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Research Article

Crustacean decapod diversity associated with four shallow meadows of *Cymodocea nodosa* meadows from the North Aegean sea

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Abstract

Seagrass meadows and the associated invertebrate fauna, are important ecological biodiversity indicators for coastal marine environments. Increasing anthropogenic stress and climate change, have made these habitats priority targets for monitoring and conservation, however, relevant information is poor for the eastern Mediterranean Sea. The present study aims at describing decapod assemblages associated with four shallow *Cymodocea nodosa* beds from the northern Aegean Sea (Greece). Physicochemical parameters were measured, and water samples were collected for nutrient and chlorophyll–a analyses. For the morphological study of *C. nodosa*, three 25 x 25 cm quadrates per site were randomly sampled. Biometric (leaf length and width, total leaf length) and structural (shoot density, above and below ground biomass and above/below ground biomass, Leaf Area Index-LAI and Leaf Area-LA) parameters were assessed, and the CymoSkew index was calculated. Finally, decapods were collected by means of a beach seine (mesh size of 2 mm), taxonomically identified and sexed with stereoscopic inspection. All examined sites were oligotrophic and exhibited similar environmental quality.The western *C. nodosa* meadows had a high ecological status (CymoSkew CV=1.70 and CymoSkew NP=1.86). A clear correlation between seagrassmorphometry and nutrient concentrations was detected, with smallest shoot size and higher densities recorded in the less impacted areas. In total, 606 crustacean decapod specimens belonging to 14 species and eight families were collected. *Hippolytes apphicaforma* A (Hippolytidae) was the dominant species. Finally, higher decapod species abundance and richness were recorded in the western stations, which host meadows of high ecological quality status.

Keywords: Biodiversity, bio-indices, Hippolytidae, *Hippolytes apphica*, seagrasses.

Introduction

Studies of crustacean decapod diversity associated with seagrass meadows are complex and require a multidisciplinary approach. These habitats are described by multifactorial and ever-changing ecological parameters, with intricate structural and functional characteristics, whereas the faunistic comparisons among the corresponding areas arise difficulties due to different sampling gears, periods, and zoogeographical regions (García Raso *et al*. 2006). Moreover, the relevant information on the macro faunal decapod communities of *Cymodocea nodosa* (Ucria) Ascherson, 1870 in the Mediterranean Sea is limited and scattered (Ledoyer 1966, 1968, Števčić 1991, Reed and Manning 2000, García Raso *et al*. 2006, Liousia *et al*. 2012, Daoulati *et al*. 2014, Papathanasiou and Orfanidis 2018).

Cymodocea nodosa is the second most abundant sea grass species in the

Mediterranean Sea, Sea of Marmara and the eastern Atlantic coasts (Den-Hartog 1970, Barberá *et al*. 2005, Cuncha and Araújo 2009), forming mono-specific or mixed species meadows with *Posidonia oceanica* or *Zostera noltei.* It is a fast growing sea grass, with high tolerance to both natural and anthropogenic stress (Papathanasiou*et al*. 2016), able to colonize unstable sandbanks (Habitat code-1110) and is protected under the Habitat Directive (92/43/EEC) and Council Regulation (1967/2006/EU) for fisheries. The species is quite common in the North Aegean Sea, where the extensive continental shelf, together with the increased turbidity in the water column that limits *P. oceanica* growth, allows it to form extensive meadows, spreading from 0 to 23m deep (Papathanasiou 2013). These meadows play a significant ecological role, providing sediment stability, trapping suspended solids within the leaf canopy and filtering the water column from excess nutrient and toxic concentrations (Duffy 2006). Moreover, they are rich biodiversity habitats, providing food, substrate and protection from predation to a numerous of organisms (Unsworth *et al*. 2015). They are also considered an ecosystem quality element according to the WFD (2000/60/EC) due to the high phenotypic plasticity and fast response rates. Many indices for the assessment of the ecological status have been based on this species, either focusing on population metrics based on ecological theories, i.e. CymoSkew (Orfanidis *et al*. 2010) and its Italian equivalent MediSkew(Orlando-Bonaca*et al*. 2015), or applying ordination methods on a number of measured parameters and measure a stations distance from theoretical pristine conditions i.e. CYMOX (Oliva *et al*. 2012). These indices are widely applied in the context of various monitoring programmes, such as those related to the Water Framework Directive (WFD) (2000/60/EC) and the Marine Strategy Framework Directive (MSFD) (2008/56/EC) (Duarte *et al*. 2017).

It is widely recognised that sea grasses around the world are in decline due to coastal anthropogenic activities, such as fishing, aquaculture, dredging, coastal development, agriculture, industrial and urban waste effluents (Orth *et al*. 2006). Even though significant efforts are being made towards the protection and management of these ecosystems, they are still under threat (Soissons 2017). As such, a more detailed description and evaluation of their ecological and economical contribution in different geographical regions would significantly help raise political and social awareness (Burgos *et al*. 2017), while exact knowledge on the decapod communities they accommodate, will allow for future biodiversity predictions under climate change and pollution scenarios. Thus, the present study aims at describing decapod assemblages associated with shallow *C. nodosa* beds from the northern Aegean Sea, as the first step towards a more systematic temporal and spatial recording from eastern Mediterranean zoogeographical areas.

Materials and Methods

Study area and Sampling sites

The study area is located in the Gulf of Kavala, at the western part of Thracian Sea and the Northeast part on the continental shelf of the North Aegean Sea (Fig. 1). It is a semi-enclosed gulf and mean depth of 32 m (max depth: 60 m) covering an area of approximately 461 m^2 (Sylaios *et al*. 2012). To the South, the gulf is connected with the North Aegean Sea through Thassos Plateau (with 20 km width and 45-50 m depth) and to the East through Thassos Passage, a narrow channel of 7.3 km width (Fig. 1). Sampling was carried out in four shallow (0.5-1.5 m) stations: Cape Vrasidas (CV, 24°19' 2.28" E, 40°49' 26.76" N), NeaPeramos (NP, 24°18' 28.44" E, 40°50' 35.16" N), NeaKarvali (NK, 24° 29' 13.92" E, 40°56' 58.2" N) and Agiasma (AG, 24° 34' 15.24" E, 40°54' 42.48" N) (Fig. 1).

Sample collection and laboratory procedures

All samples were collected in late April to early May 2017. Each station was divided into two symmetrical sites based on the seagrass meadow's surface area (i.e. CV1 and CV2 sites). The physical parameters of water quality (temperature, Dissolved Oxygen (D.O.), salinity, density and pH) were measured in situ using the portable Aquameter 200 instrument. Temperature, salinity and density values were cross-checked with a Seabird SBE-19plus CTD. The differences between those two instruments were negligible, marked only at the second digit. From all stations one water sample was taken from each site using polyethylene bottles (1.5 L), which were pre-washed with 10% diluted HNO_3 to avoid contamination. Nutrients($N\text{-}NO_3$, $N\text{-}NO_2$, $P\text{-}PO_4$, $N\text{-}NH_4$, SiO_2) were measured in 500 mL of each sample, after being passed through dried and pre-weighted nitrocellulose Whatman filters (0.45μm) with the use of a Büchi V-500 vacuum pump immediately after sampling. Total Suspended Sediment (TSS) concentrations were measured by subtracting the initial empty filter weight from the "loaded" filters after the removal of excess moisture (dry the filters in an oven at 100ºC for 24 h). Nitrates were redacted to nitrites by passing an aliquot of 10 mL sub-sample together with 10 ml of NH_4Cl through a cadmium filing column. All nutrient concentrations were determined by following the photometric methods described by Parsons *et al*. (1984). For chlorophyll-a analysis, 1000 mL of each sample were passed through 47 mm GF/F filters. All filters were placed into 15 mL test tubes filled with a 10 ml dilution of 90% acetone and 10% $MgCO_3$ (APHA 1988). The test tubes were centrifuged at 2,700 rpm for 30 minutes and chlorophyll-a determination was achieved following the trichromatic methodology according to Standard Methods (APHA 1988). The Hitachi U-2001 spectrophotometer was used for all nutrients and chlorophyll-a measurements.

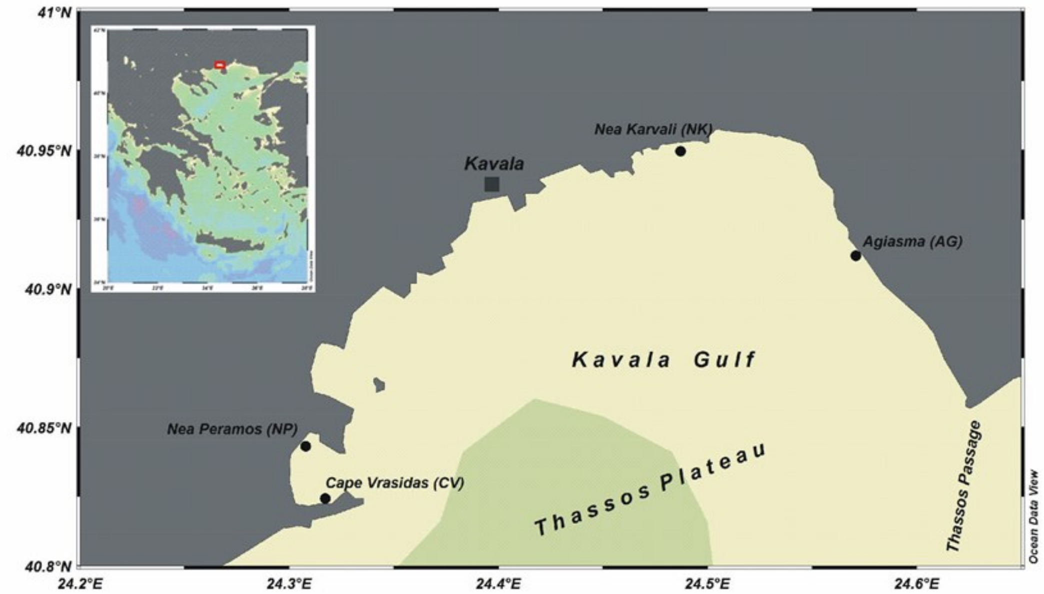

Figure 1. Map of sampling stations in the Kavala Gulf. CV: Cape Vrasidas; NP: Nea Peramos; NK: Nea Peramos; AG: Agiasma

Due to the spatial patchiness that characterizes seagrass meadows, a hierarchical nested sampling design was followed. Three random samples were quantitatively collected from each site, using a 25X25 cm metallic quadrate. Each sample was labeled and stored at -18ºC until analysis. From each quadrate 20 random shoots were chosen and separated into their leaves. Leaf and stem length, leaf width, Leaf Area Index (LAI=leaf length*width, m^2/m^2) and Leaf Area (LA=total leaf length*mean width/ shoot, cm^2/shoot) were measured for all leaves. Number of leaves per shoot and the number of all shoots in the quadrate were measured, while shoot density was calculated as shoots per m^2. Leaves were separated from roots, rinsed with water and left for 24h in 60ºC. Subsequently, above and below ground biomass were measured using a scale (Mettler PM30-K Electronic Scale), except below ground biomass that was not measured for Agiasma samples, where the meadow is typically buried during winter and a quantitative sampling of the root system is not possible. Finally, the CymoSkew index of ecosystem quality was calculated after modification of the methodological approach described by Orfanidis *et al*. (2010).

Decapod fauna was collected by means of a small beach seine (mesh size of 2 mm), which was pulled through the sea grass bed. Three replicates at about 1 m depth and covering the same distance (100 m) were taken from each study site to minimize inequality of faunal densities (Lewis and Stoner 1983). Material preserved in situ in 4% neutralized formalin solution. In the laboratory, samples were sorted and identified to the species level according to the relevant taxonomic literature (Hothuis 1987, 1993, Zariquiey Álvarez 1946, 1968, Smaldon 1993, Ingle 1996, d'Udekemd' Acoz 1996, 1999, 2007, De Grave and Fransen 2011, Appeltans *et al*. 2012). Abundances and sexes were recorded for each species. Sex identification was also verified stereoscopically through the inspection of the second pairs of pleopods in shrimps and of the gonopores' presence on the third/fifth thoracic sternites in crabs.

Data analyses

A Redundancy Analysis was conducted to test how the meadow structure differed along the sampling stations and examine which abiotic factors were responsible for any observed differences using the CANOCO 4.5 (Ter Braakand Smilauer 1999). A Detrended Correspondence Analysis (DCA) was first executed and since the axis length was significantly lower than 4, a linear ordination technique (RDA) was chosen. All abiotic parameters were log (1+x) transformed, particularly as parameter measurement units were different, and a strong linear relationship was absent (Ter Braakand Smilauer2002), while those parameters that showed high collinearity with others, were excluded from the analysis. The contribution of each abiotic parameter to the explanation of the datasets variation was evaluated using Monte Carlo permutation analysis. Since all meadows were of small to medium spatial scale, only 3 *C. nodosa* quadrats per site were sampled. CymoSkew was then calculated using an R function, that utilizes all leaf length measurements and produces via subsampling techniques 100 sub-samples of n-150 leaf length values. That distribution is further analysed and repeated another 100 times. The final CymoSkew value is then based on the distribution of the latest 100 mean values (Orfanidis *et al*. 2010).

Results and Discussion

Abiotic parameters

The values of the recorded abiotic parameters showed some variation among the sampling sites (Table 1). Sea surface temperature demonstrated the lowest value in CV (16.2°C) and the highest in NK (21.5°C) due to the intense spring fluctuations of air temperature. AG and NK stations were less saline (by 1.1 on average), while dissolved oxygen in water and pH showed similar values at all stations (average: 8.4 ± 0.5 mg/L and average: 8.2 ± 0.1, respectively; Table 1). Silicate, ammonium and nitrite depicted uniform concentrations (SD: 11.7, 7.6 and 6.1%, respectively), while the main differences between eastern and western sampling sites were detected for nitrates, ortho-phosphates, chlorophyll-a and TSS (Table 1). Nitrates almost tripled and ortho-phosphate doubled their concentrations at AG and NK stations (average: 3.9 and 0.23 μM/L, respectively), probably due to the local freshwater inputs. TSS and chlorophyll-a highest concentrations were found at NK station (Table 1). These elevated concentrations are mainly attributed to local point and non-point sources, since Nestos discharge for the week prior to the sampling was extremely low (2-25.2 m^3/s, Management Body of Nestos Delta-Ismarida-Vistonis Park), thus the river plume did not reach Kavala Gulf and enrich the area with fluvial nutrients.

***Cymodocea nodosa* metrics**

The key *Cymodocea nodosa* metrics among the four studied meadows of the N. Aegean Sea are presented in Table 2. The largest leaves were measured in AG meadow (maximum leaf length=51.4cm, maximum width=0.7cm), where the highest LAI (max LAI=454.82 m^2/m^2) and LA (max LA=43.16 cm^2/shoot) values occurred. The highest shoot density was measured in CV (max density= 100.03 shoots/m^2), while NK meadows had shoots with the highest number of leaves (max number of leaves per shoot= 7).

Table 1. Average values of physicochemical parameters (and standard deviation in brackets) and ecological status for all sampling stations.CV: Cape Vrasidas; NP: Nea Peramos; NK: Nea Karvali; AG: Agiasma.

Parameter	**CV**	**NP**	**NK**	**AG**
T (°C)	16.2 (0.1)	17.0 (0.1)	21.5 (0.0)	20.2 (0.1)
Salinity	37 (0.0)	36.4 (0.1)	35.9 (0.1)	35.4 (0.1)
σt-density (kg/m^3)	27.4 (0.0)	26.7 (0.1)	26.0 (0.0)	25.6 (0.1)
D.O. (mg/L)	8.9 (0.2)	8.5 (0.1)	7.7 (0.2)	8.3 (0.3)
pH	8.3 (0.0)	8.2 (0.2)	8.2 (0.2)	8.1 (0.1)
TSS (mg/L)	19.0 (1.8)	23.8 (4.7)	48.0 (1.4)	14.5 (0.9)
chl-a (μg/L)	0.45 (0.09)	0.25 (0.1)	2.65 (0.08)	0.45 (0.05)
N-NO_3 (μM)	1.44 (0.3)	1.96 (0.3)	3.48 (0.6)	4.29 (0.0)
N-NO_2 (μM)	0.37 (0.01)	0.42 (0.03)	0.37 (0.01)	0.39 (0.04)
N-NH_4 (μM)	0.53 (0.0)	0.53 (0.1)	0.45 (0.07)	0.49 (0.09)
P-PO_4 (μM)	0.13 (0.0)	0.15 (0.03)	0.2 (0.03)	0.27 (0.03)
SiO_2 (μM)	8.7 (0.2)	10.8 (1.1)	9.0 (0.1)	10.9 (0.04)
Ecological Status*	"good"	"good"	"moderate"	"moderate"

*Ecological status is given according to EEI (Ecological Evaluation Index) (Orfanidis and Panayiotidis 2005), and to Kamidis *et al*. 2011, Stamatis *et al*. 2006, Sylaios *et al*. 2012, Papathanasiou *et al*. 2016.

Table 2. Key *Cymodocea nodosa* metrics (mean±SE, n=6 per station) in the four meadows of the N. Aegean Sea.

Site	**Total leaf length (cm)**	**Leaf width (cm)**	**Density (shoots/m^2)**	**No leaves/shoot**	**LAI (m^2/m^2)**	**LA (cm^2/shoot)**	**CymoSkew**
CV	7.092±0.17	0.170±0.02	97.667±4.40	3.513±0.05	149.911±9.56	3.627±0.15	1.702±0.10
NP	6.792±0.17	0.184±0.02	75.833±12.85	3.133±0.06	105.909±19.72	3.057±0.34	1.863±0.23
NK	8.901±0.28	0.222±0.00	57.500±11.06	4.113±0.12	125.136±30.79	8.284±1.26	2.270±0.12
AG	23.544±0.69	0.245±0.00	54.250±7.98	3.613±0.07	325.237±45.34	21.372±1.59	2.758±0.38

According to the CymoSkew index (Table 2), all stations were sustainable, with CV (CymoSkew= 1.70) and NP (CymoSkew= 1.86) having a high and AG (CymoSkew= 2.76) and NK (CymoSkew= 2.27) a good ecological status. The RDA results showed that 96.1% of the total variance and 99.7% of the variance in the relationship between biotic metrics and the environmental parameters was explained by the first two axes. Monte Carlo analysis showed that N-NOx (p=0.01, F=30.32) and TSS (p=0.005, F=21.3) had the most significant role in explaining the variation of the dataset. Leaf width, LA and CymoSkew were correlated to N-NOx and P-PO_4, while total leaf length and LAI to SiO_2 (Fig. 2). Additionally, the above to below ground biomass ratio was correlated to TSS, while shoot density to pH. Sites from the most un-impacted meadow of CV exhibited higher DO concentration (Table 1) and higher shoot density from the rest of the sites. Significant within meadow variability was found for NK and AG, where sites from the same station were separated (Fig. 2). Site NK1 was differentiated from NK2 and AG1 to AG2 along the second axis, which is better associated to changes in DO, TSS and chl-a concentrations.

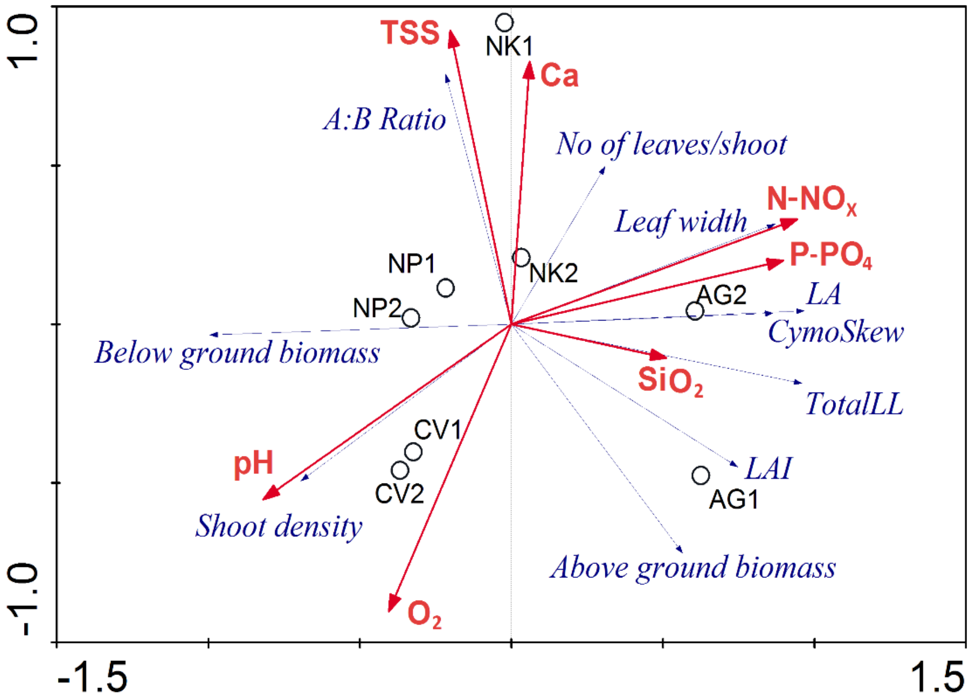

Figure 2. Redundancy analysis between abiotic parameters and key *Cymodocea nodosa* metrics sampled in four stations at the N. Aegean Sea. CV: Cape Vrasidas; NP: NeaPeramos; NK: NeaKarvali; AG: Agiasma; TotalLL:Total Leaf Length; LAI: Leaf Area Index; LA: Leaf Area; TSS: Total Suspended Sediment; A:B Ratio: Above to Below ground biomass Ratio.

Crustacean decapod diversity

In total, 606 crustacean decapod specimens belonging to 14 species and 8 families were collected, sexed and measured. The total number of individuals (Nt), the relative abundance (D %) and the total relative abundance (Dt %) are given in Table 3 for each species and sampling site. Among the families, Hippolytidae was the best represented family with four species (*Hippolyte inermis*, *H. leptocerus*, *H. niezabitowskii* and *H. sapphicaforma* A), Crangonidae, Palaemonidae and Inachidae follow with two species by family (*Philocheras fasciatus/ Philocheras trispinosus*, *Palaemon adspersus/ Palaemonel egans*, and *Macropodia rostrata/ Macropodia tenuirostris*, respectively) and Alpheidae, Thoridae, Leucosiidae and Polybiidae were represented by one species (*Athanas nitescens*, *Eualus cranchii*, *Ilia nucleus*, and *Liocarcinus depurator*, respectively). Among the study sites, the highest decapod richness and abundances were found in the western Kavala Gulf (CV and NP), with the same dominant species (*Hippolytes apphica forma* A) being the characteristic species of *Cymodocea nodosa* habitat. *Hippolytes apphica* is a Central/ Eastern Mediterranean endemic species (d'Udekemd' Acoz 1993, 1996, Koukouras and Anastasiadou 2002, Ntakis *et al*. 2010) with interesting adaptive strategies on its population structure and dynamics due to the characteristic rostral dimorphism of its two forms (*forma* A and *forma* B) (Liasko *et al*. 2015, 2017). Ramírez and García Raso (2012) reported *H. leptocerus* as the dominant species in *C. nodosa* meadows from western Mediterranean areas (Punta de Calaburras and Calahonda, Alboran Sea, Spain). Daoulati *et al*. (2014) found similar high abundances for *H. leptocerus* and *H. inermis* in La Goulette Bay in northern Tunisia, while Liousia *et al*. (2012)

found similar abundances for *H. sapphica forma* A and *H. inermis* in the eastern Ionian Sea (Port of Igoumenitsa and Amvrakikos Gulf). Our results show that that *H. sapphica* replaces *H. leptocerus* and *H. inermis* in the studied sites of the Northern Aegean Sea. Normally, *Hippolyte* species are well adapted taxa in both algal and seagrass habitats, where they camouflage, find food and shelter from predators (Ramírez and García Raso 2012). The third more abundant species was *H. niezabitowskii*, an endemic species of the Adriatic, Ionian and Aegean Seas (d'Udekemd'Acoz 1996, Koukouras and Anastasiadou 2002). This species which is occurring in moderately sheltered meadows, having a narrow ecological niche (d'Udekemd'Acoz 1993, 1996), found more abundant than *H. inermis* in the western stations (CV and NP). *Palaemon adspersus* demonstrated a total abundance of about 2.14%, while *Philocheras* species which prefer sandy bays with gravel substrata, showed higher abundances in eastern than in the western stations (NK, AG) (*P. fasciatus* Dt: 1.98% and *P. trispinosus* Dt: 2.97%). The overall sex ratio was statistically in favour to females for the two most abundant species [*H. sapphica forma* A, F:M = 1.30:1 ($\chi^2 = 5.94$, $P < 0.001$); *H. leptocerus*, F:M = 1.06:1 ($\chi^2 = 0.12$, $P < 0.001$].

Table 3. Checklist of the species collected at the four sampling stations Cape Vrasidas (CV), NeaPeramos (NP), NeaKarvali (NK) and Agiasma (AG); Nt: total number of individuals, D(%): relative abundance (%); Dt(%): total relative abundance (%).

Taxa	**CV**		**NP**		**NK**		**AG**		**TOTAL**
Caridea	Nt	D (%)	Nt	D (%)	Nt	D (%)	Nt	D (%)	Dt (%)
Family: Alpheidae									
Athanasnitescens (Leach, 1814)	2	0.43	0	0	0	0	0	0	0.33
Family Crangonidae									
Philocheras fasciatus (Risso, 1816)	5	1.07	3	3.79	0	0	4	28.57	1.98
Philocheras trispinosus (Hailstone, 1835)	0	0	1	1.26	17	38.64	0	0	2.97
Family: Hippolytidae									
Hippolyte inermis Leach, 1816	5	1.07	7	8.87	9	20.45	0	0	3.46
Hippolyte leptocerus (Heller, 1863)	102	21.75	15	18.99	15	34.1	0	0	21.78
Hippolyte niezabitowski id'Udekemd'Acoz, 1996	22	4.69	9	11.4	0	0	0	0	5.12
Hippolyte sapphica forma A d'Udekem d'Acoz, 1993	315	67.16	34	43.04	0	0	0	0	57.61
Family: Palaemonidae									
Palaemon adspersus Rathke, 1837	0	0	2	2.53	2	4.54	9	64.29	2.14
Palaemon elegans Rathke, 1837	3	0.64	0	0	1	2.27	0	0	0.66
Family: Thoridae									
Eualus cranchii (Leach, 1817 [in Leach, 1815-1875])	9	1.92	1	1.26	0	0	0	0	1.65
Brachyura									
Family: Inachidae									
Macropodia rostrata (Linnaeus, 1761)	1	0.21	0	0	0	0	0	0	0.16
Macropodia tenuirostris (Leach, 1814)	4	0.85	7	8.86	0	0	0	0	1.82
Family: Leucosiidae									
Ilia nucleus (Linnaeus, 1758)	1	0.21	0	0	0	0	0	0	0.16
Family: Polybiidae									
Liocarcinus depurator (Linnaeus, 1758)	0	0	0	0	0	0	1	7.14	0.16
TOTAL	**469**	**100**	**79**	**100**	**44**	**100**	**14**	**100**	**100**

Higher decapod species abundance and richness were significantly correlated to leaf width (correlation coefficient= -0.77 and -1.00, respectively) and to shoot density (correlation coefficient= 0.93 and 0.96, respectively). Additionally, there isa clear trend between decapod richness/abundance and the meadows' ecological status (CymoSkew index, Fig.3), with higher values of decapod diversity being sustained in stations with lower CymoSkew values (CV and NP, Table 2). It is widely accepted that good ecological status in an ecosystem sustains high biodiversity (Palumbi *et al*. 2009). The observed pattern can be attributed to the fact that dense meadows usually provide more adequate protection from natural stress (i.e. wave action), while thinner leaves allow animals to swim more freely. However, further studies are needed in order to fully map the underlying mechanisms of how these seagrass traits affect the decapod assemblies.

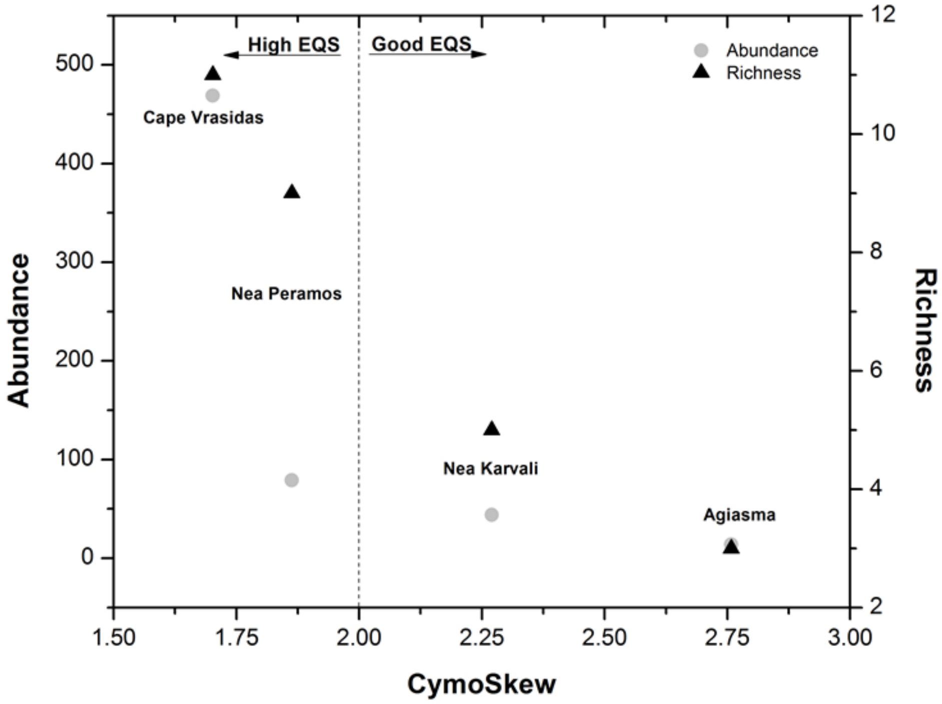

Figure 3. Decapod species abundance and richness in relation to the CymoSkew index, in four stations of Northern Aegean Sea. EQS: Ecological Quality Status.

Conclusions

The present study of decapod assemblages associated with four shallow *Cymodocea nodosa* beds from the northern Aegean Sea, recorded 14 decapod species belonging to eight families. Additionally, higher decapod richness and abundances were found in the western Kavala Gulf stations, with *Hippolyte sapphica forma* A, being the dominant species of the studied habitats. Moreover, the environmental quality for all examined sites, based on water analyses, exhibited similar values and can be characterized as oligotrophic. *C. nodosa* meadows were found to be sustainable, according to the CymoSkew index, with the western meadows of Kavala Gulf (CV and NP) having a high ecological status. Future monitoring of temporal and spatial decapodassemblages in the area will provide information on seagrasses communities, revealing the diversity and the relationships of the associated biota.

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