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RESEARCH ARTICLE

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NAMBER: A biotic index for assessing the ecological quality of mesophotic biogenic reefs in the northern Adriatic Sea

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Abstract

1. The aim of the present study was to propose a biotic index (*North Adriatic Mesophotic BiogEnic Reefs*, *NAMBER*) suitable for assessing the ecological quality of the mesophotic biogenic reefs of the northern Adriatic continental shelf based on photographic sampling.
2. At each of the 20 study sites, the degree of bioconstruction (expressed as percentage cover of crustose coralline algae), the α-diversity (expressed as the mean number of taxa), and the degree of sensitivity to human disturbance and climate change (based on literature data and expert judgement) of the benthic assemblages were selected as descriptors and combined in the *NAMBER* index, using the best values that the three metrics can currently achieve in the studied region as a reference.
3. The study highlighted that there was large spatial heterogeneity among reefs and high variability in the ecological quality values obtained by *NAMBER*, ranging from bad to high. The index indicates that reefs lying furthest from the coast, under substantially lower anthropogenic pressure, have a generally higher status of environmental quality. However, a clear geographical pattern did not emerge, as reefs close together often had different ecological qualities.
4. The *NAMBER* index, which combines three ecological descriptors, in accordance with the requirements of the European Marine Strategy Framework Directive, represents a specific adaptation to the northern Adriatic Sea of a multimetric index previously developed for the north-western Mediterranean Sea, capitalizing on previous knowledge and research efforts.
5. This multimetric biotic index provides an effective standardized tool for monitoring programmes and environmental impact assessments in the northern Adriatic mesophotic biogenic reefs and lays the foundation for ecosystem-based management and conservation in this basin.

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KEYWORDS
coralligenous assemblages, environmental status, hard bottom, Mediterranean Sea, photographic sampling, species diversity

1 | INTRODUCTION

Marine bioconstructions are biodiversity-rich three-dimensional structures resulting from the balance between the growth of calcified benthic organisms and biological and physico-chemical erosion processes (Ingrosso et al., 2018; Turicchia et al., 2022). These structures form secondary substrata that provide complex and heterogeneous habitats (Fox, 2005). In the Mediterranean Sea, coralligenous reefs represent the most important subtidal calcareous biogenic habitat in terms of extent, biodiversity, and carbon dynamics (Laborel, 1987; Martin et al., 2014; Rindi et al., 2019), primarily built by encrusting calcareous algae (i.e. rhodophytes of the orders Corallinales, Corallinapetrales, Hapalidiales, Peyssonneliales, and Sporolithales) and secondarily by calcareous invertebrates like bryozoans and scleractinians (Ballesteros, 2006). They develop over a wide range of depths, from 15 m to about 150 m, depending on environmental conditions, especially water turbidity, sunlight penetration, and currents (Martin et al., 2014), and occur in a variety of morphotypes (Bracchi et al., 2017). The two most common morphologies of coralligenous reefs are rims (i.e. vertical or near-vertical concretions that develop on steep littoral rocky cliffs) and platform banks (i.e. isolated reefs surrounded by sand or biodetritic sediments) (Ballesteros, 2006). Coralligenous rims characterize coastal rocky bottoms, whereas platform banks develop mainly offshore, on continental shelves over consolidated sediments, coalescing rhodoliths, or pre-existing rocky outcrops (Cánovas-Molina et al., 2016b). Coralligenous reefs are an iconic underwater seascape (Tribot et al., 2016; Chimienti et al., 2017) and provide multivarious ecosystem services to humans (Thierry de Ville d'Avray et al., 2019), but they are also vulnerable to both global and local disturbance (Piazzi, Gennaro & Balata, 2012; Gatti et al., 2015b; Gómez-Gras et al., 2019), such as pollution, sediment accumulation, fisheries, and stressors related to climate change (Balata et al., 2005; Piazzi & Balata, 2011; Verdura et al., 2019; Betti et al., 2020; Ponti et al., 2021). Coralligenous reefs were included as 'near threatened' in the European Red List of Habitats (Gubbay et al., 2016) and, as natural reefs (code 1170 of the European Habitats Directive, NATURA 2000–92/43/EEC; European Commission – DG Environment, 2013), these habitats should be monitored according to the European Marine Strategy Framework Directive (MSFD, 2008/56/EC). European directives require an assessment of the ecological quality status of marine areas to achieve the environmental targets and protect marine habitats from further deterioration. In this context, the adopted strategies provide for the use of bioindicators and the development of biotic indices suitable for assessing the ecological quality of coastal aquatic ecosystems (Borja et al., 2010). The 'Action Plan for the Conservation of the Coralligenous and Other Calcareous Bio-concretions in the Mediterranean Sea' (UNEP-MAP-SPA/RAC, 2017) also foresees, among the different tasks undertaken, the development and intercalibration of indices used to assess the ecological quality of these habitats.

Several methods have been proposed to assess the ecological quality status of coralligenous assemblages for both banks and rims. Coralligenous rims can be effectively surveyed by scuba diving (Teixidó et al., 2013; Piazzi et al., 2019a), whereas banks, which are generally at greater depths, are often surveyed by unmanned vehicles such as remotely operated vehicles (ROVs) (Bo et al., 2014; Casoli et al., 2017; Ferrigno et al., 2018; Piazzi et al., 2019c; Rossi et al., 2021). Ecological quality indices have been developed for both coralligenous rims (Deter et al., 2012; Gatti et al., 2012; Cecchi et al., 2014; Ruitton et al., 2014; Gatti et al., 2015a; Montefalcone et al., 2017; Sartoretto et al., 2017; Valisano et al., 2019; Piazzi et al., 2019b; Piazzi et al., 2021) and banks (Cánovas-Molina et al., 2016a; Ferrigno, Russo & Sandulli, 2017; Enrichetti et al., 2019) occurring in the north-western Mediterranean Sea. Moreover, the *MedSens* biotic index, developed to provide information on the environmental status of Mediterranean subtidal rocky coastal habitats using data collected by trained volunteer divers, has been applied to coralligenous reefs, for both rims and banks (Turicchia et al., 2021a; Turicchia et al., 2021b; Turicchia et al., 2021c).

In the northern Adriatic Sea, mesophotic biogenic reefs, locally called *tegnùe* or *trezze*, are arrayed over the muddy–sandy bottom of a large area of the continental shelf at depths ranging from 15 to 40 m (Newton & Stefanon, 1975; Spagnoli et al., 2014), where sunlight penetration is strongly affected by river run-off, sediment transport, and recurrent phytoplankton blooms (Bernardi Aubry et al., 2004), resulting in light attenuation at the sea bed of 1%–2% of the mean surface irradiance (Ponti, Fava & Abbiati, 2011). These biogenic reefs vary in size and morphology, and their upper surface may be up to 4 m above the surrounding sea bed (Gordini & Saul, 2020; Fortibuoni et al., 2020a; Ponti, 2020a; Fortibuoni et al., 2020b; Ponti, 2020b). Compared with the coralligenous rims and banks of the north-western Mediterranean Sea, many erect and fragile species such as gorgonians and large bryozoans are absent, and the composition of the encrusting calcareous algae, which are the main builders in these habitats, is also quite different: here *Lithophyllum incrustans* Philippi dominates, instead of *Lithophyllum stictiforme* (J.E. Areschoug) Hauck, *Mesophyllum alternans* (Foslie) Cabioch & M.L. Mendoza, and *Neogoniolithon mamillosum* (Hauck) Setchell & L.R. Mason (Ballesteros, 2006; Kaleb et al., 2011; Curiel et al., 2012; Falace et al., 2015; Ingrosso et al., 2018).

The assemblages inhabiting the northern Adriatic mesophotic biogenic reefs have been studied extensively (Casellato & Stefanon, 2008; Ponti, Fava & Abbiati, 2011; Curiel et al., 2012; Ponti et al., 2014; Falace et al., 2015; Fava, Ponti & Abbiati, 2016; Turicchia et al., 2022). They are affected by several factors, including sedimentation rates, hydrodynamics (Bandelj et al., 2020), water quality (Falace et al., 2015), human disturbance, especially trawling (Pranovi et al., 2000), and past episodic algal blooms, benthic mucilage, and sea-bed dystrophic crises (Zuschin & Stachowitsch, 2009; Tomašových et al., 2017). However, so far, no methods have been proposed to assess their ecological quality status. In this context, a multimetric biotic index can provide information on overall ecological quality regardless of the various local pressures, providing valuable indications for planning and monitoring conservation strategies (Ponti et al., 2009). The indices already used for the coralligenous assemblages of the north-western Mediterranean appear to be inappropriate for the Adriatic mesophotic biogenic reefs, as many common descriptors are not applicable, such as the abundance of erect and fragile anthozoan or bryozoan species, as they are absent. On the contrary, the ecological relevance, specificity, and abundance of these still poorly known Adriatic habitats require a method for assessing their ecological quality, in accordance with European Directives, and a tool for their ecosystem-based management and conservation.

The present study aims to develop a multimetric biotic index named *NAMBER* (*Northern Adriatic Mesophotic BiogEnic Reefs*) to assess the ecological quality of coralligenous reefs in the northern Adriatic Sea. The approach used to develop the index consists of evaluating descriptors already used for indices proposed for other areas in the Mediterranean Sea and selecting and combining those best suited for the Adriatic mesophotic biogenic reefs. This approach made it possible to capitalize and transfer the knowledge and experience gained in comparable, albeit different, subtidal biogenic reefs across Mediterranean sub-basins.

2 | METHODS

2.1 | Study sites and sampling

The northern Adriatic continental shelf hosts hundreds of coralligenous reefs arrayed on the sedimentary bottom (see the 'NorthernAdriatic_Reefs' compilation at the Marine Geoscience Data System, https://www.marine-geo.org/tools/search/entry.php?id=NorthernAdriatic_Reefs). In the present study the composition and abundance of epibenthic assemblages were investigated using a non-destructive photographic sampling method at 20 randomly selected sites, sampled in different summer campaigns between 2006 and 2017 from Chioggia to Grado-Trieste (over a distance of approximately 100 km) by the same research teams and using a consistent methodology. Five sites off Chioggia (i.e. AL06, MR08, P204, P213, and TQS) were sampled over a period of 12 years (2006–2017). The study sites ranged from 17 to 25 m in depth and from 240 to 46,500 m^2 in area (Figure 1; Table 1). At each site, photographs were taken randomly, a few metres apart, using a digital camera equipped with a rigid spacing frame to ensure constant distance, sampling area (21 × 28 cm = 588 cm^2), and

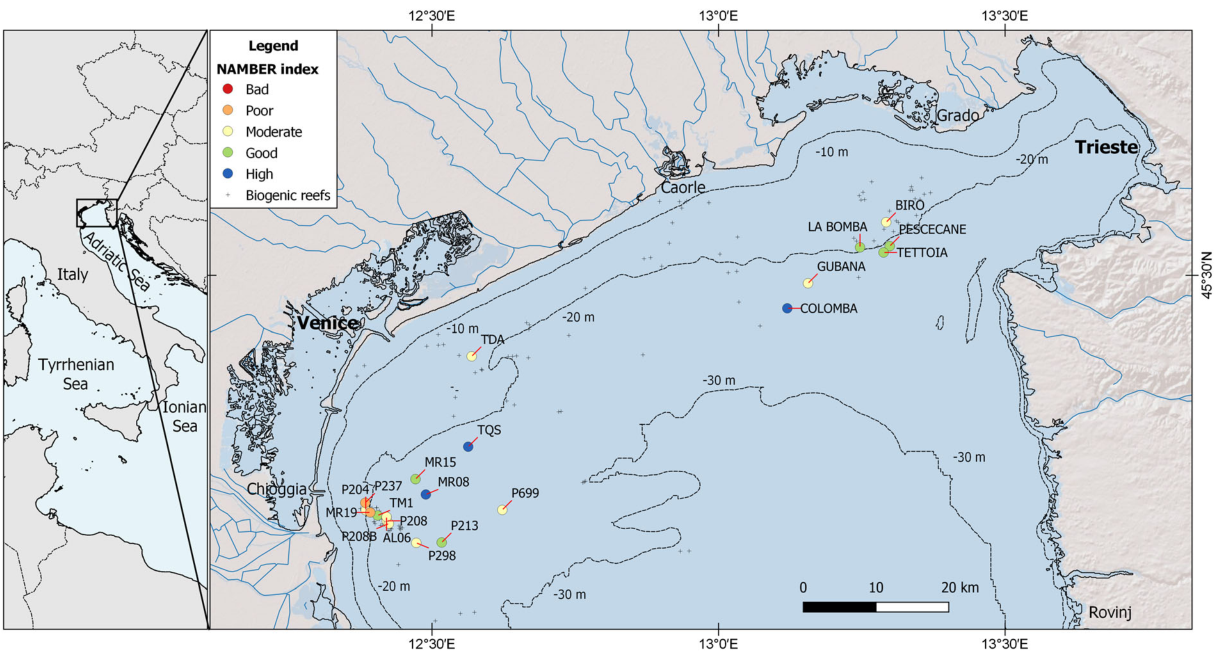

FIGURE 1 Geographic distribution and *NAMBER* classification of the biogenic reefs investigated (Mercator projection).

TABLE 1 Characteristics of the studied reefs.

Name	Latitude WGS84	Longitude WGS84	Depth (m)	Reef elevation (m)	Coast distance (km)	Site extent (m^2)	Year of sampling
AL06	45°12.170′	12°25.241′	21.9	2.1	8.07	7,000	2006 2017
BIRO	45°33.923′	13°17.546′	17.3	1.5	13.16	2,500	2017
COLOMBA	45°27.567′	13°07.193′	23.4	3.0	19.50	6,000	2017
GUBANA	45°29.414′	13°09.363′	23.4	3.0	16.71	12,000	2017
LA BOMBA	45°32.066′	13°14.809′	19.4	2.0	16.27	300	2017
MR08	45°13.831′	12°29.354′	22.2	1.5	13.79	2,100	2006 2013 2017
MR15	45°14.971′	12°28.273′	21.2	0.8	12.49	600	2006
MR19	45°12.499′	12°23.538′	21.1	1.5	6.61	8,200	2006
P204	45°12.666′	12°23.064′	20.2	2.2	6.12	16,100	2006 2013 2017
P208	45°11.876′	12°25.259′	22.0	0.6	7.81	1,900	2006
P208B	45°11.671′	12°25.482′	22.5	3.8	7.89	2,800	2013
P213	45°10.270′	12°31.013′	24.5	0.8	14.37	1,700	2006 2013 2017
P237	45°13.216′	12°23.016′	19.0	3.1	5.70	5,000	2006
P298	45°10.241′	12°28.352′	22.5	1.1	10.88	500	2006
P699	45°12.688′	12°37.360′	25.5	0.5	23.28	1,800	2006
PESCECANE	45°32.154′	13°17.915′	20.5	1.0	15.90	2,400	2017
TDA	45°24.040′	12°34.151′	20.6	4.5	7.68	3,500	2006
TETTOIA	45°31.689′	13°17.271′	20.2	1.5	16.45	2,400	2017
TM1	45°12.273′	12°24.297′	19.5	1.5	7.18	46,500	2013
TQS	45°17.347′	12°33.788′	22.0	0.6	17.99	240	2006 2013

parallelism between the focal plane and the substrate (Figure S1). As in previous studies in these habitats (e.g. Ponti, Fava & Abbiati, 2011), 10 random photographic samples from all samples available, after discarding low-quality photos, were analysed for each reef and sampling date. The percentage cover of sessile organisms was quantified by superimposing a grid of 400 equal-sized squares using the freeware software photoQuad (Trygonis & Sini, 2012). Percentage cover was based on the total readable area of each image, determined by subtracting dark and blurred areas or portions covered by motile organisms (Ponti et al., 2018); however, the unreadable area was always very small, being never greater than 20%. Organisms in the photos were identified at the lowest possible taxonomic level, based on reference collections, and then classified in morpho-ecological groups (Table 2). This grouping aims to mitigate the taxonomic uncertainty typical of photographic sampling, preserving the knowledge of the ecological role and sensitivity to environmental changes of the taxa present in the assemblages as much as possible (Garrabou, Ballesteros & Zabala, 2002; Teixidó, Garrabou & Harmelin, 2011; Casas-Güell et al., 2015).

2.2 | Data analysis of the benthic assemblages

A canonical analysis of principal coordinates (CAP; Anderson & Robinson, 2003), based on a Bray–Curtis dissimilarity matrix computed on fourth-root transformed cover data, was performed to discriminate assemblage structures among reefs and identify the morpho-ecological groups accounting for such distinction.

2.3 | Selection of descriptors

The *NAMBER* index was developed following the approach of the *ESCA* (*Ecological Status of Coralligenous Assemblages*) index (Cecchi et al., 2014), a method widely employed in the north-western

TABLE 2 Sensitivity level (*SL*) of the identified morpho-ecological groups toward human disturbance and climate change, with examples of corresponding taxa (values range from 1 to 10, where low values correspond to the most tolerant organisms and high values to the most sensitive organisms).

No.	Group	*SL*
1	Algal turf (e.g. *Antithamnion*, *Ceramium*, and *Pterothamnion*)	1
2	Ulvales	1
3	Boring organisms (e.g. *Cliona* and *Rocellaria dubia*)	1
4	Siphonous branched Chlorophyta (e.g. *Pseudochlorodesmis furcellata*)	2
5	Hydrozoans	2
6	Gelidiales	3
7	Encrusting sponges (e.g. *Antho* (*Antho*) *inconstans* and *Dictyonella incisa*)	3
8	Encrusting bryozoans	3
9	Dictyotales (e.g. *Dictyota dichotoma*)	4
10	Encrusting Phaeophyceae (e.g. *Zanardinia typus*)	4
11	Ceriantharia (e.g. *Cerianthus membranaceus*)	4
12	Clavelinidae (e.g. *Pycnoclavella communis*)	4
13	Laminar Rhodophyta without cortication (e.g. *Radicilingua*)	5
14	Peyssonneliaceae with not entirely calcified thallus (e.g. *Peyssonnelia rubra*)	5
15	Serpulids (e.g. *Protula tubularia*, *Serpula vermicularis*, and *Spirobranchus triqueter*)	5
16	Zoantharia (e.g. *Epizoanthus* and *Parazoanthus axinellae*)	5
17	Actiniaria (e.g. *Cereus pedunculatus*)	5
18	Alcyonacea (e.g. *Sarcodictyon catenatum*)	5
19	Massive lobate sponges adhering to the substrate (e.g. *Chondrilla nucula*, *Chondrosia reniformis*, *Mycale* (*Mycale*) *massa*, *Oscarella lobularis*, and *Suberites*)	6
20	Azooxantellate individual scleractinians (e.g. *Leptopsammia pruvoti*)	6
21	Small massive colonial ascidians (e.g. *Cystodytes*, *Didemnum*, and *Distaplia*)	6
22	Small erect corticated Rodophyta (e.g. *Botryocladia*)	7
23	Erect sponges (e.g. *Aplysina*, *Haliclona*, and *Ulosa digitata*)	7
24	Erect solitary ascidians (e.g. *Phallusia* and *Pyura microcosmus*)	7
25	Flattened Rhodophyta with cortication (e.g. *Halymenia floresii* and *Rhodymenia*)	8
26	Massive sponges (e.g. *Geodia cydonium*, *Ircinia*, *Petrosia*, *Polymastia*, *Sarcotragus*, and *Spongia*)	8
27	Vermetids	8
28	Crustose coralline algae (CCA, e.g. *Lithophyllum incrustans*, *Lithothamnion*, and *Mesophyllum*)	9
29	Peyssonneliaceae with fully calcified thallus (*Peyssonnelia rosa-marina* and *Peyssonnelia heteromorpha*)	9
30	Spherical colonial ascidians (e.g. *Polycitor adriaticus*)	9
31	Erect colonial ascidians (e.g. *Aplidium conicum* and *Aplidium tabarquensis*)	10

Mediterranean (Piazzi et al., 2019b). Among the descriptors reviewed by Cecchi et al. (2014) and Piazzi et al. (2019b), three were selected: the degree of bioconstruction, α-diversity, and sensitivity level of assemblages (see below). Other descriptors were excluded as they did not apply to the habitat studied (e.g. size and percentage of necrosis and epibiosis of gorgonians, absent in these habitats), were not representative of reefs surrounded by soft bottom (e.g. sediment cover) or were not sufficiently tested (e.g. β-diversity).

The degree of bioconstruction at each site was assessed by the mean percentage cover of crustose coralline algae (CCA). A value from 0 to 9 was assigned to each of the following 10 classes of percentage cover, arranged in a geometric series (Piazzi et al., 2017): 0, 0%; 1, 0 < % ≤ 0.4; 2, 0.4 < % ≤ 0.8; 3, 0.8 < % ≤ 1.6; 4, 1.6 < % ≤ 3.1; 5, 3.1 < % ≤ 6.3; 6, 6.3 < % ≤ 12.5; 7, 12.5 < % ≤ 25; 8, 25 < % ≤ 50; 9, 50 < % ≤ 100.

As a proxy, the α-diversity of each site was calculated in terms of the mean number of taxa per photographic sample.

Species sensitivity to poor ecological conditions and human impacts is a commonly used descriptor in various biotic indices for assessing marine environmental quality, as also required by recent European Directives (Ballesteros et al., 2007; Ponti et al., 2007; Borja, Ranasinghe & Weisberg, 2009; Nikolić et al., 2013; Leonardsson et al., 2015; Turicchia et al., 2021a). The sensitivity level integrates both sensitivity to disturbance (causing mortality or physical harm) and sensitivity to stress (causing physiological alteration), following Montefalcone et al. (2017). A high sensitivity value implies that the species is seldom found in altered environments; on the contrary, a low sensitivity value implies that the species can be predominantly found in altered environments (Cecchi et al., 2014). A sensitivity level (*SL*) value was assigned to each morpho-ecological group based on literature data, wherever available (e.g. Naranjo, Carballo & García-Gómez, 1996; Borja, Franco & Pérez, 2000; Ballesteros et al., 2007; Montefalcone et al., 2017; Schönberg, 2021), complemented with expert (i.e. the co-authors) judgement only when there were no alternatives (as in Turicchia et al., 2021a). A numerical scale of *SL* was established with values ranging from 1 to 10, with low values corresponding to the most tolerant organisms and high values corresponding to the most sensitive organisms (Table 2). At each site, the mean *SL* was calculated by summing the product of the percentage cover of each morpho-ecological group by its *SL* value, and then dividing by the sum of the covers:

$$SL_{mean} = \Sigma(\%Cover_{group} \times SL_{group})/\Sigma\%Cover_{group}.$$

2.4 | NAMBER *index calculation*

According to the European Water Framework Directive (WFD, 2000/60/EC), ecological status should be defined by the ecological quality ratio (*EQR*) value, which is the ratio between the value of the metrics/index calculated for the studied site (ecological quality value, *EQV*) and the corresponding value for the reference conditions (*EQR*

$= EQV_{observed}/EQV_{reference}$). The *NAMBER* index was calculated as the mean of the contribution of the three *EQR* components (i.e. percentage cover of CCA, α-diversity, and *SL*) using the formula $NAMBER = (NAMBER_{CCA} + NAMBER_{\alpha} + NAMBER_{SL})/3$, where the individual *NAMBER* contributions were calculated as the ratio of the values of the three metrics obtained at each site ($EQV_{observed}$) and the corresponding higher values found for the whole area investigated ($EQV_{reference}$). Therefore, the best actual data for each chosen metric (i.e. the highest values measured for mean *SL* and α-diversity in the northern Adriatic reefs and the highest class of percentage cover observed for CCA) were used as a proxy for the least-disturbed conditions in the northern Adriatic reefs (Stoddard et al., 2006). In agreement with Piazzi et al. (2021), five ecological status classes were defined by dividing the entire range of *NAMBER* values into five equal parts: bad, $0 < NAMBER < 0.2$; poor, $0.2 \leq NAMBER < 0.4$; moderate, $0.4 \leq NAMBER < 0.6$; good, $0.6 \leq NAMBER < 0.8$; high, $0.8 \leq NAMBER \leq 1.0$. The same scale also applies to the individual metrics that form the index.

The spatio-temporal variation of the index was analysed using data from sites sampled on multiple dates (summers 2006, 2013, 2017) by applying a randomized block two-way analysis of variance ($\alpha = 0.05$) for site and for date effects, without interaction, whereas a Tukey's test of additivity was used to detect site × date interaction, using the package 'asbio' in R (Aho, 2014).

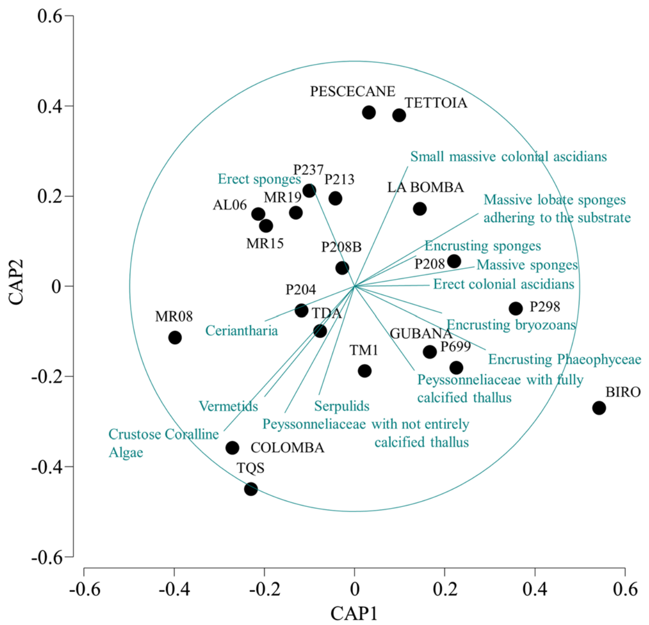

FIGURE 3 Canonical analysis of principal coordinates (CAP) showing the discriminant-type ordination of assemblages in the biogenic reefs investigated (in bold) and morpho-ecological groups accounting for such discrimination (Pearson correlation > 0.3), on the most recent sampling date.

3 | RESULTS

3.1 | Benthic assemblages

A total of 280 photographic samples were analysed from 20 sites, three of which were sampled three times and two of which were sampled twice. Ninety-eight taxa were identified and then assigned to 31 morpho-ecological groups (12 macroalgal groups and 19 macro-invertebrate groups, Table 2) to assign sensitivity levels. The mean number of taxa in the photographic samples at each study site (α-diversity) ranged from 6.4 ± 0.7 to 15.1 ± 1.2 taxa (mean ± SE, $n = 10$; Figure 2). Epibenthic assemblage structures varied greatly among reefs, apparently without a consistent geographical trend and regardless of mutual distances (Figure 3). The morpho-ecological groups that contributed most to distinguishing the assemblage were the CCA (including *L. incrustans*, *Lithothamnion* sp., and *Mesophyllum* sp.) and Peyssonneliaceae with not entirely calcified thallus (e.g. *Peyssonnelia rubra* (Greville) J.Agardh), characteristic of the COLOMBA, TQS and MR08 sites, followed by encrusting Phaeophyceae (e.g. *Zanardinia typus* (Nardo) P.C.Silva) for example at BIRO, massive lobate sponges adhering to the substrate (such as *Chondrosia reniformis* Nardo, 1847, *Oscarella lobularis* (Schmidt, 1862), *Suberites* sp., *Chondrilla nucula* Schmidt, 1862, *Mycale* (*Mycale*) *massa* (Schmidt, 1862)), and small massive colonial ascidians (like *Distaplia bermudensis* Van Name, 1902 and *Cystodytes dellechiajei* (Della Valle, 1877)).

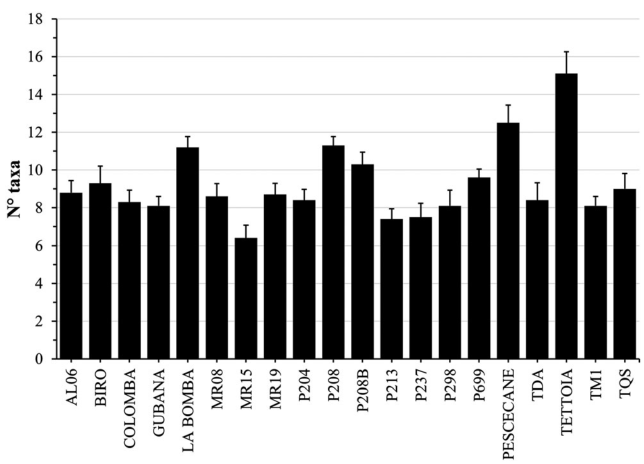

FIGURE 2 Mean number of taxa per sample (α-diversity) at each study site on the most recent sampling date (mean ± SE, $n = 10$).

3.2 | The index metrics

The percentage CCA cover, as a proxy of the degree of bioconstruction, and the corresponding metric of the index ($NAMBER_{CCA}$; Figure 4) varied widely among reefs, with the highest index values at MR08, TQS, and COLOMBA sites ($NAMBER_{CCA} = 1.00$), where their mean percentage cover exceeded 50%, and with the lowest index values at P208, P237, and BIRO sites, with a mean percentage cover lower than 0.4% ($NAMBER_{CCA} = 0.11$, corresponding to bad ecological conditions).

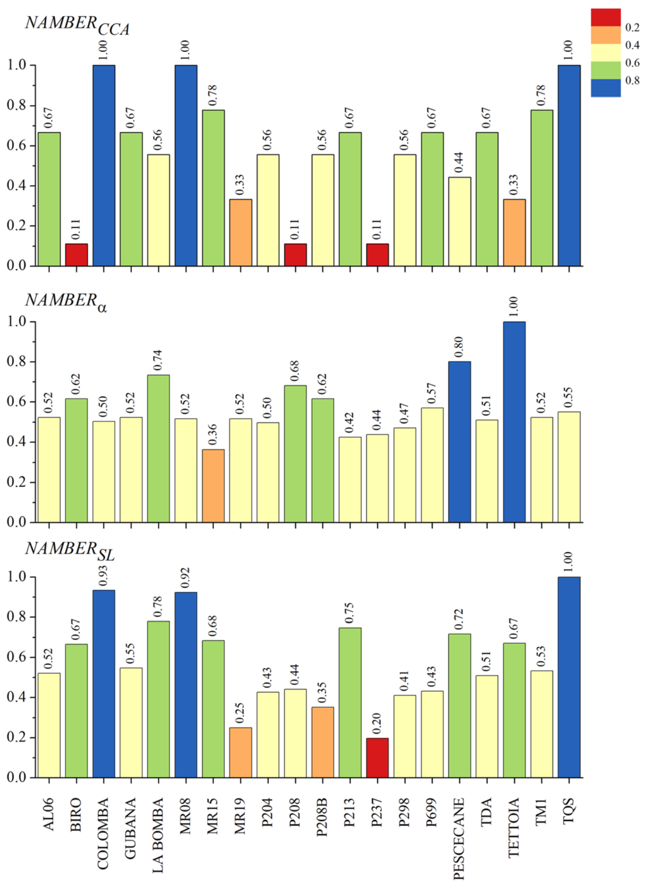

FIGURE 4 Values of the metrics included in the *NAMBER* index at each study site: CCA, crustose coralline algae; α, α-diversity; *SL*, sensitivity level (colour scale indicates the corresponding classification of the *NAMBER* index).

The α-diversity component reaches the reference value of 15.1 mean taxa per sample at TETTOIA ($NAMBER_{\alpha} = 1.00$, Figure 4) and the lower value at MR15 ($NAMBER_{\alpha} = 0.36$, corresponding to poor ecological conditions).

The mean level of sensitivity to human disturbance and climate change, and the corresponding metric of the index ($NAMBER_{SL}$; Figure 4), also varied greatly among sites. The highest mean sensitivity value, taken as a reference value, was obtained at MR08 ($SL_{mean} = 7.85$, $NAMBER_{SL} = 1.00$), but similarly high values were also achieved at COLOMBA and TQS, whereas the lowest sensitivity level was recorded at P237, in the range of bad ecological conditions ($SL_{mean} = 1.54$ and $NAMBER_{SL} = 0.20$).

The mean percentage cover of the main morpho-ecological groups, with which different levels of sensitivity are associated, varies between reefs, thus contributing to the resulting mean sensitivity of the sites (Figure 5). Reefs with lower sensitivities ($NAMBER_{SL} < 0.4$) were dominated by algal turfs and encrusting sponges. Reefs with moderate sensitivity levels ($NAMBER_{SL} = 0.4–0.6$) were characterized by a mixture of algal turf, encrusting sponges, boring organisms, and some CCA. Reefs with good sensitivity levels ($NAMBER_{SL} = 0.6–0.8$) had an increased percentage cover of massive lobate sponges adhering to the substrate, small massive colonial ascidians, and erect sponges. Finally, reefs with high sensitivity levels ($NAMBER_{SL} > 0.8$) were dominated by CCA and a relatively high abundance of boring organisms, associated with erect sponges or Peyssonneliaceae with not entirely calcified thallus.

3.3 | The *NAMBER* index

The *NAMBER* index ranged from 0.25 at P237, which was classified as poor ecological status, to 0.85 at TQS, corresponding to high ecological status (Figure 6). The three sites (TQS, COLOMBA, and MR08) with the highest mean percentage cover of CCA and the highest mean sensitivity of the assemblages, although lacking a comparatively high α-diversity, were classified as high ecological status. The ecological status of north-eastern reefs off the Gulf of Trieste were classified as moderate to high, whereas there was greater variability in the south-western reefs off Chioggia and Venice (Figures 1 and 6). This area included the reefs with the lowest classifications (i.e. poor ecological status), but also two of the three reefs with high ecological status.

Where multiple sampling dates were available, the spatio-temporal variation of the index was analysed by applying the *NAMBER* index to the same sites (AL06, MR08, P204, P213, and TQS) in different years (2006, 2013, and 2017). *NAMBER* showed small variations in the values that, when close to the threshold, may also result in slightly different classifications, as was the case with site TQS (Figure 7). However, although significant differences were found among sites regardless of time, no significant changes were found over time when disregarding sites (Table 3). Tukey's test of additivity, which for technical reasons can only be applied to sites with all sampling dates, did not detect a site × date interaction ($F = 0.9265$, denominator $df = 3$, P-value $= 0.4068$).

4 | DISCUSSION

This study investigated assemblages of northern Adriatic mesophotic biogenic reefs using proxies of bioconstruction processes, species diversity, and level of sensitivity of the main morpho-ecological groups toward human disturbance and climate change as ecological indicators. Based on these indicators, a multimetric biotic index suitable to assess the ecological quality status of these peculiar bioconstructions was defined, capitalizing on the experiences gained in the north-western Mediterranean Sea (Piazzi et al., 2021). As described in previous studies (Casellato & Stefanon, 2008; Ponti & Mescalchin, 2008; Ponti, Fava & Abbiati, 2011; Curiel et al., 2012; Ponti et al., 2014; Falace et al., 2015), the biogenic reefs of the northern Adriatic Sea are characterized by a high abundance of sponges and ascidians, revealing a community structure that differs from that of the coralligenous assemblages in the north-western Mediterranean Sea or elsewhere in

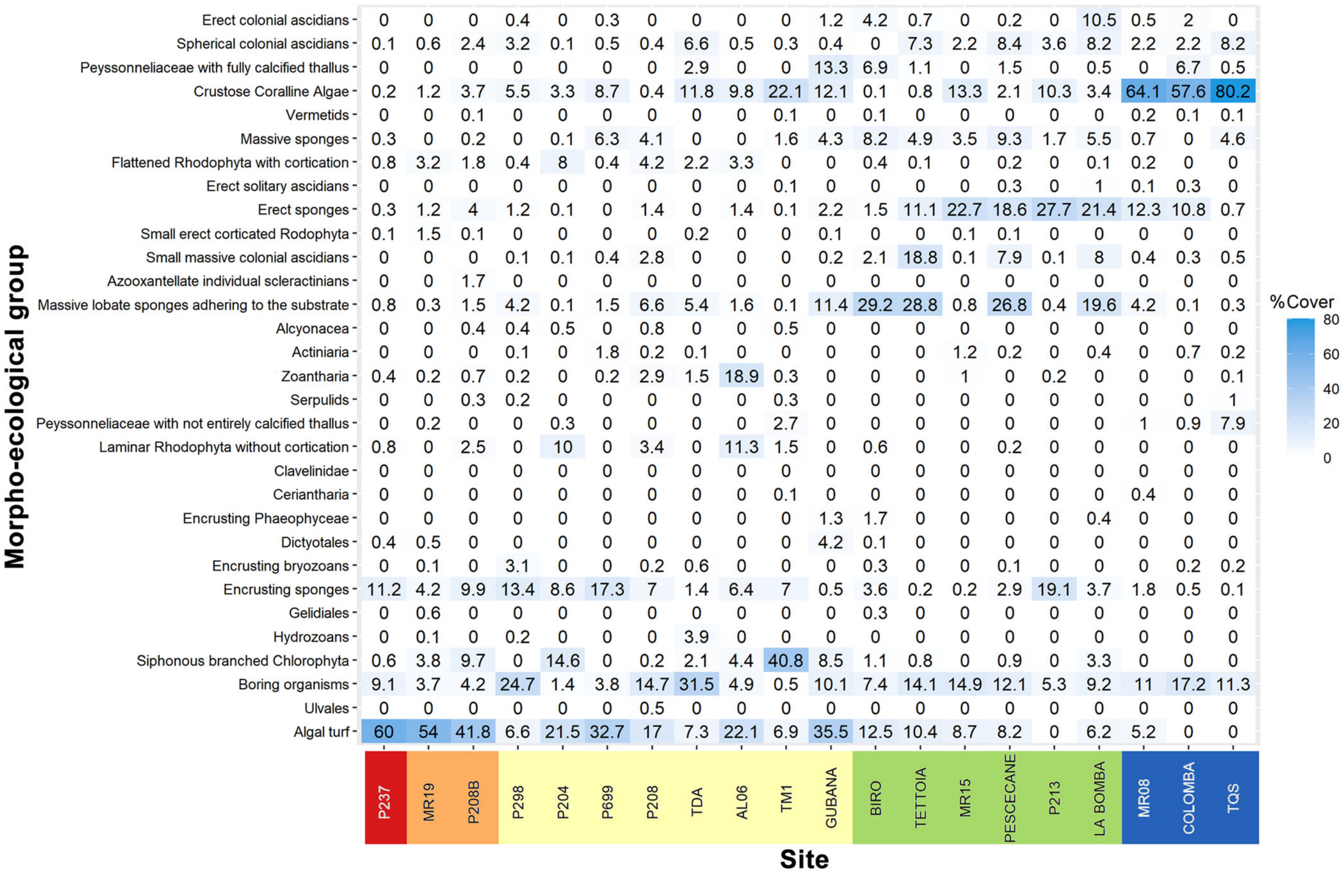

FIGURE 5 Heat map of the mean percentage cover of the main morpho-ecological groups (see Table 2) at each study site. Both groups and sites are sorted by increasing level of sensitivity toward human disturbance and climate change (colour scale for site labels indicates the corresponding classification of the $NAMBER_{SL}$ index, as presented in Figure 4).

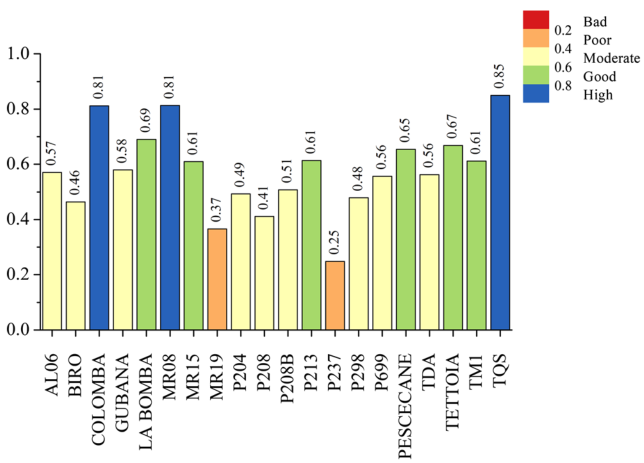

FIGURE 6 Values of the *NAMBER* index for the investigated biogenic reefs (colour scale indicate the ecological status classes).

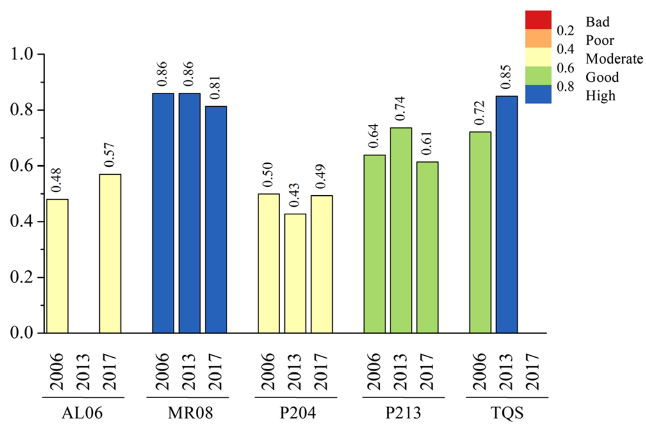

FIGURE 7 Values of the *NAMBER* index for multiple years (2006, 2013, and 2017) in a subset of biogenic reefs investigated off Chioggia (AL06, MR08, P204, P213, and TQS). Colour scale indicate the ecological status classes.

the eastern Adriatic (Kipson et al., 2015). The coralligenous assemblages in the north-western Mediterranean and eastern Adriatic are generally characterized by layered assemblages of erect and large macroalgae, anthozoans, and bryozoans (Casas-Güell et al., 2015; Doxa et al., 2016; Piazzi et al., 2016; Ponti et al., 2018), which are rare or absent in the northern Adriatic mesophotic biogenic reefs. The presence and abundance of these erect species play a relevant role in the assessment of the ecological status provided by the biotic indices developed for the coralligenous habitats of the north-western

TABLE 3 Randomized block two-way analysis of variance for difference among sites (AL06, MR08, P204, P213, and TQS) and sampling dates (2006, 2013, and 2017).

	df	SS	MS	*F*	*P*
Site	4	0.2731	0.0683	19.6925	0.0013
Date	2	0.0055	0.0028	0.7933	0.4947
Residuals	6	0.0208	0.0035	–	–

Note: Degree of freedom (*df*), sum of square (SS), and mean square (MS) were reported for each term of the model.

Mediterranean Sea, which therefore appear to be unsuitable for the mesophotic biogenic reefs of the northern Adriatic Sea. Here, it was necessary to use a different set of taxa, as well as to reselect and recalibrate the metrics for assessing ecological quality. This resulted in a new biotic index, the *NAMBER*, which is, however, based on the same principles and approaches applied in the north-western Mediterranean Sea (Cecchi et al., 2014).

The main environmental factors affecting the epibenthic assemblages inhabiting the northern Adriatic continental shelf are water turbidity, nutrients, and sediment load because of the enclosed nature of the basin and the discharge of the Po River (the largest river in Italy) and other Italian rivers (Solidoro et al., 2009; Falace et al., 2010; Cozzi & Giani, 2011; Tesi et al., 2013; Falace et al., 2015). Gradients of these factors are considered important for the differentiation of the main types of epibenthic assemblages inhabiting the northern Adriatic biogenic reefs, as identified in previous studies (Ponti, Fava & Abbiati, 2011; Curiel et al., 2012; Falace et al., 2015) and confirmed in the present investigation: (i) reefs dominated by algal turfs and encrusting sponges, usually located close to the shore; (ii) reefs dominated by calcareous red algae and colonial ascidians, usually located farthest from the coast and often small and fragmented; and (iii) reefs with intermediate conditions, where algal turfs, encrusting algae, and massive sponges are comparatively abundant. However, this general pattern can be affected by local environmental conditions and human disturbance (e.g. persistent trawling and sediment resuspension). This variability is also reflected in the values of ecological quality determined with the *NAMBER* index. On average, the index for the reefs farthest from the lagoons and river mouths showed a generally better environmental status (e.g. COLOMBA, MR08, and TQS); nevertheless, a clear geographical pattern could not be identified, as reefs that were close to each other often had different ecological quality ratings. This result suggests the occurrence of high variability in environmental conditions at different spatial scales and possible local human disturbance.

The application of *NAMBER* for different years (2006, 2013, and 2017) in a subset of five study sites (AL06, MR08, P204, P213 and TQS) showed little temporal variation and consistent differences among sites. The results indicate a capacity of the index to provide distinct classifications for the different reefs, while maintaining a general consistency over time, in accordance with the lower temporal variability of these epibenthic assemblages recognized by previous studies (Ponti, Fava & Abbiati, 2011). In the absence of environmental disasters (e.g. oil spills or dystrophic crises) or large variations in human pressures, as in this case, this stability is desirable in an index designed to discriminate between the natural spatial and temporal variability of benthic assemblages and any changes in the ecological quality of the environment (Martínez-Crego, Alcoverro & Romero, 2010). Nevertheless, relatively small variations in assemblage structures can occur over the years at any site in response to changing conditions, and the *NAMBER* index appears to capture this variability well, highlighting possible local worsening or improving trends in ecological status, as detected at the TQS site. Certainly, longer time series and the occurrence of specific natural or human disturbances are necessary to better evaluate the responsiveness of the index.

Following a previously used approach (Cecchi et al., 2014), the *NAMBER* index was developed to assess the intrinsic quality of coralligenous reefs that may depend on both local environmental conditions and human pressures. This approach can be considered particularly appropriate for the northern Adriatic Sea, where variable hydrographic conditions and the complexity of the multiple human impacts (Micheli et al., 2013; Cerrano et al., 2015; Farella et al., 2021) make it very difficult to separate the effects of natural and anthropogenic disturbances on benthic assemblages (Falace et al., 2010; Rindi et al., 2020).

The *NAMBER* index combines three ecological metrics that can be used to define seafloor integrity (indicated by the degree of bioconstruction) and biodiversity (using α-diversity), which are the general descriptors of the MSFD. Species sensitivity to human pressures is a commonly used descriptor in various ecological indices for assessing marine environmental quality under recent European Directives (Ballesteros et al., 2007; Ponti et al., 2007; Borja, Ranasinghe & Weisberg, 2009; Nikolić et al., 2013; Leonardsson et al., 2015; Turicchia et al., 2021a). A high sensitivity score is assigned to species that are intolerant of environmental degradation and human pressure and that generally thrive in communities with high diversity; conversely, a low sensitivity score is assigned to tolerant and opportunistic species that can thrive in disturbed environments (Ballesteros et al., 2007). In the northern Adriatic mesophotic biogenic reefs, some colonial ascidians (e.g. *Aplidium conicum* (Olivi, 1792) and *Polycitor adriaticus* (Drasche, 1883)) and sponges (e.g. *Geodia cydonium* (Linnaeus, 1767), *Polymastia mamillaris* (Müller, 1806), and *Tedania* (*Tedania*) *anhelans* (Vio in Olivi, 1792)), which are usually uncommon in other areas, are comparatively abundant, whereas some of the main taxa characteristic of north-western Mediterranean coralligenous reefs are rare (e.g. *Leptopsammia pruvoti* Lacaze-Duthiers, 1897) or absent (e.g. *Corallium rubrum* (Linnaeus, 1758), *Paramuricea clavata* (Risso, 1827), and *Eunicella* spp.). Therefore, different morpho-ecological groups were considered when assigning ecological values in the northern Adriatic Sea compared with in other geographical areas of the Mediterranean Sea (Piazzi et al., 2017).

In summary, this study provides the first multimetric biotic index specifically designed to assess the ecological quality of the northern Adriatic mesophotic reefs, which are increasingly threatened by local human pressures and climate change (Cerrano et al., 2015). *NAMBER* appears to have the characteristics of a good benthic biotic index:

(i) its built-in metrics, calibrated on the reefs of the northern Adriatic Sea, reflect well the biological integrity of these peculiar benthic assemblages; (ii) it is robust enough to distinguish the ecological status of different sites even in the presence of slight temporal variations in epibenthic assemblages; (iii) as it is based on photographic samples that can be easily collected in a single survey per site, it is cost-effective; and, moreover, (iv) its application is non-invasive. To date, the achievement of the conservation targets of these habitats under the European Directives has been hampered by the lack of a reliable and reproducible method to assess their ecological quality status. *NAMBER* fulfils this purpose, providing a simple and cost-effective monitoring tool that is immediately understood by managers, policymakers, and stakeholders. Thus, the authorities responsible for the conservation of the northern Adriatic mesophotic biogenic reefs should consider its implementation for regular standard monitoring to assess the ecological status and the effectiveness of enforced conservation and management plans. The regular use and reporting of this index can also be a useful tool to raise public awareness of the need for management and conservation actions for offshore marine habitats, which are generally neglected.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest associated with this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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