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IMPACT OF THE PORT OF KOPER ON *CYMODOCEA NODOSA* MEADOW

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ABSTRACT

In the Mediterranean Sea Cymodocea nodosa *is considered a valid indicator of environmental changes, due to its extensive distribution, sensitivity to different pressures, and measurability of species responses to those stressors. Navigation routes are recognized among the main pressures on the status of* C. nodosa *meadows in the Gulf of Trieste, related to a high resuspension of sediments and, in consequence, high water turbidity and reduced light levels. The MediSkew index was applied to samples collected in the* C. nodosa *meadow growing near the Port of Koper, and the ecological status was evaluated. The results were compared to those obtained for the* C. nodosa *samples from the reference site in the Gulf of Trieste, the Strunjan Nature Reserve.*

Key words: *Cymodocea nodosa,* MediSkew index, leaf lengths, Port of Koper, northern Adriatic Sea

IMPATTO DEL PORTO DI CAPODISTRIA SULLA PRATERIA DI *CYMODOCEA NODOSA*

SINTESI

Nel mare Mediterraneo Cymodocea nodosa *è considerata un valido indicatore di cambiamenti ambientali, vista la sua ampia distribuzione, sensibilità alle diverse pressioni e misurabilità delle risposte della specie a tali fattori di stress. Le rotte di navigazione vengono considerate tra le principali pressioni sullo stato delle praterie di* C. nodosa *nel Golfo di Trieste, legate a un'elevata risospensione dei sedimenti e, di conseguenza, a un'elevata torbidità dell'acqua e a livelli di luce ridotti. L'indice MediSkew è stato applicato ai campioni raccolti nella prateria di* C. nodosa *che cresce in prossimità del Porto di Capodistria per valutarne lo stato ecologico. I risultati sono stati confrontati con quelli ottenuti per i campioni di* C. nodosa *provenienti dal sito di riferimento nel Golfo di Trieste, la Riserva Naturale di Strugnano.*

Parole chiave: *Cymodocea nodosa*, indice MediSkew, lunghezza delle foglie, Porto di Capodistria, Adriatico settentrionale

INTRODUCTION

Marine angiosperms are worldwide considered as ecological engineers on shallow subtidal soft bottoms, since they create seagrass meadows, which are listed among the most valued ecosystems, providing food, shelters and nursery areas for a variety of invertebrate and fish assemblages (Heck *et al*., 2003; Wright & Jones, 2006; Como *et al*., 2008; Tuya *et al*., 2014; Espino *et al*., 2015). Additionally, seagrass meadows stabilize coastal sediments by trapping fine residues and particles that are suspended in the water column (Cabaço *et al*., 2008, 2010, 2014), provide protection against coastal erosion (Terrados & Borum, 2004), produce oxygen (Peduzzi & Vukovič, 1990) and are recognized as global carbon sinks (Duarte *et al*., 2010). For that reason, seagrass meadows are recognized as one of the priority habitats in the EU Habitat Directive (HD, 92/43/EEC).

Rapid and widespread decline of seagrass meadows were reported from many coastal areas (Orth *et al*., 2006; Tuya *et al*., 2013; Fabbri *et al*., 2015) in the last fifteen years. Coastal ecosystems are subjected to increasing anthropogenic disturbances, affecting light and nutrient resources (Hemminga & Duarte, 2000), and causing physical damage to different sea bottom types (Montefalcone *et al*., 2008; Marbà *et al*., 2014). Seagrasses have been disappearing at a rate of 110 km^2 yr^{-1} since 1980, a value equivalent to the loss rates described for mangroves, coral reefs, and tropical rainforests (Waycott *et al*., 2009). Changes observed in the global seagrass distribution point out that meadow regression phenomena should be principally attributed to the cumulative effects of local stressors, rather than to processes at basin scale, such us climate change (Telesca *et al*., 2015). The Regional Activity Centre for Specially Protected Areas (RAC/SPA) is responsible for drafting guidelines for carrying out impact studies on seagrass meadows within the Mediterranean basin. Pergent-Martini & Le Ravallec (2007) prepared such guidelines, but their real application at national levels is quite uncertain.

Four native seagrass species are present in the Adriatic Sea: *Posidonia oceanica* (Linnaeus) Delile, *Cymodocea nodosa* (Ucria) Ascherson, *Zostera marina* Linnaeus and *Zostera noltei* Hornemann (Lipej *et al*., 2006). The lesser Neptune grass, *C. nodosa*, is the most common marine angiosperm at shallow sheltered to semi-exposed sites in the Adriatic Sea, as well as along all Mediterranean soft bottom areas and at some locations in the north Atlantic (Mascaró *et al*., 2009; OSPAR, 2010). This species is known to form meadows that are mono-specific or mixed with *Z. noltei*, from the surface to 40 m of depth (Mazzella *et al*., 1993; Borum & Greve, 2004). In the Mediterranean Sea, *C. nodosa* is considered as an effective indicator of environmental changes, due to its universal distribution, sensitivity to different natural and anthropogenic pressures, and measurability of species responses to those effects (Orfanidis *et al*., 2007, 2010; Oliva *et al*., 2012; Orlando-Bonaca *et al*., 2015; Papathanasiou *et al*., 2016). Although *C. nodosa*, is characterized by a large phenotypic plasticity and is adapted to various natural and anthropogenic stressors by different physiological and morphological adaptations (Tsioli *et al*., 2018), in recent decades the species faced a severe decline in several Mediterranean and Atlantic areas (Jensen & Bell, 2001; Papathanasiou, 2013; Rosell-Fieschi & Polifrone, 2014; Fabbri *et al*., 2015).

There is still a lack of long time data series in the northern Adriatic Sea in order to support the conservation status of meadows of *C. nodosa,* while the species is currently protected only within spatially restricted Marine Protected Areas (MPAs). The ecological status of seven *C. nodosa* meadows was recently evaluated in the northern Adriatic area with the MediSkew index (Orlando-Bonaca *et al*., 2015; 2016), developed in accordance with the EU Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) requirements.

Navigation routes are recognized among the main pressures on the status of *C. nodosa* meadows (Orlando-Bonaca *et al*., 2015) in the Gulf of Trieste, related to a high resuspension of sediments and, in consequence, high water turbidity and reduced light levels. Therefore, the aim of this study was to assess the ecological status of the *C. nodosa* meadow growing near the Port of Koper, and discuss the results in comparison to those obtained for the *C. nodosa* meadow from the reference site for this species, the MPA Strunjan Nature Reserve.

MATERIAL AND METHODS

Study area, fieldwork and laboratory work

The Port of Koper is a Slovenian multi-purpose seaport, situated in the northern Adriatic Sea, connecting mainly markets of Central and Southeast Europe with the Mediterranean Sea and Far East. The marine part of the cargo port is composed of three basins (Fig. 1), associated mooring piers and 12 specialized loading terminals (Geodetski Inštitut Slovenije, 2016). The resuspension of sediments is mainly due to the vessel propulsion along navigation routes and by the assistance of tugboats within the port's basin and in front of them. The highest turbidity values were measured during the manoeuvres of large vessels (Žagar *et al*., 2014). In the last decade the Port of Koper also ordered dredging works on the sedimentary bottom along the access channels to Basin I (Luka Koper, 2015), leading to a high sedimentation/resuspension rate.

Cymodocea nodosa samples were collected in July 2018, according to the sampling protocol presented by Orfanidis *et al*. (2007). Within the seagrass meadow found near the Port of Koper, two sites (LuKp1 and LuKp2) were chosen (Fig. 1) along the same isobath

(3 m) and, within each site, two areas (LuKp1_1, LuKp1_2, and LuKp2_1, LuKp2_2) were selected that were ca. 100 m apart. Within each area, five metallic quadrats (25 cm x 25 cm) were randomly placed on the bottom by SCUBA divers. Those five quadrats were considered as replicates of one sample. All shoots of *C. nodosa* enclosed by each quadrat were carefully uprooted. Samples were labelled and individually placed in plastic bags.

To adequately assess time-based trends in the status of *C. nodosa* meadows, including evaluations of the effects of natural disturbances within MPAs, new samples were collected in July 2018 also within the Strunjan Nature Reserve (Fig. 1). The sampling site Cy2 (areas Str_3 and Str_4) was previously sampled in June 2009 and July 2013 and, according to the low score of the Pressure Index for Seagrass Meadows (PISM), the area Str_3 was chosen 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 at the laboratory of the Marine Biology Station Piran. They were slowly defrosted in a refrigerator on the day prior to analysis. Seagrass shoots were then retained in plastic washbasins with seawater. Twenty shoots from each quadrat were randomly chosen (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 sheath, length of the photosynthetic part, and its width. The age of the leaf was designated as adult (if the sheath was well-developed), intermediate (if the sheath was faintly shaped at the leaf basis), and juvenile (if the sheath was absent). The above measurements were made on at least 60 undamaged photosynthetically active leaves (adults and/or intermediates) from each quadrat. One sample was composed of five replicates of 60 leaves (300 leaves in total).

Fig. 1: Map of sampling sites for** Cymodocea nodosa **in Slovenian marine waters in 2018: near the Port of Koper (LuKp1 and LuKp2) and in the Moon Bay (Cy2), within the Strunjan Nature Reserve.
Sl. 1: Zemljevid vzorčevalnih lokalitet kolenčaste cimodoceje v slovenskem morju v 2018: blizu Luke Koper (LuKp1 in LuKp2) in v Mesečevem zalivu (Cy2) znotraj naravnega rezervata Strunjan.

Data analysis

To explore the nature of leaf lengths of *C. nodosa* in the two sampling locations, frequency histograms of ln-transformed data of lengths of the photosynthetic part of leaves (adult and intermediate) were prepared. In parallel, summary statistics for each area were examined. The normal distribution of ln-transfomed leaf lengths in every area was tested with the Kolmogorov-Smirnov test for normality (Dytham, 2003).

To quantify changes in the photosynthetic part of leaf length distribution, the MediSkew index was calculated (for details see Orlando-Bonaca *et al.*, 2015). Boundaries among status classes for the MediSkew index were set equidistantly (Table 1). Five status classes are adequate for the assessment of the Ecological Status (ES) according to the WFD. Furthermore, High and Good classes indicate a Good Environmental Status (EnS) according to the MSFD, while classes Moderate, Poor and Bad are considered as Not Good EnS.

Tab. 1: Boundaries among status classes for the MediSkew index. For the assessment of ES according to WFD, five classes should be used. For the assessment of EnS according to the MSFD, classes High and Good indicate a Good EnS, while classes Moderate, Poor and Bad are considered as 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

RESULTS

Cymodocea nodosa leaf length parameters in 2018

C. nodosa parameters per sampling area are reported in Table 2, while frequency distributions of leaf lengths in different areas are presented in Fig. 2. *C. nodosa* leaves were much shorter in the areas within the Moon Bay (Cy2) than in those near the Port of Koper, and consequently also the median values (Tab. 2, Fig. 2).

The differences in the median values of leaf lengths between sampling areas LuKp1_1 and LuKp1_2 were statistically significant (Mann-Whitney U = 52219.5, $P = 0.0003$), as well as those between sampling areas LuKp2_1 and LuKp2_2 (U = 53753.5, $P < 0.0001$), and between sampling areas Str_3 and Str_4 (U = 48975, $P = 0.0306$). Moreover, also between sampling sites LuKp1 and LuKp2 (U = 247070.5, $P < 0.0001$), between LuKp1 and Cy2 (U = 336657.5, $P < 0.0001$), and between LuKp2 and Cy2 (U = 331878, $P < 0.0001$), all the differences in the median values of leaf lengths were statistically significant.

Taking into account the frequency distributions of ln-transfomed leaf lengths of every single sample (Fig. 2), the Kolmogorov-Smirnov test for normality revealed that only the samples from areas Str_3 and Str_4 are normally distributed (D = 0.047, $P > 0.05$, and D = 0.026, $P > 0.05$, respectively), while all the other samples are not normally distributed. Accordingly, the skewness |G| was the lowest for the sample from area Str_4, while was the highest at the area LuKp2_1 (Tab. 2).

Assessment of the status of *Cymodocea nodosa*

MediSkew index values for each sampled area are presented in Table 3. The lowest value of MediSkew was found for the area Str_4, which was the closest to virtual reference conditions (MediSkew = 0). The highest values of MediSkew were calculated from samples collected at the area LuKp1_1 (Tab. 3), the nearest to the Port of Koper.

The Ecological Status (according to the WFD) and the Environmental Status (according to the MSFD) of sampling sites were assessed according to the boundaries in Table 1. Site Cy2 (and also both areas) was classified as High ES, site LuKp1 as Bad ES and LuKp2 as Poor ES (Table 3). According to MSFD requirements, only the samples collected in Cy2 (both areas) achieved a Good EnS.

DISCUSSION

The MediSkew value at site Cy2 in July 2018 was even lower than the value in July 2013 (MediSkew = 0.05) and in June 2009 (MediSkew = 0.08; for details see Orlando-Bonaca *et al.*, 2016). This result confirms that this site can still be considered as the reference site for the Gulf of Trieste. However, in 2018 the area Str_4 had a lower MediSkew value than the reference area Str_3, even if the difference between the MediSkew values for the two areas is insignificant (Tab. 3). According to PISM, site Cy2 is still without any anthropogenic pressures, apart from the impact of bathing in the summer period. However, since the site is difficult to reach from the hinterland and anchoring in the bay is not allowed, to our opinion this pressure could be considered as negligible. Light conditions for the *C. nodosa* meadow in the Moon Bay are mostly influenced by natural sedimentation rates and resuspension of sediments due to the erosion of an 80 m high flysch cliff along the coastline.

The leaves of *C. nodosa* sampled in the meadow near the Port of Koper are the longest measured in Slovenian coastal waters so far (see previous results in

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 in sites near the Port of Koper (LuKp1 and LuKp2) 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 iz lokalitet blizu Luke Koper (LuKp1 in LuKp2) in v Mesečevem zalivu (Cy2, Naravni rezervat Strunjan) v 2018. Referenčna mediana v 2018 je bila 13,95 cm.

Site	Area	Date	min length (cm)	max length (cm)	mean (cm)	median (cm)	|G|
Cy2	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	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	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

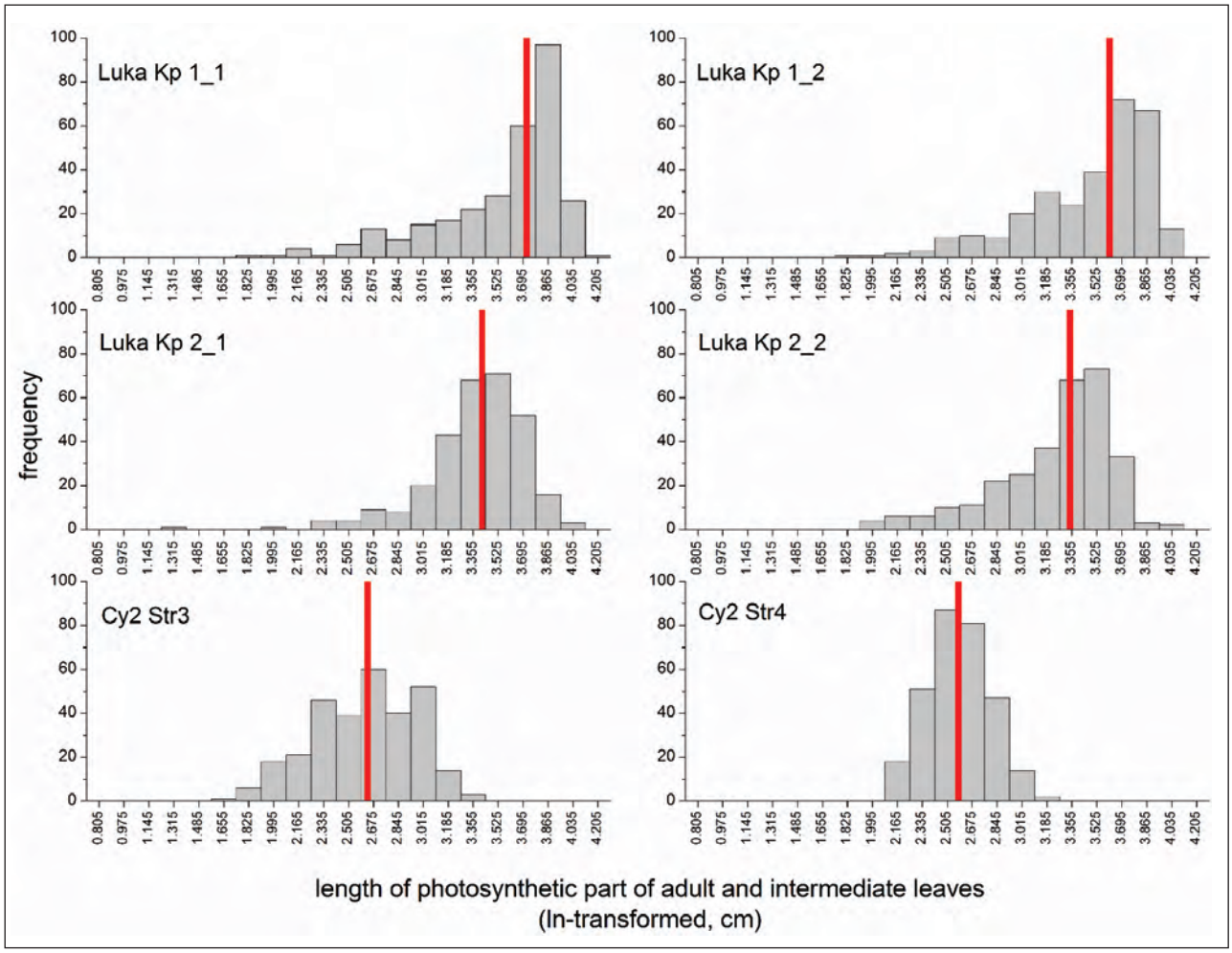

Fig. 2: Frequency histograms of ln-transformed lengths of the photosynthetic part of adult and intermediate leaves (n = 300) for *Cymodocea nodosa* ***in the four areas near the Port of Koper (LuKp1_1, LuKp1_2, LuKp2_1, LuKp2_2) and two areas in the Moon Bay (Str_3 and Str_4) in 2018. Median lengths are denoted by red vertical lines.***
Fig. 2: Frekvenčni histogrami ln-transformiranih dolžin fotosintetskega dela odraslih in srednjih listov (n = 300) za kolenčasto cimodocejo na štirih območjih v bližini koprskega pristanišča (LuKp1_1, LuKp1_2, LuKp2_1, LuKp2_2) in dveh območjih v Mesečevem zalivu (Str_3 in Str_4) v letu 2018. Mediane so označene z rdečimi navpičnimi črtami.

Orlando-Bonaca *et al.*, 2015). Seagrasses are mostly light-limited (Touchette & Burkholder, 2000) therefore, when exposed to low light levels due to high water turbidity, they respond by an increasing allocation of biomass to leaves. By prolonging leaves, marine plants can capture more light and convert it into photosynthetic production (Greve & Binzer, 2004). According to the present results, we hypothesize that the sampling area LuKp1_1, the closest to the Port of Koper, is the most impacted by sediment resuspension due to maritime traffic. Since the average maximum leaf length at site LuKp2 (55.5 cm) is lower than at site LuKp1 (61.65 cm) we can assume that with the increasing distance from the Port of Koper the sediment resuspension rate is decreasing.

In order to identify and monitor the main pressures on seagrass meadows and other benthic communities, and to correctly set threshold values, the Port of Koper should prepare and implement a long-term monitoring program inside the harbor area and in its surrounding, especially for water turbidity related to sediment resuspension/deposition. Recently, Airoldi *et al.* (2016) summarized the status and trends of the harbour of Ravenna in order to provide scientific support for the regeneration project of this area and to increase the resilience of local fragile ecosystems. The preparation of such an overview would be very useful also for the Port of Koper. Moreover, a long-term monitoring of the status of the *C. nodosa* meadow growing in the immediate vicinity of the port area is strongly recommended.

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Tab. 3: MediSkew index values for studied sampling areas of** Cymodocea nodosa **in 2018 and assessment of the Ecological Status (according to the WFD) and Environmental Status (according to the MSFD) in the Moon Bay and near the Port of Koper.
Tab. 3: Vrednosti indeksa MediSkew za raziskane lokalitete s kolenčasto cimodocejo v 2018 in opredelitev ekološkega stanja (glede na OVS) in okoljskega stanja (glede na ODMS) v Mesečevem zalivu in blizu Luke Koper.

Site	Area	Area's MediSkew	Site's MediSkew	Ecological Status	Environmental Status
Cy2	Str_3	0.065	0.04	High	Good / Achieved
	Str_4	0.024			
LuKp1	LuKp1_1	1.00	0.94	Bad	Not good / Not achieved
	LuKp1_2	0.87			
LuKp2	LuKp2_1	0.79	0.715	Poor	Not good / Not achieved
	LuKp2_2	0.64			

VPLIV KOPRSKEGA PRISTANIŠČA NA TRAVNIK KOLENČASTE CIMODOCEJE (*CYMODOCEA NODOSA*)

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POVZETEK

*Kolenčasta cimodoceja (*Cymodocea nodosa*) je zaradi široke razprostranjenosti v Sredozemskem morju, občutljivosti na razne pritiske in merljivosti odziva na te pritiske uporabna kot dober indikator okoljskih sprememb. Med glavnimi pritiski je v Tržaškem zalivu še posebej pomembna plovba na plovnih poteh, ki povzroča znatno resuspenzijo sedimentov in posledično veliko turbidnost ter slabo osvetljenost. Avtorji so vzorce, nabrane na morskem travniku kolenčaste cimodoceje blizu Luke Koper uporabili za ovrednotenje ekološkega stanja z uporabo MediSkew indeksa. Dobljene rezultate so primerjali z rezultati iz referenčnega območja v strunjanskem naravnem rezervatu.*

Ključne besede: *Cymodocea nodosa,* MediSkew indeks, dolžine listov, Luka Koper, severni Jadran

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