level of species. x = few hours, xx = few days, xxx = many days, xxxxx = many months.

Tabela 14: Primerjava metod, ki se uporabljajo na različnih skalah. <sup>1</sup> = št. ur podvodnih vzorčevanj; <sup>2</sup> = razsežnost vzorčnih enot (m<sup>2</sup>); <sup>3</sup> = skupna analizirana površina (m<sup>2</sup>); <sup>4</sup> = delež (%) taksonov, določenih na nivoju vrste. x = nekaj ur, xx = nekaj dni, xxx = mnogo dni, xxxxx = več mesecev.

	Microscale	Mesoscale photo	Mesoscale visual	Macroscale
<b>Time for sampling</b> <sup>1</sup>	x	xx	xx	Xx
<b>Time for sample and data processing</b>	xxxxx	xxx	x	x
<b>Number of sampled units</b>	25	100	45	51
<b>Sampling size</b> <sup>2</sup>	0.011 to 0.094	0.25/1	0.25/1	30/50
<b>Sampling extent</b> <sup>3</sup>	0.588	100	45	2402
<b>Accuracy of determination</b> <sup>4</sup>	80 %	59 %	79 %	100 %
<b>Repeatability</b>	no	yes	no	no

The visual census technique applied at the macroscale level is considered far superior to other typical techniques (e.g. trawling, traps, hook-and-line) in heterogeneous habitats such as coral or rocky reef barriers. This technique is non-destructive and has a minimal impact on the fish community and the environment (Sale, 1980), therefore is necessary for investigations of fish communities in sensitive habitats, such as marine reserves. According to Harmelin-Vivien and Francour (2008) this technique is particularly suitable to detect good fish swimmers (species of the family Sparidae; *Coris julis*, species of the genus *Symphodus*) and planktivorous fishes (Centracanthidae, Pomacentridae), that tend to escape trawl nets. However, it could lead to an underestimation of macrocarnivores (species of families Scorpaenidae and Serranidae), canopy-dwellers (family Syngnathidae) and benthic species (families Gobiidae and Blenniidae). Therefore comparisons between data obtained with different techniques should be considered critically.

In the present work there was no influence of successive passages along the same transects on fish counts, nor on fish/area cumulative curves. The same had been observed by De Girolamo and Mazzoldi (2001) who explained the fact that when benthic and cryptobenthic species encounter a diver, they usually remain motionless or hide in holes, whereas epibenthic species move a few meters away and then return just after the diver's passage (De Girolamo and Mazzoldi, 2001).

#### **5.2.4 Micro and macroenvironmental parameters**

The distribution of *C. caespitosa* colonies is not homogenous along the Slovenian coast. Colonies are present at different depth and with different size and abundance at different sampling sites. At site PR colonies were bigger, present with higher density and at a wider range of depth, compared with other sites. Conversely at site DR colonies were observed at shallower depth and were generally smaller and less abundant compared with other sites. Our results are consistent along the three scales analysed and with previous investigations at the same area (Schiller, 1993; Zunino, 2013; Kružić et al., 2014).

With regards to morphological characteristics of colonies there was no clear pattern among sites, probably due to low number of colonies collected and the high variability within the same site. The variation of features, such as proportion of living polyps, interstitial volume

and sediment trapped inside the colony, could exert an influence on the SAR of associated macroinvertebrates, facilitating or preventing the settlement of new species.

## 5.2 MICROSCALE

### 5.2.1 SAR

The number of invertebrate taxa increases with *C. caespitosa* colony size. The positive correlation between the number of invertebrate taxa and colony size (covered surface), together with comparison with other models, confirms that the Arrhenius (1921) equation was the best to fit SAR for *C. caespitosa* associated invertebrates. Considering the entire invertebrate community we can reasonably say that factors such as sampling sites, depths and months were not affecting the relationship between area and species richness, even if they can exert an effect on other aspects of invertebrates communities (such as presence of juveniles). In fact the pattern of the relationship between species and area, together with the density of taxa and individuals resulted to be consistent among the different sampling sites, months and depth. Moreover there was no relation between depth and taxa richness and abundance. The invertebrate community was influenced more by colony size than by sampling sites, as demonstrated also by multivariate analysis based on the entire invertebrate community.

Colony size resulted to be the best predictor of species richness. Nevertheless, in order to understand the part of the variability in species richness that was not explained by colony size, other factors need consideration. Factors independent from colony size, influencing some components of the community, could have an effect on the global relationship. These factors could increase variability in species richness, reducing the accuracy of a prediction. In the present work, considering also the amount of sediment and mud inside each colony the accuracy of the model increased. The presence of mud and sediment inside the colonies enables soft bottom dwellers, such as polychaetes of the families Capitellidae, to settle inside the colony, therefore influencing taxa composition and richness.

For dominant groups (polychaetes, molluscs and crustaceans) considered separately there was also an increase of richness with increasing colony size, so the SAR holds also for the

single dominant phyla. Nevertheless, different factors affected the relation for these three groups, making colony size less or not predictive at all.

Considering molluscs, variables other than sampling size were not related with mollusc richness and did not influence significantly SAR model. Colony size alone was highly predictive for species richness.

Considering polychaetes the colony size alone was not enough to explain taxa richness precisely. The richness of polychaetes known as soft bottom dwellers increased with the amount of sediment inside the colony. The fact that this factor exerted a bigger influence on SAR for polychaetes than for molluscs, could be explained with the rarity and scarce abundance of soft bottom molluscs inside the colonies and with the different shape of cumulative curves. The curve of polychaetes reached the asymptote faster compared with the curve of molluscs. Once the asymptote is reached, colony size is no more the most important factor influencing the assemblage, because of the reduced slope and increased noise. Cumulative curve for polychaetes reached the asymptote for a sampling volume of 1 dm<sup>3</sup>, and cluster analysis showed a difference between colonies smaller or bigger than 1 dm<sup>3</sup>, showing that polychaete assemblages in small and big colonies differed. This explains why for the analysed colony size range, total volume alone was not such a good predictor for species richness.

The SAR for crustaceans was very weak. Colony size itself was not enough for an accurate prediction of taxa richness, but none of the factors considered were related with the taxa richness of crustaceans. This was mainly because amphipods, accounting for about half the crustacean taxa, were very weakly related with colony size. They were rare and randomly distributed among colonies with few individuals of each species per colony. Taxa richness of decapods was related to colony size. However, even if the percentage of living polyps seemed to improve the model, it was not significantly related with decapod richness nor abundance.

A final consideration concerns the importance of the three dimensionality of the colony. For investigations into invertebrates strictly associated with corals, total colony volume (e.g. Abele and Patton, 1976; Koukouras et al., 1998; Martín García et al., 2008; Belmaker,

2009) and weight (e.g. Reed and Mikkelsen, 1987) are generally considered to offer the most appropriate parameters to estimate coral size. According to our calculations, there was a strict correlation between total colony volume and the area covered by colonies, suggesting both could be used as colony size descriptor. Nevertheless area covered by the colony did not give accurate predictions for the total richness of the community and of molluscs. Total colony volume resulted to be a better predictor and slightly improved the model also for polychaetes. This was due to the fact that the surface covered by colonies did not represent the real surface available for macroinvertebrates to settle, which increased with the complexity of the colony. This fact affect in particular molluscs, because they are mainly sessile and endolithic, and to a lesser extent polychaetes, because even if they are mainly free living, they are represented also by a consistent number of sessile and endolithic species. For those groups a species-volume relation (SVR, *sensu* Belmaker, 2009) could be more predictive for species richness.

Contrary to other groups, the richness of crustaceans was predicted better by the surface covered than by colony volume. Colony volume was less predictive because crustaceans, with the absence of endolithic taxa and the presence of only one sessile species, were less influenced by the three-dimensionality of the colony. Only the live part of coral heads, without the dead centre, was suitable for them, as it was observed for tropical decapods (Abele and Patton, 1976).

### **5.2.2 Mechanisms underlying SAR**

Different mechanisms were proposed to account for SAR, like habitat diversity, the area *per se*, passive sampling, resource concentration and the edge effect (Connor and McCoy, 2001). On a spatial scale of square centimetres, passive sampling is likely to contribute to the positive relationship between area and species number (Neigel, 2003). The idea, called “*the random placement hypothesis*” (Arrhenius, 1921) is that increasing area resulted in an increasing number of individuals colonising the area and consequently number of species, purely as a random process (an artefact of increasing sample size). For habitat that are colonised seasonally, such as macroinvertebrates on intertidal boulders, passive sampling proved to account for part of species richness (Connor and McCoy, 2001). Conversely, the structure of the community associated with *C. caespitosa* was much better structured

compared with intertidal areas (Pitacco et al., 2013) and probably less influenced by seasonality. Moreover, normalized abundance of invertebrates was not the same in all colonies, but there was a slight decrement with increasing colony size. All these considerations suggest us to reject the random placement hypothesis.

The present work provided additional evidences that colonies of *C. caespitosa* offer three main types of microhabitat (Pitacco et al., 2014a): hard substrate for epilithic and endolithic species, interstitial space for small motile organisms and sediment trapped for soft bottom species.

Considering the different feeding and functional groups (epilithic, endolithic, free living and soft bottom dwellers) separately, they were all highly correlated with colony size for both richness and abundance. Members of each guild should occupy similar niches, so they should be scarcely influenced by habitat heterogeneity. This could be consistent with the area *per se* hypothesis, stating that big populations increase species richness lowering the extinction probability. The same theory was supported also for tropical decapod crustaceans associated with coral heads of *P. damicornis* (Abele and Patton, 1976).

Nevertheless the present work provided also evidences that adding a microhabitat variable independent of colony size significantly improved the model, suggesting that also microhabitat diversity played a role in the increasing species richness. The presence of sediment and mud trapped inside the colony in particular exert a significant effect on invertebrate assemblages, in particular on some polychaetes.

Some groups of invertebrates, such as amphipods and some molluscs, have a patchy distribution that could be explained by factors independent of colony size. The absence of specialised molluscs, such as parasites and spongivores both in small and large colonies suggested that their presence was dependent on the presence of prey species (*e.g.* sponges for spongivore molluscs belonging to families Triphoridae and Pyramidellidae).

Many factors could influence the colonization of hard substrates, such as physical-chemical parameters (Manoudis et al., 2005), the period in which colonization starts (Anderson, 1999) and interspecific competition (Buss and Jackson, 1979). The

combination of these factors could explain the patchy distribution and diversity of assemblages among colonies.

The size of a coral head determines the size of the crannies that shelter invertebrates (Abele and Patton, 1976). In the present work interstitial volume increased with colony size and this influence in particular mobile organisms (FL and DM), which represent the majority among associated invertebrates.

But colony size is also related to coral age and consequently to the time available for associated species, adults or larvae, to colonize this type of biogenic substrate. A rough estimation of coral age, based on the maximum length of corallites and estimates of coral growth at the studied area (Lipej et al., 2013), suggested that the studied colonies ranged from 9 to 30 years old. This explains why associated invertebrate community was so rich, complex and well structured, even in small colonies.

Therefore, spatial and temporal variability probably have a synergistic influence on invertebrate richness.

All these considerations support the hypothesis, formulated by Connor and McCoy (2001) that mechanisms underlying Species-Area Relationship are not mutually exclusive and may operate in combination. In particular, area may have both a direct (*area-per se*) and an indirect effect on species richness related to microhabitat diversity.

### **5.2.3 Associated macrofaunal community**

Invertebrate community was dominated by polychaetes in term of taxa richness and abundance. Their success was probably due to their higher level of differentiation with regards to feeding modes and motility compared with other groups such as crustaceans. They were therefore able to colonize all different niches provided by coral colony. This result was consistent with previous researches in northern Adriatic (Pitacco et al., 2014a) and other areas of the Mediterranean Sea (Lumare, 1965; Koukouras et al., 1998).

Conversely in tropical scleractinian corals some researchers found Polychaeta to be the richest group (Cantera et al., 2003), but in other studies Arthropoda were reported as the most frequent, abundant and rich group (Abele and Patton, 1976; Martins Garcia et al,

2008). Even if a recent review pointed at arthropods as the most important group associated with tropical scleractinian corals (Stella et al., 2011), the total number of polychaete species could be biased by the number of studies analysed (140 for arthropods and only 10 for polychaetes). Comparison with tropical corals is made more difficult by the many still unsolved taxonomic problems, limited number of investigations and huge variety of coral species and morphology, resulting in different microhabitats for associated species (Stella et al., 2011).

All reported species associated with *C. caespitosa* (Koukouras et al., 1998; Pitacco et al., 2014a, present work) have also been found in other, mainly hard substrate communities, so by now there are no evidences of obligatory relationships. The same observation was made for species associated with another temperate coral *Oculina arbuscula* by McCloskey (1970). Conversely, obligatory relationships are quite common in tropics, involving mainly decapod crustaceans (e.g. Castro, 1978; Coles, 1980).

Some rare and uncommon species were found, together with animals whose taxonomic position is still under debate. The bivalves *Galeomma turtoni*, *Hiatella rugosa* and *Kellia suborbicularis* found in the present work were considered rare at the study area in the Gulf of Trieste (Vio and De Min, 1996; De Min and Vio, 1997). According to the most recent review (Mikac, 2015), the polychaete *Syllis columbretensis* and the sabellid *Parasabella tommasi* were found for the first time in northern Adriatic Sea and two other polychaetes, the flabelligerid *Flabelliderma cinari*, and the chysopetalid *Paleanotus chrysolepis*, are first records for the Adriatic Sea. For the flabelligerid *F. cinari* this was the first record outside its locus typicus in Turkish waters (Karhan et al., 2012).

Our research provided evidences that colonies of *C. caespitosa* constitute a nursery ground for a variety of species of invertebrates. With its three-dimensional structure, this coral offers protection from predation and wave scour, which is particularly important for organisms at the early stage of their development (Schuhmacher and Zibrowius, 1985). This explains the great number of juveniles (mainly molluscs and echinoderms) found, together with evidences of both sexual and asexual reproduction of polychaetes and decapods inside the colonies.



The proportion of juvenile molluscs found inside colonies of *C. caespitosa* was consistent with data collected in the same coastal waters within *Cystoseira* algal associations (Pitacco et al., 2014a), which represent the final stage of the succession of the photophilic algal community in the Mediterranean Sea and are well known as a nursery ground for molluscs (Poulicek, 1985; Antit et al., 2013).

Some species were found only at juvenile stage and are known to live in different environment when adult, for instance the ophiurid *Ophiothrix* sp. and the crab *Pilumnus* sp. But the majority of the most frequent and abundant species were found in both juvenile and adult forms, suggesting that most of the inhabitants complete their entire life cycle inside the colony.

Different symbiotic relationships, such as commensalism, mutualism and parasitism or prey-predator association are known between living scleractinian corals and their associated and among the associated themselves.

Invertebrates known to feed directly on *C. caespitosa* in Mediterranean Sea were not found in the present work. But corals provide microhabitats also for a large number of parasitic and commensal associates, which use the tissue and skeletons of the colonies as substrate (Floros et al., 2005). Most of these coral associates stress the coral to some degree, and some of them, particularly some sponges, polychaetes and bivalves, can do considerable harm (Sammarco and Risk, 1990; Smith and Harriott, 1998).

Some coral associates could also have a mutualistic or commensal relation with their host. Several associates benefit the hosts by preying upon the larvae of boring animals, by removing detritus and coral mucus, and even by attacking potential predators of the corals (DeVantier et al., 1986; Nogueira, 2003).

#### **5.2.4 Mollusc assemblages inhabiting colonies of *C. caespitosa***

Taxa richness and the density of molluscs associated with *C. caespitosa* were also comparable to molluscs associated with *Cystoseira* algal belts in the very same environment (Pitacco et al., 2014b). However, mollusc assemblage associated with *C. caespitosa* qualitatively differs from those associated with macroalgal belts. Macroalgal

belts are generally dominated by small gastropods with a short life cycle (Gambi and Morri, 2008, Pitacco et al., 2014b), whereas mollusc assemblages associated with the Mediterranean stony coral are dominated by bivalves, some with a long life cycle. Two examples are the Noah's Ark shell (*Arca noae*), that can live more than 16 years (Peharda et al., 2002) and the European date mussel (*Lithophaga lithophaga*), that can live more than 50 years (Galinoumitsoudi and Sinis, 1995).

These differences could be explained by considering coral and macroalgal morphologies and biological traits, which result in different opportunities for colonization and the availability of food for molluscs. In fact macroalgae offer a layer of epiphytic microalgae and diatoms for dominant grazing gastropods (Gambi and Morri, 2008), while corals offer hard substrate from which dominant epilithic bivalves filter feed (Reed and Mikkelsen, 1987).

Moreover richness and abundance of molluscs associated with *C. caespitosa* were more or less constant throughout the sampling period (from July to October), while significant seasonal fluctuations have been reported for molluscs associated with photophilic algae (Antit et al., 2013, Urra et al., 2013). This could be related with the fact that the development of *Cystoseira*'s thalli is at its maximum in spring (Falace et al., 2005), while *C. caespitosa* has a constant morphology during the year and a long life cycle (over 60 years according to Peirano et al., 2004).

The majority of species found to inhabit *C. caespitosa* in the present work were also associated with the biocoenosis of Photophilic Algae (AP) (Vio and De Min, 1996) in the study area (Gulf of Trieste). This could be due to the presence of an infralittoral macroalgal belt around the colonies (see mesoscale chapter). At the deeper site previously studied (Pitacco et al., 2014a) taxa usually associated with the Coastal Detritic biocoenosis (DC) were found inside the colonies of *C. caespitosa*, as well. This is another evidence supporting the hypothesis that the surrounding environment is probably an important factor influencing assemblages living inside the colonies (Bailey-Brock, 1976).

### **5.2.5 Polychaetes assemblages inhabiting colonies of *C. caespitosa***

In the present work 83 species of polychaetes were found. This result is comparable to results obtained by Arvanitidis and Koukouras (1994) who reported 87 species associated with *C. caespitosa* in the Aegean Sea. Only 38 co-occurred in colonies from both study areas.

The relationship between richness of polychaete taxa and colony volume showed a pattern similar to the one described for the Aegean Sea (Arvanitidis and Koukouras, 1994). The number of species increased rapidly in small colonies and then reached a plateau for the biggest ones of comparable weight.

This similar pattern found in northern Adriatic and Aegean Sea, suggests that the same SAR model could be successfully applied to polychaetes associated with *C. caespitosa* also in other areas of Mediterranean Sea, and that polychaete richness for each colony at the two study areas is comparable, even if species composition varies.

Considering different feeding guilds of polychaetes (in term of mean number of individuals per cm<sup>3</sup>) a common pattern could be found for Adriatic and Aegean Sea, as well. Sessile and tentaculate filter-feeders were the dominant group, followed by motile and discretely motile jawed carnivores, with only few individuals of deposit feeders. This pattern seems to be independent of sampling depth. No significant differences were found among two sites at different depths in the Aegean Sea (Chintiroglou, 1996), and also polychaetes associated with *C. caespitosa* in a deeper site of the Gulf of Trieste (Pitacco et al., 2014b) showed the same dominance of carnivores and filter feeders.

### **5.2.5 Crustacean assemblages inhabiting colonies of *C. caespitosa***

In the present work 43 species of crustaceans were found. This result is comparable to the results reported by Arvanitidis and Koukouras (1994), who reported 49 species associated with *C. caespitosa* in the Aegean Sea. Among them only 19 co-occurred in colonies from both study areas.

Most of the crustaceans found were free living, motile species. However, some of them, such as *Stenothoe* spp., were known to live in interstices of microcavities of biogenic

constructions (Ruffo, 1998) or were tubicolous, such as the amphipod *Corophium acutum* and tanaidaceans *Parapseudes latifrons*, *Apseudes acutifrons* and *Leptochelia savignyi* (Thiel and Watling, 2015). Some of them like amphipods, build their silk-based tube themselves, others occupy tubes already built by other organisms. The tube is very important for the animal, providing not only shelter and camouflages, but also a favourable position for filter feeding (Thiel and Watling, 2015). In the present work tanaidaceans of different sizes were found inside the same empty serpulid tubes (*pers. obs.*), suggesting an important function also for mating.

A symbiotic behaviour is known among species of the family Stenothoidae and genus *Leucothoe*, that live inside sponges, ascidians and sea anemones, probably feeding on mucus (Ruffo, 1998).

As already observed for molluscs many of the species found in the present work were known to be associated with the biocoenosis of Photophilic Algae (AP), with some of them been present also in circalittoral coralligenous habitats and among *Posidonia* meadows (Ruffo, 1998). This is an additional evidence of the influence of the macroalgal belts present around the colonies at the study sites (see mesoscale chapter).

### 5.3. MESOSCALE

#### 5.3.1 SAR

At the level of mesoscale, as result of sampling design, richness data were not independent from one another, since bigger areas depended on the smaller ones. Therefore it was not possible to apply the classical regression used for microscale, but other methods have to be employed to estimate species richness.

The incidence-based estimates calculated in the present work are based on the frequencies of rare species in a collection of sites (Oksanen et al., 2005). They are popular ways of estimating the number of unseen rare species. Adding this number to the observed number of species we obtain a better estimation of species richness (Oksanen et al., 2005). These techniques are valid ways of estimating species richness. However, they do not provide any information on how much the area is influencing species richness compared with other

factors. Therefore, other analyses, such as cumulative curves and ordination techniques, were employed to check for differences among sites and eventual interaction of factors, such as depth and *C. caespitosa* colony density, that can influence species richness.

Incidence-based estimates aim at extrapolating species richness within the sampled range. Our results showed that for a correct estimation with these techniques the same number of sample per site and species accumulation curves approaching the asymptote at each site is required. Biased results such as the ones obtained with underwater counting are misleading when setting up conservation priorities.

### 5.3.2 Megabenthic assemblages

Megabenthic assemblages were dominated by molluscs, sponges and echinoderms in terms of richness. The same had been observed for invertebrates living on the biogenic formation at cape Ronék (Pitacco et al., 2014a), in the area within the bank that surrounds colonies of *C. caespitosa*. But the composition of the community differed: in the the present study the solitary coral *Balanophylla europea* and the sponge *Aplysina aerophoba* dominated, while the sea urchin *Psammechinus microtuberculatus* was dominant on the bank. Differences were probably related to the different substrate (secondary detritic bottom mainly made of dead corallites) and depth range.

Our results showed that the stable component of benthic assemblages did not qualitative differ among sites, with the only exception of the sponge *C. nucula*, which was highly abundant at some sites and almost absent in others. Differences among sites were mainly related with different abundance of the dominant species and presence of rare or occasional species.

Macroalgal communities at the studied sites with high density of *C. caespitosa* were dominated by the highly frequent and abundant *Peyssonellia* spp., *Halimeda tuna*, *Dictyota dichotoma*, *Padina pavonia* and coralline algae (*Lithophyllum/Lithothamnion*). Therefore beds of *C. caespitosa* can be considered a zone of transition between photophilic algal assemblages, for the presence of *P. pavonia* and *D. dichotoma* (Giaccone et al., 1994), and sciaphilic algal assemblages (pre-coralligenous, *sensu* Pérès and Picard, 1964), with the presence of *H. tuna* and species of *Peyssonelia*, *Lithophyllum* and *Lithothamnion*

(Giaccone et al., 1994). The presence of coralline algae of genera *Lithophyllum* and *Lithothamnion*, characteristic of the biocoenosis of Coastal detritic bottom and molluscs such as *Thylacodes arenarius* and *Columbella rustica* characteristic of the biocoenosis of Photophile Algae (Pérès and Picard, 1964) are additional evidences of the fact that this environment is located in a transitional zone between infra and circalittoral assemblages.

## 5.4 MACROSCALE

### 5.4.1. SAR

The same incidence-based estimates calculated for the mesoscale (Oksanen et al., 2005), were also applied at level of macroscale. Those estimates suggested that globally the site STR had the lowest value of species richness (observed also with cumulative curves), despite the high density of species found in each single transect at the same site. They also suggested that other sites showed comparable values of total richness, despite the different sampling effort and density of species in each single transect. Nevertheless we cannot exclude that increasing considerably the number of samples a slight difference among sites could be observed.

Considering only the most frequent and abundant species, assemblages were quite homogeneous among sampling sites, month and depth, varying only for species abundance and not for composition. Consequently, variation of species richness with increasing sampled area was mainly related with the records of occasional and uncommon species. This confirms frequency-based estimates as a suitable tool for species estimate.

According to »random sampling hypothesis« species number increases with increasing areas just as a random process related with sampling operation. This hypothesis required a comparable fish density among sites, which is not the case of the present work. Consequently the relationship between species and area was influenced by other factors. These factors are likely to be the same factors explaining the composition of resident species in investigated area (see 5.4.2), such as depth and substrate complexity. Transects performed at sites DR and STR were shallower than others and at both sites the density of *C. caespitosa* was lower, suggesting also a less heterogeneous substrate. However, the range of variation of these potential explanatory factors in the present investigation is too

small to find a clear correlation, so additional investigations should be performed to clarify the issue.

#### 5.4.2 Fish assemblages

The Northern Adriatic ichthyofauna has lower species richness than other Adriatic areas (Orlando Bonaca and Lipej, 2005). The number of species found within the present work showed that fish assemblages associated with *C. caespitosa* beds are in accordance with average fish richness of infralittoral habitats in the Gulf of Trieste (Orlando Bonaca and Lipej, 2005). Higher substratum complexity (rocks and boulders opposed to areas in which sand or gravel predominated) is known to be related with higher species number and density of fish (Macpherson, 1994, Gratwicke and Speight, 2005) providing more shelter for adults and recruits (Guidetti, 2000; Cheminée et al., 2015) as well as more nesting sites for spawning (Lipej et al., 2009). Compared with other infralittoral habitat types in the Gulf of Trieste, our results showed lower richness compared with highly structured habitat types, such as *Cystoseira* algal belts (31 species) and higher richness compared with more homogenous habitats, such as seagrass meadow (9 species) (Orlando Bonaca and Lipej, 2005).

The most frequent and abundant species (*Serranus scriba*, *Gobius cruentatus*, *Diplodus vulgaris*, *Serranus hepatus*, *Symphodus cinereus*, *Parablennius rouxi* and *Symphodus tinca*) are considered resident species, since they are strictly related with the substrate. All these species are nektobenthic with the exception of *Chromis chromis*, which is nektonic and diurnal planktivore (Bell and Harmelin-Vivien, 1983). They are related to the substrate for feeding, since their main source of food are benthic macroinvertebrates. For blennies and gobies substrate provides also shelter, since they usually hide in holes and crevices or among boulders. Colonies of *C. caespitosa*, with their physical structure can also provide a shelter for these groups, in particular for juveniles. Compared with other blennies usually present in more shallow waters, *P. rouxi*, and the unfrequent *Parablennius gattorugine*, prefer rocks covered by precoralligenous bioformations (Orlando Bonaca et al., 2007).

Some of the most frequent species, such as *S. scriba*, *C. chromis* and *P. rouxi*, were known to be associated with rocky unvegetated areas or with short vegetation, in the lower part of

the infralittoral belt at the same depth range of the present work, from 4 to 10 m (Orlando Bonaca and Lipej, 2005). Such habitat was therefore not suitable for most species of labrids, strictly associated with vegetated areas. *Symphodus cinereus*, was the only labrid that could be considered resident. The wrasse *Symphodus roissali*, strictly related with complex algal canopies in shallow depth, such as *Cystoseira* and *Halopithys* algal belts (Orlando Bonaca et al., 2008), was found only at one site (PR) between 5 and 6 m depth.

Even if *C. caespitosa* prefers areas with rocks and big boulders, it is able to settle also on debris, colonizing areas covered by sediment and gravel (Zibrowius, 1980). This explains the occasional presence of species like *Pomatoschistus bathi* and *Gobius fallax*. *P. bathi*, first recorded in 2005, is a common littoral goby in Slovenian coastal waters (Lipej et al., 2005), showing a preference for gravel and coarse sand (Lipej et al., 2005). *G. fallax* also prefer gravel, even if it can be found also on more vegetated habitats (Orlando Bonaca and Lipej, 2005).

Fish assemblages did not differ between sites inside and outside marine protected areas. This is consistent with previous investigations failing to demonstrate a reserve effect in Slovenian waters using fish assemblage as an indicator (Lipej et al., 2003). Despite their protected status in fact, all three studied marine protected areas are subject to different types of disturbance such as fishing, angling, passing boats and SCUBA diving (Lipej et al., 2003).

## 5.5 BIODIVERSITY ASSOCIATED WITH *C. CAESPITOSA*

Despite the presence of slight differences among sites, the investigated area was homogeneous enough, to show that the stable components of fish and invertebrate assemblages associated with beds of *C. caespitosa* have specific characteristic compared with surrounding infra and circalittoral assemblages. Also communities living inside colonies of *C. caespitosa* did not varied among sites. This was probably due to the small depth range and the homogeneous characteristics of the community characterizing *C. caespitosa* beds. In the Aegean Sea in fact a difference had been found between macroinvertebrates associated with *C. caespitosa* inhabiting at different depths (3-5 vs 15-19 m), surrounded by different assemblages (photophilic algal assemblages vs biogenic



bank surrounded by gravel and sand). Given the limited differences among sites in the study area, estimates based on SAR are consistent for all sites analysed and can be reasonably extended to other sites along the Slovenian coast.

### **5.5.1 Nature conservation implication**

The present work confirmed the important role of *C. caespitosa* as a habitat builder. Beds of *C. caespitosa* in the lower infralittoral belt of the studied area host rich and diversified communities of invertebrates and fish. About 300 taxa were found, counting for about 1/7 of the almost 2000 species known (Turk and Lipej, 2002) for the Slovenian part of the Gulf of Trieste. Richness of fish associated with *C. caespitosa* beds represent 1/10 of the 259 fish species recorded in the Gulf of Trieste, 1/7 of the 184 species recorded in Slovenian waters (Marčeta, 1999) and 1/17 of the 440 species recorded for Adriatic Sea (Lipej and Dulčić, 2010).

Most of taxa richness was detected at the microscale level, which was the most time-consuming and destructive part of the work. Therefore, it's important to consider that with more fast and non-destructive monitoring techniques, the major component of biodiversity (almost 80 %) would be overlooked. Given also the few data available on fauna associated with *C. caespitosa* and the vulnerability of the coral itself (slow growth rate, mass mortality and bleaching events) estimates based on the SAR could be suitable methods to estimate species richness in areas with high coral density in a non-destructive way.

Different methods have been developed for the extrapolation of species richness. The sampling scheme for the microscale levels enabled us to use linear regression to estimate species richness associated with a colony with certain dimensions. This tool enables the addition of other variables, such as microhabitat variables, to the analysis in order to improve the extrapolation and obtain additional knowledge on biological factors influencing SAR.

The biggest colony found along the Slovenian coast (Zunino, 2013) had 68 cm of length (estimated volume = 19369 cm<sup>3</sup>). The prediction based on calculated regressions, using the total volume as size descriptor, suggests that such a colony may host a total of 130 taxa (95 % confidence intervals: 105-162), among them 39 taxa of molluscs (95 % confidence

intervals: 29-53) and 61 (95 % confidence intervals: 43-85) taxa of polychaetes. The poor fit of species/area regression for crustaceans, leave a certain uncertainty on eventual estimates of crustacean richness. Extrapolation from without the limits of a dataset should always be considered caution, but colonies larger than 50 cm in length were found to be extremely rare in the studied area (Schiller, 1993; Zunino, 2013; Kružić et al., 2014), so there's no need to push extrapolation results so far from the data range.

Moreover, the finding of rare and very poorly known species among the invertebrates associated with *C. caespitosa* in this study is an evidence of how the present knowledge on marine invertebrates is still limited and how the loss of this precious habitat builder could negatively affect certain species before they are fully known or even yet discovered.

Present work proved that SAR models can help us estimating species richness in a non-destructive way at the study area, but there's the need of additional investigations in order to improve and adapt this tool, to make it suitable for other areas of the Mediterranean Sea, as well.

Knowledge on macrofauna associated with *C. caespitosa* is still limited to few locations in the Mediterranean Sea and many questions still remains opened. For instance how factors such as depth and seasonality influence mollusc assemblages associated with *C. caespitosa*. There is a need to extend this kind of analysis in other areas of the Mediterranean Sea and also to focus investigations on some taxonomic groups that could have important roles for the health of *C. caespitosa* populations and requires dedicated sampling plans, such as coral predators and sponges.

The identification of organisms within communities to species level is one of the greatest constraints in terms of time and costs in ecological studies (Giangrande, 2003) and represents a crucial point for SAR. Some studies have suggested that working at a taxonomic level higher than species does not result in an important loss of information (e.g. taxonomic sufficiency). This type of simplification can be useful for the creation of new diversity indices, which can be a good tool for monitoring benthic community to test their response in view of well defined stressors. However, it cannot substitute good and

solid autecological knowledge of the species at the studied and monitored area, which has to be the base of the tools themselves.

It is clear that the number of species reported for a certain area or habitat is strictly linked, not only to the sampling effort, but also to the different taxonomic focus of the authors (Mastrototaro et al., 2010) and that it is very important to encourage cooperation between taxonomists and ecologists to understand the true and complex biodiversity associated with corals, and therefore improve our predictions based on SAR.

The present work also demonstrated the importance of *C. caespitosa* beds as an habitat attracting fish assemblages, even if fish richness resulted lower than highly structure habitats such as *Cystoseira* belts. It was not possible to disentangle the contribution of *C. caespitosa* and other components such as algae, sponges and presence of boulders in influencing fish assemblages, but given the longevity and sensibility of the coral it could be used as a useful indicator of the health of the system.

This species has received a special attention recently also because it can be used as a valid indicator, fulfilling the three demands: sensibility, measurability and widespread distribution. Being sensitive, sessile and long-lived it acts as a memory of environmental events. These events can be measured through the x-rays and elemental analyses of its calcareous skeleton (Silenzi et al., 2005; Montagna et al., 2007; 2008). Since the effects of stress are measurable also at a short time scale, *C. caespitosa* was selected as one of the potential indicators for the determination of Good Environmental Status (GES) in Slovenian waters according to Marine Strategy Framework Directive (Peterlin et al., 2013). The ecological status of a particular water body is a broad concept integrating both physico-chemical and biological measures, and can be defined as “good” if deviate only slightly from those normally associated with the surface water body type under undisturbed conditions. Benthos and fish are some of the biological elements of particular importance in assessing such a status (Borja et al., 2008) and species richness is a common descriptor. SAR has already been suggested as a standardisation tool to characterise ecological status with respect to species richness for lagoons (Sabetta et al., 2007).

Given its role as a bioconstructor and the interest aroused by corals among public opinion, *C. caespitosa* has the potential to become not only an indicator of good ecological status (GES) but also a flag species. Its protection would involve necessarily habitat protection with positive consequence on accompanying and associated less “catchy” taxa, such as polychaetes.

## 6 CONCLUSION

The present work was the first attempt to apply SAR models to estimate the importance of the Mediterranean stony coral for biodiversity. Our results provided evidences of the importance of *C. caespitosa* as a habitat builder.

SAR models were applied on data collected with a combination of traditional and non-destructive SCUBA diving methods at three levels: “microscale”, biodiversity inside *C. caespitosa* colonies, “mesoscale”, biodiversity of megabenthic species where density of *C. caespitosa* was high and “macroscale”, fish assemblages associated with the same area.

A total of 290 different taxa among invertebrates and fish were found, some of them were rare and poorly known. Four species were new records for the northern Adriatic. Colonies showed to be important as mating and nurse ground for invertebrates.

With fast and non-destructive techniques, the major component of biodiversity (almost 80 %) was overlooked. SAR models are suitable tools for the estimate of species richness, but they required solid autecological knowledge.

At microscale level SAR model based on linear regression held for total community, molluscs, polychaetes and crustaceans considered separately. Colony size was the best predictor of species richness. For polychaetes microhabitat represented by mud and sediment trapped inside colonies and colony three dimensional structures had an effect on species-area relation.

Different mechanisms operating in combination are probably underlying Species-Area Relationship for coral associated: area-*per se*, microhabitat diversity and age of corals.

Assemblages living inside colonies were influenced by surrounding habitat, differently structured from invertebrates associated to macroalgal belt.

Comparison with polychaetes assemblages associated with *C. caespitosa* in the Aegean Sea showed that species-area pattern was consistent in different areas of the Mediterranean.

At mesoscale level photographic technique, compared with underwater counting proved to be a better method to estimate biodiversity. It enabled a higher extent, higher number of samples, repeatability, best cost-benefit balance and provided better results. Frequency-based estimates were confirmed as suitable tools for estimate of species richness.

Sampled areas with high density of *C. caespitosa* were transitional zones between the lower part of infralittoral assemblages and the upper circalittoral ones.

At macroscale level frequency-based estimates were confirmed as suitable tools for estimate of species richness, as well. The stable component of fish assemblages was constant among sites, so variation in species richness was mainly due to rare and occasional species.

Beds of *C. caespitosa* provided food and shelter for resident fish species. Total richness values were intermediate between more structured environments, such as macroalgal belts and less structured, such as seagrass and unvegetated areas.

## 7 SUMMARY (POVZETEK)

### 7.1 SUMMARY

The Mediterranean stony coral *Cladocora caespitosa* (Linnaeus, 1767) is a native colonial, shallow-water coral, common in the whole Mediterranean it is abundant only locally. In the northern Adriatic *C. caespitosa* is more frequent and abundant along the eastern coast and in the Gulf of Trieste is particularly abundant along the Slovenian coast, where it forms “beds”, that is numerous colonies living more or less close to each other, between 4 and 10 m depth and a peculiar biogenic formation in deeper waters. *C. caespitosa* is undergoing a rapid decrease in both size and spatial distribution. Among major threats there are global warming and acidification of the ocean. Given its morphology it is capable to host a diversified faunal assemblage, but few studies are available on the macrofauna associated with this species. Moreover, coral associated species could represent an important food source for other invertebrates and benthic fishes. Nevertheless, the role of Mediterranean stony coral for benthic fishes has not being subject of investigation jet.

Species-area relationships (SARs) are among the best known and most studied patterns in ecology. They describe the pattern in which the species richness (S) increases with area (A). Models based on SAR have been proposed in conservation biology to project the expected loss of species richness from a region undergoing specified levels of habitat degradation and to estimate local species richness for hotspot identification. But despite the large number of articles published, there is still no general agreement either on the shape of the species-area curve or on its biological interpretation. To a large degree, this is because SAR models and their explanations depend on different factors such as scale, methodology and time.

The aim of this work was to check the importance of Mediterranean stony coral for marine biodiversity and to test the application of SAR in areas where *C. caespitosa* was abundant. In particular, the main goals were:

1. to test the hypothesis that the number of associated macroinvertebrate species increases in relation with the colony size, following Arrhenius power-equation model
2. to test if SAR models can be effectively applied at the levels of micro, meso and macroscale for the estimation of species richness
3. to test the importance of the Mediterranean stony coral as habitat builder for biodiversity, checking whether an increase in spatial heterogeneity is related to an increase in species richness.

Sampling surveys were performed from 2012 to 2015 with SCUBA diving at 5 sampling sites between 4 m and 9 m of depth, where *C. caespitosa* was particularly abundant. Investigations were performed with a combination of traditional methods and non-destructive SCUBA diving methods at 3 levels: “microscale”, biodiversity within *C. caespitosa* colonies at scale of square centimeters, “mesoscale”, biodiversity among colonies at scale of square meters and “macroscale”, fish assemblages associated with the area dominated by *C. caespitosa*, at scale of ten or so square meters.

At level of microscale 5 colonies of *C. caespitosa* of different dimensions were collected at each site, they were brought to laboratory, measured and then broken down. Samples were sieved, sorted and each organism determined with a stereomicroscope and a microscope. Each species was assigned to a trophic group (predators - P, parasites - Pa, omnivores - O, SF - suspension feeders - SF, deposit feeders - DF, micrograzers –MG and grazers - G) and functional groups (free living - FL, sessile epilithic species - EP, endolithic species - EN, soft bottom dwelling species – SB and discretely mobile species - DM).

At level of meso and macroscale non-destructive sampling methods were employed. At level of mesoscale two methods were employed and compared. Data were collected using quadrat sampling method. A metal frame of 1 x 1 meter separated in 4 subquadrats was placed in areas where *C. caespitosa* was more abundant. Data on invertebrate assemblages were collected *in situ* and additional data were obtained with analysis of pictures.

At levels of macroscale horizontal transects from 30 to 50 m in length were laid down at different depths. Fish were counted mostly within 2 m, 1 m to the left and 1 m to the right



of the line. Species names and abundances were marked during diving on a diver slate. A total of 51 transects were performed.

The importance of *C. caespitosa* as a habitat builder was proved. Altogether 290 taxa were found associated with *C. caespitosa* beds, 222 were detected at microscale level, 71 at mesoscale level, 25 at macroscale level. Some of them were rare and poorly known. Four species were new records for the northern Adriatic. Colonies proved to be an important nursery ground for macroinvertebrates. Half of molluscs recorded were juveniles. With fast and non-destructive techniques, the major component of biodiversity (almost 80 %) was overlooked.

Two methods were used to extrapolate species richness: linear regression for microscale and incidence-based estimates for meso and macroscale. The suitability of methods used for estimating species richness was proved at the three levels analysed. At the microscale level SAR was consistent also considering molluscs, polychaetes and crustaceans separately. At meso and macroscale level frequency-based estimates were confirmed as suitable tools for estimate of species richness. SAR models are therefore suitable tools for the estimate of species richness, but they required solid autecological knowledge.

Different mechanisms operating in combination are probably underlying Species-Area Relationship for coral associated: area-*per se*, microhabitat diversity and age of corals. Colonies of *C. caespitosa* offer three main types of microhabitat: hard substrate for epilithic and endolithic species, interstitial space for small motile organisms and sediment trapped for soft bottom species.

Assemblages living inside colonies were influenced by surrounding habitat. Assemblages associated with *C. caespitosa* were differently structured compared with invertebrates associated to macroalgal belt. The stable component of fish and benthic assemblages surrounding colonies was more or less constant among sites. Variation in species richness was mainly related with the presence of rare and uncommon species. Studied beds of *C. caespitosa* were transitional zones between the lower part of infralittoral assemblages and the upper circalittoral ones. They provided food and shelter for resident fish species.

## 7.2. POVZETEK

Sredozemska kamena korala *Cladocora caespitosa* (Linnaeus, 1767) je samonikli kolonijski ožigalkar, ki naseljuje plitve predele Jadranskega in Sredozemskega morja. V Severnem Jadranu je pogostejša in bolj številčna vzdolž vzhodne obale. V Tržaškem zalivu je številčnejša vzdolž slovenske obale, kjer se ponekod pojavlja z veliko gostoto kolonij (angl. *beds*), še posebej med 4 in 10 m globine. Znana je tudi velika biogena formacija, ki jo tvorijo mrtvi koraliti sredozemske kamene korale pri Strunjanu. Danes je sredozemska kamena korala ogrožena vrsta, ki se sooča z upadom populacije. Ogrožajo jo predvsem globalno segrevanje morij in oceanov in acidifikacija morske vode. Sredozemska kamena korala je pomembna predvsem z vidika biotske raznovrstnosti, saj je znano, da med koraliti znotraj kolonij prebiva pestra množica živali. Poleg tega je pomemben vir hrane za mnoge nevretenčarje in nekatere vrste rib. Kljub temu je danes še vedno razmeroma malo znanega o sredozemski kameni korali.

Odnos med vrstno pestrostjo in površino (*species area relationship* - SAR) je eden izmed najbolj raziskanih in pomembnih ekoloških konceptov, pri katerem se z naraščajočo površino (do neke mere) viša število vrst. SAR se je izkazal za primerno orodje za vrednotenje biotske raznovrstnosti (biodiverzitete) v danem okolju. V konzervacijski biologiji nudi uporaba SAR modela možnost ocene izgube vrstne pestrosti v okolju, ki se sooča z degradacijo habitatov ali pa za oceno lokalne vrstne pestrosti za opredelitev in prepoznavo takoimenovanih vročih točk biodiverzitete.

Namen te naloge je ugotoviti pomen sredozemske kamene korale za morsko biodiverzitetu s poskusom aplikacije SAR v okoljih, kjer kamena korala prevladuje. Glavni cilji naloge so bili:

1. z uporabo Arrheniusove enačbe testirati, ali se število nevretenčarjev povečuje z velikostjo kolonij sredozemske kamene korale,
2. testirati ali je možno uporabiti model SAR na treh nivojih površine, to so mikro-, mezo- in makro-skala, za oceno vrstne pestrosti,

3. preveriti pomen sredozemske kamene korale kot biogradnika za biodiverzitetu in ugotoviti, ali se s povečanjem prostorske heterogenosti poveča tudi vrstna pestrost.

Vzorčevanja smo opravili v obdobju med 2012 in 2015 z uporabo avtonomne potapljaške opreme (SCUBA) v globinskem razponu med 4 in 9 m, na petih vzorčevalnih postajah. Uporabili smo standardne metode za vzorčevanje pridnenih organizmov, poleg njih pa tudi nedestruktivne metode popisov obrežne ribje združbe v okoljih s prevladujočo kameno koralo.

Konceptualno smo razdelili nalogo na tri prostorske vidike in sicer na:

- mikroskalo, ki se nanaša na popisovanje in analizo pridnenih nevretenčarjev v samih kolonijah kamene korale ( $\text{cm}^2$ ),
- mezoskalo, ki se nanaša na popisovanje in analizo pridnenih nevretenčarjev med kolonijami kamene korale ( $\text{m}^2$ ), in
- makroskalo, ki se nanaša na popisovanje in analizo obrežne ribje združbe v okolju s prevladujočo kameno koralo ( $10 \text{ m}^2$ ).

Na nivoju mikroskale smo pobrali 5 kolonij različnih velikosti na vsaki od 5 vzorčevalnih lokalitet in jih prinesli v laboratorij, kjer smo opravili biometrične meritve. Nato smo kolonije razbili na posamezne koralite in s pomočjo različnih sit presortirali vse nevretenčarje na njih ali v njih. Te smo potem določili in prešteli s pomočjo stereo-lupe in mikroskopa. Vsako vrsto smo uvrstili v različne trofične razrede (plenilci, zajedavci, vsejedi, suspenzijofagi, detritivori ter pašni organizmi, mikro- in makrograzerji) in funkcionalne skupine (prostoživeče vrste, sesilne epilitske vrste, endolitske vrste, vrste mehkega dna in slabo premikajoče se vrste).

Na nivoju mezoskale smo uporabili nedestruktivne metode popisovanja. Uporabili smo dve metodi vzorčevanja s kvadratom in jih med seboj primerjali. Kovinski okvir 1 m x 1 m, dodatno predeljen na 4 podkvadrate, smo položili na morsko dno s prevladujočo kameno koralo. Nevretenčarje smo opisali in določili na mestu samem, uporabili pa smo tudi

fotografsko metodo kartiranja, pri kateri smo podatke o nevretenčarjih pridobili z analizo fotografij.

Na nivoju makroskale smo uporabili tehniko horizontalnih opazovalnih transektov, pri katerih položimo na morsko dno s prevladujočo kameno koralo v različnih globinah merilni trak dolžine 30 m ali 50 m in popisujemo obrežne ribje vrste. Ribe štejemo na razdalji 1 m levo in 1 m desno od merilnega traku in na mestu samem pridobljene podatke vnesemo na potapljaško tablico. Opravili smo 51 opazovalnih transektov.

Z dobljenimi rezultati smo potrdili pomen sredozemske kamene korale kot izjemnega biogradnika, ki nudi bivalne niše številnim vrstam nevretenčarjev.

V okolju s prevladujočo sredozemsko kameno koralo smo na vseh treh nivojih popisali 290 taksonov nevretenčarjev in rib, od tega 222 na nivoju mikroskale (v in na kolonijah), 71 na nivoju mezoskale (med kolonijami) in 25 na nivoju makroskale (v okolju z visoko gostoto kolonij).

Med 222 vrstami, ki so bile ugotovljene v kolonijah kamene korale, je bilo 95 taksonov mnogoščetincev (Polychaeta), 64 mehkužcev (Mollusca), 43 rakov (Crustacea), po 5 plaščarjev (Tunicata) in mahovnjakov (Bryozoa), 4 iglokožci (Echinodermata), 3 spužve (Spongiaria), 1 ožigalkar (Cnidaria) in 1 pršivec (Sipunculida).

Med ugotovljenimi vrstami so bile tudi nekatere redke in manj znane vrste. Štiri vrste so bile prvič potrjene za severni Jadran. Kolonije kamene korale so tudi pomembno vzrejno območje (*nursery area*) za mnoge vrste pridenih nevretenčarjev. Če bi vzorčevali le s hitrimi in nedestruktivnimi metodami, bi spregledali glavno komponento biodiverzitete (skoraj 80 %).

Za ekstrapoliranje vrstne pestrosti smo uporabili dve metodi in sicer linearno regresijo za nivo mikroskale in oceno na podlagi incidence na nivoju mezoskale in makroskale. Obe metodi sta se izkazali kot primerni na vseh treh raziskanih nivojih. Na nivoju mikroskale smo uspeli potrditi SAR model posebej za mehkužce, mnogoščetince in rake. Na nivojih mezo- in makroskale se je metoda ocene na podlagi frekvenc pojavljanja izkazala za

primerno pri ocenjevanju vrstne pestrosti. Na podlagi navedenega so se SAR modeli izkazali za primerna orodja pri ugotavljanju vrstne pestrosti, vendar le ob dobrem poznavanju avtoekologije ugotovljenih vrst.

Veliko je razlag, s katerimi so poskušali razložiti koncept modela SAR, v našem primeru, ki obravnava sredozemsko kameno koralo, pa so najpomembnejši sama površina, pestrost mikrohabitativ in starost koral. Kolonije kamene korale nudijo tri tipe mikrohabitativ in sicer: trdi substrat za epiltske in endolitske vrste, intersticielni prostor za majhne vagilne vrste in sediment, ujet med koralite, za vrste mehkega dna.

Na skupnost v kolonijah koral živečih pridnenih nevretenčarjev je vplival okoliški habitat. Struktura skupnosti nevretenčarjev v kolonijah je drugačna kot npr, v taistih skupnostih, ki prebivajo kot epibionti na makroalgah (npr. vrste iz rodu *Cystoseira*).

Glavnino ribjih vrst in vrst pridnenih nevretenčarjev okoli kolonij kamene korale na raziskanih lokalitetah sestavljajo bolj ali manj iste vrste. Variabilnost vrstne pestrosti je bila predvsem povezana s prisotnostjo redkih ali nepričakovanih vrst. Raziskana okolja s prevladujočo kameno koralo so nekakšni prehodni predeli med skupnostmi iz spodnjega infralitorala in tistimi iz zgornjega cirkalitorala. Ta okolja nudijo hrano in skrivališče rezidentnim vrstam rib.

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## ANNEXES

Annex A: Differences between sampled sites of biometrical characteristics of *C. caespitosa* (D1 = length; D2 = width; H = height; A = covered area; Vtot = total volume, % LP = percentage of living polyps), trophic guilds and functional groups (abbreviation at p. XV) tested with Kruskal-Wallis chi-squared. S – number of taxa, N – abundance. <sup>1</sup> without gregarious species

Priloga A: Razlike med lokacijami biometričnih značilnosti *C. caespitosa* (D1 = dolžina; D2 = širina; H = višina; A = površina; Vtot = skupna prostornina, % LP = delež živih polipov), prehranjevalnimi in funkcionalnimi skupinami (okrajšave str. XV) testirane z Kruskal-Wallisovim testom. S - število taksonov, N – abundanca, <sup>1</sup> brez jatnih vrst.

Groups	KW chi-squared	df	p-value	Signifi- cance	Groups	KW chi-squared	df	p-value	Signifi- cance
<b>Colonies of <i>C. caespitosa</i></b>					<b>Colonies of <i>C. caespitosa</i></b>				
D1 x site	2.403	4	0.662	NS	Is-index x site	7.361	4	0.118	NS
D2 x site	1.781	4	0.776	NS	Calyces D1 x site	32.948	4	< 0.0001	***
H x site	3.037	4	0.552	NS	Calyces D2 x site	18.126	4	0.001	**
A x site	0.705	4	0.951	NS	% LP x site	5.560	4	0.235	NS
W x site	1.945	4	0.746	NS	% int_vol x site	7.920	4	0.095	NS
V <sub>tot</sub> x site	2.545	4	0.637	NS	Algae_cov x site	4.552	4	0.336	NS
V <sub>net</sub> x site	2.249	4	0.690	NS	Cov_sponge x site	4.246	4	0.374	NS
V <sub>int</sub> x site	2.567	4	0.633	NS	Mud x site	7.964	4	0.093	NS
<b>Macrobenthic assemblages</b>					<b>Macrobenthic assemblages</b>				
<b>Trophic groups (S)</b>					<b>Trophic groups (N)</b>				
G x site	4.577	4	0.334	NS	G x site	12.321	4	0.015	NS
MG x site	4.858	4	0.302	NS	MG x site	5.293	4	0.259	NS
DF x site	1.738	4	0.784	NS	DF x site	2.370	4	0.668	NS
O x site	6.583	4	0.160	NS	O x site	6.374	4	0.173	NS
Pa x site	5.818	4	0.213	NS	Pa x site	12.321	4	0.015	*
SF x site	5.643	4	0.227	NS	SF x site	2.949	4	0.566	NS
P x site	6.658	4	0.155	NS	P x site	4.209	4	0.378	NS
<b>Functional groups (S)</b>					<b>Functional groups (N)</b>				
EN x site	8.543	4	0.074	NS	EN x site	8.686	4	0.069	NS
EP x Site	4.774	4	0.311	NS	EP x Site	2.291	4	0.682	NS
FL x site	3.266	4	0.514	NS	FL x site	0.962	4	0.916	NS

Continued

Continuation of annex A: Differences between sampled sites of biometrical characteristics of *C. caespitosa*

Groups	KW chi-squared	df	p-value	Signifi- cance	Groups	KW chi-squared	df	p-value	Signifi- cance
DM x site	9.956	4	0.041	*	DM x site	4.449	4	0.349	NS
SB x site	6.527	4	0.163	NS	SB x site	8.967	4	0.062	NS
<b>Mollusc assemblages</b>					<b>Mollusc assemblages</b>				
<b>Richness</b>					<b>Abundance</b>				
$S_{(1000\text{ cm}^3)}$ x site	2.406	4	0.662	NS	$N_{(1000\text{ cm}^3)}$ x site	4.873	4	0.3	NS
<b>Trophic groups (S)</b>					<b>Trophic groups (N)</b>				
MG x site	3.788	4	0.435	NS	MG x site	4.274	4	0.370	NS
O x site	2.195	4	0.700	NS	O x site	2.195	4	0.700	NS
Pa x site	4.000	4	0.406	NS	Pa x site	4.000	4	0.406	NS
SF x site	3.294	4	0.510	NS	SF x site	5.258	4	0.262	NS
P x site	6.695	4	0.153	NS	P x site	6.283	4	0.179	NS
<b>Functional groups (S)</b>					<b>Functional groups (N)</b>				
EN x site	2.275	4	0.685	NS	EN x site	5.526	4	0.238	NS
EP x Site	3.515	4	0.476	NS	EP x Site	5.166	4	0.271	NS
FL x site	3.812	4	0.432	NS	FL x site	2.191	4	0.701	NS
DM x site	3.222	4	0.521	NS	DM x site	3.231	4	0.520	NS
SB x site	3.133	4	0.536	NS	SB x site	3.133	4	0.536	NS
<b>Polychaetes assemblages</b>					<b>Polychaetes assemblages</b>				
<b>Richness</b>					<b>Abundance</b>				
$S_{(1000\text{ cm}^3)}$ x site	6.137	4	0.189	NS	$N_{(1000\text{ cm}^3)}$ x site	8.112	4	0.088	NS
<b>Trophic groups (S)</b>					<b>Trophic groups (N)</b>				
MG x site	5.643	4	0.228	NS	MG x site	13.074	4	0.011	*
O x site	7.040	4	0.134	NS	O x site	13.583	4	0.009	**
Pa x site	4.858	4	0.302	NS	Pa x site	13.074	4	0.011	*
SF x site	6.450	4	0.168	NS	SF x site	4.624	4	0.328	NS
P x site	5.980	4	0.201	NS	P x site	3.829	4	0.430	NS
DF x site	9.847	4	0.043	NS	DF x site	4.665	4	0.323	NS
<b>Functional groups (S)</b>					<b>Functional groups (N)</b>				
EN x site	10.903	4	0.028	*	EN x site	10.903	4	0.028	*
EP x Site	5.571	4	0.234	NS	EP x Site	2.800	4	0.592	NS
FL x site	7.557	4	0.109	NS	FL x site	3.648	4	0.456	NS
DM x site	9.885	4	0.042	*	DM x site	4.767	4	0.312	NS
SB x site	6.948	4	0.139	NS	SB x site	9.351	4	0.053	NS

Continued



Continuation of annex A: Differences between sampled sites of biometrical characteristics of *C. caespitosa*

Groups	KW chi-squared	df	p-value	Signifi- cance	Groups	KW chi-squared	df	p-value	Signifi- cance
<b>Crustacean assemblages</b>					<b>Crustacean assemblages</b>				
<b>Richness</b>					<b>Abundance</b>				
S <sub>(1000 cm<sup>3</sup>)</sub> x site	5.073	4	0.280	NS	N <sub>(1000 cm<sup>3</sup>)</sub> X site	13.019	4	0.011	*
<b>Trophic groups (S)</b>					<b>Trophic groups (N)</b>				
O x site	5.689	4	0.224	NS	O x site	12.788	4	0.012	NS
Pa x site	3.130	4	0.536	NS	Pa x site	3.130	4	0.536	NS
SF x site	5.689	4	0.224	NS	SF x site	12.788	4	0.012	NS
P x site	9.000	4	0.061	NS	P x site	10.035	4	0.040	NS
DF x site	2.670	4	0.615	NS	DF x site	3.695	4	0.449	NS
<b>Functional groups (S)</b>					<b>Functional groups (N)</b>				
EP x Site	2.400	4	0.663	NS	EP x Site	2.232	4	0.693	NS
FL x site	2.350	4	0.671	NS	FL x site	4.815	4	0.307	NS
<b>Fish assemblages</b>					<b>Fish assemblages</b>				
N fish <sub>(100m<sup>2</sup>)</sub> x site	23.766	4	< 0.0001	***	N fish <sub>(100m<sup>2</sup>)</sub> X site <sup>1</sup>	18.978	4	0.001	***
N fish <sub>(100m<sup>2</sup>)</sub> x month	6.728	3	0.081	NS	N fish <sub>(100m<sup>2</sup>)</sub> X month <sup>1</sup>	2.093	3	0.553	NS
N fish <sub>(100m<sup>2</sup>)</sub> x year	17.250	2	0.000	***	N fish <sub>(100m<sup>2</sup>)</sub> X year <sup>1</sup>	4.952	2	0.084	NS

Annex B: Average dimensions of corallites of *C. caespitosa* for each sampling site. D1 = calyces length, D2 = calyces width, H = corallite height, A = calyces surface ( $D1 * D2 * \pi$ ).

Priloga B: Povprečne dimenzije koralitov *C. caespitosa* na različnih lokalitetah. D1 = največji premer čaše, D2 = najmanjši premer čaše, H = višina koralitov, A = površina čaše ( $D1 * D2 * \pi$ ).

Site	D1	sd	D2	sd	H	sd	A	sd
<b>STR</b>	0.47	0.06	0.42	0.42	4.06	4.06	0.63	0.14
<b>PA</b>	0.51	0.07	0.44	0.44	5.72	5.72	0.71	0.16
<b>RR</b>	0.49	0.07	0.42	0.42	5.17	5.17	0.64	0.14
<b>DR</b>	0.48	0.06	0.43	0.43	3.53	3.53	0.66	0.14
<b>PR</b>	0.48	0.06	0.42	0.42	5.36	5.36	0.64	0.14

Annex C: Correlation results (abbreviation at p. XV)

Priloga C: Rezultati korrelacij (okrajšave na str. XV)

	$r_s$	p-value	signif		$r_s$	p-value	signif
Colony biometry							
total colony volume and %LP	0.947	0.108	NS	maximum axis and height	0.849	< 0.001	***
maximum and minimum axis	0.924	< 0.001	***	maximum axis and total volume	0.947	< 0.001	***
maximum axis and wet weight	0.955	< 0.001	***	surface covered and total volume	0.977	< 0.001	***
Polychaetes							
Polychaete richness				Polychaete abundance			
S and depth	-0.432	0.031	*	N and depth	-0.316	0.124	NS
S and mud	0.664	< 0.001	***	N and mud	0.595	0.002	**
S (1000 cm <sup>3</sup> ) and volume	-0.640	0.001	**	N (1000 cm <sup>3</sup> ) and volume	-0.467	0.022	*
S and A	0.711	< 0.001	***	SB and mud	0.604	0.001	**
S and volume	0.690	< 0.001	***	SB and volume	0.628	0.001	**
Functional and trophic groups (S)				Functional and trophic groups (N)			
FL and volume	0.735	< 0.001	***	FL and volume	0.808	< 0.001	***
DM and volume	0.595	0.002	**	DM and volume	0.916	< 0.001	***
C and volume	0.541	0.005	**	C and volume	0.787	< 0.001	***
DF and volume	0.406	0.044	*	DF and volume	0.866	< 0.001	***
C and volume	0.541	0.005	**	C and volume	0.787	< 0.001	***
DF and volume	0.406	0.044	*	DF and volume	0.866	< 0.001	***
Crustacean assemblage							
Crustacean richness				Crustacean abundance			
S and depth	0.086	0.684	NS	N and depth	-0.115	0.583	NS
S and mud	0.288	0.163	NS	N and mud	0.385	0.057	NS
S (1000 cm <sup>3</sup> ) and volume	-0.558	0.004	**	N (1000 cm <sup>3</sup> ) and volume	-0.429	0.032	*
Decapod richness				Decapod abundance			
S and A	0.553	0.004	**	N and A	0.64012	< 0.001	***

Continued

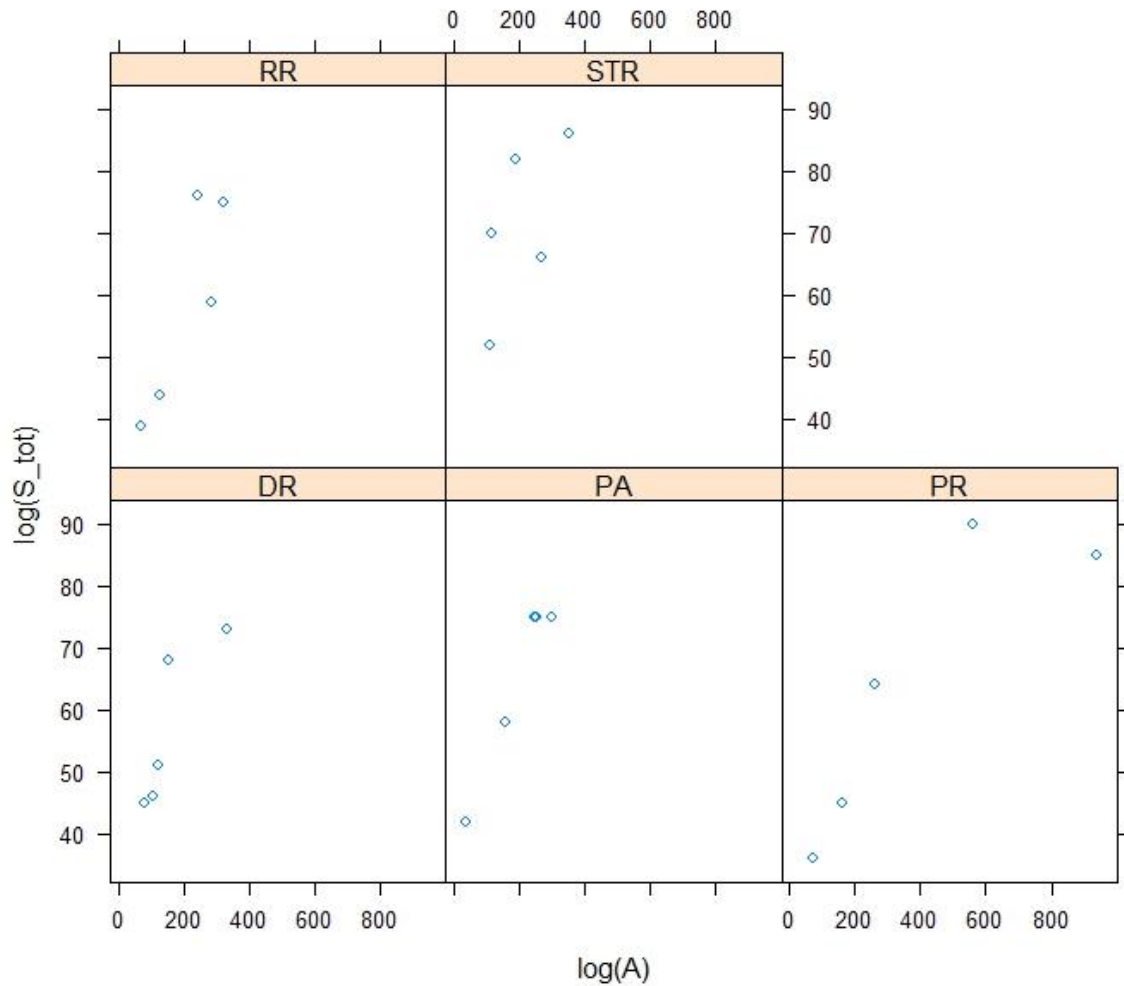
Continuation from annex C: Correlation results (abbreviation at p. XV)

	$r_s$	p-value	signif		$r_s$	p-value	signif
S and depth	0.090	0.670	NS	N and depth	-0.008	0.971	NS
S and mud	0.220	0.291	NS	N and mud	0.359	0.078	NS
S and LP	0.169	0.418	NS	N and LP	-0.020	0.923	NS
Amphipod richness				Amphipod abundance			
S and A	0.456	0.033	*	N and A	0.418	0.037	*
Trophic groups (S)				Trophic groups (N)			
C and volume	0.012	0.954	NS	C and volume	0.012	0.954	NS
DF and volume	0.466	0.019	*	DF and volume	0.362	0.076	NS
O and volume	0.408	0.043	*	O and volume	0.222	0.285	NS
SF and volume	0.408	0.043	*	SF and volume	0.222	0.285	NS
Mollusc assemblage							
Mollusc taxa richness				Mollusc abundances			
S (1000 cm <sup>3</sup> ) and volume	-0.576	0.003	**	N (1000 cm <sup>3</sup> ) and volume	-0.187	0.372	NS
S (1000 cm <sup>3</sup> ) and volume	-0.658	< 0.001	***	N (1000 cm <sup>3</sup> ) and volume	-0.599	0.002	**
S and depth	-0.185	0.375	NS	N and depth	-0.220	0.291	NS
S and depth	-0.185	0.375	NS	N and depth	-0.220	0.291	NS
Functional groups (S)				Functional groups (N)			
SB and Mud	0.270	0.192	NS				
SB and volume	-0.061	0.771	NS				
FL and volume	0.772	< 0.001	***	FL and volume	0.846	< 0.001	***
EN and volume	0.460	0.021	*	EN and volume	0.732	< 0.001	***
DM and volume	0.085	0.685	NS				
EP and volume	0.610	0.001	**	EP and volume	0.797	< 0.001	***

Annex D: AIC values (abbreviation at p. XV)

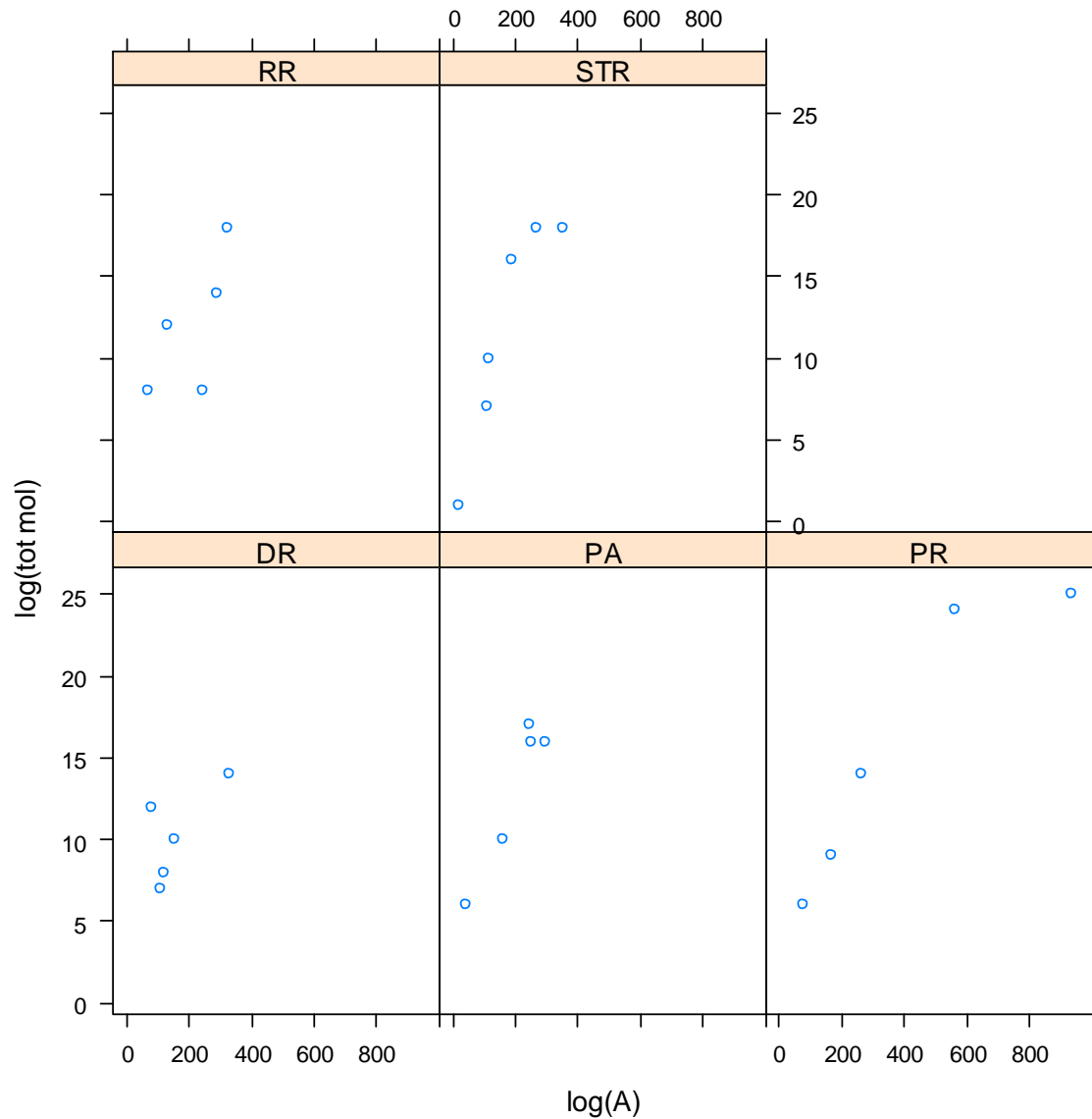
Priloga D: AIC vrednosti (okrajšave na str. XV)

<b>Model</b>	<b>Variables</b>	<b>df</b>	<b>AIC</b>
Arrhenius	S and volume	3	185.6667
Gleason	S and volume	3	183.7809
Gitay	S and volume	3	184.4852
Lomolino	S and volume	4	185.3495
Michaelis-Menten	S and volume	3	185.2392
log-log linear regression	S and volume	3	-19.919
log-log linear regression	S and A	3	-18.5215
log-log linear regression	S and volume and depth	4	-23.8049
log-log linear regression	S and volume and mud	4	-24.6308
log-log linear regression	S and volume mud and depth	5	-25.5911
log-log linear regression	mol and area	3	-1.77413
log-log linear regression	mol and volume	3	-2.68396
log-log linear regression	mol and volume and depth	4	-1.79715
log-log linear regression	mol and volume and depth and mud	5	-0.90481
log-log linear regression	poly and area	3	-0.87247
log-log linear regression	poly and volume	3	-3.07796
log-log linear regression	poly and volume and mud	4	-14.6352
log-log linear regression	poly and volume and mud and depth	5	-16.802
log-log linear regression	crust and area	3	23.6782
log-log linear regression	crust and volume	3	24.2238
log-log linear regression	crust and area and mud	4	24.6
log-log linear regression	crust and area and mud and depth	5	26.06451
log-log linear regression	crust and area and LP	4	25.67
log-log linear regression	crust and area and depth per month	8	20.38042
log-log linear regression	deca and area	3	88.86489
log-log linear regression	deca and area and mud	4	90.86252
log-log linear regression	deca and area and mud and depth	5	92.25782
log-log linear regression	deca and area and LP	4	85.31982
log-log linear regression	deca and area and depth per month	8	90.33125



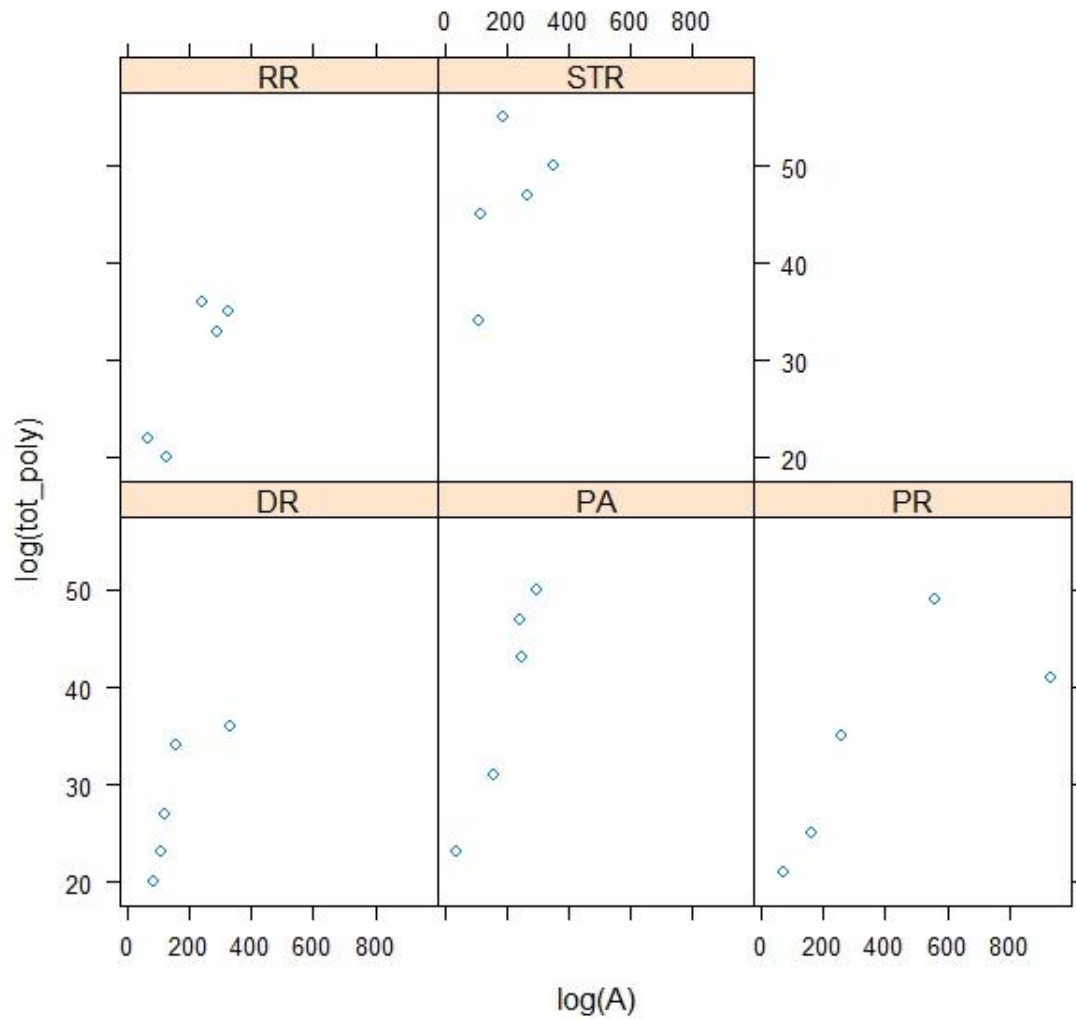
Annex E: Trellis graph showing relationship between richness of non-colonial invertebrates and area covered by colonies in each of the sampled site. A = Area covered by colonies (in  $\text{cm}^2$ ),  $S_{tot}$  = non-colonial invertebrate richness (number of taxa).

Priloga E: Trellis graf prikazuje razmerje med številom taksonov nekolonijskih nevretenčarjev in območju, ki ga posamezna kolonija pokriva na različnih lokalitetah. A = območje, ki ga pokriva kolonija ( $\text{cm}^2$ ),  $S_{tot}$  = pestrost nekolonijskih nevretenčarjev (število taksonov).



Annex F: Trellis graph showing relationship between number of taxa of molluscs and area ( $\text{cm}^2$ ) covered by colonies in each of the sampled site. A = Area covered by colonies, tot mol = total mollusc richness (number of taxa).

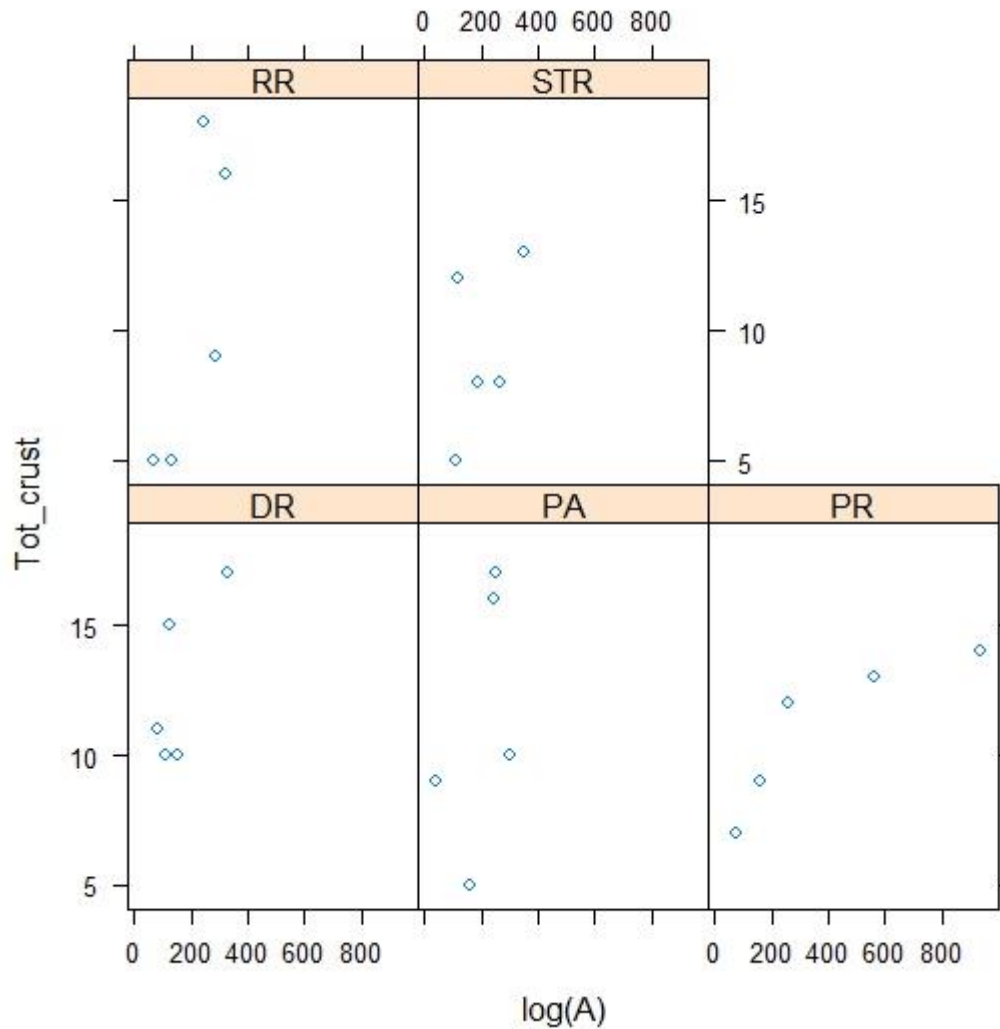
Priloga F: Trellis graf prikazuje razmerje med številom taksonov mehkužcev in območju, ki ga posamezna kolonija pokriva na različnih lokalitetah. A = območje, ki ga pokriva kolonija ( $\text{cm}^2$ ), tot mol = pestrost mehkužcev (število taksonov).



Annex G: Trellis graph showing relationship between number of taxa of polychaetes (tot\_poly) and area covered (cm<sup>2</sup>) by colonies (A) in each of the sampled site.

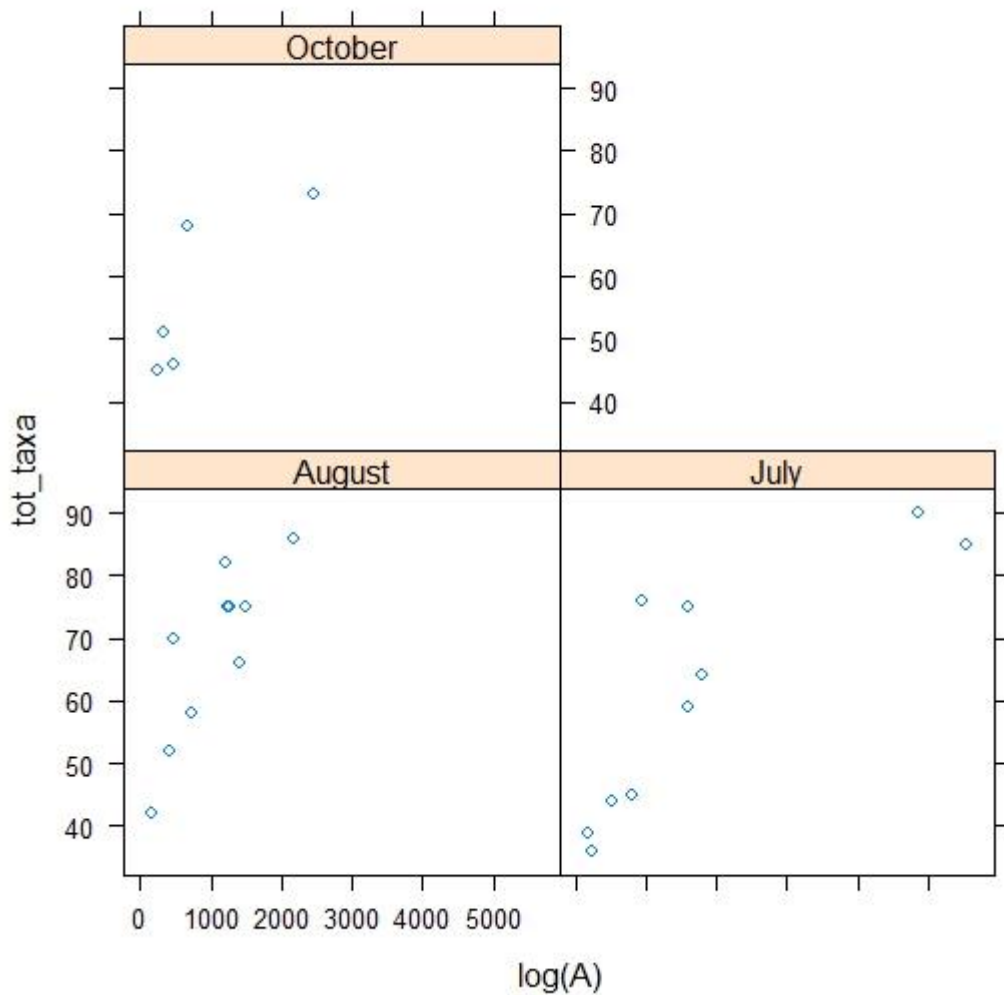
Priloga G: Trellis graf prikazuje odnos med številom taksonov mnogoščetincev (tot\_poly) in površino (cm<sup>2</sup>), ki jo posamezna kolonija pokriva na različnih lokalitetah.





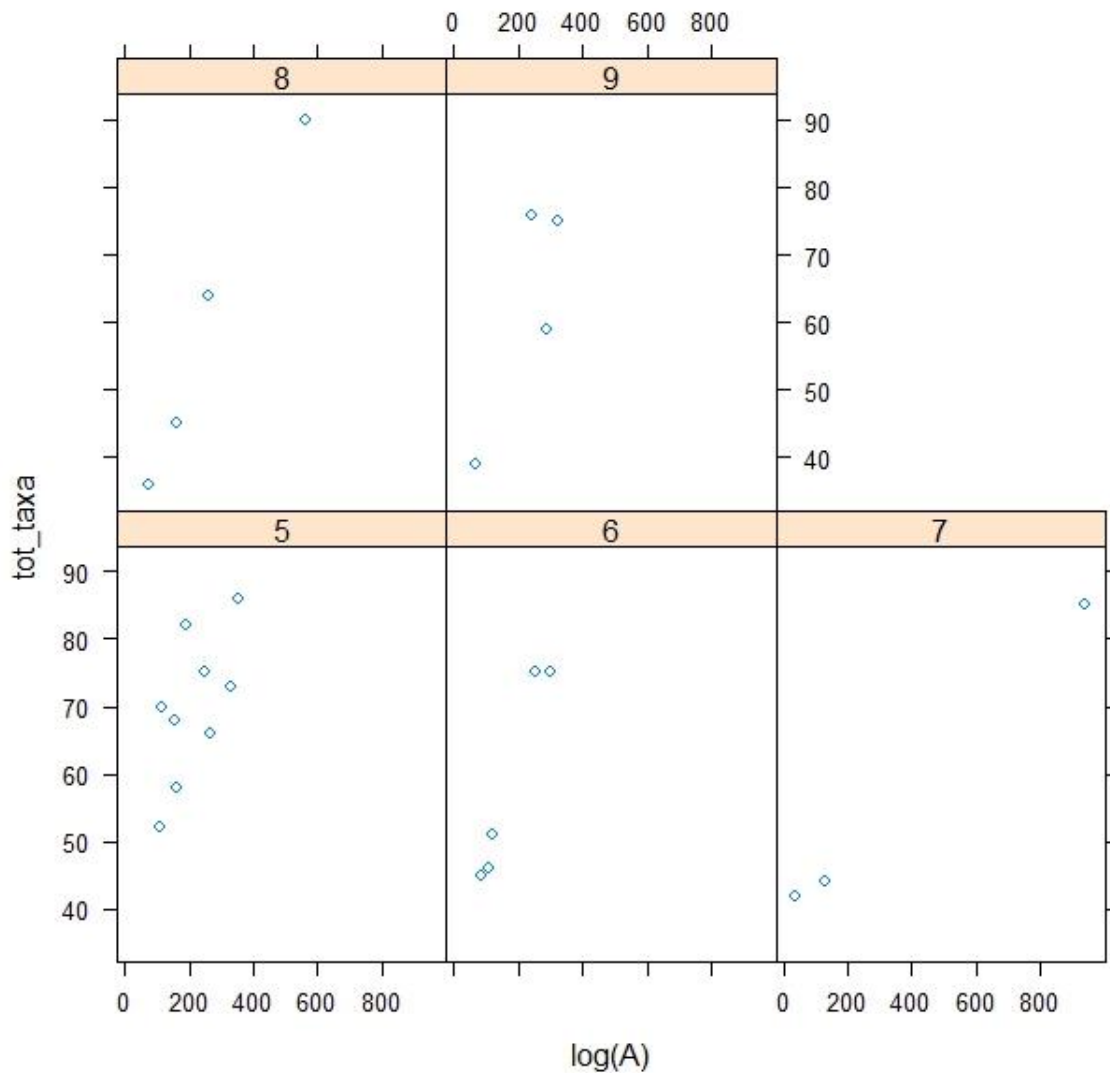
Annex H: Trellis graph showing relationship between number of taxa of crustaceans (tot\_crust) and area (cm<sup>2</sup>) covered by colonies (A) in each of the sampled site.

Priloga H: Trellis graf prikazuje odnos med številom taksonov rakov (tot crust) in površino, ki jo posamezna kolonija pokriva (cm<sup>2</sup>) (a) na različnih lokalitetah.



Annex I: Trellis graph showing relationship between richness of non-colonial invertebrates and area ( $\text{cm}^2$ ) covered by colonies for each of the sampling month. A = Area covered by colonies (in  $\text{cm}^2$ ), S\_tot = non-colonial invertebrate richness (number of taxa).

Priloga I: Trellis graf prikazuje odnos med številom taksonov nekolonijskih nevretenčarjev in površino, ki jo posamezna kolonija pokriva ( $\text{cm}^2$ ) za različne mesece vzorčenja. A = Površina, ki jo pokriva kolonija (v  $\text{cm}^2$ ), S\_tot = pestrost nekolonijskih nevretenčarjev (število taksonov).



Annex L: Trellis graph showing relationship between richness of non-colonial invertebrates and area ( $\text{cm}^2$ ) covered by colonies for each of the sampling depth (5 to 9 m). A = Area covered by colonies, S\_tot = non-colonial invertebrate richness (number of taxa).

Priloga L: Trellis graf prikazuje odnos med številom taksonov nekolonijskih nevretenčarjev in površino, ki jo posamezna kolonija pokriva ( $\text{cm}^2$ ) za vsako globino vzorčenja (od 5 do 9 m). A = Površina, ki jo pokriva kolonija (v  $\text{cm}^2$ ), S\_tot = pestrost nekolonijskih nevretenčarjev (število taksonov).