



## Article

# Species Richness of Benthic Macrofauna on Rocky Outcrops in the Adriatic Sea by Using Species-Area Relationship (SAR) Tools

Nicola Bettoso <sup>1</sup>, Lisa Faresi <sup>1</sup>, Valentina Pitacco <sup>2,\*</sup> , Martina Orlando-Bonaca <sup>2</sup> , Ida Floriana Aleffi <sup>1</sup> and Lovrenc Lipej <sup>2</sup>

<sup>1</sup> Agenzia Regionale per la Protezione dell'Ambiente del Friuli Venezia Giulia (ARPA FVG), Via Cairoli 14, 33057 Palmanova, Italy

<sup>2</sup> Marine Biology Station Piran, National Institute of Biology, Fornače 41, SI-6330 Piran, Slovenia

\* Correspondence: valentina.pitacco@nib.si

**Abstract:** In the northern Adriatic Sea, rocky outcrops called “trezze” or “tegnúe” are known as biodiversity hotspots. A total of 45 rocky outcrops were studied by using non-destructive photographic sampling during SCUBA diving. Ten invertebrate phyla with 196 taxa were recorded, 86% of which were determined at species level. Among them, 65% of the taxa were sessile, primarily represented by the phyla Porifera and Chordata. The aims of the study were: to characterize the species richness and composition of epifaunal invertebrates living on rocky outcrops; to test the efficiency of using the outcrop area as a predictor of epifauna richness, using the Arrhenius Species-Area Relationship (SAR) model; and to compare the expected richness resulting from the SAR model with the richness observed from the analysis of random photo-squares. Our results show that the SAR model describes the relationship between epibenthic species richness and outcrop size well and may have important practical applications for biodiversity estimations and nature conservation implications. It provides a useful tool, also in terms of economy and speed, to estimate the species richness of the benthic epifauna of the numerous outcrops that remain unsurveyed, based on their size.

**Keywords:** rocky outcrops; biodiversity hotspot; epifauna; Species-Area Relationship; northern Adriatic Sea



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## 1. Introduction

The Species-Area Relationship (SAR) is one of the best known and investigated empirical relationships in ecology [1,2], which still attracts considerable interest among various scientists, e.g., [3,4]. It is generally applied to studies covering large terrestrial geographical areas; however, in the last 15 years, it has also been used in marine research [5,6]. In conservation biology, models based on the SAR have been proposed to predict the expected loss of species richness in a region undergoing specified levels of habitat degradation, e.g., [7,8], and to estimate local species richness for hotspot identification, e.g., [9]. The SAR describes the pattern by which species richness increases with the increasing sampling area, and is recognized as one of the few true laws of ecology [10]. The SAR is a central argument for the theory of island biogeography [11]; however, the pattern applies not only to geographic islands, which are pieces of land surrounded by water [11,12], but also to the ‘island-like’ system [13], where similar habitat types are spatially separated by relatively unfavorable habitats [14,15]. The SAR has been widely used for terrestrial ecosystems, namely for plants [1,16] and birds [11,17]. The SAR could also be used for optimal reserve design [18]. Conversely, there are far fewer similar studies for the marine realm [18–22]. Most of the existing studies deal only with large scale patterns, e.g., [23,24], but recent papers have demonstrated the applicability of the SAR on smaller scales; for instance, to estimate the richness of invertebrates associated with colonies of the Mediterranean stony

coral, *Cladocora caespitosa* (L., 1767) [6]. The rocky outcrops, considered as the most peculiar features of the northern Adriatic Sea [25,26], are examples of small-scale island-like geomorphological elements. The northern Adriatic Sea is characterized by a rather monotonous seabed, mostly composed by mobile silty-sandy sediments. Nevertheless, this northernmost area of the Mediterranean Sea is outstanding from several points of view, due to the numerous submarine rocky substrates of biogenic concretions, irregularly scattered over the soft bottom. They are called “grébeni” or “trezze” in the Gulf of Trieste and “tegnúe” off the coast of Venice [26,27]. Similar formations are also found in the Slovenian part of the Adriatic Sea (but of different origin) and, similarly to the “grébeni” and “tegnúe”, they are known for their immense biodiversity [28]. The first report about these rocky outcrops at the bottom of the Adriatic Sea arose to Olivi (1792), who labelled them as tegnúe in his book “Zoologia Adriatica”; however, they were truly documented by underwater exploration more than 50 years ago [29]. At present, the geological origin of these outcrops is still widely debated. Originally, they were interpreted as beachrocks [30–32], whereas the current hypothesis about their genesis suggests the presence of processes linked to methane seeping, cementation, and lithification [33–37]. In detail, these outcrops originate from the sediment and are not associated with the bedrock matrix of the continental plate [34]. Regardless of their geological origin, these rocky substrates are suitable for the settlement and subsequent development of specific floristic and faunistic assemblages. In the Mediterranean Sea, such habitats are mainly formed by the accumulation of calcareous incrusting algae that develop in sciaphilic environments [25,27]. These coralline algae are also called “bioengineers” as they create new niches for many invertebrates and other algae. Such complex biostructures give a typical appearance to the colorful underwater landscape [28] and are also referred to as “coralligenous” [38]. However, the northern Adriatic outcrops differ from the classic “coralligenous” because they have very small concretions of coralline algae, the faunal component dominating throughout the year [39–41]. Casellato and Stefanon [26] estimated 480 species of macrofaunal invertebrates, both epibenthos and endobenthos, belonging to 11 phyla. Most of the living biomass consists of suspension-feeders, while herbivores and carnivores were far less abundant; however, it is noteworthy that the abundance of sponges often reach considerable sizes [25].

The depth of these habitats ranges between 9 and 40 m, at different distances from the coast (3 to 13 nautical miles), with extremely variable morphology and structure. Their size ranges from a single small block of 1 m<sup>2</sup> up to a few 1000 m<sup>2</sup>, and their height rarely exceeds 4 m [26]. At present, up to 4000 outcrops have been recorded, mostly located off the Venetian coast (tegnúe), whereas in the Gulf of Trieste, approximately 250 are known on the Italian side (grébeni or trezze) [42].

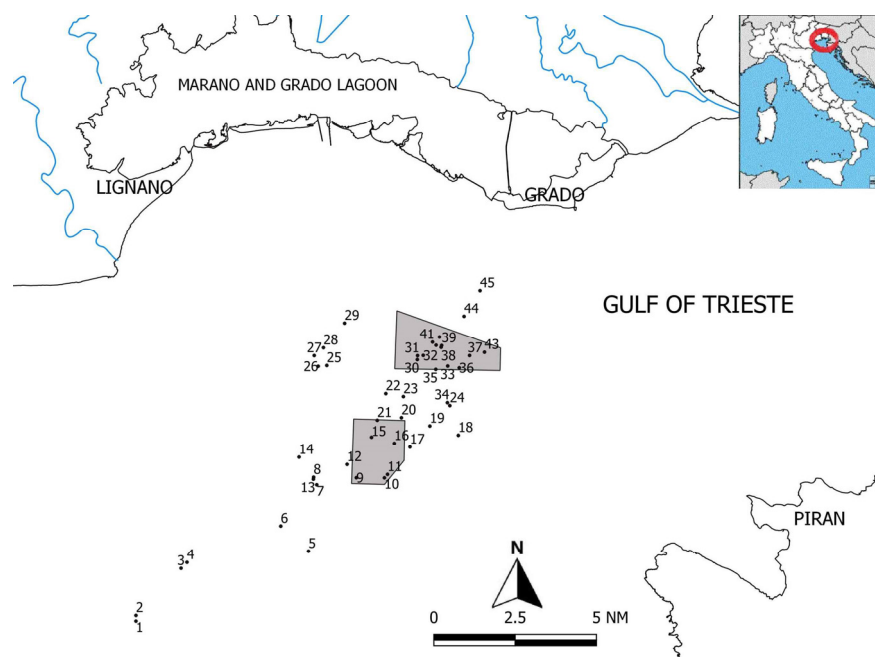
The aim of this research was to: (1) characterize the species richness and composition of epifaunal invertebrates living on rocky outcrops; (2) test the efficiency of using the outcrop area in relation to sample size as a predictor of epifauna richness, using the Arrhenius SAR model; and (3) compare the expected richness resulting from the Arrhenius SAR model with the richness observed in the analysis of 30 random photo-squares.

## 2. Materials and Methods

### 2.1. Study Area

The outcrops were studied in the Gulf of Trieste, the northernmost basin of the Adriatic Sea (Figure 1). The Gulf of Trieste is a shallow semi-enclosed basin (max depth 25 m), characterized by the largest tidal amplitudes and the lowest winter temperatures in the Mediterranean Sea [43], by high temperature and salinity variations, and important stratification of the water column [44]. The hydrodynamism is mainly related to the ascending eastern current from the Istrian coast. The general circulation pattern is predominantly counter-clockwise in the lower layer and clockwise in the surface layer. This circulation, particularly in the surface layer, can be modulated by prevailing winds from eastern quadrants such as the Bora [44]. The Gulf of Trieste is characterized by various environmental and anthropogenic pressures, affecting benthic communities, such as the periodic occurrence of

the phenomenon “mare sporco” (mucilage aggregates), episodes of hypoxia and anoxia, substantial riverine inflows, intensive maritime transport, intensive fishery, mariculture and others [45,46]. These pressures could inflict certain changes in the soft and hard bottom animal communities, at least in terms of succession [47]. The sediments are quite varied, ranging from sands with patches of beachrocks to muds, predominantly detritic, so that the associated biocoenoses of the Gulf traditionally belong roughly to the DC (Détritique Côtier), DE (Détritique Envasé) and VTC (Vases Terrigènes Côtières) biocoenoses, as defined by Pérès and Picard [48,49]. The rocky outcrops are mostly located off the Marano and Grado Lagoon, at a distance from the coast of between 3 and 10 nautical miles (nm) and a depth ranging between 13 and 25 m (Table 1). The sediment is constituted by sand and pelitic sand [50], where the DC biocenosis is prevailing [49,51]. This area is characterized by the presence of sub fossil rhizomes of *Posidonia oceanica* (L.) Delile, sometimes bordering the outcrops. The occurrence of the biocenosis of coarse sands and fine gravels under the influence of bottom currents is also noteworthy (SGCF: Sables Grossiers et des fines graviers sous l’influence des Courants de Fond, sensu Pérès and Picard) [48]; this primarily occurs due to the presence of the cephalochordate *Branchiostoma lanceolatum* (Pallas, 1774) [51]. On the other hand, in the zone influenced by the Tagliamento estuary, the presence of characteristic species of VTC biocoenosis can be detected [49,51].



**Figure 1.** Study area with outcrops investigated and Sites of Community Importance (in grey).

**Table 1.** Names of the 45 rocky outcrops investigated by SCUBA diving, sampling depth, distance from the coasts, and number of invertebrates’ taxa. \* = outcrops in which a 30 × 30 cm square was investigated to compare the expected richness from SAR model; SCI indicates the outcrop inside the sites of community importance.

	Outcrop Name	Sampling Date	Coast Distance (nm)	Depth (m)	n. Point Intercepts	n. Taxa
1	COLOMBA *	17 September 2014	10	24	55	53
2	COLOMBA 2 *	24 September 2014	10	24	72	53
3	STRUCOLO *	24 September 2014	9.5	25	59	51
4	GUBANA *	17 September 2014	9.5	24	63	60
5	BARDELLI *	18 September 2014	10	23	24	49

Table 1. Cont.

	Outcrop Name	Sampling Date	Coast Distance (nm)	Depth (m)	n. Point Intercepts	n. Taxa
6	NICOLA *	9 July 2013	9	23	101	54
7	DAVE *	5 July 2013	9	21	130	71
8	LA LONGA	4 July 2014	8.6	20.5	33	58
9	AGENORE * <sup>SCI</sup>	29 August 2014	8.8	21	41	61
10	PESCECANE * <sup>SCI</sup>	31 October 2014	9.5	21	59	79
11	SCARPENE * <sup>SCI</sup>	19 January 2015	8.6	19	50	64
12	ALDEBARAN *	5 July 2013	9.1	19	37	52
13	LA BOMBA *	4 July 2014	8.3	20	80	65
14	SARATOGA *	1 August 2013	8.3	18	138	73
15	DORSALE * <sup>SCI</sup>	6 August 2013	9.4	18	107	67
16	SUDPIASTRA * <sup>SCI</sup>	29 August 2014	7.7	21	43	65
17	SPARI *	1 August 2013	7.9	20	103	57
18	LE GATTE	29 August 2014	7.1	20	99	65
19	ALBERT	31 October 2014	7	19	64	58
20	MISTO *	5 July 2013	7.2	18	103	55
21	BIRO * <sup>SCI</sup>	29 August 2014	7.6	18	53	62
22	PESCHERIA *	29 August 2014	6.6	17	50	71
23	S. PAOLO *	19 January 2015	6.6	17	94	59
24	MORO *	22 December 2014	6.2	21	69	43
25	SALIENT *	4 July 2014	6.5	13	99	71
26	SARGASSI	27 May 2014	6.5	15	92	55
27	PINNACOLI *	9 July 2013	6.5	14	113	63
28	NORDALTI *	6 August 2013	6	13	87	59
29	MUSSOLI	27 March 2013	4.5	13	27	43
30	W FUMAPIL <sup>SCI</sup>	10 April 2015	9.9	17	105	82
31	FUMAPIL <sup>SCI</sup>	22 December 2014	5.2	16.5	42	55
32	TIMOTEO * <sup>SCI</sup>	26 January 2015	5.3	16	55	72
33	SEPA <sup>SCI</sup>	21 May 2014	5.2	18	184	82
34	CP *	26 January 2015	5.3	17	82	60
35	AREA 4 <sup>SCI</sup>	3 April 2014	5	17.5	45	52
36	PALO LARGO <sup>SCI</sup>	3 July 2014	4.8	19	85	75
37	PALI * <sup>SCI</sup>	11 July 2013	4.5	18	52	30
38	S. PIETRO <sup>SCI</sup>	18 September 2014	4.6	16	29	62
39	S. PIEROTERRA <sup>SCI</sup>	10 August 2014	4.6	17	83	70
40	CORVINE * <sup>SCI</sup>	6 August 2013	4.6	15	84	70
41	SPERLIG <sup>SCI</sup>	10 April 2015	8.6	16.5	105	79
42	AMERIGO * <sup>SCI</sup>	10 May 2014	4.4	17	184	94
43	MALUSA <sup>SCI</sup>	26 August 2014	3.5	16.5	102	70
44	MENEGHEL *	11 July 2013	3.4	14	25	24
45	AREA 3	1 April 2014	3	14	44	43

Since 2015, a limited number of biogenic outcrops in the Gulf of Trieste have been under the legal protection of the European Habitats Directive (92/43/EEC) [52]. Their inclusion in the European Natura 2000 network as Sites of Community Importance (SCIs) (Decision EU 2015/69 of 3 December 2014) recognizes the need to protect these habitats in order to preserve their unique assemblages and mitigate the effects of climate change and local stressors (i.e., dystrophic crises, mucilage, dredging, fishing, anchoring) on them [41,53]. This site is called “Trezze San Pietro e Bardelli” (IT3330009) (Figure 1) and includes 19 of the investigated outcrops (Table 1). At present, a regional legislation (D.G.R. of the Friuli Venezia Giulia Region n. 1701 of 4 October 2019) establishes the conservation measures for the site. In particular, anchoring, trawling and hydraulic dredge fishery on the outcrops within the SCI is prohibited.

## 2.2. Fieldwork and Photo-Analysis

A total of 45 rocky outcrops (Figure 1, Table 1) were investigated by SCUBA diving to assess the richness of the epibenthic fauna and to test the application of the Arrhenius SAR model by photographic sampling. The length of the outcrops varied between 10 and 100 m, the width between 3 and 20 m and the height between 0 and 3 m. The “point intercept transect” method [54] was performed along the entire perimeter and across the major axis of each outcrop.

The SCUBA photographic samples were collected over a three-year period (2013–2015), mainly during the spring and summer period (Table 1). A photographic sample was taken every 2 m along the transect through a photo-square of 400 cm<sup>2</sup> taken at a focal distance of 0.3 m (Canon PowerShot G10 model equipped with SEA&SEA YS110α strobe). Thus, the amount of point intercepts along the transect is proportional to the sample size (A), which is a function of the outcrop area. In addition, epibenthic megafauna species were recorded, mostly within 2 m, 1 m to the left and 1 m to the right of the transect line. Finally, following the transect path, a 30 × 30 cm (900 cm<sup>2</sup>) square (divided into 4 sub-squares) was randomly positioned in the centre of 30 outcrops for additional photographic sampling, which was used for comparison with the expected richness from the SAR model.

A total of 3571 images were analyzed (3451 from transects and 120 (30 × 4) from squares). Animals that were readily identifiable in the photographs were identified to the lowest possible taxonomic level, whereas those not easily recognizable at the species level were collected and stored for determination at the laboratory.

## 2.3. Data Analysis

The recorded taxa in the point intercept transects were listed following the WoRMS nomenclature (World Register of Marine Species—[www.marinespecies.org](http://www.marinespecies.org), accessed on 30 June 2021): species, genus, family, order, class and phylum (Table S1). The permanency of the epibenthic community on the outcrops, in term of the number of times the taxon was found in the total of samples, was evaluated through a constancy index [55,56], as follow:

$$C_{ij} = (n_{ij}/n_j) \times 100 \quad (1)$$

where  $n_{ij}$  is the number of occurrences of taxon  $i$  in outcrops group  $j$  and  $n_j$  is the number of outcrops investigated. Thus, every taxon was termed as Permanent when  $C = 100\%$ , Constant ( $100\% > C \geq 50\%$ ), Frequent ( $50\% > C \geq 25\%$ ) or Temporary ( $C < 25\%$ ). Every taxon was classified on the basis of its mobility into sessile or vagile. The characteristic species of coralligenous biocoenosis and those common in this habitat were identified on the basis of Ballesteros’ [57] review on Mediterranean coralligenous assemblages. Following Ballesteros [57], the species considered as an animal builder and bioeroders were also identified. The protected species were recorded on the basis of the list in the Annexes of the SPA/BD protocol of the Barcelona Convention [58], as well as those whose exploitation is regulated (Annex III).

The relationship between the total number of taxa (Stot) along the transect and the outcrop area (A) was first tested with Spearman’s coefficients for nonparametric distri-

butions [59]. The same analysis was used to test the relation between A and taxa richness of each of the dominant phyla (Porifera-Spor, Mollusca-Smol, Chordata-Scho and Cnidaria-Scni), of the motility mode (sessile-Sses and vagile-Svag), of the constancy classes (permanent-Sper, constant-Scon, frequent-Sfre, temporary-STEM) and of the characteristic/common species of coralligenous biocoenosis (Scor).

The linear regression model for log-transformed data (Arrhenius model [1]) was used to fit the SAR data. The regression lines were calculated to describe the relationship between the A and S of the total taxa, dominant phyla, motility modes, constancy classes and taxa of coralligenous biocoenosis, when they resulted in being significantly correlated. Analyses on residuals were performed to check against strong deviations from the assumption of normality, homogeneity, independence and absence of pattern in the residuals for validation of regression models. Adjusted R-squared was used to validate the linear regression for the Species-Area Relationship. It is defined by the equation:

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

where n is sample size, SS<sub>residual</sub> is a sum of the squared deviations of the observed values from the fitted values, SS<sub>total</sub> is a sum of the squared deviations of the observed data from the mean, and K is the number of parameters. The calculations were performed using the vegan package [60] for R.

The coefficients of the regression lines (slope and intercept) were used to estimate the expected richness for a 30 × 30 cm square and the results were compared with the average observed richness values obtained from the random 30 × 30 cm photo-squares. The Wilcoxon two-sample paired test was applied in order to test the null hypothesis that the median of the paired differences between the expected richness by Arrhenius model and the average observed value is 0. A  $p < 0.05$  was chosen as the significance threshold. All of the calculations were performed using R version 3.6.1 [61].

### 3. Results

A total of 196 taxa of epibenthic invertebrates were identified by photo-sampling on the 45 outcrops, including 169 species (full list in Table 2), 152 genera, 119 families, 57 orders, 14 classes, 10 phyla (Table S1). The recorded phyla (Figure 2A) were: Porifera with 58 taxa, followed by Mollusca (47), Chordata (29), Cnidaria (17), Arthropoda (15), Annelida (11), Echinodermata (11), Bryozoa (6), finally Phoronida (1) and Platyhelminthes (1). The phylum Porifera was represented by the class Demospongiae, the Mollusca were mainly formed by the class Gastropoda (34 taxa, 18 of which were Nudibranchia), the class Ascidiacea represented the phylum Chordata, Anthozoa represented the phylum Cnidaria (with the exception of the scyphozoan *Nausithoe punctata*), Malacostraca Decapoda the phylum Arthropoda, and Polychaeta the phylum Annelida (one species was the echiurid *Bonellia viridis*). The phylum Echinodermata was more or less equally represented by Asterozoa, Echinozoa, and Ophiurozoa; the phylum Bryozoa was constituted by the class Gymnolaemata. The phylum Phoronida was represented by the species *Phoronis muelleri*, and finally an undetermined specimen for the phylum Platyhelminthes (further taxonomic details are shown in Table S1).

**Table 2.** List of species determined for the present work.

Taxonomic list		
Porifera	<i>Phymanthus pulcher</i> (Andres, 1883)	<i>Protula tubularia</i> (Montagu, 1803)
<i>Agelas oroides</i> (Schmidt, 1864)	<i>Calliactis parasitica</i> (Couch, 1842)	<i>Bispira volutacornis</i> (Montagu, 1804)
<i>Axinella polypoides</i> Schmidt, 1862	<i>Sagartia elegans</i> (Dalyell, 1848)	<i>Myxicola infundibulum</i> (Montagu, 1808)
<i>Axinella cannabina</i> (Esper, 1794)	<i>Anemonia viridis</i> (Forsskål, 1775)	Arthropoda
<i>Axinella damicornis</i> (Esper, 1794)	<i>Cornularia cornucopiae</i> (Pallas, 1766)	<i>Paguristes eremita</i> (Linnaeus, 1767)
<i>Raspailia (Raspailia) viminalis</i> Schmidt, 1862	<i>Maasella edwardsii</i> (de Lacaze-Duthiers, 1888)	<i>Dromia personata</i> (Linnaeus, 1758)
<i>Dictyonella incisa</i> (Schmidt, 1880)	<i>Cladocora caespitosa</i> (Linnaeus, 1767)	<i>Galathea strigosa</i> (Linnaeus, 1761)



Table 2. Cont.

<i>Bubaris vermiculata</i> (Bowerbank, 1866)	<i>Caryophyllia</i> ( <i>Caryophyllia</i> ) <i>inornata</i> (Duncan, 1878)	<i>Galathea intermedia</i> Lilljeborg, 1851
<i>Acanthella acuta</i> Schmidt, 1862	<i>Leptopsammia pruvoti</i> Lacaze-Duthiers, 1897	<i>Lysmata seticaudata</i> (Risso, 1816)
<i>Chondrilla nucula</i> Schmidt, 1862	<i>Cerianthus membranaceus</i> (Gmelin, 1791)	<i>Maja crispata</i> Risso, 1827 in [Risso, 1826–1827]
<i>Chondrosia reniformis</i> Nardo, 1847	<i>Epizoanthus arenaceus</i> (Delle Chiaje, 1836)	<i>Homarus gammarus</i> (Linnaeus, 1758)
<i>Cliona viridis</i> (Schmidt, 1862)	<i>Parazoanthus axinellae</i> (Schmidt, 1862)	<i>Pagurus anachoretus</i> Risso, 1827 in [Risso, 1826–1827]
<i>Cliona celata</i> Grant, 1826	Mollusca	<i>Pagurus prideaux</i> Leach, 1815 [in Leach, 1815–1875]
<i>Cliona rhodensis</i> Rützler & Bromley, 1981	<i>Philinopsis depicta</i> (Renier, 1807)	<i>Periclimenes amethysteus</i> (Risso, 1827 in [Risso, 1826–1827])
<i>Aplysilla sulfurea</i> Schulze, 1878	<i>Haliotis tuberculata</i> Linnaeus, 1758	<i>Pilumnus hirtellus</i> (Linnaeus, 1761)
<i>Dysidea avara</i> (Schmidt, 1862)	<i>Thylacodes arenarius</i> (Linnaeus, 1758)	<i>Scyllarus arctus</i> (Linnaeus, 1758)
<i>Ircinia variabilis</i> (Schmidt, 1862)	<i>Rapana venosa</i> (Valenciennes, 1846)	Bryozoa
<i>Sarcotragus spinosulus</i> Schmidt, 1862	<i>Muricopsis cristata</i> (Brocchi, 1814)	<i>Schizobrachiella sanguinea</i> (Norman, 1868)
<i>Dysidea fragilis</i> (Montagu, 1814)	<i>Hexaplex trunculus</i> (Linnaeus, 1758)	<i>Chartella papyracea</i> (Ellis and Solander, 1786)
<i>Sarcotragus foetidus</i> Schmidt, 1862	<i>Bolinus brandaris</i> (Linnaeus, 1758)	<i>Reteporella beaniana</i> (King, 1846)
<i>Pleraplysilla spinifera</i> (Schulze, 1879)	<i>Felimare villafranca</i> (Risso, 1818)	Phoronida
<i>Scalarispongia scalaris</i> (Schmidt, 1862)	<i>Edmundsella pedata</i> (Montagu, 1816)	<i>Phoronis muelleri</i> Selys-Lonchamps, 1903
<i>Spongia</i> ( <i>Spongia</i> ) <i>officinalis</i> Linnaeus, 1759	<i>Felimida krohni</i> (Vérany, 1846)	Echinodermata
<i>Cacospongia mollior</i> Schmidt, 1862	<i>Dendrodoris grandiflora</i> (Rapp, 1827)	<i>Marthasterias glacialis</i> (Linnaeus, 1758)
<i>Haliclona</i> ( <i>Reniera</i> ) <i>mediterranea</i> Griessinger, 1971	<i>Paradoris indecora</i> (Bergh, 1881)	<i>Echinaster</i> ( <i>Echinaster</i> ) <i>sepositus</i> (Retzius, 1783)
<i>Petrosia</i> ( <i>Petrosia</i> ) <i>ficiformis</i> (Poiret, 1789)	<i>Felimare tricolor</i> (Cantraine, 1835)	<i>Sphaerechinus granularis</i> (Lamarck, 1816)
<i>Haliclona</i> ( <i>Haliclona</i> ) <i>simulans</i> (Johnston, 1842)	<i>Trapania maculata</i> Haefelfinger, 1960	<i>Paracentrotus lividus</i> (Lamarck, 1816)
<i>Haliclona</i> ( <i>Soestella</i> ) <i>mamillata</i> (Griessinger, 1971)	<i>Dendrodoris limbata</i> (Cuvier, 1804)	<i>Echinus melo</i> Lamarck, 1816
<i>Haliclona</i> ( <i>Soestella</i> ) <i>mucosa</i> (Griessinger, 1971)	<i>Flabellina affinis</i> (Gmelin, 1791)	<i>Holothuria</i> ( <i>Holothuria</i> ) <i>tubulosa</i> Gmelin, 1791
<i>Haliclona</i> ( <i>Halichoelona</i> ) <i>fulva</i> (Topsent, 1893)	<i>Paraflabellina ischitana</i> (Hirano and T. E. Thompson, 1990)	<i>Ocnus planci</i> (Brandt, 1835)
<i>Oscarella lobularis</i> (Schmidt, 1862)	<i>Tethys fimbria</i> Linnaeus, 1767	<i>Holothuria</i> ( <i>Panningothuria</i> ) <i>forskali</i> Delle Chiaje, 1823
<i>Clathria</i> ( <i>Clathria</i> ) <i>compressa</i> Schmidt, 1862	<i>Crimora papillata</i> Alder and Hancock, 1862	<i>Ophioderma longicaudum</i> (Bruzelius, 1805)
<i>Tedania</i> ( <i>Tedania</i> ) <i>anhelans</i> (Vio in Olivi, 1792)	<i>Discodoris stellifera</i> (Vayssière, 1903)	<i>Ophiura ophiura</i> (Linnaeus, 1758)
<i>Mycale</i> ( <i>Aegogropila</i> ) <i>tunicata</i> (Schmidt, 1862)	<i>Doriopsilla areolata</i> Bergh, 1880	Chordata
<i>Phorbas tenacior</i> (Topsent, 1925)	<i>Doris pseudoargus</i> Rapp, 1827	<i>Aplidium tabarquensis</i> Ramos-Espla, 1991
<i>Crella</i> ( <i>Yvesia</i> ) <i>rosea</i> (Topsent, 1892)	<i>Felimare fontandraui</i> (Pruvot-Fol, 1951)	<i>Aplidium conicum</i> (Olivi, 1792)
<i>Phorbas fictitius</i> (Bowerbank, 1866)	<i>Paraflabellina gabinierei</i> (Vicente, 1975)	<i>Polycitor adriaticus</i> (Drasche, 1883)
<i>Crambe crambe</i> (Schmidt, 1862)	<i>Polycera quadrilineata</i> (O. F. Müller, 1776)	<i>Didemnum commune</i> (Della Valle, 1877)
<i>Antho</i> ( <i>Antho</i> ) <i>inconstans</i> (Topsent, 1925)	<i>Berthella ocellata</i> (Delle Chiaje, 1830)	<i>Didemnum coriaceum</i> (Drasche, 1883)
<i>Myxilla</i> ( <i>Myxilla</i> ) <i>roseacea</i> (Lieberkühn, 1859)	<i>Calliostoma zizyphinum</i> (Linnaeus, 1758)	<i>Diplosoma listerianum</i> (Milne Edwards, 1841)
<i>Ulosa digitata</i> (Schmidt, 1866)	<i>Bolma rugosa</i> (Linnaeus, 1767)	<i>Aplidium elegans</i> (Giard, 1872)
<i>Mycale</i> ( <i>Aegogropila</i> ) <i>contarenii</i> (Lieberkühn, 1859)	<i>Tylodina perversa</i> (Gmelin, 1791)	<i>Polysyncraton lacazei</i> (Giard, 1872)
<i>Hemimycale columella</i> (Bowerbank, 1874)	<i>Elysia viridis</i> (Montagu, 1804)	<i>Cystodytes dellechiaiei</i> (Della Valle, 1877)
<i>Mycale</i> ( <i>Carmia</i> ) <i>macilentia</i> (Bowerbank, 1866)	<i>Arca noae</i> Linnaeus, 1758	<i>Didemnum lahillei</i> Hartmeyer, 1909
<i>Polymastia mamillaris</i> (Müller, 1806)	<i>Glycymeris glycymeris</i> (Linnaeus, 1758)	<i>Polyclinella azemai</i> Harant, 1930

Table 2. Cont.

<i>Suberites domuncula</i> (Olivi, 1792)	<i>Rocellaria dubia</i> (Pennant, 1777)	<i>Aplidium nordmanni</i> (Milne Edwards, 1841)
<i>Terpios fugax</i> Duchassaing and Michelotti, 1864	<i>Limaria hians</i> (Gmelin, 1791)	<i>Aplidium turbinatum</i> (Savigny, 1816)
<i>Halichondria (Halichondria) semitubulosa</i> (Lamarck, 1814)	<i>Mytilus galloprovincialis</i> Lamarck, 1819	<i>Clavelina sabbadini</i> Brunetti, 1987
<i>Suberites massa</i> Nardo, 1847	<i>Ostrea edulis</i> Linnaeus, 1758	<i>Diazona violacea</i> Savigny, 1816
<i>Tethya aurantium</i> (Pallas, 1766)	<i>Pinna nobilis</i> Linnaeus, 1758	<i>Phallusia fumigata</i> (Grube, 1864)
<i>Tethya citrina</i> Sarà and Melone, 1965	<i>Mimachlamys varia</i> (Linnaeus, 1758)	<i>Phallusia mammillata</i> (Cuvier, 1815)
<i>Geodia cydonium</i> (Linnaeus, 1767)	<i>Talochlamys multistriata</i> (Poli, 1795)	<i>Ascidia aspersa</i> (Müller, 1776)
<i>Aplysina aerophoba</i> (Nardo, 1833)	<i>Pecten jacobaeus</i> (Linnaeus, 1758)	<i>Ascidia mentula</i> Müller, 1776
<i>Aplysina cavernicola</i> (Vacelet, 1959)	<i>Venus verrucosa</i> Linnaeus, 1758	<i>Distomus variolosus</i> Gaertner, 1774
<i>Hexadella racovitzai</i> Topsent, 1896	<i>Octopus vulgaris</i> Cuvier, 1797	<i>Halocynthia papillosa</i> (Linnaeus, 1767)
Cnidaria	<i>Sepia officinalis</i> Linnaeus, 1758	<i>Styela plicata</i> (Lesueur, 1823)
<i>Nausithoe punctata</i> Kölliker, 1853	Annelida	<i>Botrylloides leachii</i> (Savigny, 1816)
<i>Cereus pedunculatus</i> (Pennant, 1777)	<i>Bonellia viridis</i> Rolando, 1822	<i>Botryllus schlosseri</i> (Pallas, 1766)
<i>Aiptasia mutabilis</i> (Gravenhorst, 1831)	<i>Serpula vermicularis</i> Linnaeus, 1767	<i>Botrylloides violaceus</i> Oka, 1927
<i>Condylactis aurantiaca</i> (Delle Chiaje, 1825)	<i>Sabella spallanzanii</i> (Gmelin, 1791)	
<i>Phymanthus pulcher</i> (Andrès, 1883)	<i>Protula tubularia</i> (Montagu, 1803)	

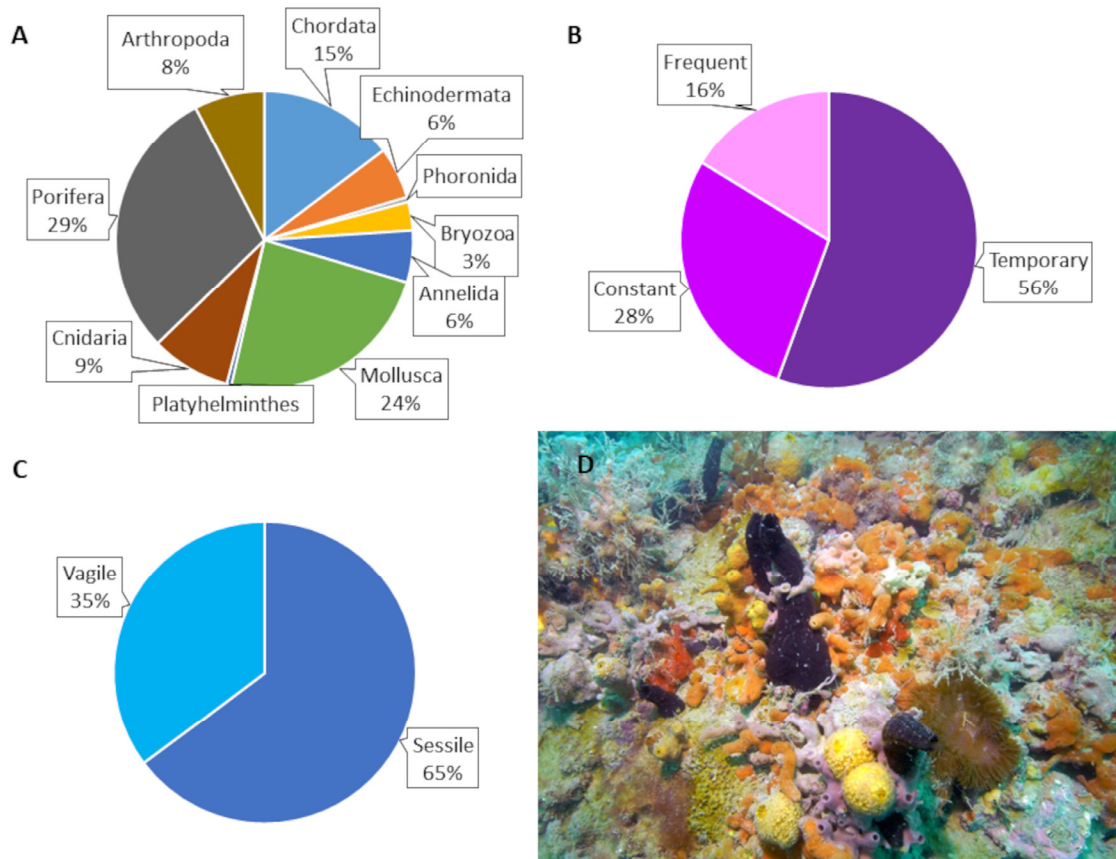


Figure 2. Percentages of phyla (A), classes of constancy (B), motility modes (C) and a picture (D) of epibenthic invertebrates found on rocky outcrops. Photo by L. Faresi.

With regard to the constancy index, 55 taxa were assessed as constant, 32 as frequent and 109 as temporary; no taxon was recorded as permanent (Figure 2B). Among the constant taxa, phylum Porifera was dominant (24 taxa), followed by Mollusca (7), Chordata (7), Annelida (6), Echinodermata (5), Bryozoa (3), Cnidaria (2) and Arthropoda (1); four constant species are protected (Porifera: *Sarcotragus foetidus*, *Tethya aurantium*, *Geodia cydonium*



and *Aplysina aerophoba*), one species was characteristic of coralligenous biocenosis (Porifera: *Haliclona mediterranea*) and eleven were considered as common in this habitat (Porifera: *Chondrosia reniformis*, *Cliona viridis*, *Dysidea avara*, *Ircinia variabilis*, *Phorbast fictitious*, *G. cydonium*; Mollusca Gastropoda: *Muricopsis cristata*, *Calliostoma zizyphinum*; Annelida: *B. viridis*; Chordata: *Aplidium conicum*, *Pyura* sp.) (Table S1).

Regarding the frequent taxa, Porifera were dominant (10 taxa), followed by Chordata (7), Mollusca (5), Arthropoda (4), Cnidaria (3), Annelida (2) and Bryozoa (1); four were protected species (Porifera: *Aplysina cavernicola*; Cnidaria: *Cladocora caespitosa*; Mollusca Bivalvia: *Pinna nobilis*; Arthropoda: *Homarus gammarus*), one species was characteristic of coralligenous biocenosis (Chordata: *Cystodytes dellechiaiei*) and six were considered as common for this biocenosis (Porifera: *Pleraplysilla spinifera*, *Oscarella lobularis*; Cnidaria: Scyphozoa *Nausithoe punctata* and Anthozoa *Epizoanthus arenaceus*; Arthropoda: *Dromia personata*; Chordata: *Polysyncraton lacazei*) (Table S1).

Finally, for the temporary taxa, Mollusca dominated (35 taxa), followed by Porifera (24), Chordata (15), Cnidaria (12), Arthropoda (10), Echinodermata (6), Annelida (3), Bryozoa (2), Platyhelminthes (1) and Phoronida (1); six are protected species (Porifera: *Axinella polypoides*, *Axinella cannabina*, *Spongia officinalis*, *Tethya citrina*; Arthropoda: *Scyllarus arctus*; Echinodermata: *Paracentrotus lividus*), two species were characteristic of coralligenous (Porifera: *Agelas oroides* and *Axinella damicornis*) and four were common species (Porifera: *Scalorispongia scalaris*; Cnidaria: *Cornularia cornucopiae*, *Maasella edwardsii*; Mollusca Gastropoda: *Dendrodoris grandiflora*) (Table S1). Regarding the presence and persistence of animal builders and bioeroders, eight species were identified as builders and five bioeroders. In detail, the builders *G. cydonium*, *Serpula vermicularis*, *Schizomavella* sp., and the bioeroders *C. viridis*, *Cliona celata* and *Sphaerechinus granularis* were constantly recorded; *E. arenaceus*, *Serpula* sp., *C. caespitosa* as builders and *Rocellaria dubia* as bioeroder were frequently found; in the temporary taxa, the builders *Caryophyllia inornata*, *Leptopsammia pruvoti* and the bioeroder *Echinus melo* were recorded (Table S1).

Finally, considering the mobility (Figure 2C), 127 taxa were sessile species (i.e., Porifera, Cnidaria, Bryozoa and Chordata), whereas the remaining 69 taxa were vagile (i.e., Mollusca Gastropoda, Arthropoda and Echinodermata) (Table S1).

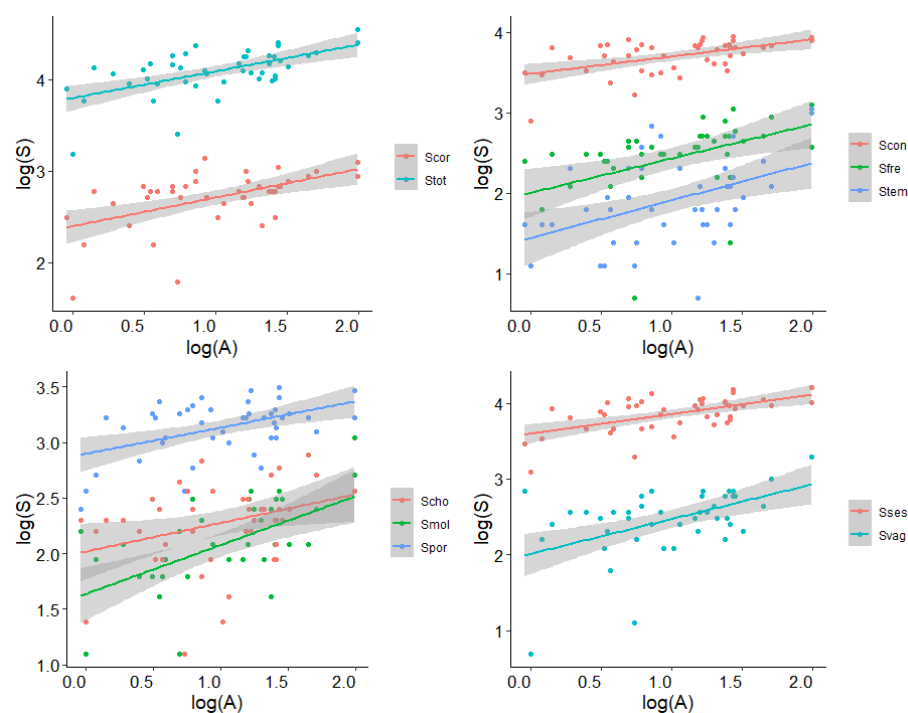
The sample size (A) in each outcrop ranged between 24 point intercepts in the outcrop 5 (Bardelli) and 184 in the outcrops 33 and 42 (Sepa and Amerigo) (Table 1); the median value was 72. The number of taxa (S) found on every outcrop ranged between 24 (outcrop 44 Meneghel) and 94 (42 Amerigo) (Table 1), with a median value of 61 taxa. The relationship between the S and A when applying the Spearman correlation coefficient was significant for all of the groups considered, with the exception of Cnidaria; therefore, this phylum was excluded from further analysis (Table 3).

The linear regressions of the log-transformed variables (S and A) according to the Arrhenius model showed that the sample size (A), as the function of outcrop area, could be a predictor of the epifauna richness (Figure 3; Table 3). Considering the different taxonomic groups, the curve with the highest slope was that of Mollusca, followed by Chordata and Porifera (Table 4). Regarding the constancy groups, the highest slope was that of the temporary and frequent species (Table 4), whereas for motility, the curve of the vagile species showed a higher slope compared to the sessile species (Table 4).

On this basis, and considering the coefficients of the regression lines (slope and intercept), the expected richness for a surface of 0.09 m<sup>2</sup> (30 × 30 cm) is compared with the average richness values observed from the 30 × 30 cm photo-squares, randomly positioned in the centre of 30 outcrops (Table 4). The Wilcoxon two-sample paired test showed no significant differences between the expected and observed richness values (Table 4); therefore, the null hypothesis that the median of the paired differences is 0 cannot be rejected.

**Table 3.** Results of Spearman correlation between sample size and taxa richness: correlation coefficient ( $r_s$ ) and its significance ( $p$ ). n.s. = non-significant.

	$r_s$	$p$
Total	0.57	<0.05
Porifera	0.32	<0.05
Mollusca	0.56	<0.05
Chordata	0.35	<0.05
Cnidaria	0.28	n.s.
Constant	0.46	<0.05
Frequent	0.60	<0.05
Temporary	0.44	<0.05
Sessile	0.51	<0.05
Vagile	0.44	<0.05
Coralligenous	0.51	<0.05



**Figure 3.** Linear regressions on log-transformed variables: Stot—total species; Scor—coralligenous species; Scon—constant species; Sfre—frequent species; Stem—temporary species; Scho—Chordata species; Smol—Mollusca species; Spor—Porifera species; Sses—sessile species; Svag—vagile species, A—sample size.

**Table 4.** Adjusted R-squared,  $p$ -value, slope and intercept values of linear regression in Figure 3; number of species expected by Arrhenius model for a sample of 30 × 30 cm and average number of species observed in the 30 × 30 cm squares.

Groups	Adj R-Squared	$p$ -Value	Slope	Intercept	Expected	Observed
Total	0.338	<0.001	0.291	3.796	22	21 ± 7
Porifera	0.210	<0.001	0.236	2.896	10	9 ± 3
Chordata	0.091	<0.001	0.257	2.017	4	5 ± 2

Table 4. Cont.

Groups	Adj R-Squared	p-Value	Slope	Intercept	Expected	Observed
Mollusca	0.297	<0.001	0.563	1.45	1	2 ± 2
Constant	0.246	<0.001	0.214	3.481	19	17 ± 6
Frequent	0.176	<0.01	0.426	2.003	3	3 ± 2
Temporary	0.179	<0.01	0.466	1.443	1	1 ± 1
Sessile	0.312	<0.001	0.258	3.597	20	18 ± 6
Vagile	0.234	<0.001	0.459	2.012	4	3 ± 2
Coralligenous	0.259	<0.001	0.312	2.395	5	6 ± 2

#### 4. Discussion

From our literature review, the richness of the macro-zoobenthic community on the hard substrata in the northern Adriatic Sea has been estimated in the past based on faunal censuses. Casellato et al. [25] reported a total of 317 taxa belonging to 11 phyla (collected in tegrúe off the Venetian coast). A list of 480 species followed in an overview on the northern Adriatic coralligenous habitat [26], in which Mollusca and Annelida prevailed in term of recorded species. Subsequently, Falace et al. [41] reported 573 taxa for coralligenous outcrops located off the Venetian coast and in the Gulf of Trieste (many of them are investigated in the present paper), including a relatively high number of macroalgae. Recently, Nesto et al. [62] published a review in which the macro-zoobenthic biodiversity was analyzed on hard bottoms between natural and artificial reefs, experimental fields and wrecks along the coast of the Veneto Region. In these publications, both the epibenthos and the endobenthos were considered, the latter being represented mainly by molluscs, crustaceans and polychaetes [26]. The present study focused only on the epibenthic fauna identifiable by photo-samples; nevertheless, 10 phyla with 196 taxa were recorded, of which 169 were determined at species level (86%). Almost two thirds (65%) of these taxa were sessile species, represented mainly by the phylum Porifera and the class Ascidiacea (phylum Chordata). As for Porifera, the number of taxa detected in this study (58 taxa) was very similar to the number reported by Falace et al. [41] (59 taxa), accounting for 25% of the whole Adriatic Sea (230 species) and 8.5% of the Mediterranean Sea (681 species) [63]. According to Falace et al. [41], the high number of sponges appears to be a common feature of the eastern Mediterranean, most probably due to the absence of alcyonarians and gorgonians [64], while the latter are most representative of the coralligenous habitat in the central and southern Adriatic Sea [65]. The taxa of the sponges, recorded on 45 outcrops in the Gulf of Trieste, were common to 73% of those listed by Falace et al. [41] based on the bibliographic data for the northern Adriatic outcrops. Moreover, it is now possible to add information on the permanence of the Porifera species (and other taxa) on the outcrops: 41% (24 taxa) were found constantly (50–100% of the investigated outcrops) and, among these, four species were found to be protected (see results and Table S1). Of the nine taxa of the protected Porifera listed in the Annexes of the SPA/BD protocol of the Barcelona Convention [58], six were recorded on the outcrops, whereas *Spongia officinalis* (recorded as temporary taxa) is included in the list of species whose exploitation is regulated (Annex III). The phylum Mollusca followed Porifera in terms of taxa richness, representing more than 50% of the vagile epibenthos; 75% of the species were classified as temporary, being found on the outcrops with a frequency <25% (see Table S1). Almost three quarters (72%) of mollusc species were represented by class Gastropoda, of which the order Nudibranchia was the richest. As the diet of many nudibranchs is taxon-specific and sometimes also species-specific, the high diversity of nudibranchs is related to the diversity of many hydrozoans, sponges, bryozoans, and some others. It is worth mentioning the frequent occurrence of the pen shell *Pinna nobilis* during the study period at the outcrops (a total of 46 specimens settled in coarse sand on 19 outcrops), this species also being, at present, heavily stressed in the northern Adriatic Sea [66]. The widespread mass mortality in the

Mediterranean Sea, known to be caused by the protozoan *Haplosporidium pinnae*, brings this endemic bivalve to the brink of extinction [67,68].

In the class Ascidiacea, 45% of the recorded species were common to the 27 taxa in the list of Falace et al. [41], with seven taxa being constantly found on the outcrops. Among these, the occurrence of *Aplidium conicum*, *Aplidium tabarquensis* and *Polycitor adriaticus*, characteristic of different habitat types in the northern Adriatic calcareous bio-concretions, should be highlighted based on an onshore-offshore gradient: the outcrops closest to the coastline are more affected by coastal currents and river inputs, whereas the outcrops in deep offshore waters are dominated by unarticulated calcareous macroalgae and, to a lesser extent, by the tunicate *P. adriaticus* [41].

Our results show that the log-log Arrhenius model for the SAR [1] describes the relationship between epibenthic species richness and the size of the outcrops in the northern Adriatic Sea well. According to the theory of island biogeography [69], larger areas provide greater habitat diversity and enhanced resources, leading to larger populations of more species with a reduced risk of local extinction. Although it is increasingly recognized that other factors, such as island age and environmental heterogeneity, also play an important role [70,71], the ecological conditions on islands are thought to primarily be a product of the island area and isolation [72]. Early studies of marine habitats focused on natural and artificial marine ‘islands’, such as wooden panels [73], denuded reefs [74] or asbestos panels [75]. Those experiments were mostly small-scale, short-lived studies of patch dynamics in ephemeral habitats, and this is likely the reason why most of the results were inconclusive or contrary to the equilibrium theory of island biogeography [14]. However, with the increasing knowledge on marine habitats, increasing evidence of the possible applications of this theory arose. The same SAR model tested in the present work has been successfully applied to other marine island-like habitats, such as coral colonies [6,76,77], off-shore banks [78] and true Island [79]. The comparison among different island-like systems is a challenging task as different factors influence their degree of insularity and isolation, therefore affecting the applicability of biogeographic theories such as the SAR [15]. Marine Island-like Systems are often not as isolated as true islands, and the level of isolation depends on the “permeability of the matrix”; that is, on the contrast between the targeted habitat and the habitat surrounding it [15]. Moreover, the extent to which such systems can be island-like also depends on the characteristics of the system itself (e.g., isolation, age, currents and larval dispersal), and also varies in relation to the different taxa on the basis of larval duration [80–82]. The scale of the analysis can also influence the results [83,84].

Our results confirm the taxa-dependence of the SAR when applied to benthic invertebrates. In the present work, the same SAR model held for the dominant phyla (Porifera, Chordata and Mollusca) considered separately, with the exception of cnidarians, which are represented by a smaller number of recorded taxa. Molluscs showed the steepest curve and the best fits compared to the other benthic taxa. The same features of the SAR curves for molluscs were reported in other works [6,78,79]. A higher slope of the curve generally corresponds to greater isolation [85]; therefore, some authors tested whether the slope of the curve and the significance of the relationship could be a function of adult motility and larval dispersal ability [78]. The taxa with sessile adult life stages and short larval duration exhibited a steep and significant SAR slope [78,79]. Conversely, higher slopes were observed for the vagile with respect to sessile species, and for Mollusca compared to Porifera and Chordata, in the present work. A possible explanation could be related to the different phyla focused on the different work: Stortini et al. [78] compared molluscs/Cirripedia with echinoderms and crustaceans, while Hachich et al. [79] compared molluscs/seaweed with fishes. In accordance with the schematic interpretation proposed by Triantis et al. [85], several factors (isolation, dispersion capability, habitat diversity, etc.) influence the characteristics of the SAR, and the relative importance of those factors changes between systems and according to the scale analyzed. At small scales, as in the case of coral colonies [6,76,77], the random placement and habitat diversity play an important role, while at larger scales, such in the case of real island [79], evolutionary history could play a major role [85]. The

scale at which the northern Adriatic outcrops were analyzed could be considered as intermediate between these two cases. The northern Adriatic coralligenous assemblages seem to be the most isolated and poorly connected among the different subregions of the central Mediterranean Sea [27]. Bandelj et al. [52] argued that hydrodynamic connectivity is an important factor affecting the macrobenthic assemblages of outcrops. Therefore, this network of outcrops arranged in a scattered mosaic on the seafloor, also referred to as Mesophotic Biogenic Habitats, can be considered as a regional-scale meta-community resulting from the local community interactions and spatial dynamics [41,52]. However, similarly to other mesophotic environments, such as the *Neopycnodonte* bioconstruction [86], with the progressive decrease in light intensity, there is a decreasing input of calcareous algae, probably due to the turbid waters and hyper sedimentation in the northern Adriatic Sea, and the greater depths in the southern Adriatic/Ionian Sea.

From a conservation perspective, the species groups with steeper curves increase rapidly in diversity with the increasing area, but decrease equally rapidly with the shrinking area. Therefore, this parameter is a strong indicator of the group's sensitivity to habitat and climate-space loss [83]. In the studied area, molluscs would therefore be the group most sensitive to habitat loss. Indeed, this taxon seems to play an important role in structuring the macrozoobenthic community in hard substrates in the northern Adriatic, as it is more sensitive than the other groups to the anthropogenic stressors [62].

The rocky outcrops, although rather limited in size, represent distinctive geomorphological elements with outstanding numbers of epifauna and endofauna. Such areas are inhabited by a significantly higher number of benthic invertebrates than the adjacent sublittoral muddy, soft bottoms [87–89]. In fact, rocky outcrops are geological features which include a plethora of microhabitat features, such as holes, cracks, crevices, cavities, overhangs and others. Such microhabitats in rocky outcrops provide shelter, feeding grounds and reproductive potential for many benthic invertebrates. Densely vegetated outcrops, on the other hand, attract many fish and other predators that approach such habitats. Based on the current knowledge, rocky outcrops, known for their diversified epifaunal community, can be used as a bioindicator to check the quality of entire communities and to verify the presence of impacts of natural or anthropogenic origins [90]. In this context, richness estimation through a photo survey using  $30 \times 30$  cm squares randomly positioned on rocky substrates can be considered as a useful monitoring tool, also in term of economy and speed. In fact, a significant deviation of the observed richness from the expected richness calculated using the SAR model could indicate a disturbance affecting the benthic outcrops communities. Therefore, models based on the SAR could be proposed to forecast the expected loss of species richness in a region and/or to estimate local species richness for hotspot identification, as in the case of the northern Adriatic rocky outcrops.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w15020318/s1>, Table S1: Taxonomic list of epibenthic fauna with mobility, function, status, and permanency. S-sessile, V-vagile, B-builder, E-eroder, COR-characteristic of coralligenous sensu [56], cor-common in coralligenous sensu [56], P-protected, C-constant, F-frequent, T-temporary.

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