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IMPROVEMENT OF THE ECOLOGICAL STATUS OF THE *CYMODOCEA NODOSA* MEADOW NEAR THE PORT OF KOPER

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ABSTRACT

Seagrass beds are more or less the marine counterpart of tropical rainforests, and their health is related to different anthropogenic stressors, including navigation routes and port activities. In the Mediterranean Sea, Cymodocea nodosa *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 impacts. The aim of this study is to present the changes in the assessment of the ecological status of the* C. nodosa *meadow near the port of Koper, which was evaluated as Bad in 2018. The results show a significant improvement in the ecological status of the meadow, which can be attributed to a reduction in anthropogenic stressors.*

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

MIGLIORAMENTO DELLO STATO ECOLOGICO DELLA PRATERIA DI *CYMODOCEA NODOSA* VICINO AL PORTO DI CAPODISTRIA

SINTESI

Le praterie di fanerogame marine vengono considerate la controparte marina delle foreste pluviali tropicali, e la loro salute è legata a diversi fattori di stress antropogenici, tra cui le rotte di navigazione e le attività portuali. Nel Mediterraneo, Cymodocea nodosa *è considerata un indicatore efficace del cambiamento ambientale, a causa della sua distribuzione universale, della sensibilità a varie pressioni naturali e antropogeniche, e della misurabilità delle risposte della specie agli impatti. Lo scopo di questo studio è di presentare i cambiamenti nella valutazione dello stato ecologico della prateria di* C. nodosa *vicino al porto di Capodistria, che è stato valutato come Cattivo nel 2018. I risultati mostrano un miglioramento significativo dello stato ecologico della prateria, che può essere attribuito a una riduzione dei fattori di stress antropogenici.*

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

INTRODUCTION

Seagrass meadows are among the most productive environments in the seas and oceans worldwide (Spalding *et al.*, 2003; Brodersen *et al.*, 2018). They provide habitat niches, food, and protection from predators for many different organisms in lagoons and marine ecosystems (Hemminga & Duarte, 2000; Como *et al.*, 2008; Tuya *et al.*, 2014; Espino *et al.*, 2015). These environments are also important for human well-being (Nordlund *et al.*, 2018; Unsworth *et al.*, 2018), as they provide a range of ecosystem services, including moderating wave action and thus protecting the coastline from erosion (Ondiviela *et al.*, 2014; Cabaço & Rui Santos, 2014), stabilising sediments (Terrados & Borum 2004; Widdows *et al.*, 2008), regulating nutrient cycling and sequestering carbon (Duarte *et al.*, 2010; Luisetti *et al.*, 2013), purification of seawater (Richir *et al.*, 2013), and providing a system for education and research (Effrosynidis *et al.*, 2018). For these reasons, they have been included as priority habitats in a number of legal regulations, including the European Habitats Directive (HD, 92/43/EEC).

Seagrass beds are more or less the marine counterpart of tropical rainforests, and their health is associated with various types of anthropogenic stressors. These pressures include navigation routes and port activities, seabed dredging, commercial and recreational activities such as fishing and mooring, runoff from urban and agricultural areas, wastewater, and more recently, increasing climate change and ocean acidification (Short et al., 2011; Tuya et al., 2002; Marbà et al., 2014; Orlando-Bonaca et al., 2015, 2019; Repolho et al., 2017). Such pressures affect light and nutrient resources (Hemminga & Duarte, 2000), and cause physical damage to different sea bottom types (Montefalcone *et al.*, 2008; Marbà *et al.*, 2014). Rapid and widespread declines in seagrass meadows have been reported from many coastal areas over the past fifteen years (Orth *et al.*, 2006; Tuya *et al.*, 2013; Fabbri *et al.*, 2015). Seagrasses have disappeared at a rate of 110 km^2 per year since 1980, a value similar to the rates of loss described for mangroves, coral reefs, and tropical rainforests (Waycott *et al.*, 2009). In terms of cover, one third of the world's seagrass meadows are reported to have already disappeared (Waycott *et al.*, 2009).

Four native seagrass species are found in the Adriatic Sea: *Posidonia oceanica* (Linnaeus) Delile, *Cymodocea nodosa* (Ucria) Ascherson, *Zostera marina* Linnaeus and *Zostera noltei* Hornemann (Lipej *et al.*, 2006). In the Mediterranean Sea, *C. nodosa* 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; Oliva *et al.*, 2012; Orlando-Bonaca *et al.*, 2015; Papathanasiou *et al.*, 2016). Although *C. nodosa* exhibits great phenotypic plasticity and can adapt to various natural and anthropogenic stressors through physiological and morphological adaptations, a sharp decline has been reported in coastal areas (Orth *et al.*, 2006; Short *et al.*, 2011; Tuya *et al.*, 2013, 2014; Fabbri *et al.*, 2015; Mačić & Zordan, 2018; Najdek *et al.*, 2020) in recent decades.

In the northern Adriatic Sea, there is still a lack of long-time data series to support the conservation status of *C. nodosa* meadows, which is included in Annex II (List of Endangered or Threatened species) of the Convention for the Protection of the Mediterranean Sea Against Pollution (the Barcelona Convention). 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 evaluated in 2018 (Orlando-Bonaca *et al.*, 2019), and subsequently monitored in 2020 and 2021. An annual monitoring programme is planned for the future, as shipping routes and port activities are considered one of the main pressures on the

Tab. 1: Boundaries among status classes for the MediSkew index (classes High and Good indicate a Good Environmental Status).

Tab. 1: Meje med posameznimi razredi stanja za MediSkew indeks (razreda Zelo dobro in Dobro označujeta Dobro okoljsko stanje).

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

status of *C. nodosa* meadows (Orlando-Bonaca *et al.*, 2015). The aim of this study is to present the changes in the assessment of the ecological status of the *C. nodosa* meadow near the port of Koper from 2018 using the MediSkew index.

MATERIAL AND METHODS

Study area, fieldwork and laboratory work

The Port of Koper is a Slovenian multi-purpose port on the northern Adriatic Sea, mainly connecting markets in Central and South-eastern Europe with the Mediterranean Sea and the Far East. The marine part of the cargo port consists of tree basins, associated mooring piers and specialized loading terminals. The highest water turbidity values were measured during manoeuvres of the large ships (Žagar *et al.*, 2014). Dredging of the sedimentary bottom was carried out in the Port of Koper along the access channels to Basin I (Luka Koper, 2015). Moreover, construction works, including dredging for the construction of a new RORO berth in the Basin III, were officially opened on May 27, 2019, and completed on March 31, 2020 (Franka Cepak, *pers. comm.*), resulting in a high sedimentation/resuspension rate.

The seagrass meadow located near the Port of Koper was sampled in July 2018, 2020 and 2021. Two sites (LuKp1 and LuKp2) were selected (Fig.

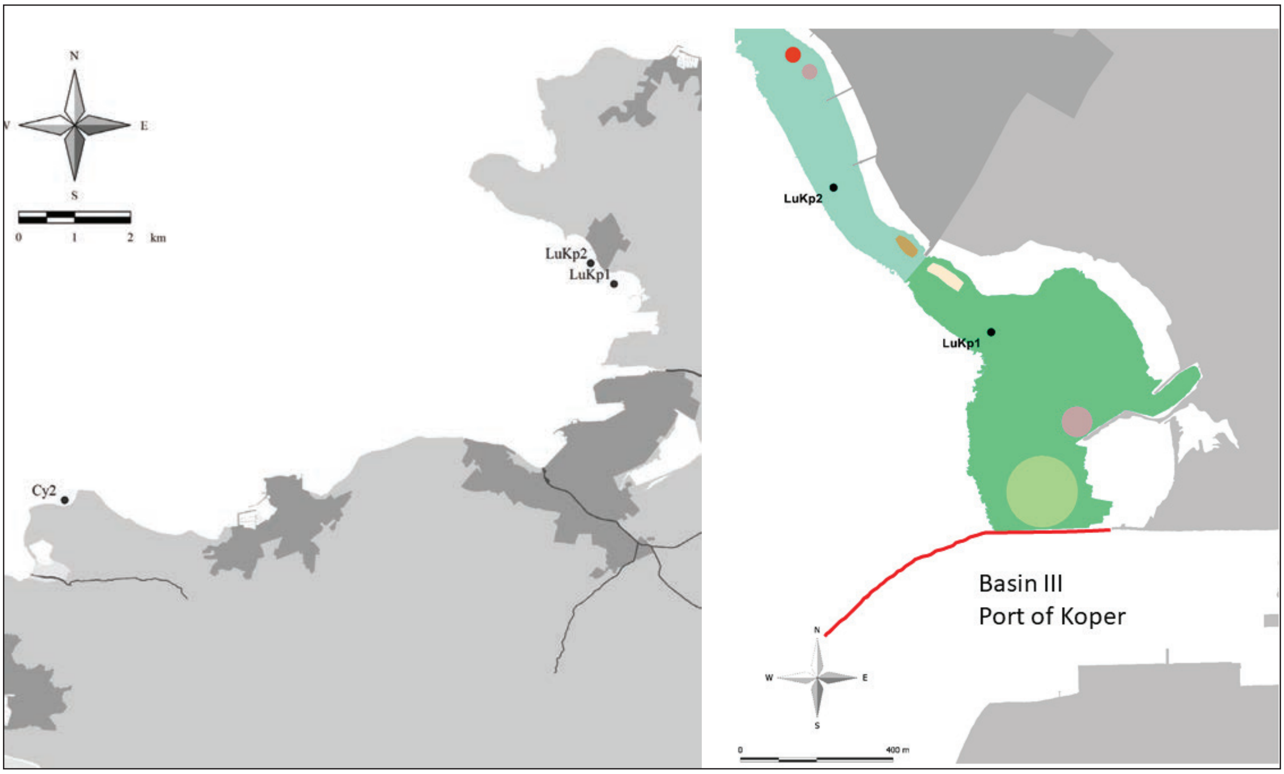

Fig. 1: Map of sampling sites for* Cymodocea nodosa *near the Port of Koper (LuKp1 and LuKp2) and the reference site in the Moon Bay (Cy2). The site LuKp1 is located in the part of the* C. nodosa *meadow with a higher density, while the site LuKp2 is located in a less dense part of the meadow. Other colours on the map: the largest, light green circle =* Zostera noltei*; grey circles = boulders with* Dictyota dichotoma*,* Padina pavonica *and turf; red circle = boulders with* Cystoseira compressa *and* P. pavonica*; dark brown area = boulders with* P. pavonica*,* Halopteris scoparia *and turf; light brown area = boulders with* Padina pavonica *and turf.

Sl. 1: Zemljevid mest vzorčenja kolenčaste cimodoceje blizu Luke Koper (LuKp1 in LuKp2) in referenčno mesto vzorčenja v Mesečevem zalivu (Cy2). LuKp1 se nahaja v zelo gostem delu travnika kolenčaste cimodoceje, medtem ko se LuKp2 nahaja v manj gostem delu. Druge barve na karti: največji, svetlozeleni krog =* Zostera noltei*; sivi krogi = skale prekrite z vrstama* Dictyota dichotoma *in* Padina pavonica *ter turfom; rdeči krog = skale prekrite z vrstama* Cystoseira compressa *in* P. pavonica*; temno rjavo območje = skale prekrite z vrstama* P. pavonica *in* Halopteris scoparia *ter turfom; svetlo rjavo območje = skale prekrite z vrsto* Padina pavonica *in turfom.

Tab. 2: Statistic parameters (minimum, maximum, mean, median) and absolute value of skewness (|G|) of ln-transformed lengths of photosynthetically active parts of* Cymodocea nodosa *leaves from the sampling areas near the Port of Koper (LuKp1 and LuKp2) in 2018, 2020 and 2021, and in Moon Bay (Cy2, Strunjan Nature Reserve) in 2018. The reference median value in 2018 was 13.95 cm.

Tab. 2: Statistični parametri (minimum, maksimum, povprečje, mediana) in absolutna vrednost koeficienta asimetrije (|G|) ln-transformiranih dolžin fotosintetsko aktivnega dela listov kolenčaste cimodoceje* (C. nodosa) *na točkah vzorčenja blizu Luke Koper (LuKp1 in LuKp2) v 2018, 2020 in 2021 ter v Mesečevem zalivu (Cy2, Naravni rezervat Strunjan) v 2018. Referenčna mediana v 2018 je bila 13,95 cm.

Area	Date	Min length (cm)	Max length (cm)	Mean (cm)	Median (cm)	|G|
Str_3	12.7.2018	5.4	30.5	14.5	**13.95**	0.261
Str_4	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	27.33	25.90	0.355
LuKp1_2	1.7.2021	7.3	57.1	28.12	27.20	0.442
LuKp2_1	1.7.2021	11.5	47.7	24.72	22.95	0.142
LuKp2_2	1.7.2021	5.7	46.2	24.15	23.15	0.659

1) along the same isobath (3 m) and, within each site, two areas (LuKp1_1, LuKp1_2, and LuKp2_1, LuKp2_2) were chosen, approximately 100 m apart. In each area, five metallic frames (25 cm x 25 cm) were randomly placed on the bottom by SCUBA divers. These five squares were considered replicates of one sample. All shoots of *C. nodosa* located in each frame were carefully uprooted. The samples were labelled and individually placed in plastic bags.

In July 2018, samples of *C. nodosa* were also collected in the Strunjan Nature Reserve (sampling site Cy2, areas Str_3 and Str_4). Due to the low Pressure Index for Seagrass Meadows (PISM) value, the area Str_3 was selected as the reference area for *C. nodosa* in the Gulf of Trieste in 2009 (Orlando-Bonaca *et al.*, 2015), and it has to be sampled and assessed every 5 years.

The samples of *C. nodosa* were stored in a freezer at −20°C in the laboratory of the Marine

Tab. 3: MediSkew index values for the sampling areas of* Cymodocea nodosa *in the Port of Koper and in the Moon Bay (Strunjan) and assessment of the Ecological Status (according to the WFD) and Environmental Status (according to the 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 morski travnik ob Luki Koper in v Mesečevem zalivu (Strunjan).

<table>
<tr><th>Year</th><th>Area</th><th>Area's MediSkew</th><th>Site's MediSkew</th><th>Meadow's MediSkew</th><th>Ecolog. Status</th><th>Environ. Status</th><th>N of leaves</th><th>N of adult leaves</th></tr>
<tr><td rowspan="6">2018</td><td>Str_3</td><td>0.065</td><td rowspan="2">0.04</td><td rowspan="2">-</td><td rowspan="2">High</td><td rowspan="2">Good / Achieved</td><td>300</td><td>213</td></tr>
<tr><td>Str_4</td><td>0.024</td><td>300</td><td>218</td></tr>
<tr><td>LuKp1_1</td><td>1.00</td><td rowspan="2">0.935</td><td rowspan="4">0.825</td><td rowspan="4">Bad</td><td rowspan="4">Not good / Not achieved</td><td>300</td><td>225</td></tr>
<tr><td>LuKp1_2</td><td>0.87</td><td>300</td><td>204</td></tr>
<tr><td>LuKp2_1</td><td>0.79</td><td rowspan="2">0.715</td><td>300</td><td>247</td></tr>
<tr><td>LuKp2_2</td><td>0.64</td><td>300</td><td>218</td></tr>
<tr><td rowspan="4">2020</td><td>LuKp1_1</td><td>0.71</td><td rowspan="2">0.635</td><td rowspan="4">0.640</td><td rowspan="4">Poor</td><td rowspan="4">Not good / Not achieved</td><td>251</td><td>181</td></tr>
<tr><td>LuKp1_2</td><td>0.56</td><td>300</td><td>223</td></tr>
<tr><td>LuKp2_1</td><td>0.62</td><td rowspan="2">0.645</td><td>300</td><td>246</td></tr>
<tr><td>LuKp2_2</td><td>0.67</td><td>300</td><td>222</td></tr>
<tr><td rowspan="4">2021</td><td>LuKp1_1</td><td>0.39</td><td rowspan="2">0.415</td><td rowspan="4">0.37</td><td rowspan="4">Good</td><td rowspan="4">Good / Achieved</td><td>300</td><td>238</td></tr>
<tr><td>LuKp1_2</td><td>0.44</td><td>300</td><td>207</td></tr>
<tr><td>LuKp2_1</td><td>0.26</td><td rowspan="2">0.325</td><td>300</td><td>231</td></tr>
<tr><td>LuKp2_2</td><td>0.39</td><td>300</td><td>212</td></tr>
</table>

Biology Station Piran. The day before the analysis, they were slowly defrosted in a refrigerator. Seagrass shoots were then kept in plastic wash basins containing 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 (when the leaf sheath was well-developed), intermediate (when the leaf sheath was weakly developed at the leaf base), and juvenile (when the leaf sheath was absent). The above measurements were made 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).

Additionally, in May 2020, the meadow and other vegetation types in the area were checked by applying a field method based on visual observation of sea-bottom segments covered with vegetation in the infralittoral belt. The survey consisted of a cruise along the coastline in a small boat. Sublittoral communities were identified using a large Aquascope Underwater Viewer and directly annotated in a graphic display. This graphic support was prepared at an appropriate small scale and was suitable for use in the field. The final result is a division of the shoreline into several sectors, each identified by a community category (see Fig. 1). The information obtained on the distribution of communities was transcribed into a georeferenced graphic support in a Geographical Information System. All vegetation types between 1 and 4 m depth, were mapped.

Data analysis

To quantify changes in the photosynthetic part of the leaf length distribution for each *C. nodosa* sampling area near the Port of Koper, 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) according to the WFD. In addition, High and Good classes indicate Good Environmental Status (EnS) according to the MSFD, while the classes Moderate, Poor, and Bad are considered Not Good EnS.

RESULTS AND DISCUSSION

The surveyed *C. nodosa* meadow near the Port of Koper can be considered as a part of the biocoenosis of superficial muddy sands in sheltered waters. The part of the meadow closest to the Port of Koper has a higher density of shoots than the part to the north (different green colours in Fig. 1). Within the meadow, rocky biotopes were also found, which include small communities dominated by *Padina pavonica* (Linnaeus) Thivy, *Dictyota dichotoma* (Hudson) J.V. Lamouroux*, Halopteris scoparia* (Linnaeus) Sauvageau and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin. A monospecific patch of *Zostera noltei* was also found close to the Port (see Fig. 1).

The parameters of *C. nodosa* per sampling area are shown in Table 2. The leaves of *C. nodosa* were significantly shorter in the areas within the reference site in the Moon Bay (Cy2) than in the areas near the Port of Koper in all years, and consequently so were the median values (Tab. 2). The skewness |G| was the highest in the LuKp2_1 area in 2018 (Tab. 2). However, the results show that mean and median leaf length values decreased in all sampled areas in the Port of Koper in 2020 and additionally in 2021. Leaf lengths were still much longer than those at the reference area, but there is a very clear trend of decreasing leaf lengths since 2018 near the Port of Koper (Tab. 2).

It should be emphasized that in 2020, many adult leaves of *C. nodosa* at LuKp1_1 were broken, without apical parts, and therefore we could not measure 300 undamaged leaves for this area (Tab. 3), as indicated in the methodology. All samples collected in 2021 had fewer damaged leaves and the number of adult leaves of *C. nodosa* exceeded 200 per sample (Tab. 3).

The ES (according to WFD) and the EnS (according to MSFD) of sampling areas and sites were assessed according to the boundaries in Table 1. The MediSkew index values for each sampled area in the Port of Koper are presented in Table 3. The two areas of the sampling site LuKp2, furthest from the Port Basin III, improved the ES from Poor in 2018 and 2020 to Good in 2021.

The improvement in the status of the LuKp1 sampling site is also impressive (Tab. 3). The area LuKp1_2 was assessed as Bad ES in 2018, while it remained Moderate in 2020 and 2021. The area LuKp1_1 improved from Bad ES in 2018 to Poor in 2020 and to Good in 2021. The ES of the entire meadow of *C. nodosa* near the Port of Koper was evaluated as Good in 2021, which is two orders of magnitude better than in 2020 (Tab. 3).

The results obtained from 2018 to 2021 show a significant improvement in the ES of the *C. nodosa* meadow. The Good ES achieved in 2021 may be related to the reduction of anthropogenic pressures, as the construction of the new RORO berth was completed in March 2020. This construction resulted in higher sediment resuspension in recent years, leading to increased turbidity and consequently less light. Seagrasses are generally light-limited (Touchette & Burkholder, 2000). Thus, when exposed to low light levels due to high water turbidity, they respond by increasing biomass distribution to the leaves. The increase in leaf size allows marine plants to capture more light and convert it into photosynthetic production (Greve & Binzer, 2004). That resuspension of sediments and water turbidity are critical to the health of *C. nodosa* meadows is confirmed by recent research (Orfanidis *et al.*, 2020). Additionally, the decrease in anthropogenic pressures near the port area in 2020 was also influenced by the Covid-19 pandemic, which led to a decrease in maritime traffic, especially cruise ship traffic in the Port of Koper, as reported in many local media. March et al. (2021) have attempted to assess the impact of the pandemic on maritime traffic globally, which in turn has implications for the blue economy and ocean health.

The results of the present study are very encouraging, and as the Port of Koper has prepared a long-term monitoring programme in the harbour area and its surroundings, we hope to confirm the improved ES of the *C. nodosa* meadow near the Port in the long term.

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IZBOLJŠANJE EKOLOŠKEGA STANJA MORSKEGA TRAVNIKA KOLENČASTE CIMODOCEJE (*CYMODOCEA NODOSA*) V BLIŽINI KOPRSKEGA PRISTANIŠČA

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POVZETEK

Morski travniki so bolj ali manj morski ekvivalent tropskega deževnega gozda, njihovo zdravje pa je povezano z različnimi antropogenimi dejavniki, vključno s plovnimi potmi in pristaniškimi dejavnostmi. V Sredozemskem morju je kolenčasta cimodoceja (Cymodocea nodosa) *zaradi svoje univerzalne razširjenosti, občutljivosti na različne naravne in antropogene pritiske ter merljivosti odzivov vrste na vplive, učinkovit kazalnik okoljskih sprememb. Namen študije je predstaviti spremembe v oceni ekološkega stanja morskega travnika kolenčaste cimodoceje v bližini koprskega pristanišča, ki je bilo 2018 opredeljeno kot Zelo slabo. Rezultati kažejo bistveno izboljšanje ekološkega stanja travnika, kar lahko pripišemo zmanjšanju antropogenih pritiskov.*

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

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