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The most suitable time and depth to sample *Cymodocea nodosa* (Ucria) Ascherson meadows in the shallow coastal area. Experiences from the northern Adriatic Sea

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The Lesser Neptune grass, Cymodocea nodosa*, is the most common seagrass species in shallow sheltered to semi-exposed sites along the Mediterranean soft bottom. The MediSkew index was recently developed as an improvement of the CymSkew index, in order to assess the status of* C. nodosa *meadows correctly in view of the implementation of three European Directives. The index takes into account the length of the photosynthetic part of* C. nodosa *leaves, which increases from the less degraded meadow to the most degraded meadow. To adequately assess temporal and spatial trends in the status of* C. nodosa *meadows, including estimates of the effects of natural disturbances within marine protected areas, the MediSkew index was applied to new samples collected at 3 m and at 6 m of depth, during two months (in July and in September). The analyses are discussed in view of monitoring and conservation of* C. nodosa *meadows. According to the results of this study, the monitoring programme in the northern Adriatic Sea should be conducted in July, with the collection of samples at 3 m of depth. The presented sampling and assessing methodology proved to be time- and cost-effective for the evaluation of the status of* C. nodosa *meadows and of human-induced pressures.*

Key words: *Cymodocea nodosa*, MediSkew index, leaf lengths, sampling time, sampling depth, northern Adriatic Sea

INTRODUCTION

Seagrasses are amongst the most valuable ecosystems on shallow subtidal soft bottoms worldwide (COSTANZA *et al*., 1997; TUYA *et al*., 2014). They are considered as ecological engineers (WRIGHT & JONES, 2006), providing food, shelters and nursery areas for a variety of fish and invertebrates (HEMMINGA & DUARTE, 2000; COMO *et al*., 2008; ESPINO *et al*., 2015). Moreover, seagrass beds stabilize sediments by trapping fine sediments and particles that are suspended in the water column (CABAÇO *et al*., 2010), provide protection against coastal erosion (TERRADOS & BORUM, 2004), produce oxygen (PEDUZZI & VUKOVIČ, 1990) and play an important role as global carbon sinks (DUARTE *et al*., 2010). Therefore, they are recognized as one of the priority habitats in the EU Habitat Directive (HD, 92/43/EEC).

Since coastal ecosystems are subjected to very intense human pressures, affecting light and nutrient resources (HEMMINGA & DUARTE, 2000), as well as causing mechanical damage to the sea bottom by anchoring, dredging and filling, as part of coastal urban development (SHORT & WYLLIE-ECHEVERRIA, 1996; MONTE-

FALCONE *et al.*, 2008; MARBÀ *et al.*, 2014), rapid and widespread decline of seagrass meadows has been reported from many areas (ORTH *et al.*, 2006; TUYA *et al.*, 2013; FABBRI *et al.*, 2015). According to the global assessment provided by WAYCOTT *et al.* (2009), seagrasses have been disappearing at a rate of 110 km^2 yr^{-1} since 1980, a value comparable to the loss rates reported for mangroves, coral reefs, and tropical rainforests. Nowadays, the changes observed in seagrass distribution point out that phenomena of meadow regression should be attributed to the cumulative effects of local stressors, rather than to processes at basin scale, such us climate change (TELESCA *et al.*, 2015). In the Mediterranean Sea area, the Regional Activity Centre for Specially Protected Areas (RAC/SPA) is responsible for drafting guidelines for carrying out impact studies; therefore, PERGENT-MARTINI & LE RAVALLEC (2007) have prepared such guidelines for Impact Assessment on Mediterranean seagrass meadows, but their actual application at national level is quite questionable.

In the Adriatic Sea, four native species of seagrass are present: *Cymodocea nodosa* (Ucria) Ascherson*, Posidonia oceanica* (Linnaeus) Delile*, Zostera marina* Linnaeus and *Zostera noltei* Hornemann (LIPEJ *et al.*, 2006). Among them, *C. nodosa* (the Lesser Neptune grass or seahorse grass) is the most common species at shallow sheltered to semi-exposed sites, not only in the Adriatic Sea, but along all Mediterranean soft bottoms and at some locations in the north Atlantic, including the Canary Islands and Madeira (DEN HARTOG, 1970; MASCARÓ *et al.*, 2009; OSPAR, 2010). *C. nodosa* forms meadows that are mono-specific or mixed with *Zostera noltei* (MAZZELLA *et al.*, 1993), and can be found down to 40 m of depth (BORUM & GREVE, 2004). Despite the confirmed global regression of seagrass meadows, a recent paper reported that the population trend of *C. nodosa* along the North Atlantic and Mediterranean coasts could be considered "*stable*" and categorized the species as "*least concern*" according to the IUCN Red List of Threatened Species criteria (SHORT *et al.*, 2011). However, in the Mediterranean Sea, the species is considered a valid indicator of environmental changes, due to its widespread distribution, sensitivity to a variety of natural and anthropogenic impacts, and measurability of species responses to those impacts (REIZOPOULOU & NICOLAIDOU, 2004; ORFANIDIS *et al.*, 2007, 2010; OLIVA *et al.*, 2012; ORLANDO-BONACA *et al.*, 2015; PAPATHANASIOU *et al.*, 2016).

In the northern Adriatic Sea, there is still a lack of data to support the conservation status of *C. nodosa* meadows, and the species is protected only within Marine Protected Areas (MPAs). The status of seven *C. nodosa* meadows (representing dominant communities on shallow soft bottoms, in the depth range of 1 m to approx. 10 m) was recently evaluated for a first assessment in the northern Adriatic with the newly developed MediSkew index (ORLANDO-BONACA *et al.*, 2015). The index was developed in accordance with the EU Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) requirements. The MediSkew index is an improvement of the CymSkew index (ORFANIDIS *et al.*, 2007, 2010) that takes into account the length of the photosynthetic part of *C. nodosa* leaves, which increases from the less degraded meadow to the most degraded meadow.

To adequately assess temporal trends in the status of *C. nodosa* meadows, including estimations of the effects of natural disturbances within MPAs, new samples were recently collected in the Strunjan Nature Reserve, which is considered to be the reference site for this species in the Gulf of Trieste (ORLANDO-BONACA *et al.*, 2015). For this study, the MediSkew index was applied not only to samples collected at 3 m of depth, as proposed in the sampling methodology, but also at 6 m of depth. Moreover, sampling was carried out twice, in July and in September. The results of the analysis are discussed in view of monitoring and conservation of *C. nodosa* meadows in the northern Adriatic Sea.

MATERIAL AND METHODS

Study area

The Gulf of Trieste is a shallow marine basin (average depth of around 17 m), which constitutes the northernmost part of the Mediterranean Sea (STRAVISI, 1983). It is influenced by numerous freshwater inflows, high bottom sediment resuspension and densely populated coastal areas. Sea temperature ranges from 6 °C in February to over 25 °C in summer, while salinity fluctuates between 33 and 39 (BOGUNOVIĆ & MALAČIČ, 2008). Recent studies have underlined the oligotrophication of the basin over the last decade (MOZETIČ *et al*., 2010; 2012).

The surface sediments of the coastal zone that is a few dozen meters wide and coincides with the 5 m isobath, consist of silt and sandy silt, with approximately 30% carbonate (OGORELEC *et al*., 1991).

Field and Laboratory Work

Sampling was carried out within the *Cymodocea nodosa* meadow located in Moon Bay (Mesečev zaliv), a part of the Strunjan Nature reserve, the largest marine protected area in Slovenia. In this MPA, the coastal area is still in its near-natural state, characterized by Eocene flysch cliffs composed of interbedded sandstone and marlstone, with small gravel/pebble beaches at the base (OGORELEC *et al*., 1991). The sampled *C. nodosa* meadow can be considered part of the biocoenosis of superficial muddy sands in sheltered waters (LIPEJ *et al*., 2006).

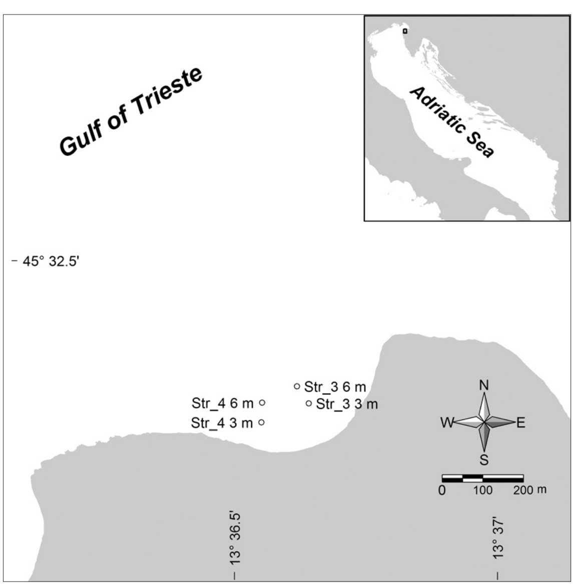

Fig. 1. Map of sampling site Cy2 for Cymodocea nodosa *in Moon Bay (Strunjan Nature Reserve, Gulf of Trieste). Two areas (Str_3 and Str_4) were sampled at two depths (3 m and 6 m)*

Samples were collected in July and September 2013, according to the sampling protocol presented by ORFANIDIS *et al.* (2007). The sampling site Cy2 (Fig. 1) was previously sampled in June 2009 and, according to the low score of the Pressure Index for Seagrass Meadows (PISM), was chosen as the reference site for *C. nodosa* in the Gulf of Trieste (ORLANDO-BONACA *et al*., 2015). At this site two areas (Str_3 and Str_4), ca. 100 m apart, were sampled. In both areas, five metallic quadrats (25 cm x 25 cm) were randomly placed on the bottom by SCUBA divers at two depths: 3 m and 6 m. All shoots of *C. nodosa* within each quadrat were manually uprooted carefully. Samples were placed individually in labeled plastic bags.

At the laboratory, the samples of *C. nodosa* were stored in a freezer at −20°C. They were slowly thawed in a refrigerator on the day prior to analysis. Seagrass shoots were then kept in plastic washbasins 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 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).

Data analysis

To explore the nature of leaf lengths of *C. nodosa* in Moon Bay, 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 sample was tested with the Shapiro-Wilk (W) test (RAHMAN & GOVIDARAJULU, 1997).

To quantify changes in the photosynthetic part of leaf length distribution, the MediSkew index (ORLANDO-BONACA *et al*., 2015) was calculated using the following sequence of steps. First, the skewness for each sample was calculated with the adjusted Fisher-Pearson standardized moment coefficient (Eq. 1):

$$G = \frac{n}{(n-1)(n-2)} \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{s} \right)^3$$

where n is the sample size (= 300), x_i is the ln-transformed length of the photosynthetic part of the single seagrass leaf, $\bar{x}$ is the sample mean (arithmetic mean of ln-transformed lengths of photosynthetic parts of 300 leaves) and s is the sample standard deviation. The skewness G of pristine meadows calculated with ln-transformed leaf lengths is close to zero, while that of disturbed meadows shows different degree of deviation from zero.

Secondly, the MediSkew index was calculated, using the following equation (Eq. 2):

$$MediSkew = \frac{|(Md_{area} - Md_{RC})|/20 \text{ cm} + |G|/2}{2}$$

where Md_{area} is the median length in cm of the photosynthetic part of adult and intermediate *C. nodosa* leaves in a certain area (n = 300), Md_{RC} is the median length of the photosynthetic part of adult and intermediate *C. nodosa* leaves in the reference area Str_3 at 3 m depth in June or July (in 2009, Md_{RC} was 11.5 cm, while in 2013 it was 15.1 cm), and |G| is the absolute value of skewness of *C. nodosa* ln-transformed leaf lengths for a certain area (n = 300), calculated using Eq. 1.

Moreover, since both skewness and the difference between medians needed an appropriate weight to guarantee the index an ecologically sound output, in Eq. 2 they were divided by a number high enough to obtain a similar range of values (e.g. from 0 to 1). For differences between medians, a round number (20 cm) close to the maximum difference obtained for our areas was chosen, while the absolute value of skewness was divided by 2, which was calculated as a theoretical maximum value for |G| (ORLANDO-BONACA *et al*., 2015). This weighting gave the deviation from the reference median a double importance in comparison with |G|.

As the MediSkew index is based on median values and shape of data distribution (skewness), non-parametric tests were chosen for the quantification of differences of leaf length parameters between the two sampling areas (Str3 and Str4), two sampling depths (3 m and 6 m) and two sampling months (July and September). The differences between medians were tested with the Mann-Whitney test (U), while differences between distributions of ln-transformed data were tested with the Kolmogorov-Smirnov test (D) (DYTHAM, 2003).

Boundaries among status classes for the MediSkew index are set equidistantly (Table 1, ORLANDO-BONACA *et al*., 2015). Five equal classes are appropriate in case of a linear relationship between pressures and impacts (URIARTE & BORJA, 2009; OLIVA *et al*., 2012). Such a linear relationship was demonstrated between the PISM and the MediSkew in the Gulf of Trieste (ORLANDO-BONACA *et al*., 2015). These five 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.

Table 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 (Orlando-Bonaca et al.*, 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 2013

C. nodosa parameters per sampling area are reported in Table 2, while frequency distributions of leaf lengths in different areas, depths and months (samples) are presented in Fig. 2. There were no substantial differences in the median values of leaf lengths between the two sampling areas (U = 720495, P = 0.1848), while the differences were statistically significant between sampling depths (U = 394094.5, P < 0.0001) and months (Mann-Whitney U = 887841, P < 0.0001). At the depth of 3 m, the leaves were substantially shorter (median value of all leaf lengths for both months was 12.95 cm) than at the depth of 6 m (median value of all leaf lengths for both months was 18.0 cm). As regards month of sampling, the leaves were shorter in September (median value of all leaf lengths for both depths was 13.5 cm) than in July, when the overall median length was 16.1 cm. Accordingly, the shortest leaves with a vis-

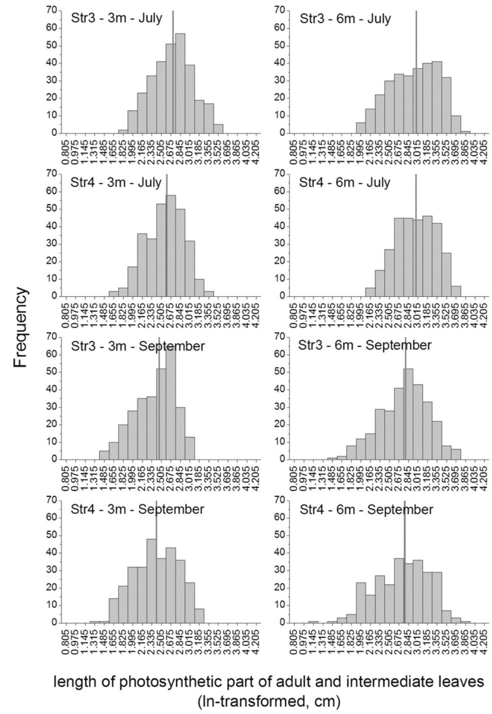

Fig. 2. Frequency histograms of ln-transformed lengths of the photosynthetic part of adult and intermediate leaves (n = 300) for Cymodocea nodosa *in the two areas of Moon Bay (Gulf of Trieste) in 2013. Median lengths are denoted by red vertical lines*

Table 2. Length parameters (minimum, maximum, mean, median) and absolute value of skewness (|G|) of the photosynthetically active part of Cymodocea nodosa *leaves from the sampling areas in Moon Bay (Strunjan Nature Reserve, Gulf of Trieste). The MdRC in 2009 was 11.5 cm, while in 2013 it was 15.1 cm*

Area	Year	Month	Sampling depth (m)	min length (cm)	max length (cm)	mean (cm)	median (cm)	|G|
Str_3	2009	June	3	3.4	25.5	12.3	**11.5**	0.269
Str_4	2009	June	3	5.2	26.1	12.5	12.3	0.307
Str_3	2013	July	3	5.9	33	15.9	**15.1**	0.014
Str_3	2013	July	6	7.1	46.5	20.9	19.8	0.190
Str_4	2013	July	3	5.4	29.4	13.8	13.5	0.254
Str_4	2013	July	6	8.8	40.5	20.9	19.7	0.126
Str_3	2013	September	3	4.3	21.5	11.6	11.8	0.489
Str_3	2013	September	6	4.8	42.2	17.4	16.4	0.296
Str_4	2013	September	3	4.0	26.3	12.1	11.2	0.135
Str_4	2013	September	6	3.1	46.1	17.2	16.1	0.329

ible sheath at the base were 3.1 cm long and were collected in September at 6 m of depth in area Str_4. Moreover, the average and maximum lengths of the photosynthetic parts of *C. nodosa* leaves were the lowest in September at 3 m of depth, but in area Str_3. On the contrary, the longest adult leaves were more than 46 cm and were measured in July at 6 m of depth in area Str_3.

Differences between shape of distributions were also tested: as with the median, the frequency distributions of all data were not significantly different between the two sampling areas (D = 0.033, $P = 0.526$). Differences in distribution were found between sampling depths (D = 0.331, $P < 0.0001$) and months (D = 0.193, $P < 0.0001$). Taking into account the frequency distributions of ln-transfomed leaf lengths of every single sample (Fig. 2), the Shapiro-Wilk test for normality revealed that only the sample from area Str_3 at 3 m depth in July is normally distributed (W = 0.991, $P > 0.05$), while all the other samples are not normally distributed (W, $P < 0.05$). Accordingly, the skewness |G| was the lowest for the July sample from area Str_3 at 3 m depth, while in the same area and depth in September, the skewness |G| was the highest compared to all the other samples (Table 2).

Cymodocea nodosa leaf length parameters in 2009

When comparing the samples collected in summer 2009 and 2013, it was found that in June 2009 *C. nodosa* leaves were significantly shorter than in July 2013 in both areas (U = 28782.5, $P < 0.0001$ for Str_3 and U = 37468.5, $P = 0.0004$ for Str_4) (Figs. 2 and 3). In 2009, as in 2013, also only the ln-transformed leaf lengths from area Str_3 were normally distributed (W = 0.991, $P = 0.06$). The shape of the distributions in both years were significantly different for both areas (D = 0.283, $P < 0.0001$ for Str_3 and D = 0.147, $P = 0.0025$ for Str_4). The skewness |G| of the 2009 samples was higher than in the 2013 (Table 2; Fig. 2).

Assessment of the status of *Cymodocea nodosa*

MediSkew index values for each studied area, sampling month and depth are presented in Table 3. The lowest value of MediSkew was found for area Str_3 in July at 3 m of depth, which was closest to virtual reference conditions (MediSkew = 0). At site Cy2 scale, the value in July 2013 at 3 m of depth was lower

Table 3. MediSkew index values for studied areas (Str_3 and Str_4) of Cymodocea nodosa *in different years, months and depths, and assessment of the Ecological Status (according to the WFD) and Environmental Status (according to the MSFD) in Moon Bay (Strunjan Nature Reserve, Gulf of Trieste)*

Area	Year	Month	Sampling depth (m)	Area's MediSkew	Cy2 site's MediSkew	Ecological Status	Environmental Status
Str_3	2009	June	3	0.067	0.08	High	Good/Achieved
Str_4	2009	June	3	0.095			
Str_3	2013	July	3	0.003	0.05	High	Good/Achieved
Str_4	2013	July	3	0.104			
Str_3	2013	July	6	0.165	0.16	High	Good/Achieved
Str_4	2013	July	6	0.146			
Str_3	2013	September	3	0.205	0.17	High	Good/Achieved
Str_4	2013	September	3	0.131			
Str_3	2013	September	6	0.105	0.11	High	Good/Achieved
Str_4	2013	September	6	0.107			

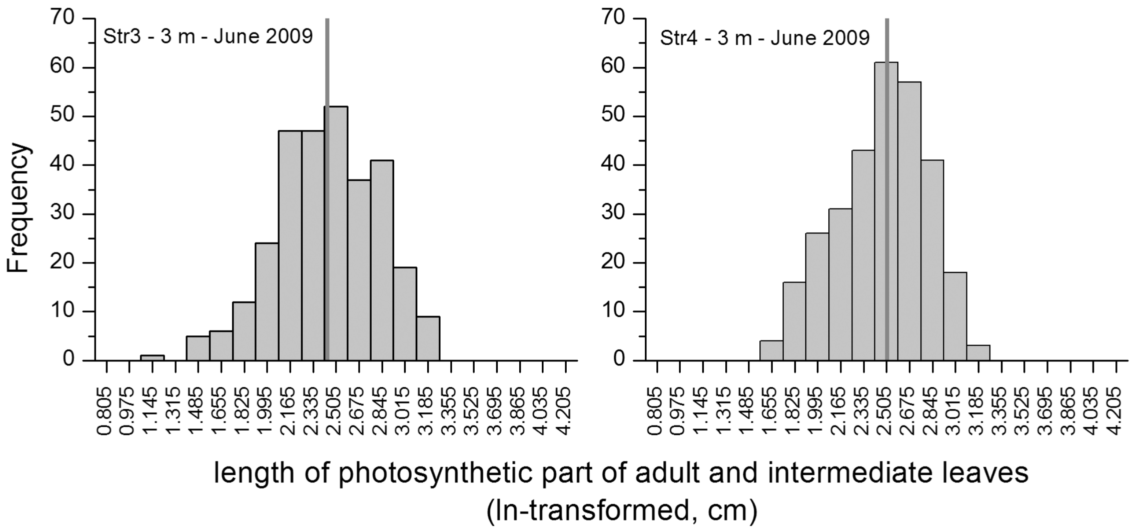

Fig. 3. Frequency histograms of ln-transformed lengths of the photosynthetic part of adult and intermediate leaves (n = 300) of Cymodocea nodosa *in the two areas of Moon Bay (Gulf of Trieste) in June 2009. Median lengths are denoted by red vertical lines.*

that the value in June 2009, while the value for July 2013 at 6 m of depth was higher. The highest values of MediSkew were calculated from samples collected in September 2013 at 3 m of depth (Table 3).

The Ecological Status (according to the WFD) and the Environmental Status (according to the MSFD) of site Cy2 in different months and depths were assessed according to the boundaries in Table 1. Site Cy2 was classified as High ES (Table 3) at both sampling depths and months. According to MSFD requirements, all collected samples achieved a Good EnS in Moon Bay.

DISCUSSION

Four years after the first sampling of *Cymodocea nodosa* in the Strunjan Nature Reserve in 2009, the evaluation of the status in Moon Bay using data from 2013 confirmed that the site is justifiably considered a reference site for this species in the Gulf of Trieste, as proposed by ORLANDO-BONACA *et al.* (2015). This site still has no anthropogenic pressures according to PISM, which means absence of: artificial coastline, direct leaching, mariculture, rainwater discharges, industrial wastewater discharges and navigation routes (see methodology in ORLANDO-BONACA *et al.*, 2015). Among natural pressures that can impact the status of *C. nodosa* meadow in this bay, the most important is the presence of a high flysch cliff on the coast, causing higher natural sedimentation rates and resuspension of sediments in coastal waters, and consequently affecting light conditions. Knowing that seagrass leaf length is the parameter most influenced by different environmental pressures (DANGER *et al.*, 2008), and that light-limited marine plants are usually responding by increasing allocation of biomass to leaves when exposed to low light levels (TOUCHETTE & BURKHOLDER, 2000; PAPATHANASIOU *et al.*, 2016), the evidence that *C. nodosa* leaves are longer at 6 m than at 3 m of depth is not an unexpected result (Table 2). By lengthening the leaves, more light can be captured and converted into photosynthetic production (LONGSTAFF & DENNISON, 1999; GREVE & BINZER, 2004). However, even with statistically significant longer leaves, samples collected at 6 m of depth at this reference site still presented a High ES (Table 3). Therefore, the information added from the analysis of samples collected at 6 m of depth were not relevant (or not necessary) for a correct evaluation of the status of *C. nodosa* meadows using the MediSkew index.

The High ES achieved by samples collected in September had not been hypothesized from the beginning of the study, since very little is known about the growth cycle of *C. nodosa* leaves in the northern Adriatic Sea. During a research project conducted from April 1987 to March 1988 in the Gulf of Trieste, the maximum leaf length of *C. nodosa* was observed in August (PEDUZZI & VUKOVIČ, 1990); however there is no available data regarding the rates of leaf fall for this area. Our hypothesis of lower maximum leaf length in September, compared to July (Table 2), is related to the probable loss of the oldest leaves of each *C. nodosa* shoot right at the turn of August and September. This hypothesis is supported by the results of CUNHA & DUARTE (2007) who registered a maximal rate of *C. nodosa* leaf loss in Ria Formosa lagoon (South Portugal) in August. Data from coastal waters of Ischia Island (Tyrrhenian Sea) also showed that the monthly mean length of *C. nodosa* leaves decreased from August to September of the same year (CANCEMI *et al.*, 2002). Considering all data collected in 2009 and 2013, it can be concluded that July is the best month for sampling *C. nodosa* leaves in the northern Adriatic Sea, in order to obtain the oldest completely developed leaves, before they start to fall. Since human-induced and natural pressures on *C. nodosa* meadows could vary each year, it is recommended to sample the reference area (in order to obtain the Md_{RC} value for use in the MediSkew equation) in the same month and year as other sites of *C. nodosa* meadows in the same geographic area.

Even if the *C. nodosa* meadow in Strunjan Nature Reserve proved to be in good health, with a Good EnS achieved according to MediSkew values, a regular monitoring programme should be established as soon as possible, outside and inside MPAs. A scale-based sampling approach, including MPAs (with almost no anthropogenic pressures), will help in separating variability related to natural and human-induced pressures. Also, assuming that the level of anthropogenic pressures in MPAs will remain negligible, natural disturbance events (such as heavy storms or increasing sedimentation rates that can lead to light reduction) can have negative consequences on a meadow's status. TUYA *et al.* (2013) already reported that the recovery of *C. nodosa* meadows after large meteorological disturbance events may take a long time, considering the low resilience of this seagrass species to such natural pressures. According to the results of this study, the monitoring programme in the northern Adriatic should be conducted in July, with the collection of samples at 3 m of depth. However, the application of legislative instruments, such as the implementation of a monitoring programme, should be accompanied by the scientific evaluation of key coastal ecological and human-induced factors, in order to mitigate the impact of multiple pressures and develop effective management solutions. The presented sampling and assessment methodology proved to be time- and cost-effective for the evaluation of the status of *C. nodosa* meadows and of human-induced pressures.

The conservation of *C. nodosa* meadows makes these coastal ecosystems the right choice for future research on poorly known climate-related changes, above all along the coastline of the Gulf of Trieste, where marine organisms are unable to move northwards.

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Najprikladnije vrijeme i dubina za uzorkovanje livada morske cvjetnice *Cymodocea nodosa* (Ucria) Ascherson, u plitkom obalnom području. Iskustva iz sjevernog Jadrana

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SAŽETAK

Čvorasta morska resa, *Cymodocea nodosa*, najučestalija je vrsta morske cvjetnice u plitkim zaklonjenim i poluzaklonjenim uvalama duž mediteranskog mekog dna. MediSkew indeks je nedavno razvijen kao poboljšanje indeksa CymSkew u cilju ispravne procjene stanja livada vrste *C. nodosa* u skladu s provedbom triju europskih direktiva. Indeks u obzir uzima duljinu foto sintetskog dijela lišća *C. nodosa*, koji se povećava od manje do najviše degradirane livade. Kako bi se na odgovarajući način procijenili vremenski i prostorni trendovi stanja livada vrste *C. nodosa,* uključujući procjene učinaka prirodnih poremećaja unutar zaštićenih morskih područja, MediSkew indeks primijenjen je na nove uzorke prikupljene na 3m dubine i na 6m dubine, tijekom srpnja i rujna. Analize su rađene s obzirom na praćenje i očuvanje livada vrste *C. nodosa.* Prema rezultatima ovog istraživanja, program praćenja u sjevernom Jadranu trebao bi biti proveden u srpnju, a uzorci bi se prikupljali na 3m dubine. Prikazana metodologija uzorkovanja i procjenjivanja pokazala se vremenski i troškovno učinkovita za procjenu stanja livada vrste *C. nodosa* i ljudskog utjecaja na njih.

Ključne riječi: *Cymodocea nodosa*, MediSkew indeks, dužina listova, vrijeme uzorkovanja, dubina uzorkovanja, sjeverni Jadran