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A new index (MediSkew) for the assessment of the *Cymodocea nodosa* (Ucria) Ascherson meadow's status

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ABSTRACT

In the Mediterranean region *Cymodocea nodosa* is widely distributed throughout shallow sites. Therefore, a correct assessment of the status of its meadows is of great importance for the implementation of the Water Framework Directive (WFD), the Marine Strategy Framework Directive (MSFD), and the Habitat Directive (HD), especially for areas where *Posidonia oceanica* meadows (the most frequently used indicator in the Mediterranean Sea) are rare or not present. The previously proposed index for the evaluation of the status of *C. nodosa* meadows (CymoSkew) is based on ln-transformed relative frequencies of photosynthetic leaf lengths, which we believe is statistically questionable. Therefore, we further improved the methodology and developed a new index named MediSkew, where ln-transformation is applied to raw data of leaf lengths. More specifically, the index is a combination of two metrics, both based on *C. nodosa* leaf length: deviation from the reference median length (Medi-) and skewness of the length frequency distribution (-Skew), though greater importance was assigned to the first. To develop the classification criteria for the assessment of the status, also a Pressure Index for Seagrass Meadows (PISM), for the evaluation of pressure–impact relationships, was developed. The MediSkew is meant to be a rapid screening method for wide areas, therefore the index should be tested for the assessment of the status of *C. nodosa* meadows throughout Mediterranean coastal waters.

1. Introduction

Seagrass communities play a major role worldwide in coastal environments, forming extensive and highly productive habitats, providing food, shelter, and essential nursery areas for fish and invertebrates (Hemminga and Duarte, 2000). Moreover, seagrass beds are known to trap fine sediments and particles that are suspended in the water column, which increases water transparency, and provide protection against coastal erosion (Terrados and Borum, 2004).

Because of the key ecological services they provide to coastal zones, seagrass meadows rank among the most valuable ecosystems in the biosphere (Costanza et al., 1997) and, therefore, they are recognized as one of the priority habitats in the EU Habitat Directive (HD, 92/43/EEC). Their growth and distribution are controlled by the physical, chemical and biological properties of their living environment (Greve and Binzer, 2004; Orth et al., 2006; Waycott et al., 2009; Short et al., 2011). However, seagrass meadows are experiencing a significant widespread decline due to heavy anthropogenic impacts affecting light and nutrient resources (Hemminga and Duarte, 2000) as well as mechanical damage caused by anchoring, dredging, etc.

In European marine waters four native species of seagrass are present: *Posidonia oceanica* (Linnaeus) Delile, *Cymodocea nodosa* (Ucria) Ascherson, *Zostera marina* Linnaeus and *Zostera noltei* Hornemann (Borum and Greve, 2004). All four are present along the coastline of the Gulf of Trieste in the Adriatic Sea (Lipej et al., 2006). Among them, the Lesser Neptune grass or seahorse grass (*C. nodosa*) is the most common species in shallow sheltered to semi-exposed Mediterranean sites (Den Hartog, 1970). *C. nodosa* has a tropical origin and is nowadays restricted to the Mediterranean Sea and some locations in the north Atlantic, from southern Portugal and Spain to Senegal, including the Canary Islands and Madeira (Borum and Greve, 2004; OSPAR, 2010). It forms meadows

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that are mono-specific or mixed with *Z. noltei* (Mazzella et al., 1993), and can be found in deep waters (40 m) (Borum and Greve, 2004). *C. nodosa* is considered a pioneer species which can quickly colonize bare areas of the sea floor (Borum and Greve, 2004). Its rhizome may grow several metres per year (Duarte and Sand-Jensen, 1990; Boudouresque et al., 1994).

As a consequence of the worldwide alteration and deterioration of coastal areas and the need to assess the status of the environment at different levels regulated by European Commission (EC) directives, a growing body of research is focused on biotic indices for coastal waters (reviewed by Martínez-Crego et al., 2010). One of the ecological quality elements commonly used are seagrasses (Martínez-Crego et al., 2008; Mascaró et al., 2013). The high sensitivity of seagrasses to environmental conditions (e.g. water transparency, nutrient levels, erosion, water temperature) and the widespread geographical distribution of these plants make seagrasses useful indicators of coastal sea deterioration (Orth et al., 2006). Marbà et al. (2013) reviewed seagrass indicators used in European coastal waters, and found altogether 49 indices, including 51 metrics. However, for the Mediterranean region, the majority of them assess *P. oceanica* meadows and just two indicators deal with *C. nodosa* directly. Many of them were developed following European Water Framework Directive (WFD, 2000/60/EC) requirements and need to be improved to respond to all Marine Strategy Framework Directive (MSFD, 2008/56/EC) necessities, as was also stressed by Van Hoey et al. (2010) for benthic indicators in general.

Following WFD requirements, the Ecological Status (ES) of the Slovenian meadow of *P. oceanica* was assessed (Lipej et al., 2007). As this meadow is restricted in size (0.64 ha; Turk and Vukovič, 1998), and is the only one of this species in the Gulf of Trieste (besides the small patch of *P. oceanica* near Grado), it was not considered as representative for the area. The ES of *C. nodosa* meadows in Slovenian coastal waters was not assessed, since the MED-GIG group of experts for macrophytes in coastal waters worked mainly on the intercalibration of methods for the assessment of the ES of macroalgae (MED-GIG, 2011). Therefore, it was decided that, according to MSFD, the status of *C. nodosa* meadows should be assessed. MSFD obligates Member States (MS) to achieve or maintain Good Environmental Status (GEnS) of marine waters by 2020. Specifically, each MS shall, in respect of the marine region or subregion concerned, make an initial assessment, determine a set of characteristics for GEnS and establish a set of environmental targets and associated indicators to guide them towards achieving GEnS. Within the initial assessment, the condition of *C. nodosa* meadows was examined (Orlando Bonaca et al., 2012). These meadows represent dominant communities in shallow soft bottoms throughout the Slovenian coastal belt, extending from 1 m to approx. 10 m of depth. The correct assessment of the status of those meadows is of crucial importance for the assessment of the EnS according to Descriptors D1 (Biodiversity), D5 (Eutrophication) and D6 (Sea-floor integrity). In fact, *C. nodosa* has all the ecological characteristics that make a species a valid indicator, such as a large area of distribution (which makes possible the comparison throughout a broader area), sensitivity to certain natural and anthropogenic impacts, and measurability of species responses to those impacts (Reizopoulou and Nicolaidou, 2004; Orfanidis and Panayotidis, 2005).

The only two indices dealing directly with *C. nodosa*, CYMOX and CymoSkew, were chosen as candidates for seagrass status evaluation (Marbà et al., 2013). The CYMOX index (Oliva et al., 2012), used along Spanish Mediterranean coasts, is composed of several metrics on different organisation levels plus some pollution descriptors, such as $\delta^{15}N$, $\delta^{34}S$, root weight ratio and heavy metal content, etc. On the other hand, CymoSkew (Orfanidis et al., 2007, 2010) is based on one single metric and takes into account *C. nodosa* leaf length, specifically its photosynthetic part, which according to Orfanidis et al. (2010) increases from the less degraded meadow to the most degraded meadow. Such phenotypic plasticity was attributed to various interactions among limiting resources by different theories (Gleeson and Tilman, 1992; Danger et al., 2008), mainly to nutrients (Short, 1983; Mvungi and Mamboya, 2012; Marbà et al., 2013) and light (Abal et al., 1994; Orfanidis et al., 2010) availability. Under conditions of intermediate levels of nutrients, typically occurring in the Gulf of Trieste (Mozetič et al., 2010), seagrasses normally increase the uptake and assimilation of nutrients which eventually leads to production of more biomass, i.e. leaf elongation (Fabbri et al., 2015; Tuya et al., 2002). More precisely, seagrasses invest more into below-ground tissues under nutrient limited conditions and, in contrast, more biomass is produced in above-ground tissues when more nutrients are available (in Lee et al., 2007). However, longer exposure to high nutrient concentrations would lead to meadow degradation, but mainly because of the light limitation induced by increased growth of phytoplankton and epiphytes (Papathanasiou et al., 2015; Tuya et al., 2014). The CymoSkew is simpler and less economically demanding for a rapid screening of large areas in comparison to CYMOX. When CymoSkew was applied to our data from the Gulf of Trieste, we observed some inconsistencies in the statistical approach used by the authors. More precisely, the authors made a ln-transformation of relative frequencies of photosynthetic leaf lengths and used these values in the skewness formula (Orfanidis et al., 2007, 2010). Since we believe that the ln-transformation has to be applied to raw leaf length data, we decided to improve the statistical analysis, and develop a new index named MediSkew, with the addition of a second metric based on *C. nodosa* leaf length.

The aim of the study was to provide a rapid screening method for wide areas in order to determine the health of *C. nodosa* meadows. Eventually, in order to be able to classify the Ecological Status (ES) and the Environmental Status (EnS) according to MediSkew values, we developed also a Pressure Index for Seagrass Meadows (PISM) for the evaluation of pressure–impact relationships. Applying the region-specific pressure indices, such as PISM, to develop the classification criteria, we assure that the assessment method (MediSkew) is valid over a variety of geographically different areas, with a broad applicability in the context of all the three European Directives above mentioned.

2. Material and methods

2.1. Study area

The Gulf of Trieste is a shallow marine basin, located at the northernmost part of both the Adriatic and the Mediterranean Sea, with an average depth of around 21 m. It is influenced by freshwater inflows, bottom sediment resuspension and highly populated coastal areas (Ogorelec et al., 1991). The circulation of water masses in the Gulf of Trieste is variable, but generally follows the anticlockwise circulation system of the Northern Adriatic and is mainly influenced by the tidal regime (with an amplitude of approx. 0.5 m) and by wind-driven currents (Ogorelec et al., 1991; Malačič and Petelin, 2009). Sea temperatures range from 6 °C in February to more than 25 °C in summer, and the salinity fluctuates between 33 and 39 (Ogorelec et al., 1991; Bogunović and Malačič, 2008). Recent studies concerning the reduction of chlorophyll *a* concentrations all over the Northern Adriatic, consistent with the decrease in concentrations of phosphate and ammonia (Solidoro et al., 2009; Mozetič et al., 2012), underlined the oligotrophication of the basin over the last decade (Mozetič et al., 2010).

The sediments of the south-eastern coast of the Gulf of Trieste are composed of detrital material which originates from flysch in