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Long term succession of engineering species Gongolaria barbata (Stackhouse) Kuntze (Fucales: Ochrophyta) along the Romanian Black Sea coast

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Abstract

Background and purpose: Cystoseira sensu lato are of great interest due to their primary role in maintaining high biodiversity and the functioning of rocky habitats, and as provider of crucial ecosystem services. Conspicuous historical declines have been reported in many regions, Romanian Black Sea coast included. Gongolaria barbata (Stackhouse) Kuntze (Fucales: Ochrophyta) is the only remaining representative of Cystoseira s. l. along the Romanian coast and currently the most important habitat-forming species, with extended to patchy canopies in the southern rocky coasts. To better understand the status of such ecological important species, we analyse and compare the succession of Gongolaria infralittoral populations during reference (60s), decline (70s) and more recent period (2009 – 2022), based on an extensive literature review (historical quantitative data) and recent information from annual monitoring program.

Material and methods: Quadrats $(20 \times 20 \text{ cm})$ were used for sampling both in the past and present, with a total number of 144 samples collected between 2009 - 2022. Both historical and recent data were statistically analysed using non – parametric tests due to the absence in normality and homogeneity of data sets.

Results: Following sea ice from 1972 and violent storms as the main drivers of local Gongolaria decline, out of the thirteen known development areas from the reference period, nowadays only five are left. However, recent wet biomass and density values are appropriate to those of the reference period (1962 – 1971) and much higher compared to the maximum decline period (1972 – 1979).

Conclusions: G. barbata has been in a fragile balance along the Romanian coast in the last decade, highly sensitive in front of increasingly anthropogenic activities.

INTRODUCTION

Macroalgae in shallow and sheltered parts of coastal areas are subject to great pressure from various human activities, as well as being at risk due to climate change *(1). Cystoseira sensu lato* (Fucales, Ochrophyta) was recently subdivided into three genera: *Cystoseira, Ericaria* and *Gongolaria (2).* Similarly to kelp forests, *Cystoseira* species are a habitat "engineer" on temperate reefs, as their canopies modify local environmental conditions and provide habitats and refugia to a wide range of benthic and nektonic species (3), widely recognized as bioindicators for their restricted habitat in the sub-coastal zone and low tolerance to pollution (4). As Schenk (5) mentioned "It does not matter what the question is, algae are the answer." Indeed, the three-dimensional structure of such habitats provides food sources, substratum for settlement and shelter for many smaller algae, invertebrates, and fish (6). They also provide a wide range of crucial ecosystem services, including food provision, biodiversity enhancement, nutrient cycling, gas and climate regulation, bioremediation, leisure, and recreation, to name a few (7), along with the production of several bioactive metabolites with antioxidant, anti-inflammatory, antifungal, antiviral and antibacterial effects (8).

Cystoseira species have their maximum diversity in the Mediterranean Sea, with two thirds of all species described found there. Outside the Mediterranean Sea, they are mainly found in the Northeast Atlantic (4, 9). For the Black Sea, these are among the most important foundation species (10). The decline or disappearance of habitat building species, either by natural or anthropogenic disturbances, is leading to severe habitat transformations, generally results in barrens with an overall loss of biodiversity or an increase of filamentous turf algae (1). In the last decades, the general decline of Cystoseira populations has become a global phenomenon, directly or indirectly connected to human mediated activities (11), that is likely to intensify under climatic change (10), with the Romanian Black Sea coastal area not being an exception. Coastal urbanization (triggering habitat damage), nutrient enrichment and chemical pollution have been recognized among the major stressors (12). Habitat loss, due to coastal development and urbanization, or even natural storms are among the perturbations frequently associated with fragmentation and loss of Cystoseira populations (1). Without light limitations, the main factor modifying the abundance of coastal communities is nutrient availability, which depends largely on hydrodynamism. Geomorphological characteristics of the substrate (lithology, slope, depth) may account for the environmental heterogeneity of the system, a key factor in algal distribution. Previous studies indicate that the type of substrate and depth can also affect the distribution of Cystoseira. Decimetric blocks and pebbles displaced by storms can affect these communities and lead to their replacement by other species (4), generally smaller and less complex turf-forming algae (6), unable of supporting a quantitatively rich and diverse associated fauna. Since Cystoseira follows long term periodicity, its disappearance from shallow rocky bottoms is considered as indicative of severe environmental degradation. In order to prevent shifts from canopy to turf-forming algae, priority should be given to the management of nutrient levels, especially in enclosed bays and estuaries, since it has been demonstrated that Fucoids are stronger competitors for space than algal turfs in oligotrophic water conditions (6). An important feature of *Cystoseira* settlements is that their zygotes sink rapidly, so that they fall and stick to the substrate near the parent algae. Due to their limited dispersal ability and low growth rates of *Cystoseira* zygotes, the colonisation of new or damaged areas is therefore difficult, so habitat fragmentation has a major impact on these species (7, 8) and when *Cystoseira* species disappear from large geographical areas, their populations can recover significantly only by restoration activities that increase recruitment (12).

Cystoseira barbata (Stackhouse) C. Agardh, 1820, currently the only representative of Cystoseira sensu lato along the Romanian Black Sea coast, a major habitat-forming species in the upper - infralittoral creating dense canopies, is recently known as Gongolaria barbata (Stackhouse) Kuntze. Nomenclature was checked by Guiry and Guiry (13). Local populations of G. barbata have suffered from spectacular regression in several areas of the Black Sea (including the Romanian coast), where formerly it was a dominant species (14). The particularly severe winter of 1971 – 1972, with much more active local frost compared to the winters of 1953 - 1954 or 1959 - 1960, had a disastrous effect on infralittoral life, hence on G. barbata (15). Nowadays, the species is considered 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, in Romania).

This species is severely threatened both by land – based and marine human impacts, such as the artificialization of the coastline which likely appears more important in sheltered bays than in exposed shores. The decrease in water quality, including pollution, eutrophication and increase in sediment loads, provides an additional threat whose effects are particularly important in enclosed bays with low water renewal (14).

Cystoseira species are currently of great interest inside the scientific community due to their high ecological value and major decline suffered worldwide. Numerous ecological reconstruction operations are taking place in several countries to support the recovery of these marine forests. G. barbata is currently the most important ecological species for the Romanian coast. According to Gann et al. (16), to make restoration interventions consistently successful, a detailed knowledge of present and past distribution of lost habitats, the individuation of donor populations, and the identification of the stressors that caused their decline or disappearance, together with the evaluation of their mitigation state, should be considered prerequisites to select putative restoration sites. In this regard, to support future ecological restoration activities, the first step is to provide a baseline study by presenting the specific situation from the Romanian

Black Sea coast and possible donor areas for in situ or ex situ experiments. To this end and to better understand status of such ecological important species, this study aims to: (i) compare the available historical data of the 1960s - 1970s with the last fourteen years monitoring data (2009 – 2022); (ii) analyse the population dynamics in order to assess losses, gains or stability; (iii) provide an up-to-date assessment of the current distribution and extent of *G. barbata* along the Romanian Black Sea coast.

MATERIAL AND METHODS

Study area and sampling design

Current field surveys were carried out between 2009 and 2022, during the maximum development of *G. barbata* along the Romanian Black Sea coast (beginning of summer – early autumn). Our study area consisted of six sampling stations with algal forest of the canopy - forming G. barbata. Sampling covered all known distribution ranges of G. barbata, explored by boat and a diving team. Local populations of G. barbata are nowadays substantially fragmented, occurring especially in the southern part, from Jupiter - towards Vama Veche, but also at Cazino Constanta, separated by patches of turf-forming algae mainly of Ulva spp., Cladophora spp. and Ceramium spp. (Figure 1). SCUBA sampling surveys were performed by collecting one replicate per depth (from 0 to 3 m) from the upper infralittoral zone, at each sampling station, using quadrats (20×20 cm). Given the highly sensitive biological material and patchy appearance of the canopy, we sampled only a single quadrat per depth. A total number of 144 fresh samples were collected between 2009 -2022. Brought to laboratory, samples were studied using



Figure 1. G. barbata sampling areas along the Romanian Black Sea coast

a stereomicroscope (OLYMPUS SZX10), further submitted to a qualitative (frond length, identification of epiphytes and associated species) and quantitative analysis (calculation of wet biomass and density). Wet biomass values were reported to square meters and density data to individuals per square meters (*17*). Historical data showed that same sampling period (August – September) and same "square method" were also used in the 1960s – 1970s (*18, 19*), allowing a comparison of data.

During the 1960s and 1970s, mapping was performed between Vama Veche and Cap Midia. Thirteen areas were delimited from south to north, numbered from I to XIII, where area I is the southernmost point of the Romanian coast, as follows: Vama Veche (Area I), 2 Mai (Area II), Saturn – Mangalia (Area III), Cap Aurora – Jupiter (Area IV), Olimp (Area V), 23 August (Area VI), Costinesti (Area VII), Tuzla (Area VIII), Tuzla – Eforie Sud (Area IX), Eforie Sud (Area X), Agigea (Area XI), Est Constanta (Area XII), Constanta Nord (Area XIII) (Figure 1). During that period, a total area of 34,100 m² was mapped and sampled between 1 to 4 m depth. Samples were collected from areas I to XII, since area XIII was no longer populated with G. barbata since the 60s (Müller, 1970). For data comparability, current sampling stations were assimilated to these twelve areas from the reference period, as follows: Vama Veche (Area I); 2 Mai (Area II); Saturn - Mangalia (Area III); Jupiter (Area IV) and Cazino Constanta (Area XII) (Figure 1). Maps were generated using ArcGIS Desktop 10.7 software (20).

Data source

In order to analyse the long-term patterns in the distribution and quantitative succession of G. barbata and to reconstruct historical distributions, all available data were collected. In this regard, we performed an extensive literature review, including grey literature, NIMRD (National Institute for Marine Research and Development) historical reports from the late 1960s and 1970s and PhD thesis of former Romanian algologists. A total of 783 raw historical data belonging to our institute's archive were digitized as follows: 261 wet biomass historical data, 249 density historical data, 63 data related to the degree of coverage and area occupied by G. barbata populations during the 60s - 70s, 210 data on average frond length. Several population parameters were considered: wet biomass (g/m²), frond density (no. of individuals/m²), frond length (cm), degree of coverage (%), area occupied by populations with exploitable potential (m²) and stocks (tons). The common parameters for reference, decline and present were wet biomass (g/m^2) and frond density (no. of individuals/m²). Nowadays, we can no longer talk about the concept of exploitability of G. barbata since its main role along the Romanian coast is strictly ecological. All these historical data, along with data collected during 2009 - 2022, were statistically analysed. Three major time periods were defined in the succession of G. barbata along the Romanian coast: reference (1962 - 1971), decline (1972 - 1979) and recent / present period (2009 - 2022). For a more complete and detailed statistical analysis and considering sea ice from 1972 winter as a phenomenon with a major impact on the succession of *G. barbata*, five sub-periods were also defined, as follows: reference (1962 - 1969), reference - before sea ice (1970 - 1971), decline - sea ice (1972), decline - after sea ice (1979) and recent period/present (2009 - 2022).

Statistical analysis

Statistical analyses were carried out using XLSTAT 2021.3.1. (21), and PRIMER (v.7.0) (22, 23). Normal distribution and homogeneity of variances were established before each statistical analysis using Shapiro – Wilk Normality Test and Levene's test (two - tailed test). Since normality and homogeneity criteria were not met, the non - parametric Kruskal - Wallis (two - tailed test) was selected and applied to wet biomass and density data corresponding to the five sub - periods defined above. Spearman's Rho, a non-parametric one-tailed correlation test, was performed to investigate the relation between four significant parameters measured for G. barbata: wet biomass, density, frond length and degree of coverage. The significance level was set at p < 0.05. However, if higher significance levels were achieved (p < 0.01 and p < 0.001), levels were presented separately. Prior to PRIMER analyses, data were fourth - root transformed to eliminate inappropriate weighting of some measurements. Principal Component Analysis (PCA) was used to establish the spatio - temporal variation pattern of biomass and density values during the five sub - periods. PCA was performed on a matrix consisting of mean values of biomass and density. A One – Way crossed ANOSIM (analysis of similarities) applied on average fourth – root transformed biomass data was used to test the null hypothesis: no differences between past and current biomass values. Average biomass variation among different areas and time periods was displayed using "Shade Plot task" in PRIMER software. Shade plots were built to clearly visualize the differences between sampling stations and time periods in clusters. Square root transformed data were used for this purpose.

RESULTS

The Shapiro – Wilk's test showed that wet biomass (W = 0.835, p = 0.0001, p < 0.05) and density data (W = 0.769, p = 0.0001, p < 0.05) significantly deviate from a normal distribution in the five sub – periods. Levene's test, used to assess homogeneity of variances of the variables (wet biomass and density), showed that data differed markedly among the study periods (F (1, 495) = 3.860, p = 0.0001). Therefore, Kruskal – Wallis combined with a multiple pairwise comparisons (Dunn's post-hoc test), were applied to test different scenarios: to highlight the

Table 1. Non-parametric Kruskal – Wallis (two-tailed) and post-hoc test (Dunn's test for pair-wise comparisons). n = 144 for biomass; n = 108 for density (n = number of observations); K1 = observed value; K2 = critical value; df = 4 (df = degree of freedom); p = 0.0001 (p = p - value (one-tailed)). P = the five sub – periods statistically analysed between 1960 and 2022, as follows: P1 = reference (1962 – 1969); P2 = reference – before sea ice (1970 – 1971); P3 = decline - sea ice (1972); P4 = decline - after sea ice (1979); P5 = present / recent period (2009 – 2022).

Р	Mean rank	K1	K2	Pair-wise comparisons				
				P1	P2	P3	P4	P5
Wet biomass								
P1	131.50	95.88	9.49			106.67	110.30	-57.15
P2	142.69					117.86	121.49	-45.95
Р3	24.83			-106.67	-117.86			-163.81
P4	21.20			-110.30	-121.49			-167.44
P5	188.65			57.15	45.95	163.81	167.44	
Dens	ity							
P1	72.51	59.12	9.49		-67.90		-83.15	-52.18
P2	140.42			67.90				
Р3	115.67							
P4	155.66			83.15				30.97
P5	124.69			52.18			-30.97	

Values in bold letters show significant correlations at p < 0.05. Values in italic letters show significant correlations at p < 0.001. Bonferroni corrected significance level: 0.005.

differences between the five defined sub – periods, between common development areas in the last fifty years and among sampling sites monitored during 2009 – 2022.

Non-parametric Kruskal – Wallis's test showed statistically significant differences during past and recent period regarding *G. barbata* wet biomass (p = 0.0001, p < 0.05) and density (p = 0.0001, p < 0.05). For biomass, Dunn's post-hoc test showed statistically significant differences (p < 0.05) between the current study period (2009 – 2022) and past decades: reference period (p = 0.0001), decline – sea ice (p = 0.0001) and decline – after sea ice (p = 0.0001) (Table 1).

Regarding density, statistically significant differences (p < 0.05) were observed between present and reference period (p = 0.0001) and between present and decline pe-



Figure 2. Multivariate analysis of similarities of wet biomass values in the 1960s, 1970s and 2009 - 2022.



Figure 3. Shade plot of the dispersion – weighted square – root transformed biomass data for G. barbata across different areas and different years with spectrum scale intensity. Dendrogram on y-axis derived by subjecting a Bray – Curtis resemblance matrix. Romanian Black Sea coast: Area I = Vama Veche; Area II = 2 Mai; Area III = Saturn – Mangalia; Area IV = Cap Aurora – Jupiter; Area V = Olimp; Area VI = 23 August; Area VII = Costinesti; Area VIII = Tuzla; Area IX = Tuzla – Eforie Sud; Area X = Eforie Sud; Area XI = Agigea; Area XII = Est Constanta.

riod – after sea ice (p = 0.032). Other significant differences were recorded between the 60s and early 70s (p = 0.005) and between the reference period and the maximum decline period from late 70s (p = 0.0001) (Table 1).

One – Way ANOSIM applied on wet biomass data showed similar results to Kruskal – Wallis's test, rejecting the null hypothesis that there are no differences between past and current *G. barbata* biomass values. In other words, ANOSIM analysis (fourth root transformed and Bray – Curtis resemblance matrix) demonstrated significant differences regarding the quantitative assessment of *G. barbata* in the last fifty years (p = 0.001, r = 0.908) (Figure 2).

The shade plot is a visualization of the transformed and averaged data matrix, where white denotes the absence of the species, while the depth of shading, ranging from grey shades through black, represents increasing values for species' biomass during that period. Notable is the species' total absence starting with the end of the 70s from entire areas between Est Constanta (Area XII) and 23 August (Area VI) (Figure 3).

Only three distribution areas of *G. barbata* are considered common in the last 50 years, namely: Vama Veche (Area I), 2 Mai (Area II) and Cap Aurora – Jupiter (Area IV). Among these areas, Kruskal – Wallis showed significant differences referring to biomass data (p = 0.015, p < 0.05). Dunn's post hoc highlighted statistically significant differences between Vama Veche and Cap Aurora – Jupiter (p = 0.004, p < 0.05, n = 66). Bonferroni corrected significance level was 0.0167. Regarding density, no statistical difference was noticed between these common areas (p > 0.05).

In the last fourteen years (2009 – 2022), the species developed forest-like assemblages especially in the southern part (Jupiter, Saturn, Mangalia, 2 Mai, Vama Veche), but also at Cazino Constanta (Figure 1). In this regard, data collected from these sampling sites were statistically analysed in term of species' biomass and density. Regard-

Table 2. <i>S</i>	pearman's correlation	s between different	parameters measured	for G. barba	ta during reference	, decline and	l current time perioa
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Parameters	Wet biomass (g/m ²)	Density (no. of individuals/m²)	Frond length (cm)	Degree of coverage %
Wet biomass (g/m²)	1			
Density (no. of individuals/m ²)	0.153	1		
Frond length	0.555***	-0.258**	1	
Degree of coverage %	0.456***	0.191*	0.144	1

* Significant at p < 0.05, ** significant at p < 0.01, *** significant at p < 0.001, n = 108



Figure 4. Principal component analysis (PCA) between sampling areas and time periods on the basis of wet biomass (a) and density (b). The length of the vectors indicates the contribution of each parameter. Romanian Black Sea coast: Area I = Vama Veche; Area II = 2 Mai; Area III = Saturn – Mangalia; Area IV = Cap Aurora – Jupiter; Area V = Olimp; Area VI = 23 August; Area VII = Costinesti; Area VIII = Tuzla; Area IX = Tuzla – Eforie Sud Area X = Eforie Sud; Area XI = Agigea; Area XII = Est Constanta.

ing biomass, Kruskal - Wallis (two - tailed test) demonstrates no significant differences between these stations (p > 0.05), hence the hypothesis of species regeneration along the Romanian coast in the past decade in comparison with the 70s. The fact that G. barbata wet biomass remained relatively constant during 2009 – 2022 is considered very important, taking into consideration species' sensitivity to anthropogenic disturbing factors. Kruskal - Wallis's test applied on density data showed statistically significant differences between sampling stations (p = 0.027, p < 0.05, n= 21). In addition, Dunn's post-hoc test, underlined significant differences between Mangalia and 2 Mai (p = (0.014) and between Mangalia and Vama Veche (p =0.001). Bonferroni corrected significance level was 0.0033. Spearman's correlation test was employed to see the correlation between four significant population parameters, both in the past (60s - 70s) and last decade (2009 - 2022). These parameters are essential in monitoring the health

status of *G. barbata* canopies. Significant positive correlations were observed between wet biomass and frond length and between wet biomass and degree of coverage (p < 0.001). Positive correlations were also recorded between density and degree of coverage (p < 0.05), as expected. Significant negative correlations were observed between density and frond length (p < 0.01) (Table 2).

Principal Component Analysis was performed on wet biomass and density data. For biomass, data showed that PC1 and PC2 explained 73.9% of the total variance in the data matrix, where PC1 explained 51.2% and PC2 22.7% of the data variability. The eigenvalues for the first two principal components were 60.9 (PC1) and 27 (PC2). The PCA analysis shows a clear separation of the three analysed time periods (reference, decline and present). Area III (Mangalia) and Area II (2 Mai) have the highest contribution in terms of biomass (Figure 4a). For density, PCA showed that PC1 and PC2 explained 69.3% of the total variance in the data matrix, where PC1 explained 39.7% and PC2 29.6% of the data variability. The eigenvalues for the first two principal components were 8.05 (PC1) and 5.99 (PC2). Similar to biomass situation, in the spatio – temporal distribution of density data, PCA shows the same clear separation of the three analysed time periods (reference, decline and present). Agigea (Area XI), with an average density of 190 individuals/m², has the highest contribution for the reference time, while Mangalia (Area III) contributed the most to PCA analysis for 2009 – 2022, with an average density of 173 individuals/m² (Figure 4b). Both for biomass and density, PCA shows some overlapped results due to high similarity between sampling areas.

DISCUSSION

Even during the reference period, along Romanian coast *G. barbata* thrived only in shallow waters, in varying densities at depths of about 0 to 6 *meters (24)*. During the last decade in situ observations showed that *G. barbata* developed only between 1 and 5 m. According to Berov *et al. (25)*, the species can be found along the Bulgarian coast at depths between 3 to 5 m and 7 to 10 m. By comparison, the Romanian coast is characterized by higher load of nutrients (as a consequence of Danube's influence) and a more reduced water transparency comparing to other seas. Accordingly, all these factors hinder the development of *Gongolaria* in lower infralittoral.

To better understand the succession of G. barbata along the Romanian coast and the causes of its inclusion in the Red List, it is imperative to present its trajectory over the decades, highlighting the main causes of its decline. Historical studies reported the presence of G. barbata from Constanta to Vama Veche (Figure 1). In the 60s, dense canopies were found in Vama Veche, 2 Mai, Cap Aurora, Olimp and 23 August. The highest wet biomasses were identified in areas I - V (the coastal area between Vama Veche and Olimp). Gongolaria degradation has been noted since the end of 60s, when it was observed that area XIII (north of Constanta), once sheltering extended canopies, no longer harboured any population with more than 10% coverage, but only small clumps or isolated threads. Area XII (Est Constanta) presented a similar situation due to silting of the rocky bottom by suspended matter. The populations disappeared over a distance of 2 km, from the new pier of Constanta port to Agigea. Although with a lower intensity, such phenomena could also be observed along Agigea towards Eforie Sud. At the beginning of the 70s, it was already considered that these brown algal forests were in a regression' process, mainly affected by increasing sediment loads and water pollution consisting of extremely toxic compounds like detergents discharged together with household water, chlorinated hydrocarbons such as DDT or fluoride (18). Under favourable weather conditions of 1971, Gongolaria stock increased in some areas from 4300 tonnes to 5350 tonnes (26). In the 1970s, the total biomass of *Cystoseira* (*Ericaria crinita* and *G. barbata*) for the whole Black Sea was estimated to be approximately 2×10^6 tonnes, with the majority of biomass found along the Caucasus coast of Russia and Georgia (25).

Along the Romanian coast, thermal anomalies from winter 1972 led to the formation of massive ice blocks during the first decade of February to mid - March 1972, subsequently fragmented under winds and strong waves. These structures acted with a destructive mechanical effect on shallow areas. As a result, both flora and fauna, including populations of Gongolaria, suffered a shocking destruction of 45 up to 100%. These canopy-forming species were obviously the first affected since major part of the thallus was caught in the ice mass. Regardless the sampling profile, between 2 to 2.5 m, in situ observations showed the massive 100% destruction suffered by this species, with loose rocks more affected than bed - rocks. It was estimated that after this destructive episode, Gongolaria stock decreased to 900 - 1000 tons (26). The instability of rocky bedrocks, due to hydrodynamic forces (waves, currents, seasonal storms), can abrade and destroy canopy macroalgae on rock surfaces, significantly affecting their distributions (27). Seawater temperature is known as a limiting factor in the natural spread of Cystoseira populations. Thus, an abnormal increase in water temperature during the winter season, along with severe storms, lead to serious biological anomalies, affecting the reproductive potential of G. barbata, disrupting its physiological cycle. More recent weather anomalies were also mentioned for the Gulf of Trieste (Adriatic Sea), where, not only increasing winter temperatures in the Gulf, as reported for 2019, but also exceptional wind periods, like in spring of 2020, lead to serious biological anomalies and the loss of the reproductive potential of G. barbata: periods when the species was not found to be fertile, or prematurely fertile periods as in winter of 2019 after a marine heatwave (12).

Mapping performed between 1972 and 1973 from 0.5 to 2 m at Cap Midia, Agigea, Eforie Sud, Costinesti, Mangalia and Vama Veche, highlighted that these algal canopies were seriously damaged, at the point of becoming locally extinct in some areas. Increased anthropogenic activities from the 70s (e.g., extensive hydro technical constructions meant to consolidate the coastline) correlated with intense phytoplankton blooms events and particularly violent storms, hampered the natural recovery of Gongolaria populations. According to Perkol - Finkel and Airoldi (27), in early 2000s, along the Conero Riviera (Marche, Italy) a regression of G. barbata was also reported, as a consequence of the same extreme events. The decline of the Cystoseira forests was rather sudden, with 70% of the canopies lost during 2002 - 2005. The proximate trigger appears to be related to combined acute disturbance from severe storms and beach nourishments (27). Furthermore, Cystoseira species (including G. barbata) are low-dispersal species whose propagules do not have a planktonic stage, and reproductive drifting thalli in floating rafts are the main mechanism of connectivity between populations. Therefore, if connectivity is limited, the subsequent smaller population sizes render populations more vulnerable to threats (11). In the early 80s, the only significant stocks (approx. 120.7 tons of wet biomass) were those from the southern part, between 23 August and Vama Veche, while the total disappearance of *Gongolaria* specimens between Cap Midia and Agigea was recorded. Narrow belts, i.e., as scattered patches with underdeveloped branches were observed between Agigea and 23 August (24). The decline was devastating at Vama Veche, where the loss of *G. barbata* population reached 83.1%. This fact is due to species' distribution on a smooth calcareous platform that did not provide a strong adhesion (comparing to hard rocky substrate) to support specimens over 40 cm high in front of massive ice blocks (15). In the last decade, an extensive canopy can be found at Vama Veche, in the upper infralittoral, from 1 to 3 m. Comparing past and current situation, it was noticed that for some sampling areas average wet biomass values were appropriate to those of the reference period (1962 – 1971) and much higher compared to the period of maximum decline (1972 – 1979) (Figure 5).

Regarding density, the situation was similar, with current values close to those of the reference period (Figure 6). When we make these statements, we are strictly referring to the six areas where the species developed nowa-



Figure 5. Comparative aspects of average wet biomass values during reference, decline and present.



Figure 6. Comparative aspects of average density values during reference, decline and present.

days: Cazino Constanta, Jupiter, Saturn, Mangalia, 2 Mai, Vama Veche. Nevertheless, current maps exhibit reduced areas occupied by *G. barbata* comparing to historical distribution, meaning a clear regression.

Statistical significance differences showed by Kruskal – Wallis's test between Mangalia, 2 Mai and Vama Veche, regarding species density, are due to the fact that higher proportion of young plants develops at Mangalia comparing to other areas. The average density reported for Mangalia in the last decade is approx. 173 individuals/m², much higher compared to 2 Mai (58 individuals/m²) or Vama Veche (65 individuals/m²), areas where the specimens are generally mature with a sparse distribution. For

the Romanian coast, in some areas, this is a consequence of natural selection that intervenes by eliminating small individuals that no longer receive enough light necessary for their own development, which explains the negative correlations between density and frond length (Table 2). In Mangalia, *G. barbata* develops in sheltered areas at shallow depths (0.5 to 2 m), under a favourable light and temperature regime that allows an optimal species' development, without disruptions of the reproductive cycle or photosynthetic process. Here, comparing to other zones, the beginning of the reproductive period (presence of mature receptacles) is the earliest (at the end of May), particularly important when considering in situ or ex situ ecological reconstruction activities.



Regression of average wet biomass by average density

Figure 7. The relation between wet biomass and density during reference and decline for areas I (Vama Veche), II (2 Mai), IV (Cap – Aurora – Jupiter) and VI (23 August).

During reference and decline period, in areas I, II, IV and VI, an inverse proportionality relationship is observed between wet biomass and density (Figure 7). Although a contradictory aspect at first sight, the explanation relies in species' ability to follow its natural physiological pattern. *G. barbata* continued its reproductive process and apparently succeeded in populating an available substrate after the massive denudation that occurred following the sea ice from 1972. However, the wet biomass values were very reduced, lower compared to the reference period, showing that although there was a high density, it was generated by recruits, particularly sensitive to environmental changes and unable to support a rich associated fauna.

CONCLUSIONS

In conclusion, although sea ice phenomena have been reported after, the same intensity as the one from the 70s was no later reported. For the Romanian coast, sea ice was the main driver of species' decline. Since 1973, the recommendations of the romanian researchers were to manage the remaining stocks and avoid exploitation, precisely to prevent species' extinction (19). It can be stated that in the last fourteen years *G. barbata* has been in a fragile balance along the Romanian coast. The species naturally recovered in some areas due to reduced nutrient load comparing with high eutrophication period of the 70s and improved water quality. Though, its distribution is mainly patchy considering the particular nature of the substrate and other limiting factors, so the species is highly sensitive to anthropogenic impact.

The provided information (both historical and more recent data) can be used as a basis for improving the evaluation of the conservation status of *G. barbata*, an

ecologically important species. Taking into consideration recent data and evidence of the regional decline, we proposed to still consider the species as being Critically Endangered along the Romanian Black Sea coast. According to Benedetti-Cecchi *et al. (28)*, it can be stated that future anthropogenic disturbances from coastal area will negatively affect natural populations and assemblages by interacting with fundamental ecological processes. As recommended by Mancuso *et al. (29)*, extensive baseline monitoring is needed to describe how *Cystoseira* populations are changing and implement a management framework for the conservation of these valuable but vulnerable habitats.

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