

Assessing the Ecological Status of *Gongolaria Barbata* (Stackhouse) Kuntze (Fucales, Ochrophyta) Habitat Along the Romanian Black Sea Coast - A Source of Multiple Ecosystem Services

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Abstract. *Gongolaria barbata* (Stackhouse) Kuntze (formerly known as *Cystoseira barbata* (Stackhouse) C. Agardh, 1820) builds essential habitats for marine biodiversity and ecosystem optimal functioning along the Romanian Black Sea coast. *G. barbata* forms so-called brown algal forests especially in the southern part of the Romanian Black Sea shore, providing all categories of ecosystem services, at the same time being a source of potentially bioactive metabolites. Over the last decades, *Cystoseira sensu lato* have suffered a general decline due to anthropogenic pressure and the Romanian Black Sea coast is not an exception. *G. barbata* is the only remained representative of *Cystoseira s. l.* from the Romanian coast and currently the most important habitat - forming species, being a suggestive indicator of environmental degradation and loss of habitats. The study aims to present the last fourteen years ecological status assessment of the sensitive habitat Upper-infralittoral rock dominated by *G. barbata*. Sampling was conducted between 2009 – 2022 (summer seasons) and a total number of 144 samples were collected using the “quadrat method” (20 x 20 cm). Data were statistically analyzed, and the specific Ecological Index (EI) was applied to evaluate the ecological status. The results of this study showed that this vulnerable habitat reached good ecological status during 2009 - 2012, except for 2012 and 2014. Nevertheless, the current distribution of *G. barbata* habitat is sparse, and the species remains highly sensitive to increasing anthropogenic activities in coastal zones.

Key words: *Gongolaria barbata*, canopy-forming algae, MSFD, ecological status, Romania.

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1. Introduction

Canopy-forming seaweeds *Cystoseira sensu lato* (further mentioned as *Cystoseira s.l.*) (Fucales, Ochrophyta) form diverse and productive habitats along temperate rocky coasts of the Mediterranean Sea [1] and Black Sea. Are considered among the most productive assemblages in the shallow coastal area

[2]. In the last decades, canopy-forming species are being gradually replaced by turf-forming smaller algae in many coastal areas, as a consequence of multiple anthropogenic pressures, comprising coastal urbanisation, eutrophication and increasing sediment loads. Since algae of the order Fucales are long living species that follow long-term periodicity, their loss from rocky bottoms is indicative of environmental degradation and loss of habitats [2]. *Cystoseira s.l.* macroalgal forests are being lost at an alarming rate as a result of degradation drivers including eutrophication, overgrazing (by sea urchins and herbivorous fish present in high densities), increasing coastal sediment loads, and the impacts of urbanisation. Remaining populations are experiencing increasing range contraction and fragmentation, often resulting in coastal ecosystems shifting from complex, productive systems to less structured assemblages (e.g., dominated by turf algae) [1, 3]. In recent years, many studies have highlighted that macroalgae represent a key marine resource for ecological and sustainable living, thus helping to address today's global problems, such as water pollution, ocean acidification, and global warming. Macroalgae show the potential to provide innovative, eco-friendly, and nutritious food sources and natural compounds for various industries, such as biomedical, food, agricultural, and pharmaceutical industries [4]. There is significant evidence that seaweeds play critical roles in supplying all categories of ecosystem services. On a worldwide scale, seaweeds habitats (such as kelp forests and seaweed beds) are the most widespread and productive coastal ecosystems and are one of the principal photosynthesis organisms on planet Earth. Macroalgal forests provide a variety of ecological services, including direct support for commercial, recreational, and subsistence fisheries and aquaculture. Erosion management and temperature change are examples of indirect ecological services. Cultural and religious importance, biodiversity, and scientific worth are all examples of intrinsic ecological services. Humankind and human societies have repeatedly turned to seaweeds in times of crisis to take advantage of what this diverse and ancient, polyphyletic assemblage of marine, photosynthetic organisms can offer in order to meet basic sustenance needs, alleviate disease suffering, and secure health, well-being, and survival at critical stages of human history. An example from history explains how people tried to use algae for their own benefit. Abd al-Rahmn, an Alexandrian, discovered how to extract "algin" from the brown alga *Gongolaria barbata* (formerly *Cystoseira barbata*), which he subsequently used to fireproof fabric. He used this material to shelter the ships from the catastrophic so-called Greek Fire used by the Byzantine navy to set enemy boats on fire. With the terrible extent of global challenges that people and civilizations are currently confronted with, such as pandemics, climate change, and the need for sustainable food sources and supplies, we may turn our collective and concentrated attention to seaweeds once more [5]. As Schenk (2021) said "It does not matter what the question is, algae are the answer" [6].

Macroalgae are used as food and feed, and also as sources of bioactive metabolites. In particular, Phaeophyceae have high contents of polysaccharides, minerals, polyunsaturated fatty acids and vitamins. Furthermore, these organisms contain high levels of secondary metabolites with pharmacological interest, such as terpenoids, phenolic compounds and alkaloids, which have been linked to interesting biomedical activities, including antitumoral and neuroprotective. Phytochemical studies have revealed that species belonging to *Cystoseira s.l.* are rich in phlorotannins, sterols, meroditerpenoids and sesquiterpenoids, some of which exhibiting antioxidant, antitumoral, antifouling and/or antimicrobial activities with potential applications in the pharmaceutical industry. *Cystoseira* are also known to contain molecules with antioxidant properties [7]. The benefits of *Cystoseira s.l.* to humans are highlighted by the fact that they produce numerous potentially bioactive metabolites, i.e., fatty acids, steroids, phlorotannins, polysaccharides and terpenoids [2]. For example, the major saturated fatty acid in *G. barbata* was palmitic acid followed by stearic and myristic acids. Besides oleic acid isomers, significant amounts of arachidonic acid were found among polyunsaturated fatty acid [8]. Vizetto - Duarte and colab. (2016) concluded that overall, *Cystoseira s.l.* can be considered a valuable source of bioactive secondary metabolites and a promising source of health products [7].

Cystoseira s.l. display a complex structure with an arborescent thallus. Where well-developed, these species can form forests, which play an important role as ecosystem engineers, supporting a highly structured tri-dimensional and diversified macroalgal assemblage with high biodiversity and productivity and providing shelter (refuge) and food for marine invertebrates and for juvenile fish [9]. The three-dimensional structure of such habitats provides food sources, substrata for settlement and shelter for many smaller algae, invertebrates, and fish. Moreover, *Cystoseira* spp. produce several potentially bioactive metabolites, i.e., fatty acids, steroids, phlorotannins, polysaccharides, and terpenoids, which have diverse benefits for humans [11]. Indeed, antiviral, antibacterial, antioxidant, anti-inflammatory, and antifungal activities have been confirmed for numerous *Cystoseira* species [10]. A reduction or loss of *Cystoseira* forests could probably trigger bottom-up effects in rocky shores habitats, with consequences for the whole ecosystem structure, functioning and services provided to humans [1]. Molecular data confirm the morphological identification of *G. barbata* and support the transition of *G. barbata* to the genus *Gongolaria* (from *Cystoseira barbata*, genus *Cystoseria*), which was previously proposed based solely on genetic data [11]. Nowadays, along the Romanian Black Sea coast, *G. barbata* is considered to be Critically Endangered (according to IUCN criteria) and is included in the List of Endangered Marine Species (according to Order no. 488/2020 published in the Official Gazette no. 300 of April 9, 2020).

Marine Strategy Framework Directive (MSFD, 2008/56/EC) and its interaction with other policies provide coverage of specific descriptors of the marine environment to achieve Good Environmental Status (GES) of European marine waters by 2020. Among these descriptors, "sea-floor integrity" is an important goal [12]. The Directive defines Good Environmental Status (GES) as: "The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive" [13]. Although all marine ecosystems have been impacted by humans, rocky reefs are amongst the most affected as they have multiple pressure stressors acting synergistically [14]. Benthic macroalgae are considered a valid biological element for ecological status evaluation in agreement with the MSFD requirements. Numerous macroalgal indicators have been designed to assess ecological quality, each tailored to different biogeographic provinces [15]. Along shallow rocky reefs, canopy-forming brown macroalgae belonging to *Cystoseira* are listed among the most sensitive species for ecological quality assessment.

The study aims to present the last fourteen years ecological status assessment of the sensitive habitat Upper-infralittoral rock dominated by *G. barbata*, emphasizing the importance of this engineer canopy - forming species for the marine environment.

2. Material and Methods

2.1. Sites and sampling procedure

The brown algae *G. barbata* forms the vulnerable habitat Upper-infralittoral (3-10 m) rock dominated by *G. barbata*, part of the broad habitat type Infralittoral rock and biogenic reef. Its vulnerability is accentuated by its patchy distribution - at Cazino Constanța and towards the southern part of the Romanian Black Sea coast (Jupiter – Saturn - Mangalia – 2 Mai – Vama Veche). Thus, the survey covered six sites located along the Romanian Black Sea coast (see Figure 1).

Sampling was conducted between 2009 – 2022, summer seasons. Monitoring samples were collected in a depth range of 0 to 3 m. At each sampling site, one sample, in each depth range, was randomly scratched off the rocky bottom, using randomly positioned quadrats (20 cm × 20 cm). A total number of 144 samples were collected between 2009 – 2022. Additional non-destructive SCUBA visual surveys were performed at each sampling site. All samples were placed in plastic bags and transported to the laboratory, where they were qualitative (using the stereomicroscope OLYMPUS SZX10) and quantitative analysed [16]. The Algaebase website was used as an up-to-date source of nomenclatural information for macroalgae identification [17]. Sorted macroalgae were blotted on filter paper. Wet biomass was calculated for *G. barbata*, as the main species, but also for the associated and epiphytic species. Values were reported to square meter as g/m².

Density (total number specimens in a quadrat of 20 x 20 cm) was also calculated, further expressed as indv/m^2 . Sampling map was generated using ArcGIS Desktop 10.7 software [18].

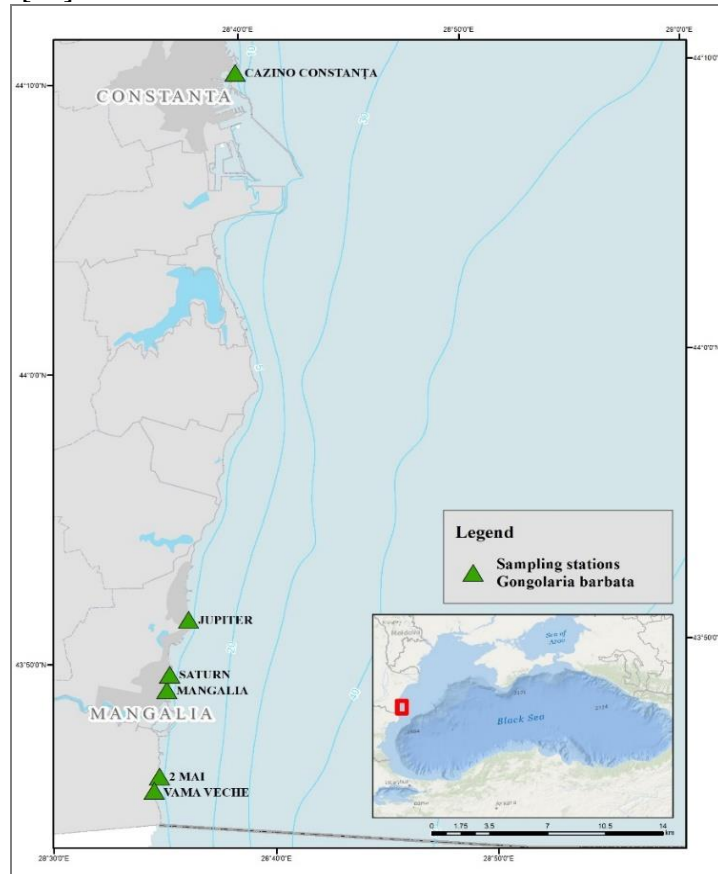


Fig. 4 *G. barbata* sampling sites along the Romanian Black Sea coast

2.2.Data analysis

Statistical analyses were carried out using JASP 0.16.4.0. [19]. Normal distribution and homogeneity of variances were established before each statistical analysis using Shapiro-Wilk Normality Test and Levene Test. After checking the normal distribution of data and the homogeneity of variances, parametric or non-parametric statistics were accordingly applied. The level of significance was adjusted to 0.05 for all statistical analyses. Spearman correlation coefficient (ρ) and Pearson correlation coefficient (r) were used to determine the relationships between significant population parameters, namely wet biomass (g/m^2) and density (indv/m^2) and Ecological Index (EI).

2.3. Evaluation of the ecological status

To evaluate the ecological status of the vulnerable habitat Upper-infralittoral rock dominated by *G. barbata*, Ecological Index (EI) was applied, subsequently reported to EQR (Ecological Quality Ratio) for standardization with the other biological elements, according to MSFD requirements. Ecological Index (EI) is a multiparametric index based on the proportion of sensitive and tolerant to eutrophication species, including aspects of wet biomass and specific diversity. EI_EQR takes values from 0 to 1, with a threshold value of 0.644 to define a habitat as being in good ecological status (GES). In other words, when the index shows a value lower than 0.644, the habitat is in a bad ecological status (non-GES), while a value greater than 0.644 indicates a good ecological status (GES) (see Table 1). According to the tolerance to environmental conditions, species were defined as follows: late-successional (ESG I) and opportunistic species (ESG II):

ESG IA, ESG IB, ESG IC – perennial species indicative of good ecological status (including here species of *Phyllophora*, *Cystoseira s.l.*, *Zostera*);

ESG IIA – species with a high adaptability capacity;

ESG IIB, ESG IICa, ESG IICb - opportunistic species, capable of developing in eutrophic areas, with a high reproductive capacity (*Ceramium* spp., *Ulva* spp., *Cladophora* spp.) - indicative of bad ecological status.

ESG IA species are considered the most sensitive, while those included in ESG IICb, the most tolerant [20]. Main criteria in differentiating the species into sensitivity groups was species morphology, biology and growth rates, as well as observational and experimental evidence of their sensitivity to eutrophication in the specific conditions of the Black Sea [21, 22]. *G. barbata* is included in ESG IB. A series of specific formulas were applied to define the ecological status:

EI-bad (0-1) = [ESGIICa/ESGII], ESGII=ESGIICa+ESGIICb (define areas included in bad ecological status);

EI-bad (1-2) = [(ESGIIA/ESGII) x 0.6+(ESGIIB/ESGII) x 0.8] +1 (define areas included in bad ecological status);

EI poor (2-4) = 5 x [(ESGIA/ESG) x 1+(ESGIB/ESG) x 0.8+(ESGIC/ESG) x 0.6] +2 (define areas included in bad ecological status);

EI high, good, moderate (4-10) = 10 x [(ESGIA/ESG) x 1+(ESGIB/ESG) x 0.8+(ESGIC/ESG) x 0.6] (define areas included in good ecological status).

A background dataset including wet biomass data and Ecological State Group (ESG I or ESG II) for every species identified in each sample, was prepared. To ensure comparability in accordance with the WFD, the EI values ranging from 0 to 10 can be transformed into Ecological Quality Ratios (EQR) from 0 to 1, meaning the ratio between EI value and the expected value under the reference conditions, as follows:

$EI_EQR = (EI \text{ obtained value} / RC \text{ value})$, where the referent value (RC) is 9.32.

The ecological status of Upper-infralittoral rock dominated by *G. barbata* was assessed by evaluating the ecological status of each sampling station within this particular habitat. An average value was then calculated. Finally, the obtained value is compared to the limits presented in Table 1 and the ecological status is defined.

Table 2. EI and EI_EQR limits for defining the ecological status

Biomass proportions of sensitive species (ESG I)	EI	EI_EQR	Ecological status
80-100 % ESGI	7.8 - 10	0.837 – 1	GES
60-80 % ESGI	6 - 7.8	0.644 – 0.837	
40-60 % ESGI	4 - 6	0.429 – 0.644	non - GES
0-40 % ESGI	2 - 4	0.214 – 0.429	
0 % ESGI	< 2	< 0.214	

Note: GES = Good Environmental Status; non – GES = Good Environmental Status not reached; ESG I = Ecological Status Group I species (i.e., sensitive species as *G. barbata*).

3. Results and Discussions

During 2009 – 2022, Shapiro-Wilk test shows strong deviations from the assumption of normality for the Ecological Index EI_EQR ($W = 0.691$; $p = 0.0001$; $p < 0.05$) and density data ($W = 0.781$; $p = 0.001$; $p < 0.05$). In contrast, wet biomass data follow a normal distribution ($W = 0.947$; $p = 0.057$; $p > 0.05$) (see Table 2). Levene’s test, used to assess homogeneity of variances of the variables (EI_EQR, wet biomass and density) showed that data differed markedly among the study period ($F(2, 96) = 3.091$; $p = 0.0001$; $p < 0.05$).

Table 2. *G. barbata* - Descriptive Statistics for measured parameters

	EI_EQR	average wet biomass (g/m²)	average density (indv/m²)
Valid	41	41	17
Median	0.824	5976.500	75.000
Mean	0.780	6578.852	99.588

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	EI_EQR	average wet biomass (g/m ²)	average density (indv/m ²)
Std. Deviation	0.114	3159.174	69.056
Shapiro-Wilk	0.691	0.947	0.781
p-value of Shapiro-Wilk	< 0.001	0.057	0.001
Minimum	0.410	1116.867	25.000
Maximum	0.858	14145.833	325.000

Habitat loss and fragmentation have long been considered the primary cause for biodiversity loss and ecosystem degradation worldwide. Habitat fragmentation often refers to the reduction of continuous tracts of habitat to smaller, spatially distinct remnant patches, and habitat loss typically occurs concurrently with habitat fragmentation. Although some habitats are naturally patchy in terms of abiotic and biotic conditions, human actions have profoundly fragmented landscapes across the world altering the quality and connectivity of habitats [23]. Although nowadays *G. barbata* has highly limited distribution areas along the Romanian coast, incomparable with those from the 1960s, the fact that wet biomass did not show major fluctuations from one year to another during 2009 – 2022, is extremely important, considering that the species is highly sensitive to anthropogenic disturbing factors.

The average wet biomass varied between a minimum of 1100 g/m², recorded in Mangalia, in 2014, and a maximum of 14100g/m², recorded on 2 Mai during summer 2020. Regarding the average density, the variation was between a minimum of 25 ind/m², recorded at Vama Veche, in 2022 and a maximum of 145 ind/m², counted at Mangalia in 2019. Regarding density, outliers were also recorded, such as 325 ind/m² registered in Mangalia in 2018 (see Figure 2, b). In general, Mangalia is characterized by a high density of this brown algae, since the area is highly favorable to its development (shallow waters, shelter area, high transparency). Juvenile specimens, in a higher density compared to 2 Mai and Vama Veche (areas represented by mature specimens forming forest-like assemblages) are commonly found here. Wet biomass showed no outliers during the last decade (see Figure 2, a). The ecological index EI_EQR varied between 0.410 and 0.858, with various extreme values depending on the development of opportunistic epiphytic/associated species (i.e., ESG II species – *Ulva* spp. and *Cladophora* spp.) (see Figure 2, c).

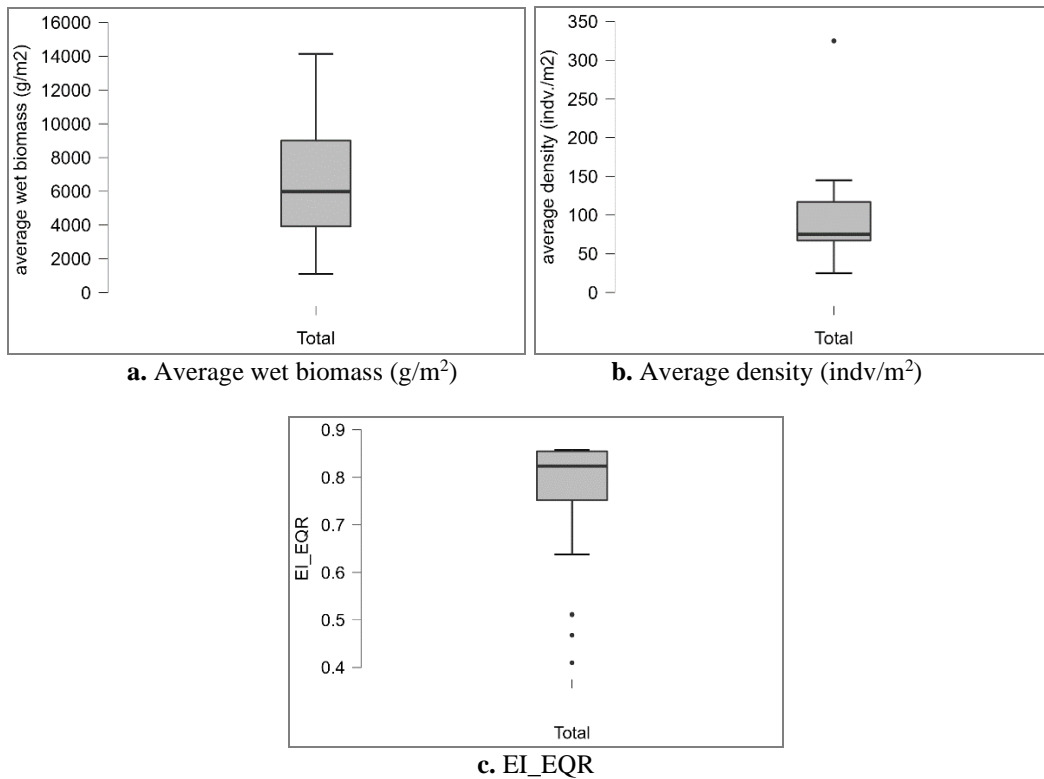


Fig. 2. Box plot showing the variation of average wet biomass (g/m²), average density (indv./m²) and EI_EQR during 2009 – 2022 (summer seasons)

Correlation matrix showed a positive correlation ($p = 0.042$; $p < 0.05$) between average wet biomass and Ecological Index EI_EQR and a significant negative correlation ($p = 0.009$; $p < 0.05$) between average density and EI_EQR (see Table 3). For the Romanian coast, a good ecological status is characterized by the presence of large specimens, with a sparse distribution. In the absence of adult thalli in a given area, the natural recovery of *Gongolaria* population is hindered by the very limited spread of this algae, due to the rapid fertilization and the sinking of zygotes [10]. In other words, the zygote (from which a new plant will develop) will get attached in the immediate vicinity of the mature specimen that generated it. The existence of an available substrate is extremely important for an optimal subsequent evolution of this new plant.

Hence, a slightly reduced density, along with the presence of healthy reproductive individuals and favorable rocky substrate, will determine a good ecological status of *G. barbata* habitat. This fact explains this negative correlation of the EI_EQR index and density along the Romanian coast. The progressive growth of branches and adventitious ramifications, the highest spatial complexity of the frond implies an enrichment of available habitats [24]. If connectivity is

limited, the subsequent smaller population gene pools and sizes render populations more vulnerable to threats [25].

Maintaining populations of foundation species depends on the persistence of adults and the successful recruitment and growth of early life-history stages to replace any adults lost from the population [26].

Table 3. Correlation matrix for wet biomass, density and EI_EQR

Variable	EI_EQR	
EI_EQR	Pearson's r	
	<i>p</i> -value	
	Spearman's rho	
	<i>p</i> -value	
average wet biomass (g/m ²)	Pearson's r	0.320 *
	<i>p</i> -value	0.042
	Spearman's rho	0.257
	<i>p</i> -value	0.104
average density (indv/m ²)	Pearson's r	-0.433
	<i>p</i> -value	0.083
	Spearman's rho	-0.611 **
	<i>p</i> -value	0.009

Note: * $p < 0.05$, ** $p < 0.01$.

Referring to other countries, many similarities regarding the regression of *Cystoseira* forests, were observed between the Romanian Black Sea coast and other seas. For instance, along the western Croatian Istrian coast, for the period 2009 – 2013, a composition of *Cystoseira s.l.* forests comparable to that evaluated during the 1950s, was reported. Starting from 2015, a large regression of *Cystoseira s.l.* canopies was observed at sites that are not directly threatened by anthropogenic pressures, thus allowing researchers to hypothesize a determining role of high summer temperatures and benthic mucilage (micro- and macroalgal blooms) on the decline of *Cystoseira* forests. Considering the hypothesis stipulated by Orlando – Bonaca and colab (2021), future research on the thermal responses of canopy forming species in the context of climate change should be planned also for the Romanian Black Sea coast [2]. The canopy of *G. barbata* diminished near industrialised areas and got replaced by simpler communities, dominated by stress-resistant and ephemeral species such as *Ulva* spp. Community shifts from canopy-forming fucoids to bushy, turf or fleshy opportunistic species have been widely reported across gradients of impact around the Mediterranean Sea, but a study from 2018 report this for the first time in oligotrophic waters of Cyprus [14].

When considering last decade situation and the reference period from the 1960s, a regime shift from marine forests to areas dominated by opportunistic species and even barren grounds devoid of erect macroalgae has been also reported for the Romanian coast and is generally linked to increased anthropogenic pressures (see Figure 3).



Fig. 3. A climax community with *G. barbata* (left picture); perennial *G. barbata* co-existing with opportunistic *Ulva* species (middle picture); leafy and filamentous green opportunistic algae (right picture)

In the last decade, the vulnerable habitat Upper-infralittoral rock dominated by *G. barbata* reached good ecological status, excepting 2012 and 2014 (summer period), when the index was below the threshold value of 0.644 indicating good ecological status (see Table 1 and Figure 4). This fact was due to the intense development of some opportunistic species, especially in the southern areas where this habitat is also present. Regarding the quantitative evolution of the opportunistic species, four scenarios have emerged during 2009 – 2023. Thereby, in accordance with these scenarios, between 2010 and 2013, *Cladophora* spp. experienced an abundant development along the entire coastal zone. The southern part of the Romanian coast was not an exception. At 2 Mai, *C. vagabunda*, associated and epiphytic species on *G. barbata*, recorded high biomasses of over 1600 g/m² (average value for 0 – 3 m depth range). Starting with 2014 until 2019, a massive development of *Ulva* spp. was recorded. During summer 2014, the green algae *U. rigida* and *U. intestinalis* developed abundantly, with biomasses of over 1300 g/m² (for *U. rigida*) and 900 g/m² (for *U. intestinalis*) [27].

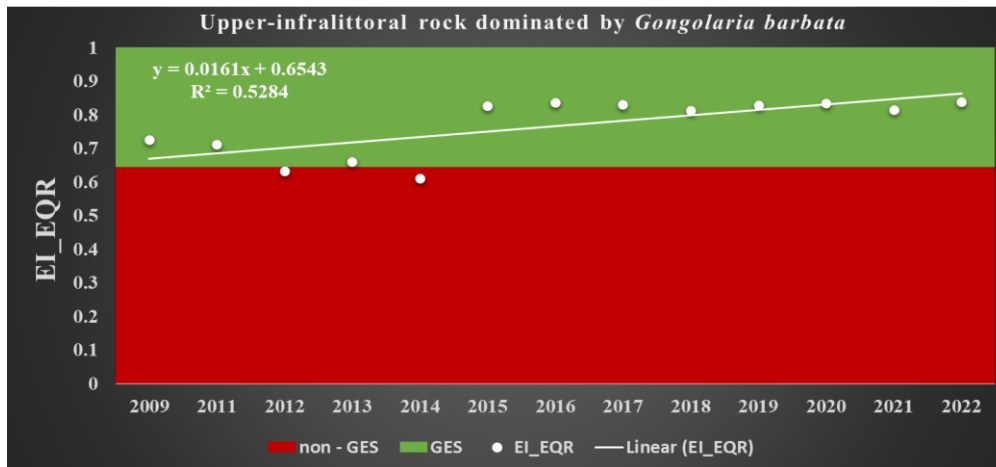


Fig. 4. The evolution of ecological status for the Upper-infralittoral rock dominated by *G. barbata* during 2009 - 2022

Currently, the ecologically important habitat - Upper-infralittoral rock dominated by *G. barbata* hosts numerous floristic and faunistic elements. Although, along with the decline of the host species, numerous epiphytic species have disappeared [28], however, nowadays rare algal species such as the epiphytic *Acrochaetium secundatum* or the encrusted red alga *Phymatolithon lenormadii* can still be found within this habitat.

Conclusions

(1) Although the results are promising and the general ecological status of this vulnerable habitat was predominantly high in the last decade, we must consider that the current distribution of this habitat foundation species is nowadays extremely sparse, and the species remains highly sensitive to increasing anthropogenic activities in coastal zones.

(2) The maintenance of the status quo in coastal areas is needed to protect not only the ecosystem but also human existence. Furthermore, the rehabilitation of coastal zones in damaged ecosystems employing various native seaweeds (referring to ecological reconstruction activities) provides significant advantages to humans. When ecosystems are subjected to human effects (e.g., eutrophication, species invasions), seaweeds lose their ability to supply ecological services and, in certain cases, can even provide “dis-services” to humans. Finally, protection of seaweed biodiversity should be addressed since there is evidence that most of the ecological services that can be outlined are more efficient in areas with richer and more functionally varied ecosystems. In an era where various stresses threaten ecosystems and human life, the conservation of seaweed variety and their usage as suppliers of benefits to people are both a problem and a necessity [5]. In conclusion, as stated by Orlando – Bonaca and colab (2021 a), habitat types dominated by *Cystoseira* s.l. deserve more research efforts and conservation

actions to maintain their still good ecological status, immense microhabitat diversity, and rich invertebrate and ichthyofauna [2]. The same recommendation came from Kletou and colab. (2018). The authors suggested that *Cystoseira* forests receive more attention when coastal developments are evaluated in Cyprus. The authors highlighted that shallow reefs around parts of Cyprus are still covered in luxuriant *Cystoseira* forests, but this habitat is threatened by coastal developments [14].

(3) The situation is similar for the Romanian Black Sea coast, the protection being more important as *G. barbata* is currently the only representative of *Cystoseira sensu lato*.

(4) In summary, more researchers underline that is not too late to conserve *Cystoseira* forests by raising public awareness and mitigating human impacts on coastal ecosystems. The disappearance of these furoid forests leads to systems with lower biodiversity and reduced ecosystem services to humanity [14].

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