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FIVE-YEAR MONITORING OF THE ECOLOGICAL STATUS OF THE *CYMODOCEA NODOSA* MEADOW NEAR THE PORT OF KOPER

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ABSTRACT

Seagrass meadows are among the most productive ecosystems in marine environments worldwide and are often considered to symbolise near-pristine conditions on sedimentary bottoms, but their condition is associated to various types of anthropogenic stressors. In the Mediterranean Sea, Cymodocea nodosa *is considered an effective indicator of environmental change, due to its universal distribution, its sensitivity to various natural and anthropogenic pressures, and the measurability of the species' responses to these impacts. The aim of this study is to present the improvement of the ecological status of the* C. nodosa *meadow near the port of Koper over a period of five years and to compare these results with the reference site in the northern Adriatic Sea.*

Key words: *Cymodocea nodosa*, MediSkew index, Port of Koper, status assessment, northern Adriatic Sea

MONITORAGGIO QUINQUENNALE DELLO STATO ECOLOGICO DELLA PRATERIA DI *CYMODOCEA NODOSA* PRESSO IL PORTO DI CAPODISTRIA

SINTESI

Le praterie di fanerogame marine sono tra gli ecosistemi più produttivi negli ambienti marini di tutto il mondo e sono spesso considerate il simbolo di condizioni quasi incontaminate sui fondali sedimentari, ma il loro stato è associato a vari tipi di stress antropico. Nel Mediterraneo, Cymodocea nodosa *è considerata un efficace indicatore del cambiamento ambientale, grazie alle sue: distribuzione universale, sensibilità a varie pressioni naturali e antropiche e misurabilità delle risposte a questi impatti. Lo scopo di questo studio è presentare il miglioramento dello stato ecologico della prateria di* C. nodosa *vicino al porto di Capodistria in un periodo di cinque anni e confrontare questi risultati con il sito di riferimento nell'Adriatico settentrionale.*

Parole chiave: *Cymodocea nodosa*, indice MediSkew, Porto di Capodistria, valutazione dello stato, Adriatico settentrionale

INTRODUCTION

Seagrass meadows are among the most productive ecosystems in marine environments worldwide (Brodersen *et al*., 2018) and are often considered emblematic of near-pristine conditions on sedimentary bottoms (Sfriso *et al*., 2021). Seagrass meadows cover large areas of the seabed, and despite their relatively low floral diversity, they support a rich marine fauna (*e.g.* fish and invertebrates) and provide a range of ecosystem services, including habitat provision, biodiversity conservation, food security, sediment stabilization, protection from coastal erosion, carbon sequestration and potentially mitigation of climate change impacts (Cullen-Unsworth & Unsworth, 2013; Espino *et al*., 2015; Unsworth *et al*., 2018; Rodil *et al*., 2022; Traganos *et al*., 2022). They are listed as priority habitats in several legislations, including the European Habitats Directive (HD, 92/43/EEC).

Seagrass meadows are among the best-studied coastal vegetated habitats due to their worldwide occurrence and relative accessibility in shallow waters. They are more or less the marine counterpart of tropical rainforests, and their condition is linked to various types of anthropogenic stressors. These pressures include shipping routes, vessel traffic, port activities, nutrient loading, siltation, mechanical disturbance (*e.g.* seabed dredging), pollution, aquaculture, introduction of new competitors (like non-indigenous organisms), commercial and recreational activities, runoff from urban and agricultural areas, and increasing climate change and ocean acidification (Marbà et al., 2014; Orlando-Bonaca et al., 2015, 2019; Repolho et al., 2017; Sfriso *et al.,* 2023). These stressors cause physical damage to the seabed (Marbà *et al*., 2014), limit the light available for photosynthesis and impair nutrient resources (Hemminga & Duarte, 2000). Since the mid-17th century, the global cover of seagrasses has decreased by about 29% (51,000 km^2) and the annual loss of seagrass habitats adds about 300 Tg of carbon per year to the global active carbon pool (Capistrant-Fossa & Dunton, 2024).

In the Mediterranean Sea, *Cymodocea nodosa* (Ucria) Ascherson is considered an effective indicator of environmental change, due to its universal distribution, sensitivity to various natural and anthropogenic pressures, and the measurability of the species' responses to these impacts (Orfanidis *et al*., 2007, 2010; Orlando-Bonaca *et al*., 2015; Papathanasiou *et al*., 2016; Nadzari *et al*., 2022). Although *C. nodosa* exhibits great phenotypic plasticity and can adapt to various natural and anthropogenic stressors through physiological and morphological adaptations, a strong decline has been reported in coastal areas in recent decades due to direct and indirect effects of multiple stressors (Fabbri *et al*., 2015; Najdek *et al*., 2020; Stockbridge *et al*., 2020).

There is still a lack of long-term data series to support the conservation status of *C. nodosa* meadows in the northern Adriatic Sea*,* while the species is still only protected in spatially restricted Marine Protected Areas (MPAs). The ecological status of *C. nodosa* meadows in the Gulf of Trieste was assessed using the MediSkew index (Orlando-Bonaca *et al*., 2015; 2016), which was developed in accordance with the requirements of the EU Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC). The ecological status of the *C. nodosa* meadow growing near the Port of Koper was first assessed in 2018 (Orlando-Bonaca *et al*., 2019), and then monitored annually from 2020 to 2023, as shipping routes and port activities are considered one of the main pressures on the status of *C. nodosa* meadows (Orlando-Bonaca *et al*., 2015). The aim of this study is to investigate the changes in the ecological status of the *C. nodosa* meadow near the port of Koper during five years of monitoring compared to the reference area for *C. nodosa* in the Gulf of Trieste.

MATERIAL AND METHODS

Study area, fieldwork and laboratory work

The Port of Koper is the only Slovenian port, connecting the markets of Central and South-Eastern Europe with the Mediterranean and the Far East. Today, the marine part of the cargo port consists of three basins, associated jetties, and specialised loading terminals. The main impacts on seabed habitats in the vicinity of the port are caused by high water turbidity due to manoeuvres of large ships (Žagar et al., 2014), dredging and other activities (Luka Koper, 2015, 2020a), which lead to a high sedimentation/resuspension rate.

The investigated *C. nodosa* meadow near the Port of Koper can be considered as part of the biocoenosis of superficial muddy sands in sheltered waters (Orlando-Bonaca *et al*., 2015, 2022). The meadow was sampled in July 2018, 2020, 2021, 2022 and 2023. Two sites (LuKp1 and LuKp2) were selected (Fig. 1) along the same isobath (3 m). Site LuKp1 (45°34.350'N; 13°44.183'E) is about 500 m away from the water area of the Port of Koper, while site LuKp2 (45°34.551'N; 13°43.861'E) is about 1000 m away from the Port. Within each site, two areas (LuKp1_1, LuKp1_2, and LuKp2_1, LuKp2_2) were selected, approximately 100 m apart. In each area, five metal frames (25 cm x 25 cm) were randomly placed on the bottom by SCUBA divers. These five squares were considered replicates of a sample. All shoots of *C. nodosa* found in each frame were carefully uprooted. The samples were lbelled and individually packed in plastic bags.

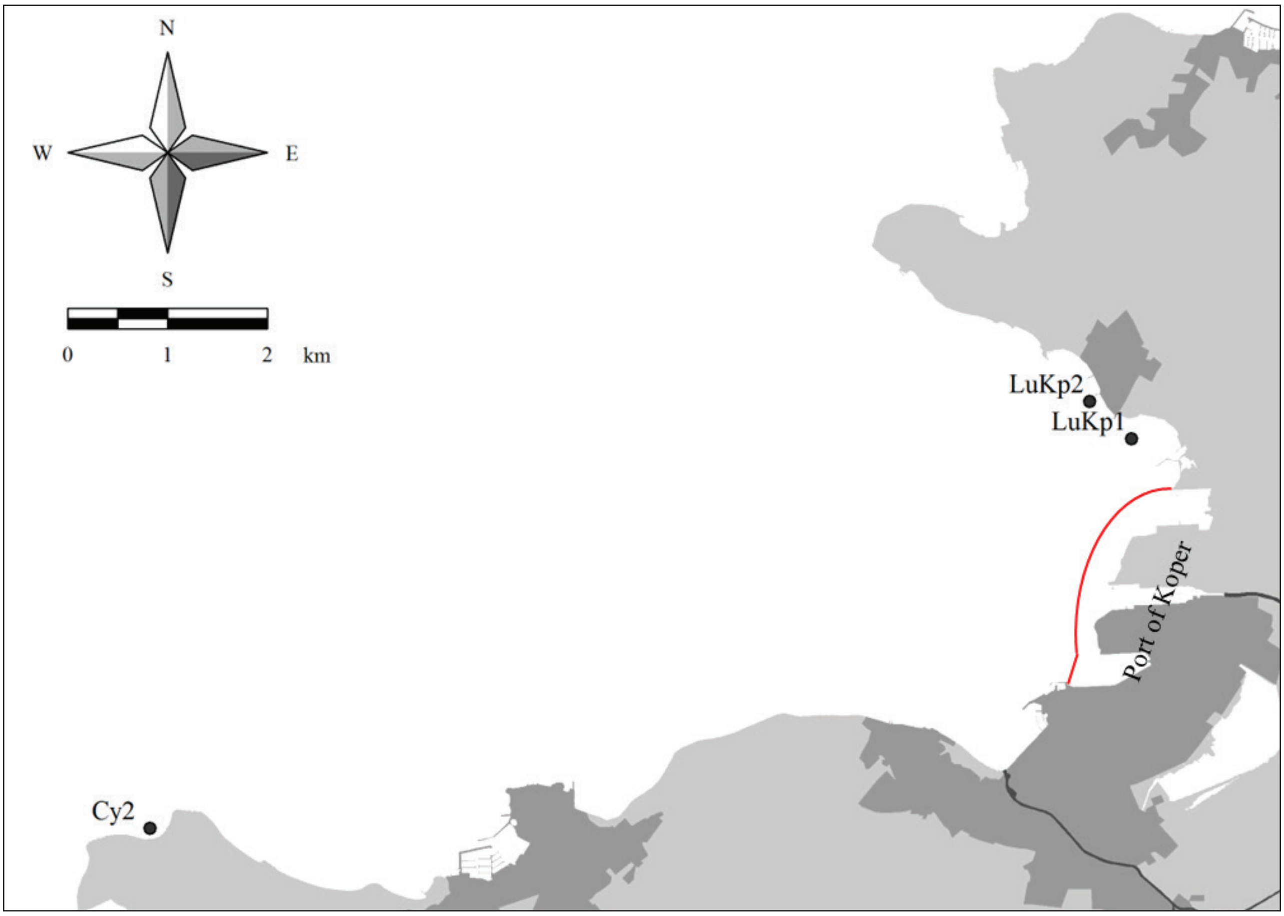

Fig. 1: Map of sampling sites for** Cymodocea nodosa **in Slovenian marine waters: near the Port of Koper (LuKp1 and LuKp2) and in the Moon Bay (St. Cross Bay, Cy2), within the Strunjan Nature Reserve. The redline indicate the water area of the Port of Koper.

Sl. 1: Zemljevid vzorčnih lokalitet kolenčaste cimodoceje v slovenskem morju: ob Luki Koper (LuKp1 in LuKp2) in v Mesečevem zalivu (Zalivu Sv. Križa, Cy2), v Naravnem rezervatu Strunjan. Rdeča črta označuje akvatorij Luke Koper.

In July 2018 and July 2023, samples of *C. nodosa* were also collected in the Strunjan Nature Reserve (sampling site Cy2, areas Cy2_1 and Cy2_2). According to the low value of the Pressure Index for Seagrass Meadows (PISM), the area Cy2_1 was selected as the reference area for *C. nodosa* in the Gulf of Trieste (Orlando-Bonaca *et al.*, 2015).

The samples of *C. nodosa* were stored in a freezer at -20°C in the laboratory of the Marine Biology Station Piran. The day before the analysis, they were slowly defrosted in a refrigerator. The seagrass shoots were then stored in plastic wash basins with seawater. Twenty shoots from each quadrat were randomly selected (Orfanidis *et al.*, 2007). For each leaf (usually 5-6 leaves per shoot), the following parameters were measured to the nearest mm: length of the leaf sheath, length of the photosynthetic part and its width. The age of the leaf was designated as adult state (when the leaf sheath was well-developed), intermediate state (when the leaf sheath was weakly developed at the leaf base), and juvenile state (when the leaf sheath was absent). The above measurements were performed on at least 60 undamaged, photosynthetically active leaves (adult and/or intermediate) from each frame. One sample consisted of five replicates of 60 leaves (300 leaves in total).

Data analysis

Summary statistics were examined for each *C. nodosa* sampling area near the Port of Koper. 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 boundaries among the status classes for the MediSkew index were set equidistantly (Tab. 1). Five status classes are sufficient for the assessment of the Ecological Status (ES)

Tab. 1: Boundaries among the status classes for the MediSkew index. Five classes should be used for the assessment of ES according to the WFD. For the assessment of EnS under the MSFD, the classes High and Good indicate a Good EnS, while the classes Moderate, Poor and Bad are considered Not Good EnS (see Orlando-Bonaca et al., 2015).
Tab. 1: Meje med posameznimi razredi stanja za MediSkew indeks. Za opredelitev ekološkega stanja po Evropski vodni direktivi (OVS) smo uporabili 5 razredov. Za opredelitev okoljskega stanja po Okvirni direktivi o morski strategiji (ODMS), razreda Zelo dobro in Dobro označujeta Dobro okoljsko stanje, medtem ko razredi Zmerno, Slabo in Zelo slabo opredeljujejo Slabo okoljsko stanje (po Orlando-Bonaca in sod., 2015).

Status classes	Absolute values of MediSKew
High	0 ≤ MediSKew < 0.2
Good	0.2 ≤ MediSKew < 0.4
Moderate	0.4 ≤ MediSKew < 0.6
Poor	0.6 ≤ MediSKew < 0.8
Bad	0.8 ≤ MediSKew ≤ 1

under the WFD. In addition, the classes High and Good indicate Good Environmental Status (EnS) according to the MSFD, while the classes Moderate, Poor and Bad are considered as Not Good EnS.

RESULTS AND DISCUSSION

The parameters of *C. nodosa* per sampling area are listed in Table 2. The data show that the mean and median leaf lengths at all four sampling areas near the Port of Koper were lower in 2023 than in 2022 and in all previous years for which data are available. At sampling areas LuKp1_1 and LuKp1_2, the maximum leaf length values in 2023 were also lower than in the previous year, while at sampling areas LuKp2_1 and LuKp2_2 they were similar to 2022.

The maximum leaf length, mean and median values at the reference area Cy2_1 were lower in 2023 than in 2018 (Tab. 2), confirming a High ecological status of *C. nodosa*. In both years, the leaves of *C. nodosa* in the areas within the reference site in the Moon Bay (Cy2) were significantly shorter than in the areas near the Port of Koper in all 5 sampling years, as were the median values (Tab. 2). However, there has been a clear trend towards decreasing leaf lengths near the Port of Koper since 2018 (Tab. 2).

All samples of *C. nodosa* near the Port of Koper had fewer damaged leaves in 2023 than in 2020 and 2021 (*pers. obs.*). In particular, at LuKp1_1 *C. nodosa* had so many broken leaves, without apical parts, in 2020 that it was not possible to measure 300 leaves per sampling area (Tab. 3), as stated in the methodology.

The ES (according to WFD) and EnS (according to MSFD) of the sampling areas and sites were assessed according to the boundaries in Table 1. The MediSkew index values for each sampled area near the Port of Koper and for areas Cy2_1 and Cy2_2 at the reference site Cy2 are presented in Table 3. The sampling site LuKp1, which is closest to the Port of Koper, improved the ES from Bad in 2018 to Poor in 2020, then Moderate in 2021 and 2022 to Good in 2023. At the sampling site LuKp2, which is furthest from the Port Basin III, the ES value improved from Poor in 2018 to Good in 2023, with a decrease to Moderate in 2022 (Tab. 3). These data also indicate that the negative impact on the *C. nodosa* meadow decreases with increasing distance from the Port, and that this impact can be observed and monitored within a radius of one kilometer from the port area. It should also be noted that the sampling sites are located away from the Port in the direction of the sea current, which flows counterclockwise in the northern Adriatic (Ogorelec *et al.*, 1991).

The ES of the entire meadow of *C. nodosa* near the Port of Koper was evaluated as Bad in 2018, while it achieved a Good ES in 2021 and 2023 (Tab. 3).

The results obtained from 2018 to 2023 show a significant improvement in the ES of the *C. nodosa* meadow. The Good ES in 2021 and 2023 could be related to the reduction of local anthropogenic pressures, such as construction works within the harbour area, dredging and maritime traffic, which play a key role in the regression of seagrass meadows (Orfanidis *et al.*, 2020; Salinas *et al.*, 2020; Stockbridge *et al.*, 2020). Such pressures lead to increased sediment resuspension, resulting in higher turbidity and consequently less light for the light-limited seagrasses (Touchette & Burkholder, 2000). Marine plants react to low light conditions by increasing the distribution of their biomass on their leaves. By enlarging the leaves, they can capture more light and convert it into photosynthetic production (Greve & Binzer, 2004). Since the construction works for the new RO-RO berth in Basin III were completed in March 2020 (Luka Koper, 2020a) and other construction works interfering with the seabed were not carried out later (Luka Koper, 2023), most of the sediment resuspension is probably due to ship traffic, which mainly occurs when entering and leaving the Port by manoeuvring

Tab. 2: Statistical parameters (minimum, maximum, mean, median) and absolute value of the skewness (|G|) of the ln-transformed lengths of the photosynthetically active parts of the leaves of* Cymodocea nodosa *from the sampling areas near the Port of Koper (LuKp1 and LuKp2) in 2018, 2020–2023, and in the Moon Bay (St. Cross Bay, Cy2, Strunjan Nature Reserve) in 2018 and 2023. The reference median value in 2023 was 10.95 cm.

Tab. 2: Statistični parametri (minimum, maksimum, povprečje, mediana) in absolutna vrednost koeficienta asimetrije (|G|) ln-transformiranih dolžin fotosintetsko aktivnega dela listov kolenčaste cimodoceje na točkah vzorčenja blizu Luke Koper (LuKp1 in LuKp2) v 2018, 2020–2023 ter v Mesečevem zalivu (Zalivu Sv. Križa, Cy2, Naravni rezervat Strunjan) v 2018 in 2023. Referenčna mediana v 2023 je bila 10,95 cm.

Area	Date	Min length (cm)	Max length (cm)	Mean (cm)	Median (cm)	|G|
Cy2_1	12.7.2018	5.4	30.5	14.5	**13.95**	0.261
Cy2_2	12.7.2018	8.1	22.7	13.5	13.20	0.022
LuKp1_1	17.7.2018	5.9	66.2	37.8	41.25	1.423
LuKp1_2	17.7.2018	6.0	57.1	34.7	37.05	1.162
LuKp2_1	17.7.2018	3.7	58.8	30.7	30.45	1.533
LuKp2_2	17.7.2018	6.9	52.2	27.3	28.25	1.130
LuKp1_1	14.7.2020	5.4	62.5	32.0	31.90	1.044
LuKp1_2	14.7.2020	7.4	57.7	29.9	29.25	0.706
LuKp2_1	14.7.2020	5.1	61.3	29.2	28.90	0.979
LuKp2_2	14.7.2020	7.3	55.9	31.4	31.25	0.955
LuKp1_1	1.7.2021	8.7	55.8	27.3	25.90	0.355
LuKp1_2	1.7.2021	7.3	57.1	28.1	27.20	0.442
LuKp2_1	1.7.2021	11.5	47.7	24.7	22.95	0.142
LuKp2_2	1.7.2021	5.7	46.2	24.2	23.15	0.659
LuKp1_1	11.7.2022	9.1	57.1	30.7	30.45	0.762
LuKp1_2	11.7.2022	9.8	42.3	27.1	27.40	0.675
LuKp2_1	11.7.2022	9.0	42.4	26.7	26.80	0.738
LuKp2_2	11.7.2022	6.4	45.1	26.0	24.95	0.461
Cy2_1	12.7.2023	5.1	20.4	11.0	**10.95**	0.347
Cy2_2	12.7.2023	3.5	19.5	11.7	11.80	0.497
LuKp1_1	5.7.2023	6.6	39.8	21.1	20.60	0.397
LuKp1_2	5.7.2023	6.7	38.3	21.9	21.60	0.731
LuKp2_1	5.7.2023	5.2	45.7	21.1	20.15	0.382
LuKp2_2	5.7.2023	8.5	46.4	22.2	21.35	0.391

Tab. 3: MediSkew index values for the sampling areas of* Cymodocea nodosa *in the Port of Koper and assessment of the Ecological Status (according to WFD) and Environmental Status (according to MSFD).
Tab. 3: Vrednosti indeksa MediSkew na točkah vzorčenja s kolenčasto cimodocejo in opredelitev ekološkega stanja (glede na OVS) in okoljskega stanja (glede na ODMS) za travnik ob Luki Koper.

Year	Area	Area's MediSkew	Site's MediSkew	Meadow's MediSkew	Ecolog. Status	Environ. Status	N of leaves	N of adult leaves
2018	Cy2_1	0.065	0.04		High	Good / Achieved	300	112
	Cy2_2	0.024					300	123
	LuKp1_1	1.00	0.935	0.825	Bad	Not good / Not achieved	300	225
	LuKp1_2	0.87					300	204
	LuKp2_1	0.79	0.715				300	247
	LuKp2_2	0.64					300	218
2020	LuKp1_1	0.71	0.635	0.640	Poor	Not good / Not achieved	**251**	181
	LuKp1_2	0.56					300	223
	LuKp2_1	0.62	0.645				300	246
	LuKp2_2	0.67					300	222
2021	LuKp1_1	0.39	0.415	0.37	Good	Good / Achieved	300	238
	LuKp1_2	0.44					300	207
	LuKp2_1	0.26	0.325				300	231
	LuKp2_2	0.39					300	212
2022	LuKp1_1	0.60	0.550	0.50	Moderate	Not good / Not achieved	300	279
	LuKp1_2	0.50					300	286
	LuKp2_1	0.51	0.450				300	276
	LuKp2_2	0.39					300	269
2023	Cy2_1	0.087	0,12		High	Good / Achieved	300	177
	Cy2_2	0.146					300	186
	LuKp1_1	0.34	0.395	0.37	Good	Good / Achieved	300	232
	LuKp1_2	0.45					300	223
	LuKp2_1	0.33	0.342				300	263
	LuKp2_2	0.36					300	272

with tugboats (*pers. obs.*). The data in Table 4 (Luka Koper, 2020b, 2023, 2024) show that the number of ships in the Port of Koper decreased from 1,899 ships in 2018 to 1,433 in 2020 (24.5%) due to the Covid-19 pandemic. The number of ships in the Port then increased until 2022 and then fell slightly in 2023. This means that the number of ships decreased by 13.5% in 2023 compared to 2018. These data hypothesize that the reduction in the number of ships arriving at the Port could have a positive impact on improving the condition of the nearby *C. nodosa* meadow.

In addition, climate change has been a growing concern in recent years, as sea level rise and rising seawater temperatures may further contribute to the decline of seagrass beds (Duarte *et al.*, 2018;

Tab. 4: The number of ships arriving in the Port of Koper for the period 2018 to 2023 (data from Luka Koper, 2020b, 2023, 2024).
Tab. 4: Število ladij, ki so vplule v Luko Koper v obdobju od 2018 do 2023.

Year	Number of ships
2018	1.899
2019	1.664
2020	1.433
2021	1.551
2022	1.659
2023	1.642

Fortes *et al.*, 2018; Tsioli *et al.*, 2022; Llabrés *et al.*, 2023). The Mediterranean Sea is warming three times faster than the oceans (Savva *et al.*, 2018) because it is a more enclosed sea. Moreover, the northern Adriatic was hit by a severe heatwave in 2023. The temperature of the surface layers of the sea in the Gulf of Trieste exceeded 30 °C in summer, which has only been measured twice in the past. In addition, the seawater temperature on the seabed exceeded 24 °C, 1 °C higher than at any time in the last 20 years (data from the oceanographic buoy VIDA, https://www.nib.si/mbp/en/oceanographic-data-and-measurements). This places a great pressure on the organisms living on the seabed, which are unable to move. According to Savva *et al.* (2018), *C. nodosa* meadows have a higher tolerance to heat waves than *Posidonia oceanica* meadows, which is probably due to the tropical origin of the genus *Cymodocea*.

Ocean acidification (Repolho et al., 2017) and infections by protists of the genus *Labyrinthula* (Olsen & Duarte, 2015) are also already having a lasting impact on seagrass meadows in other parts of the Mediterranean. Considering all the aforementioned pressures on such ecosystems, the results of a recent study focusing on the dynamics of seagrass meadows along the Slovenian coastline (Ivajnšič *et al.*, 2022) are of great importance. The temporal perspective showed a stable cover of seagrass meadows in the study area (282.4 ha in 2014 and 283.5 ha in 2020). However, the spatial perspective showed a different development of the current extent of seagrass meadows. In some areas, *C. nodosa* meadows has almost completely disappeared (marine area of the Strunjan Landscape Park), while in other areas along the Slovenian coast it has been re-established (Ivajnšič *et al.*, 2022).

The results of the present study indicate a positive trend in the ES of the *C. nodosa* meadow near Koper. Since the Port authority has planned a long-term monitoring programme in the harbour area and its surroundings, we recommend that in addition to the assessment of the ES with the MediSkew index, the distribution of the meadow (in terms of cover) and the detection of possible signs of disease on the leaves of *C. nodosa* should also be evaluated.

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PETLETNO SPREMLJANJE EKOLOŠKEGA STANJA TRAVNIKA KOLENČASTE CIMODOCEJE (*CYMODOCEA NODOSA*) V BLIŽINI KOPRSKEGA PRISTANIŠČA

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POVZETEK

Morski travniki so med najbolj produktivnimi ekosistemi v morskih okoljih po vsem svetu in pogosto veljajo za simbol skoraj čistih razmer na sedimentnem dnu, vendar je njihovo stanje povezano z različnimi antropogenimi pritiski. V Sredozemskem morju velja kolenčasta cimodoceja (Cymodocea nodosa) *za učinkovit kazalnik okoljskih sprememb zaradi svoje univerzalne razširjenosti, občutljivosti na različne naravne in antropogene pritiske ter merljivosti odzivov vrste na te vplive. Namen te študije je predstaviti izboljšanje ekološkega stanja travnika kolenčaste cimodoceje v bližini koprskega pristanišča v obdobju petih let in te rezultate primerjati z referenčnim območjem v severnem Jadranu.*

Ključne besede: *Cymodocea nodosa,* MediSkew indeks, Luka Koper, ocena stanja, severni Jadran

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