

First implementation of the MediSkew index in the *Cymodocea nodosa* meadows of the Boka Kotorska Bay (Montenegro) and assessment of the ecological status

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ABSTRACT

The seagrass *Cymodocea nodosa* is a protected species and one of the most important habitat engineers in the Mediterranean Sea. Therefore, the assessment of the status of its meadows is of great importance for the planning of conservation measures, and the implementation of the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD). This is of special importance in the areas where *Posidonia oceanica* is not present or does not dominate, as it is the case of the Boka Kotorska. The MediSkew index which has already been successfully implemented in the northern Adriatic Sea was for the first time tested in the Boka Kotorska Bay (southern Adriatic Sea). First results show that the ecological status at five sites is very good and good, while the status at one site is moderate. Furthermore, the MediSkew index is applicable in different geographical areas and it is sensitive to different environmental conditions, so the application of this methodology is strongly recommended for further monitoring of *Cymodocea* meadows and assessment of the ecological status.

Keywords: *Cymodocea nodosa*, status evaluation, MediSkew index, southern Adriatic Sea

INTRODUCTION

Seagrasses are important habitat engineers in the Mediterranean Sea and play a key role in coastal areas providing many different ecological services like primary production, nutrient cycling, food, shelter and nursery areas for different species, sediment stabilization and protection from the erosion (Den Hartog, 1971; Orth *et al.*, 2006; Short *et*

al., 2007). Because of all that they are recognized as one of the priority habitats by the EU Habitat Directive (EU 92/43/EEC) and they are protected by different international conventions as well as national legislation in Montenegro (Barcelona Convention 1976; Bern Convention 1979; Official Gazette of Montenegro No. 76, 2006).

The most widespread and important seagrass after *Posidonia oceanica* (L.) Del in the Mediterranean Sea is *Cymodocea nodosa* (Ucria) Ascherson. It is present in many coastal areas of the Mediterranean and in several locations in the North Atlantic from Southern Portugal and Spain to Senegal, including the Canary Islands and Madeira (Ayala, 2010). Unfortunately, the Boka Kotorska Bay could be considered a small model for the Mediterranean Sea with all the negative anthropogenic stressors impacting seagrasses worldwide, and recent studies show a significant regression of *Cymodocea* meadows in the inner part of the Boka Kotorska Bay (Mačić & Zordan, 2018). The reasons for the decline are multiple stressors such as wastewater discharges, decline in water transparency, increase of sedimentation, beach nourishment and anchoring (Papathanasiou, 2013). With the aim to protect aquatic ecosystems, the Water Framework Directive (EU WFD, 2008/56/EC) and the Marine Strategy Framework Directive (EU MSFD, 2000/60/EC) require to ensure a good ecological and environmental status. As a consequence of the need to evaluate the ecological status (ES) of different water bodies according to WFD and to compare it between different countries and regions, different methodologies have been developed (Marba *et al.*, 2013). Furthermore, according to MSFD requirements, EU Member States are obligated to carry out an initial assessment, determine the Good Environmental Status (GEnS) and provide a set of measures to maintain or achieve GEnS. Montenegro is a candidate country for EU accession and various documents have already been provided to fulfil the requirements of the EU directives. An initial assessment has been carried out and a set of measures for GEnS have been proposed (GM-MESPU, 2021a; 2021b). Although there have been several studies related to *C. nodosa*

meadows in the Boka Kotorska Bay, they were related mainly to seagrass meadow mapping, and anatomical and physiological study of *C. nodosa*, but morphological parameters of this seagrass have not been monitored so far and an assessment of the ecological condition of *Cymodocea* meadows is still missing (Karaman & Gamulin Brida, 1971; Stjepčević & Parenzan, 1980; Mačić & Sekulić, 2001; Mačić *et al.*, 2002; Mačić, 2014).

Having in mind that this seagrass species is a good bioindicator due to its large distribution area and its sensitivity to different stressors, several indices have been developed as possibilities to evaluate and compare seagrass meadows from different regions and their environmental status (Marba *et al.*, 2013; Orfanidis, 2010; Orlando Bonaca *et al.*, 2015, 2022; Papathanasiou *et al.*, 2016). However, we should keep in mind that the ecological quality of an ecosystem is assessed by comparing it to a pristine status (reference condition), and the reference conditions may be different for various regions and countries. In comparison to other indices geographically closest to the region of Montenegro and the only one developed for the Adriatic Sea is the MediSkew index that has been successfully implemented in the northern Adriatic Sea (Orlando Bonaca *et al.*, 2015; 2022). The aim of this paper is to test the MediSkew index in the Boka Kotorska Bay and to evaluate the possibility of implementing this methodology for further monitoring of *Cymodocea* meadows and assessment of the status in the southern Adriatic.

MATERIAL AND METHODS

Study area

Boka Kotorska Bay is a fjord-like bay on the south-eastern coast of the Adriatic Sea. It is covering an area of 87.3 km², while the

coastline of the bay is 105 km, contributing almost up to 1/3 of the Montenegrin coast (Joksimović *et al.*, 2017). The water circulation in the bay is very slow and depends heavily on the inflow of freshwater from small rivers, runoff from surrounding mountains and submarine springs („vruljas“). It is a naturally eutrophicated area and the sea bottom of the bay is mostly sandy-muddy, providing suitable substrate for seagrasses. The dominant seagrasses are *C. nodosa* and *P. oceanica*, while *Nanozostera noltei* (Hornemann) Tomlinson & Posluszny is present only in mixed meadows with *C. nodosa* (Mačić & Krivokapić, 2017; IBMK-UCG, 2023). Because of different very important, protected and endangered habitats and species, the inner part of the Bay (Kotor-Risan Bay) is listed as one of the UNESCO Natural and Cultural Heritage Sites (Official Gazette of Montenegro No. 56, 2013), proclaimed also an Emerald Site and most likely at least part of it will become a Natura 2000 site (GM-MESPU, 2021b; Canessa *et al.*, 2024). Unfortunately, the whole Boka Kotorska Bay is also a densely populated coastal area under strong anthropogenic impacts. Some of the main stressors are municipal untreated sewage discharges, beach nourishment, anchoring, cruising and water pollution from the sea (RAC/SPA - UNEP/MAP, 2013; Bataković *et al.*, 2017).

Sampling sites and methodology

Sampling was carried out in the Boka Kotorska Bay, at six sites (Fig. 1) according to the sampling protocol presented by Orlando Bonaca *et al.* (2015). At the sites of Dobrota, Prčanj and the island of Sv. Marko, sampling was done in the summer of 2019, while at Risan, Tivat and Herceg Novi it was done in the summer of 2020. According to the aforementioned methodology, two areas were selected within each site, which are about 100

m apart. For the Dobrota and Prčanj sites, the areas within a site were spaced further apart, as we wanted to avoid areas with small patches and degraded seagrass meadows. Within each area, five randomly placed quadrats 25 cm x 25 cm were placed on the bottom and all shoots of *C. nodosa* from the quadrats were uprooted and placed in plastic bags.



Figure 1. Map of sampling sites of *C. nodosa* in the Boka Kotorska Bay (Montenegro).

All samples were frozen at -20°C until the analysis, then rinsed in seawater and approximately twenty shoots, randomly selected from each quadrat, were used to measure the following parameters: length of leaf sheet, length of the photosynthetic part and its width. The measurements were performed on 60 undamaged adult leaves (when the leaf sheath was well developed) and intermediate leaves (when the leaf sheath was weakly developed at the leaf base), while juvenile leaves (when the leaf sheath was absent) were not considered. A sample for each area thus consists of five replicates of 60 leaves (300 leaves in total).

Data elaboration

To determine the ES and GEnS of seagrass meadows, it is very important to identify the reference conditions, *i.e.* those sites that are closest to pristine ecosystems. Boka Kotorska Bay has been under anthropogenic influences

for centuries and has been designated as a natural eutrophic area (Krivokapić *et al.*, 2011). The Pressure Index for Seagrass Meadows (PISM) (for details, see Orlando Bonaca *et al.*, 2015) was used to evaluate the different intensity and typology of pressures at the sites where *C. nodosa* was sampled. An improvement was made to PISM, adding a third category to the intensity of rainwater discharge. We think that this is important for the assessment of the status since Boka Kotorska Bay is an area with recorded periods of heavy rainfall (the highest rainfall in Europe was recorded in the hinterland of Boka Kotorska Bay (Joksimović *et al.*, 2017), so the inflow from the surrounding mountains could be very intense and significant for the marine environment.

To quantify changes in the photosynthetic part of the leaf length distribution, the MediSkew index was calculated (for details, see Orlando-Bonaca *et al.*, 2015). The reference area was selected based on the results of the leaf length measurements and the PISM evaluation. Its median value of the length of the photosynthetic part of *C. nodosa* leaves was then used in the MediSkew equation.

RESULTS AND DISCUSSION

For the first time, leaf dimensions of *C. nodosa* were measured in Montenegro, and the minimum, maximum and mean values of the photosynthetically active part of the leaf length are shown in Fig. 2. The minimum values of length ranged from 6.1 cm to 15.9 cm, while the maximum values ranged from 34.5 cm to 62 cm. The greatest differences in leaf length were recorded at sites Risan 1 and 2, Dobrota 2 and Prčanj 1.

It has been documented several times that the length of the leaves of this seagrass depends significantly on the different environmental conditions, and that the leaves are shorter in pristine marine areas than in areas with different and more intense pressures (Ferdin & Forqurean, 2004; Orfanidis *et al.*, 2007; Orlando Bonaca *et al.*, 2015). In the Boka Kotorska Bay, the lowest length values of the photosynthetically active part of the leaves were measured at the Sv. Marko 1 area. Also, the skewness, *i.e.* the ln-normal distribution of photosynthetic leaf lengths $|G|$, was very close to 0 at this area in particular (Tab. 1).

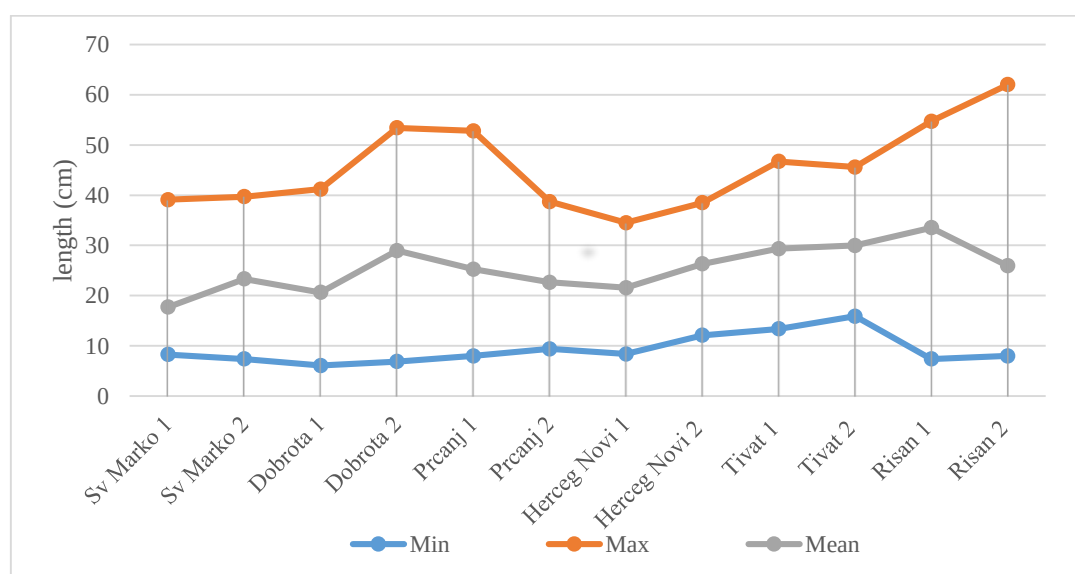


Figure 2. Length parameters of the photosynthetically active part of *C. nodosa* leaves (minimum, maximum, mean).

Unfortunately, at the Sv. Marko site, only 290 undamaged leaves were measured in area 1 instead of a total of 300 leaves, while only 120 leaves were measured for the area 2. We can only assume that the high number of damaged leaves is due to more intensive grazing in this area or careless thawing of the sample. Anyhow, this may cause problems in further calculations, and sampling and measurements for the reference area should be done very carefully in future surveys, as required by the methodology (Orlando Bonaca *et al.*, 2015), and preferably over several years.

The assessment of the different intensity and typology of pressures at the areas where *C. nodosa* was sampled is summarized in Table 2. According to the low value of the Pressure Index for Seagrass Meadows, the area Sv. Marko 1 was confirmed as the reference area for *C. nodosa* in the Boka Kotorska Bay (Tab. 2) and therefore its reference median value of the length of the photosynthetic part of *C. nodosa* leaves was used for the MediSkew calculation. The MediSkew index values for the individual areas and sites are shown in Table 1. The best values, *i.e.* high ecological

status values, were calculated for both areas in Sv. Marko and Prčanj and for the Dobrota 1 area, while the worst status (poor) was assessed for the Risan area 1. The summarized ecological status for the surveyed sites was high for Sv. Marko and Prčanj and good for Dobrota, Tivat and Herceg Novi. According to the MSFD, all of them meet the criteria for achieving GEnS. Risan was the only site where the moderate ecological status was assessed, and according to the MSFD, GEnS is not achieved. One of the assumptions for this situation could be the discharge of untreated municipal wastewater in the bay of Risan as well as the occasional intensive inflow of rainwater that reduces the water transparency. Having in mind that this site is located in the inner part of the Boka Kotorska Bay and that the water exchange with the open sea is significantly reduced, we believe that this could have a significant impact on the overall ES and GEnS of the Risan Bay. Furthermore, we are certain that the wastewater treatment measures recently implemented will contribute significantly to the improvement of the overall environmental status.

Table 1. Median value of the length of the photosynthetically active part of the leaves, absolute value of the skewness of *C. nodosa* and MediSkew values, the assessed Ecological Status according WFD (five classes) and Environmental Status according MSFD (two classes).

Sampling areas	Median	Skewness G	MediSkew index	Ecological status (WFD)	Environmental status (MSFD)
Sv. Marko 1	16.90	0.076144	0.02	0.075	Good/achieved
Sv. Marko 2	23.20	0.51891	0.13		
Dobrota 1	20.20	0.49609	0.13	0.27	Good/achieved
Dobrota 2	28.90	0.76847	0.41		
Prčanj 1	24.10	0.27062	0.17	0.16	Good/achieved
Prčanj 2	22.00	0.41478	0.15		
Herceg Novi 1	21.65	0.85234	0.25	0.32	Good/achieved
Herceg Novi 2	26.70	0.87576	0.39		
Tivat 1	29.55	0.76602	0.43	0.39	Good/achieved
Tivat 2	29.75	0.41968	0.35		
Risan 1	34.10	1.10784	0.63	0.515	Not good/ Not achieved
Risan 2	26.10	1.00284	0.40		

Table 2. Assessment of the main anthropogenic pressures at areas with *C. nodosa* meadows according to the Pressure Index for Seagrass Meadows (see Orlando-Bonaca *et al.*, 2015 for the criteria used to assess the level of six pressures).

Cymodocea areas	artificial coastline	direct leaching	mariculture	rainwater discharge	industrial wastewater	navigation routes	sum	confinement	final pressures
Sv. Marko 1	0	2	0	0	0	0	2	1.25	2.5
Sv. Marko 2	0	2	0	0	0	1	3	1.25	3.75
Dobrota 1	3	2	0	2	1	1	9	1.25	11.25
Dobrota 2	3	2	0	2	2	2	11	1.25	13.75
Prčanj 1	3	2	0	1	1	2	9	1.25	11.25
Prčanj 2	3	2	0	1	1	1	8	1.25	10
Herceg Novi 1	1	2	0	1	1	0	5	1.25	6.25
Herceg Novi 2	1	2	0	1	1	0	5	1.25	6.25
Risan 1	1	2	0	2	2	0	7	1.25	8.75
Risan 2	1	2	0	2	2	0	7	1.25	8.75
Tivat 1	1	2	0	1	1	1	6	1.25	7.5
Tivat 2	1	2	0	1	1	1	6	1.25	7.5

In conclusion, we should emphasise that this is the first test of the MediSkew index in the southern Adriatic and that the first results show that the methodology meets the possibilities of application in different geographical areas, that it is relatively easy to apply and sensitive to different environmental conditions. Montenegro is a candidate country for EU accession and is obliged to plan a monitoring programme that will assess the environmental status according to the EU directives (WFD and MSFD) and evaluate the success of the set targets for achieving a GEnS every 6 years. For these reasons, the application of the MediSkew index is strongly recommended for further testing, primarily for a more accurate evaluation of reference values. Our expectation is that this method will be an effective tool for further monitoring, providing a scientific basis for the adoption of appropriate protection measures.

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Prva primjena MediSkew indeksa u livadama *Cymodocea nodosa* u Bokokotorskom zalivu (Crna Gora) i procjena ekološkog statusa

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SAŽETAK

Morska trava *Cymodocea nodosa* je zaštićena vrsta i jedan od najvažnijih graditelja staništa u Sredozemnom moru. Stoga je procjena statusa njenih livada od velikog značaja za planiranje mjera očuvanja, te implementaciju Okvirne direktive o vodama (VFD) i Okvirne direktive o morskoj strategiji (MSFD). Ovo je od posebnog značaja u područjima gde nema ili ne dominira *Posidonia oceanica*, kao u slučaju Bokokotorskog zaliva. MediSkew indeks je već uspješno primijenjen u sjevernom Jadranu, a prvi put smo testirali metodologiju u Bokokotorskom zalivu (južno Jadransko more). Prvi rezultati pokazuju da je ekološki status na pet lokaliteta veoma dobar i dobar, dok je samo na jednoj lokaciji umjeren. MediSkew indeks je primjenljiv u različitim geografskim oblastima i osjetljiv je na različite uslove životne sredine, pa se primjena ove metodologije preporučuje za dalje praćenje livada *Cymodocea* i procjenu statusa.

Ključne riječi: *Cymodocea nodosa*, procjena statusa, MediSkew index, južno Jadransko more