

RESEARCH ARTICLE

## Body size descriptor of *Cymodocea nodosa* indicates anthropogenic stress in coastal ecosystems

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### Abstract

- 1 - We studied skewness (asymmetry) of log-transformed relative frequencies of *Cymodocea nodosa* leaf length (SkLnRfLL) as an easily measurable indicator of anthropogenic stress in selected biotopes of Eastern Macedonia, North Aegean, Greece.
- 2 - Since the spatial variation of the skewness coefficient was not known, a random nested sampling design within a hierarchy of spatial scales ranging from 10's of metres (area) to 100's of metres (site) to kilometres (biotope) was implemented. Three *Cymodocea nodosa* biotopes of the eastern Kavala Gulf coasts (Nea Karvali, Erateino, Agiasma), with that of Nea Karvali being the most degraded (close to an industrial area), were sampled during the main seagrass growth season in July 2004. Fifty five quantitative samples with 60 or more *Cymodocea* leaves were analysed in total, using non-parametric and parametric nested ANOVA.
- 3 - Statistically significant variation ( $p < 0.01$ ) was observed at the biotope scale, showing maximum mean value in the most degraded biotope of Nea Karvali (3.7) and minimum mean values in the less impacted biotopes of Agiasma (2.3) and Erateino (2.7). This pattern was also confirmed by post-hoc comparison.
- 4 - SkLnRfLL, based on the theory of normal population distribution and metabolic theory of ecology, seems to be a promising indicator of anthropogenic stress in North Aegean coastal ecosystems.

**Keywords:** Angiosperms, leaf length, nested analysis, Water Framework Directive-WFD.

### Introduction

The shallow waters of the Mediterranean Sea and worldwide oceans host extensive and highly productive meadows of seagrasses, which are fundamental for most trophic levels, considerably influencing ecosystem structure and function (Den Hartog, 1970; Hemminga & Duarte, 2000). *Cymodocea nodosa* (Ucria) Ascherson is a very common seagrass of shallow sheltered to semi-exposed Mediterranean coasts (Den Hartog, 1970) forming meadows that are mono-specific or mixed with *Zostera noltii* Hornem. (Mazzella et al., 1993). It is a perennial fast-growing plant of sandy to muddy pristine as well as degraded coastal environments (Cancemi et al., 2002; pers. observ.). Along the Greek coasts, *Cymodocea* is a dominant element of

Macedonian, North Aegean coasts, often forming meadows mixed with *Z. noltii* (Orfanidis et al., 2005). Our knowledge of this widely distributed species, however, is very limited, especially in comparison to the other common Mediterranean seagrass *Posidonia oceanica* (Linnaeus) Delile (Cancemi et al., 2002). Both *Posidonia* and *Cymodocea* seagrass meadows are declining today in the Mediterranean Sea due to heavy anthropogenic stress affecting light and nutrient resources (Hemminga & Duarte, 2000; Short et al., 2001). Although great effort is being made to use *Posidonia*'s structural and dynamic features as water quality indicators (see Pergent-Martini et al., 2005), similar research on *Cymodocea* lags behind. Ecological assessment of the critical ecosystem parameters trends and status requires

the use of biotic component metrics, especially of resident habitat-forming organisms such as seagrasses. Metabolic theory in ecology (Basset, 1995; Brown et al., 2004), which combines organism body size distribution with functions and dynamics of ecological systems, provides a new theoretical context in which to use body size descriptors in the assessment of ecosystem quality (Basset et al., 2004).

In order to develop new practical indicators of anthropogenic stress in coastal waters, the skewness of log-transformed relative frequencies of *Cymodocea nodosa* leaf length (SkLnRfLL) was studied along a gradient of anthropogenic stress in Kavala Gulf, Eastern Macedonia, North Greece. Since the spatial variation of this skewness coefficient was not known, a random nested sampling design within a hierarchy of spatial scales, ranging from 10's of metres (area) to 100's of metres (site) to kilometres (biotopes), was implemented. Regarding ecosystems as hierarchically organized systems (O'Neil, 1988), such a sampling approach can indicate the underlying causal ecological processes of variation.

## Methods

### Study area.

Kavala Gulf (40.52° N; 24.25° E) is a semi-enclosed coastal water body of Eastern Macedonia, North Greece, which is linked to the northern Aegean Sea through the Thassos Island channel and plateau. Temperature ranges from 11° C in winter to 26° C in summer. Salinity strongly depends on Black Sea waters, which are less saline than Aegean waters, and ranges from 33 PSU in spring and summer to 35 PSU in winter. The study area comprises three biotopes located on the eastern coasts of the gulf (Figure 1). The biotope of Nea Karvali is located close to the area where a phosphorus fertilizer plant, wastewater treatment works and crude oil de-sulphurization factory exist. The biotopes of Erateino and Agiasma are located in the seaward area of the Nestos Delta lagoons, where mussel cultivation in deeper waters than sampled in this study is carried out. The Nestos Delta was suggested for inclusion in the

European Natura 2000 network (code GR1150010). Table 1 shows mean values of key water and sediment environmental parameters sampled close to the studied biotopes at 10 m isobath (Sylaios et al., 2004). Mean total dissolved inorganic nitrogen (TDIN) and mean soluble reactive phosphorus (SRP) concentrations increased from Agiasma (TDIN=5.7  $\mu\text{mol l}^{-1}$ , SRP=1.49  $\mu\text{mol l}^{-1}$ ) and Erateino (TDIN=5  $\mu\text{mol l}^{-1}$ , SRP=1.61  $\mu\text{mol l}^{-1}$ ) towards Nea Karvali (TDIN=11.9  $\mu\text{mol l}^{-1}$ , SRP=2.57  $\mu\text{mol l}^{-1}$ ). A similar trend (except Pb) was evident for certain heavy metal concentrations in sediments. The maxima of mean total concentrations (in ppm) were measured in Nea Karvali as follows: Cu (39.9), Ni (43.8), Zn (102.9), Cd (1.5), Cr (162.3). Mean total maximum concentration of Pb were measured in Agiasma (40.2). All metal concentration values were produced by strong acid digestion of <1mm sediment fraction.

### Sampling design and collection of data.

The Kavala Gulf biotopes of Nea Karvali, Erateino, and Agiasma, dominated by mixed *Cymodocea nodosa* and *Zostera noltti* meadows, were sampled in July 2004. In each sampling site, profiles of temperature (T °C) and salinity (PSU) were measured using portable meters (WTW instruments). Light extinction coefficient (k) was determined by measuring vertical profiles of photosynthetic active radiation (PAR;  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; Li-Cor instruments) at 0.5m intervals using the formula (JERLOV, 1970):

$$k = (\ln I_0 - \ln I_z) / z$$

[( $I_0$  = PAR at 0.05 m depth;  $I_z$  = PAR at z m depth; k = extinction coefficient ( $\text{m}^{-1}$ )]

The variability of the *Cymodocea nodosa* leaf length skewness coefficient was examined on three spatial scales ranging from 10's of metres (area) to 100's of metres (site) to kilometres (biotope) (Figure 2).

Sampling was realized in three biotopes that were ca. 5-8 km apart. In each biotope, 2 sites were randomly chosen along the same isobath (3 to 4 m) that were ca. 500-800 m apart. In each site, two areas were randomly selected that were ca. 50-80 m apart.

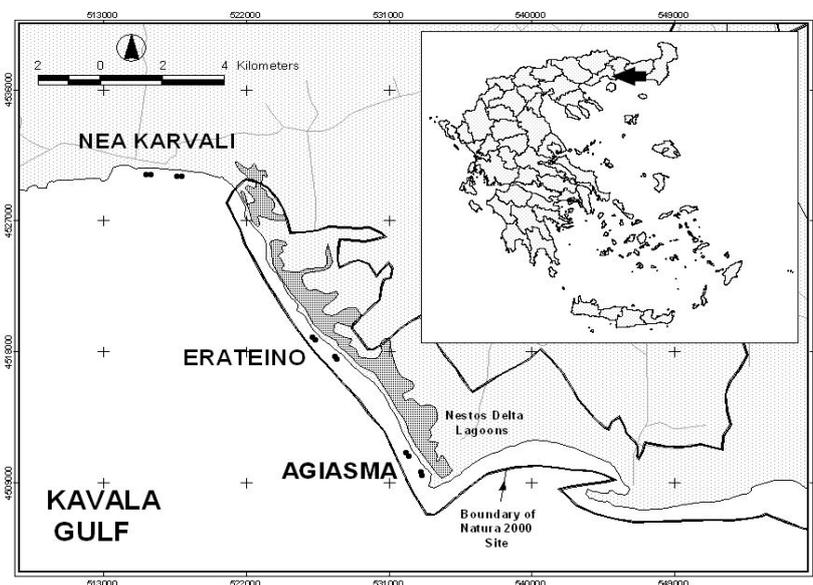


Figure 1. Map of the studied area.

In each area, five metallic quadrates (25 x 25 cm) were randomly placed by divers on the bottom and subsequently all the shoots within a quadrate were very carefully uprooted with a knife. Samples were labelled and placed individually in plastic bags. During sampling and transportation to the laboratory, samples were kept in a cool box. In the laboratory samples were stored in a freezer at  $-20^{\circ}\text{C}$ . In the laboratory the leaves (total leaf = sheath + photosynthetic part) of 20 shoots from each quadrate were randomly selected and measured to the nearest mm.

*Analysis of data.*

Skewness of log-transformed relative frequencies of *Cymodocea nodosa* leaf length (SkLnRfLL) was analysed using parametric nested analysis of variance (ANOVA) with biotope (3 levels) and site (2 levels) within each biotope, and area (2 levels) within each site and biotope. All factors were treated as random in the analyses. Fifty five quantitative samples with equal or more than 60 *Cymodocea* leaves were analysed in total. According to Glass and

Table 1. Mean values of seawater nutrients and sediment heavy metals (strong acid, <1mm) sampled at 10m isobath close to the studied biotopes (from Sylaios et al., 2004).

Abiotic Parameters	Nea Karvali	Erateino	Agiasma
<b>Seawater (surface)</b>			
N-NO <sub>3</sub> (μmol/l)	8.8	3.8	4.9
N-NO <sub>2</sub> (μmol/l)	0.18	0.07	0.14
N-NH <sub>4</sub> (μmol/l)	2.89	1.13	0.66
P-PO <sub>4</sub> (μmol/l)	2.57	1.61	1.49
<b>Sediment</b>			
Cu (ppm)	39.9	6.5	16.6
Ni (ppm)	43.8	12.7	28.5
Zn (ppm)	102.9	58.3	90.1
Pb (ppm)	31.97	27.7	40.2
Cd (ppm)	1.5	0.3	0.1
Cr (ppm)	162.3	54	93.7

Hopkins (1996), ANOVA homogeneity tests are not necessarily very robust and we repeated the key analyses using Kruskal-Wallis non-parametric ANOVA. A pattern was accepted only when non-parametric and parametric nested ANOVA gave similar results. Post-hoc tests (unequal N HSD) were used to identify statistically significant ( $p < 0.05$ ) differences between biotopes. All ANOVA and variance tests of non-transformed data were carried out using the STATGRAFICS v. 7.1 software package.

### Results

Temperature decreased from surface (25.3-26.2° C) to depth (24.4-25.5° C), while the opposite happened for salinity, which increased from the surface (31.4 to 32.1 PSU) to the depth (32-33.1 PSU), indicating the existence of a shallow thermocline. Figure 2 shows the profiles of irradiance across the studied biotopes: Nea Karvali (A1, A2), Erateino (B1, B2), Agiasma (C1, C2).  $k$  slightly decreased from Nea Karvali (0.252-0.288  $m^{-1}$ ) to Agiasma (0.239-0.244  $m^{-1}$ ), indicating an increase of water transparency.

SkLnRfLL showed significant differences in its mean values on the biotope scale (kilometres) of the nested parametric and non-parametric ANOVA (Table 2). Figure 3 shows the variation of mean SkLnRfLL values for the biotopes Nea Karvali, Erateino, and Agiasma. Higher mean values were estimated at the most degraded biotope of Nea Karvali (3.7), while lower values were estimated at the less degraded biotopes of Erateino (2.7) and Agiasma (2.3). Post-hoc tests indicated significant differences between Nea Karvali on the one hand and the Erateino and Agiasma biotopes on the other (Table 3).

### Discussion

SkLnRfLL calculation is based upon skewness of leaf length log-transformed relative frequencies, which is an easily measurable morphological feature of the common Mediterranean seagrass *Cymodocea nodosa*. When this metric was estimated across a water quality gradient sampled at different spatial scales from meters to kilometres, it showed

statistical significant variation only on the kilometre (biotope) scale (Figure 3, Table 2). Such a relatively broad spatial scale variance pattern seems very likely to be related to biotope-specific environmental factors or to chronic pollution (Underwood, 1997; Benedetti-Cecchi et al., 2001).

Table 2. Comparisons between biotope, site and area of skewness of log-transformed relative frequencies of leaf length of *Cymodocea nodosa* (SkLnRfLL) using non-parametric and parametric nested ANOVA for the biotopes Nea Karvali, Erateino, and Agiasma. \* $p < 0.05$ , \*\* $p < 0.01$ .

Source of variation	df	Parametric		Non-Parametric	
		MS	F	df	$\chi^2$
Biotope	2	7.64	37.22**	2	10.11**
Site(Biotope)	3	0.2	0.49	5	12.32*
Area(Site*Biotope)	6	0.41	0.61	11	14.79
Error	43	0.68			

Table 3. Post-hoc comparisons of the SkLnRfLL index of *Cymodocea nodosa* between the biotopes Nea Karvali, Erateino, and Agiasma. Numbers in bold indicate statistical differences for  $p < 0.05$ .

	Agiasma	Erateino
Agiasma		
Erateino	0.263	
Nea Karvali	<b>0.000</b>	<b>0.008</b>

The metric is based on the theory of normal population distribution, where any divergence quantified by skewness can be regarded as indicative of stressed ecosystems (Gray, 1979). Asymmetry of *Posidonia* populations size distribution has often been associated with changes during stand development and in the degree of competition (Panayotidis et al., 1981). Such an explanation is in agreement with the general view that body size-abundance distribution descriptors seem to respond directly to resource availability-related environmental pressures (Basset et al., 2004). High and low

skewness values in this study indicated higher and lower divergence from normal leaf length distribution characterizing the most (Nea Karvali) and less degraded (Erateino, Agiasma) biotopes respectively. These results are in accordance with environmental monitoring data available for the Kavala Gulf (Sylaios et al., 2004, 2006; Stamatis et al., 2006) indicating dissolved nutrients and heavy metals in sediments as the main pollutants of the area, with the Nea Karvali biotope being one of the most contaminated areas of the gulf. Similarly,  $k$  slightly decreased from Nea Karvali (0.252-0.288  $\text{m}^{-1}$ ) to Agiasma (0.239-0.244  $\text{m}^{-1}$ ), indicating an increase in water transparency which may correlated with increasing water quality.

Skewness has also been used to describe stress in ecosystems elsewhere. For example, very similarly to the present study, the skewness of log-transformed frequency of bare size patches in desert rangeland ecosystems has been used as

an indicator of ecosystem health (Whitford, 1998). Hackney and Durako (2004) indicated that size-frequency distributions of morphometric and biomass features of the seagrass *Thalassia testudinum* Banks ex König could be indicative of physical and chemical environments in Florida Bay. Reizopoulou and Nicolaidou (2007) suggested a new biotic index (Index of Size Distribution-ISD) to assess water quality in coastal lagoons, calculated as the skewness of the size frequency distribution of macrobenthic assemblages.

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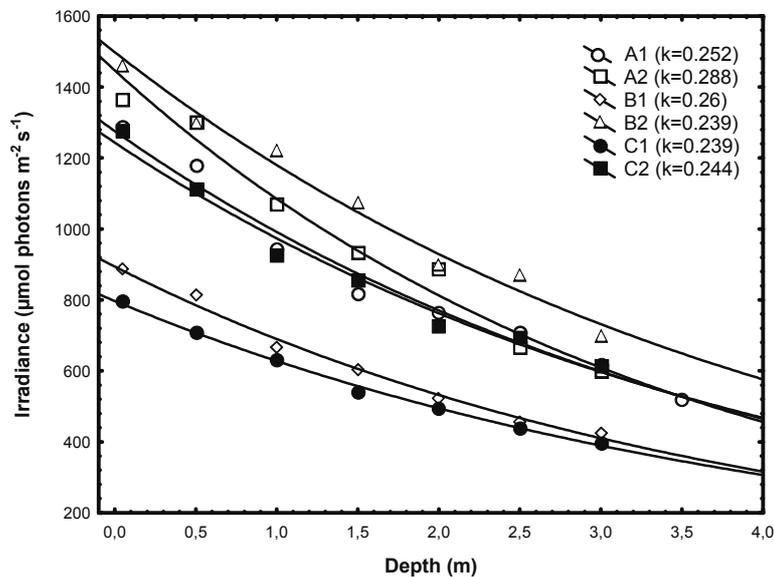


Figure 2. Irradiance profiles fitted exponentially and attenuation coefficient ( $k$ ) in the studied biotopes: Nea Karvali (A1, A2), Erateino (B1, B2), Agiasma (C1, C2).

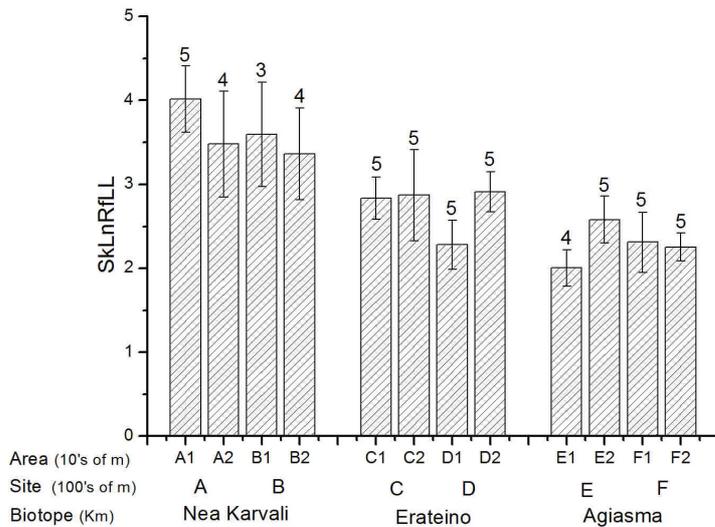


Figure 3. Variance of mean values ( $\pm$  SE; numbers indicate n) of skewness of log-transformed relative frequencies of leaf length of *Cymodocea nodosa* (SkLnRfLL) for the biotopes of Nea Karvali, Erateino, and Agiasma.

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