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Opportunistic seaweeds replace *Cystoseira* forests on an industrialised coast in Cyprus

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Abstract

Seaweeds are affected by humans worldwide, although no studies have assessed this in Cyprus. The Water Framework Directive requires ecological assessments of European coastal waters with biological indicators. We investigated macroalgal community metrics in the upper subtidal across *ca* 10 km of shoreline, encompassing undeveloped areas with limited human access as well as the most industrialised and impacted coast of Cyprus (Vasiliko Bay). Quadrats (20 x 20cm) were used to survey the algal communities in summer 2012 and spring 2013. Of the 51 taxa, we recorded, *Cladophora nigrescens* and *Laurencia caduciramulosa* (a non-native species) are new records for Cyprus. Brown algae of the genus *Cystoseira*, e.g., *Cystoseira barbatula*, formed dense forests covering rocky substrata on shorelines with limited human access. *Cystoseira* spp. decreased in abundance around bathing waters and was very rare in heavily industrialised parts of the bay. In impacted areas, fleshy and filamentous opportunistic species such as opportunistic *Ulva* and *Chaetomorpha* species with lower biomass than perennial species, proliferated in spring. The Ecological Evaluation Index (EEI-c) we used was a robust biotic index reflecting anthropogenic stress. Without improved management, the Marine Strategy Framework Directive targets may not be met on some coastlines of Cyprus since seaweed forests are in decline and are further threatened by imminent development.

Keywords: Ocean sprawl; eastern Mediterranean; macroalgae; biological indicators; *Ulva*; ecological assessment; Marine Strategy Framework Directive.

Introduction

The human ecological footprint is growing worldwide, and this is especially obvious on rocky shores (Halpern *et al.*, 2008). Although the coastal zone is less than 3% of the Earth's surface, it is home to about 60% of the world's population, and this is expected to rise to 80% by 2050 (Hyun *et al.*, 2009). The policy responses to this reality in Europe are the Marine Strategy Framework Directive (MSFD, 2008/56/EC), which is an attempt to achieve or maintain 'good environmental status' by 2020, and the Water Framework Directive (WFD, 2000/60/EC) that aims to achieve 'good ecological status' in coastal waters. A range of biological indicators have been developed to assess environmental and ecological status based on biological quality elements.

Studies worldwide have shown that seaweeds integrate the effects of water quality; in degraded conditions long-lived species tend to be replaced by short-lived, opportunistic species that form less complex habitats (Murray & Littler, 1978). Their responsiveness to anthropogenic disturbances makes macroalgae a key element used to classify coastal waters into different ecological status classes (ESC). There are no studies of seaweed communities across gradients of human impacts in Cyprus, and this setting is interesting since it is so highly oligotrophic (Kletou & Hall-Spencer, 2012). Numerous macroalgal indicators have been designed to assess ecological quality, each tailored to different biogeographic provinces (Neto *et al.*, 2014). The Ecological Evaluation Index continuous formula (EEI-c) has been adopted in the central and eastern Mediterranean to assess the ESC's of coastal waters using benthic macrophytes (Orfanidis *et al.*, 2001; 2011). Here we tested this index in our surveys of coastal waters off Cyprus.

Although all marine ecosystems have been impacted by humans, rocky reefs are amongst the most affected as they have multiple pressure stressors acting synergistically (Firth *et al.*, 2016). Undeveloped shores of the Mediterranean often have a continuous belt of *Cystoseira* spp. 'forests' that support a diverse range of associated species (Bulleri *et al.*, 2002; Cheminée *et al.*, 2013; Pitacco *et al.*, 2014). *Cystoseira* forests can host richer and more abundant juvenile fish assemblages compared to turf algae or barren reefs (Thiriet *et al.*, 2016; Cheminée *et al.*, 2017). There are *ca.* 40 species of *Cystoseira* described so far and all these perennial brown fucoids, except *C. compressa*, are included in the Barcelona Convention as they are of high marine conservation importance. There has been a major global loss of canopy-forming algae and of *Cystoseira* forests throughout the Mediterranean; urbanisation, nutrient enrichment, sediment loading, physical disturbance, invasive species, overfishing and marine heat waves have all contributed to these losses (Strain *et al.*, 2014; Mineur *et al.*, 2015).

Cyprus is presently undergoing very rapid changes in coastal use (Hadjimitsis *et al.*, 2016) but there are no published studies about the impact of this expansion in resource exploitation on marine ecology. Baseline information on marine biota and sensitive ecosystems is lacking. A few macroalgal investigations were carried out at pristine locations of Cyprus for WFD and MSFD, which resulted in high ecological assessments (Stavrou & Orfanidis, 2012). Here, we conducted surveys along a 10 km stretch of coast to assess whether ocean sprawl is being managed effectively to maintain this good ecological status. We analysed seaweed assemblages on natural and modified hard substrata in the upper sublittoral zone across a gradient of anthropogenic pressures. Our surveys covered shores with limited human access, bathing waters and the most industrialised parts of Cyprus – the aim was to describe seaweed communities on shores with low to high levels of human influence, to identify patterns of change in macrophytic coverage and biomass that might be relevant to coastal pressures, substratum type and seasonality, and assess the ESC's using the biotic index EEI-c that has been developed for Mediterranean waters.

Materials and Methods

Study Area

Some areas to the west of Vasiliko Bay have not been developed and access is limited to recreation. At the western side of the bay there are restaurants and fish farms offshore, but they have clean 'Blue Flag' bathing waters. By stark contrast, the east of the bay has a completely developed foreshore; there is a naval base, a crude oil import terminal, the main power station in the region, a desalination plant and a large cement plant. The recent discovery of major gas reserves in the eastern Levantine (Ruble, 2017) has triggered further developments in eastern Vasiliko Bay; infrastructure has been built including a 1.2 km long offshore jetty and fuel storage facilities on land. Further coastal disruption is underway, such as land reclamation west of Vasiliko port and construction of a liquefied petroleum gas and bitumen storage area east of the port, where heavy dredging is anticipated to create an approach canal to the berth.

Sixteen rocky coastal sampling sites were selected along *ca* 10 km of coastline extending from Agios Georgios westwards to Zygi eastwards and encompassing Vasiliko Bay (Fig. 1). Conglomerate is the dominant substratum at sites 14-16. All other sites were limestone bedrock, the dominant intertidal and shallow sublittoral substratum. Sites 11 and 13 were exceptions, as they were breakwaters made of quarried limestone boulders that have been in place for several decades. Coastal defence boulders were also present at sites 9, 10 but sampling here occurred on natural submerged hard substrata.

Field and Laboratory work

At each sampling site, four to six replicate macroalgal samples (n = 4-6) were taken from smooth horizontal surfaces in the upper subtidal (0.3-1.5 m below the water level), in the summer (June-July of 2012) and spring (March-April of 2013). Each sampling unit was a 0.04 m^2 photoquadrat (20 cm x 20 cm) placed haphazardly over vegetated hard substrata. Macroalgae within the quadrat were then scraped off with a chisel and transported to the laboratory. Vertical photographs were also taken of the scraped area that allowed an estimation of coverage of small and encrusting species (e.g., coralline algae). To minimise the adverse impacts of scraping to *Cystoseira* forests, parts of the holdfast attached to the substratum were left behind to allow regeneration. In the laboratory macroalgae were sorted to the lowest possible taxonomic level, and the abundance of each taxon was estimated as percent coverage of the sampling surface. The surface covered by each sorted taxon in vertical projection was quantified within a transparent cuboidal container filled with seawater and having at its bottom a square 20 x 20 cm matrix divided in 100 squares, where each square represented 1% of sampling surface. *In situ* photographs of quadrats were processed to estimate percent coverage of obvious species and where appropriate modify the estimations made in the laboratory. Sorted macroalgae were blotted on filter paper and weighed (wet weight) and then dried and reweighted (dry weight). Photomicrographs to aid identification of macroalgae species were taken using Olympus CX41 microscope and Olympus SZ stereoscope fitted with a Q. Imaging Micropublisher 5.0 RTV camera. For nomenclature the AlgaeBase taxonomic database was used (Guiry & Guiry, 2018). To assess ecological quality, the abundance of the two Ecological Status Groups (ESG I and ESG II), the Ecological Evaluation Index continuous formula (EEI-c) and the Ecological Quality Ratio (EQR) were calculated for each site following Orfanidis *et al.* (2011).

Statistical Analysis

Preliminary analysis indicated that where macroalgae formed dense canopies, calculations of percent coverage based on *in situ* vertical photographs underestimated short, encrusting and sciophilic macroalgae species that develop under the dense canopy of taller photophilic species.

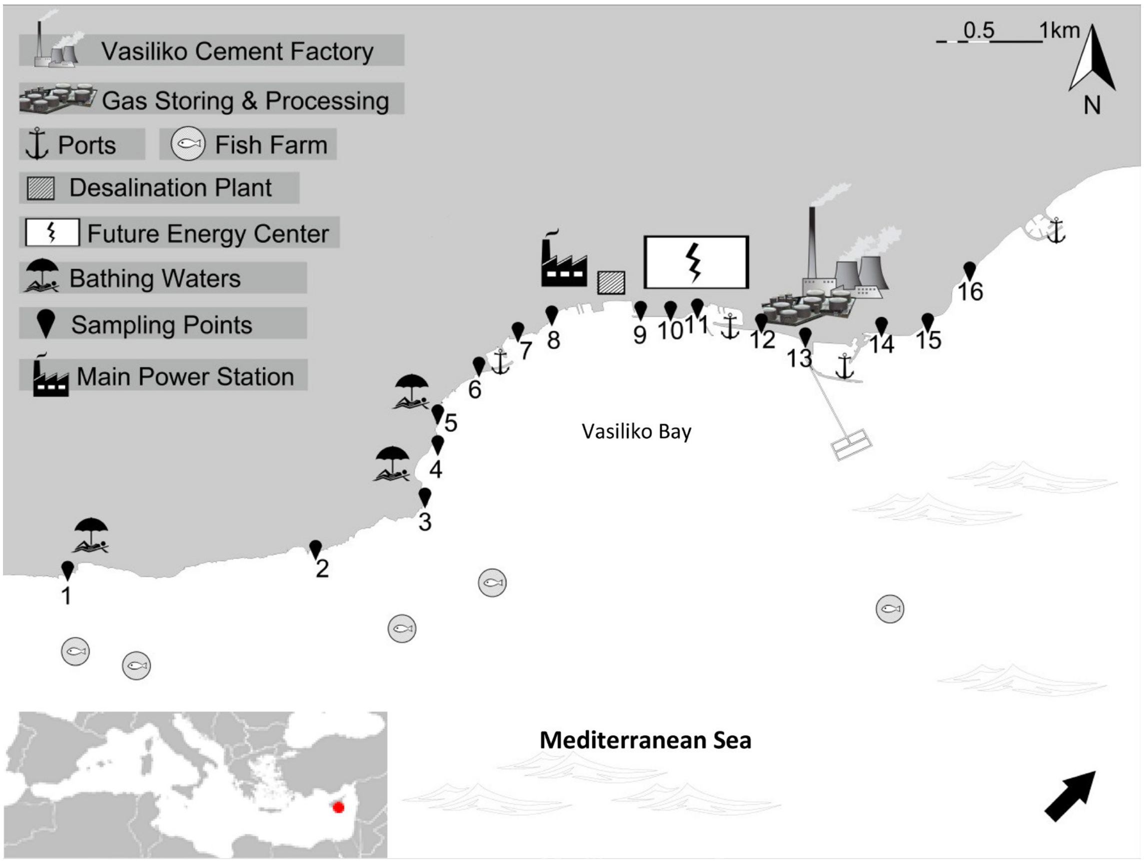

Fig. 1: Coastal developments in Vasiliko Bay, south Cyprus in the eastern Mediterranean Sea (red dot). There are bathing waters in the west with small natural beaches surrounded by limestone bedrock. The coastline in central-eastern parts of the bay is heavily industrialised, whereas east of the bay there is little coastal modification. The arrow below represents the dominant surface current direction.

Thus, data obtained using the scraping method and quantified in the lab were combined with those from *in situ* photo-quadrats of scraped substrata to add coverage of encrusting algae. Average seaweed coverage (%) and dry biomass (g m^{-2}) was calculated for all late-successional ESG I and opportunistic ESG II species. To identify the main drivers of change and potential interaction effects in coverage (%) and dry mass (g m^{-2}) for both ESG I and ESG II between sites and time, a two-way analysis of variance (ANOVA) was computed. The fixed factor sites comprised 16 site levels and the fixed factor time comprised the two sampling seasons: summer 2012 and spring 2013.

The seasonal macroalgal abundance data % coverage were square-root transformed and analysed using PRIMER v7.0.13. A non-metric multidimensional scaling (nMDS) analysis based on Bray Curtis dissimilarity was undertaken (number of restarts: 100) and a Similarity Profile Analysis (SIMPROF) was used to distinguish statistical differences in macroalgal communities among sites. In addition to this, a one-way and a two-way analysis of similarity (ANOSIM) were performed as complementary analysis based on sites, time and the crossing of the two.

The level of anthropogenic stress at each sampling site was calculated using the MALUSI index (Papathanasiou & Orfanidis, 2018). The MALUSI stress index considers different intensities of indirect and direct pressures (such as agriculture, urbanisation, industrialisation, sewage outfall, aquaculture, fresh water and sediment run off) around a 3 km radius of the study site. Sampling sites were then grouped into three categories based on the MALUSI index scores (2-4 = low stress; >4-8 = medium stress, and >8 = high stress). Sampling sites were also grouped based on the substratum type (natural limestone, natural conglomerate and ‘modified’). The ‘modified’ sampling sites were those on the external side of port breakwaters or where there was coastal hardening. Comparisons of the macroalgal community structure were conducted using one-way ANOSIM. Similarity percentage procedure (SIMPER) analysis was used to identify the species that contributed most to the dissimilarities between different levels of each category and the top three species that contributed to the similarity within each level of category across the two seasons (Clarke *et al.*, 2014).

To assess differences in ecological quality between grouped sites, Ecological Quality Ratio scores were analysed using a Kruskal-Wallis test followed by a Dunn’s pairwise comparison with a Bonferroni correction for the substratum type and stress category (Dinno, 2016). A Mann-Whitney test was used for seasonal comparisons. Main and interaction effects between sites and time were identified using a two-way ANOVA and to see how EEI-c score matched with the MALUSI index scores, a Pearson's Correlation was computed.

For all two-way ANOVA analyses, the normality of errors and homogeneity of variances were visually inspected and tested via a Shapiro-Wilk test and Levene's test, respectively. To achieve the normal distribution of errors and homogeneity of variances, the data were square-root transformed and the analysis was repeated, without requiring proceeding to a non-parametric test. For the Pearson's Correlation analysis, a power transformation was conducted, and normality of data and equal variances were verified with a Shapiro-Wilk test and F-test, respectively. For all the statistical analyses the significance level α was set at 0.05. Graphical material was generated with R-studio v3.4.2 package: ggplot2 (Wickham, 2016).

Results

Macroalgal abundance and biomass

A diverse range of macroalgal taxa was sampled from the upper subtidal, including 21 Ochrophyta, 15 Rhodophyta and 11 Chlorophyta (Table 1, Table 1 in supplementary material). *Cladophora nigrescens*, *Chondrophycus* cf. *glandulifera* and the alien *Laurencia caduciramulosa* are new records for Cyprus. Two more non-native species were sampled (*Caulerpa cylindracea* and *Stypopodium schimperi*), though in small proportions. Two seagrasses (*Posidonia oceanica*, *Cymodocea nodosa*), a Chrysophyte and Cyanobacteria were also found in samples from hard substrata.

There were around 4-10 macroalgal taxa per sampling site with the lowest diversity recorded at site 10, a heavily industrialized area (MALUSI stress index score = 10). At all sites, there was >100% algal coverage due to multiple layers of vegetation, except at site 7, near the naval port, and on conglomerate substrata (sites 14 – 16, Fig. 2). Canopy-forming *Cystoseira* and other ESG I species dominated on undeveloped shores, but their abundance was low in industrialised areas. For example, total coverage of ESG I species at site 2 was 121 ± 15%, while at site 11 coverage of the same group dropped to 15 ± 4%. The total coverage of opportunistic ESG II species on industrialised coasts matched the coverage of *Cystoseira*-dominated sites (e.g., ESG II coverage was 111 ± 9% at site 13) but their biomass was lower (ESG I biomass at site 2 was 1.11 ± 0.27 kg m^{-2}, while ESG II biomass at site 13 was 0.37 ± 0.07 kg m^{-2}) (Fig. 2). The highest total biomass was found at site 2, which also had the highest coverage of *Cystoseira*.

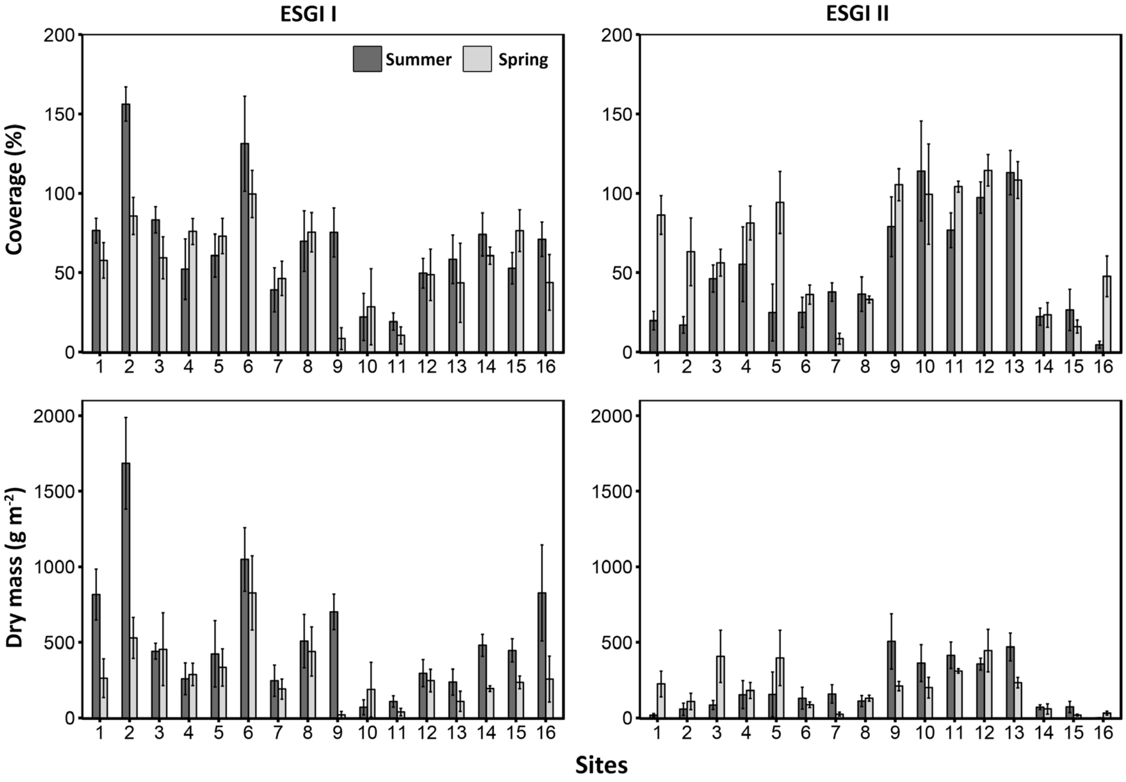

Fig. 2: Contribution to average total coverage (top panel) and average dry mass (bottom panel) of macroalgae separated into Ecological Status Group I (left) and ESG II (right), and season of sampling (error bars = SE, n= 4-6), for sampling sites across Vasiliko Bay, Cyprus in 2012-2013.

Table 1. Taxa recorded, and % coverage in 134 quadrats (0.04 m^2) sampled on hard substrata at 0.3 - 1.5 m depth across Vasiliko Bay in late summer 2012 and early spring 2013. Late-successional (Ecological Status Group I) and opportunistic species (Ecological Status Group II) are separated in five categories based on their sensitivity to pressures (Orfanidis et al., 2011). Taxa with an asterisk correspond to non-native introductions. New records for Cyprus appear in bold.

Taxa	Ecological Status Group	Summer Coverage (%)	Spring Coverage (%)
CYANOBACTERIA			
Cyanobacteria	*IIB*	0.21	0.02
CHLOROPHYTA			
Acetabularia acetabulum (Linnaeus) P.C.Silva	*IC*	0.19	0
Anadyomene stellata (Wulfen) C.Agardh	*IC*	0.97	0.39
**Caulerpa cylindracea* Sonder	*IIA*	0.21	0.75
Chaetomorpha aerea (Dillwyn) Kützing	*IIB*	0.08	3.71
Chaetomorpha linum (O.F.Müller) Kützing	*IIB*		
Cladophora laetevirens (Dillwyn) Kützing	*IIB*	5.91	2.8
***Cladophora nigrescens* Zanardini ex Frauenfeld**	*IIB*		
Dasycladus vermicularis (Scopoli) Krasser	*IIA*	4.1	4.28
Flabellia petiolata (Turra) Nizamuddin	*IC*	0.01	0
Ulva intestinalis Linnaeus	*IIB*	4.59	7.16
Ulva linza Linnaeus	*IIB*		
CHRYSOPHYTA			
Chrysophyte sp.	*IIB*	0.14	0
OCHROPHYTA			
Cladostephus spongiosum (Hudson) C.Agardh	*IIA*	6.6	3.02
Cystoseira barbata (Stackhouse) C. Agardh	*IB*	0.86	2.38
Cystoseira barbatula Kützing	*IA*	26.04	29.44
Cystoseira cf. *elegans* Sauvageau	*IA*	0	0.08
Cystoseira compressa (Esper) Gerloff & Nizamuddin	*IB*	1.57	1.14
Cystoseira crinitophylla Ercegovic	*IA*	2.09	0
Cystoseira foeniculacea (Linnaeus) Greville f. *foeniculacea*	*IA*	8.88	0.36
Dictyopteris polypodioides (A.P.De Candolle) J.V.Lamouroux	*IIA*	1.41	1.88
Dictyota dichotoma (Hudson) Lamouroux	*IIA*	1.06	4.96
Dictyota implexa (Desfontaines) J.V.Lamouroux	*IIA*	0	0.73
Dictyota mediterranea (Schiffner) G. Furnari	*IIA*	5.41	17.55
Feldmannia irregularis (Kützing) Hamel	*IIB*	0	0.97
Feldmannia simplex (P. Crouan & H. Crouan) Hamel	*IIB*	0.12	0
Halopteris scoparia (Linnaeus) Sauvageau	*IIA*	15.1	13.55
Hydroclathrus clathratus (C. Agardh) M. Howe	*IIA*	0.03	0.04
Padina pavonica (Linnaeus) Thivy	*IB*	5.95	11.17
Sargassum vulgare C. Agardh	*IB*	0.04	1.66
Scytosiphon lomentaria (Lyngbye) Link	*IIB*	0	0.07
Sphacelaria cirrosa (Roth) C. Agardh	*IIA*	4.7	3.71
**Stypopodium schimperi* (Kützing) M.Verlaque & Boudouresque	*IIA*	0.31	0.09
Taonia atomaria (Woodward) J. Agardh	*IB*	0	0.1

(continued)

Table 1 continued

Taxa	Ecological Status Group	Summer Coverage (%)	Spring Coverage (%)
		RHODOPHYTA	
Botryocladia botryoides (Wulfen) Feldmann	*IIA*	0.01	0
Chondria dasyphylla (Woodward) C. Agardh	*IIA*	0	0.1
***Chondrophycus* cf. *glandulifera* (Kützing) Lipkin & P.C Silva**	*IIA*	0.01	0.13
Dasya corymbifera J. Agardh	*IIB*	1.81	1.25
Herposiphonia secunda (C.Agardh) Ambronn	*IIB*	0.04	0
Jania rubens (Linnaeus) J.V.Lamouroux	*IC*	17.46	6.67
Jania virgata (Zanardini) Montagne	*IC*		
***Laurencia caduciramulosa* Masuda & Kawaguchi**	*IIA*	0	0.16
Laurencia obtusa (Hudson) Lamouroux	*IIA*	0.17	0.02
Corallinaceae	*IC*	0.91	0.8
Peyssonnelia sp.	*IC*	0.18	0
Polysiphonia sp.	*IIB*	0.1	0
Rytiphlaea tinctoria (Clemente) C. Agardh	*IB*	0.49	0.85
Spermothamnion flabellatum Bornet	*IIB*	0.03	0
Wrangelia penicillata (C. Agardh) C. Agardh	*IIB*	0.39	0.43
		TRACHEOPHYTA	
Cymodocea nodosa (Ucria) Ascherson	*IB*	0.54	0.14
Posidonia oceanica (Linnaeus) Delile	*IA*	0.41	0.31

Table 2. Two-way ANOVA of Coverage (%) and Dry mass (g m^{-2}) for two ecological macroalgal groups: ESG I and ESG II, based on sites, season and the interaction of the two. The "sd" denotes significant different and "ns" denotes not significant.

Variable	Effects	df	Sum of squares	Mean square	F value	*p*-value
ESG I						
Coverage (%)	Site	15	91511	6101	7.016	0.0000 sd
	Season	1	4797	4797	5.517	0.0208 sd
	Site x Season	15	23281	1552	1.785	0.0468 sd
Dry mass (g m^{-2})	Site	15	5416	361	6.508	0.0000 sd
	Season	1	1189	1189	21.427	0.0000 sd
	Site x Season	15	1792	120	2.153	0.0127 sd
ESG II						
Coverage (%)	Site	15	661	44	11.636	0.0000 sd
	Season	1	49	49	12.87	0.0005 sd
	Site x Season	15	183	12	3.229	0.0002 sd
Dry mass (g m^{-2})	Site	15	3300	220	7.309	0.0000 sd
	Season	1	5	5	0.182	0.6704 ns
	Site x Season	15	1042	69	2.307	0.0072 sd

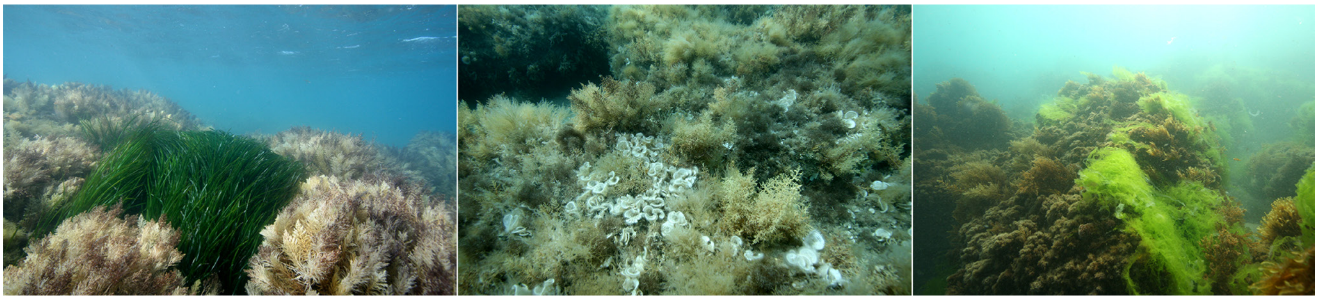

Fig. 3: Macroalgal community shifts across Vasiliko Bay, southern Cyprus in 2013. A climax community with *Cystoseira* spp. and *Posidonia oceanica* with several layers of vegetation covered limestone rocky shores with limited human access (left picture). Perennial species co-existed with bushy opportunists, at sites with moderate anthropogenic impact (middle picture). On heavily industrialised coasts opportunistic species dominated (right picture).

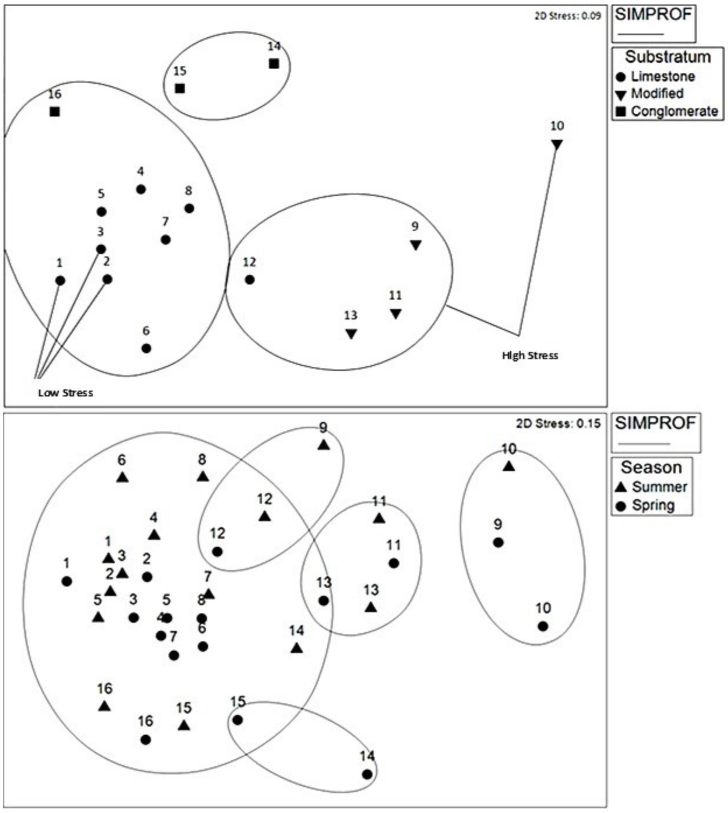

Fig. 4: Macroalgal community similarities tested with a SIMPROF test (significant different groups are assigned with a SIMPROF line) and displayed as a non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis similarities. The top panel was run with the average macroalgal % coverage at each site, separated by substratum type and stress level (high stressed and low stressed sampling sites are noted; all others were classified as medium stressed based on MALUSI index scores). The bottom panel was run with the average seasonal macroalgal % coverage at each site, separated by season of sampling.

Abundance and biomass were significantly correlated (R = 0.943, $p = 0.001$), as expected.

There was a significant interaction between the effects of the sites and time on the ESG I coverage and biomass. The effect of time was observed in most sites, and although ESG I coverage and biomass were reduced at some sites (i.e., 2, 6, and 9) between samplings, it increased in other sites (Table 2). The interactive effects between sites and time were also significant on the ESG II coverage, whereby all sites showed a change in coverage from one sampling period to the other. Significant interaction between the effects of sites and time were notable in biomass of ESG II species as well. Although the biomass of opportunistic macroalgae was different between

Table 3. Pairwise differences in macroalgal community composition across Vasiliko Bay, southern Cyprus, calculated using ANOSIM (R statistic and Significance level). The average dissimilarity and main taxa responsible for these differences calculated by SIMPER analysis are given as well as their average percent coverage. The “sd” denotes significant different and “ns” denotes not significant.

Pairwise groups of sites	R statistic	Significance level	Average Dissimilarity	Main taxa responsible for dissimilarity	Av. Coverage %
			Seasons		
Summer, Spring	0.069	0.001 sd	74.96	*Jania* spp.	17.5, 6.7
			Stress Category		
Low, Medium stressed	-0.063	0.899 ns	63.71	*D. mediterranea*	33.5, 8.5
Low, High stressed	0.557	0.001 sd	83.85	*C. barbatula*	58.8, 6.2
Medium, High stressed	0.494	0.001 sd	84.11	*H. scoparia*	0.4, 42.7
			Rocky Substratum		
Limestone, Modified	0.714	0.001 sd	85.76	*H. scoparia*	3.2, 49
Limestone, Conglomerate	0.421	0.001 sd	74.51	*P. pavonica*	4.9, 21.3
Modified, Conglomerate	0.649	0.001 sd	87.92	*H. scoparia*	49, 0.6

the sites, it only changed at a few sites between the two sampling periods and overall time did not affect the biomass of ESG II species (Table 2).

Community structure

Shifts in macroalgal community structure were observed across a gradient of stress (Fig. 3; Table 3). Late-successional species, particularly *Cystoseira barbatula* and to a lesser extent *Cystoseira foeniculacea* f. *foeniculacea,* formed dense aggregations at the sites with limited human influence. Their canopy was often partly covered by epiphytes (e.g., the *Jania* spp., *Dictyota mediterranea*, *Sphacelaria cirrosa* and *Wrangelia penicillata*) and there was a diverse understorey of Corallinaceae and fleshy seaweeds (e.g., *Padina pavonica*, *Dasycladus vermicularis, Anadyomene stellata*, *Rytiphlaea tinctoria, Cladophora* spp.). On modified coasts *Cystoseira* forests were almost absent, here opportunistic seaweed (*Halopteris scoparia*, *Cladostephus spongiosus*, *Dictyopteris polypodioides*, *Dictyota dichotoma* and nitrophilous green algae *Ulva* spp. and *Cladophora* spp.) dominated.

Macroalgal community structure differed across sites (one-way ANOSIM, R = 0.6, $p < 0.05$) depending largely on levels of stress and substratum type and to a lesser extent on the time of sampling (Fig. 4; Table 3). The macroalgal community at highly stressed sites was different compared to medium and low stressed sites. The macroalgal communities were also affected by substratum type (Table 3), for example *Padina pavonica* was more abundant on conglomerate than on limestone substrata and *Cystoseira barbatula* was the most abundant species on natural substrata but was absent from modified substrata where it was replaced by *Halopteris scoparia* turf. The macroalgal assemblages within the Vasiliko Bay changed between the two sampling periods, though the effect of time was not strong (Table 3), mainly because it was only prominent in some sites (two-way ANOSIM, R = 0.4, $p < 0.05$; Fig. 4). Spring blooms of green algae were recorded at some sites; for example, *Ulva* spp. increased from 0% to 54% coverage at the industrial site 9 and *Chaetomorpha* spp. increased from 0-2% to 11-52% coverage on conglomerate substrata (Table 4).

Ecological status classes

Shifts in macroalgal communities across the study area were well reflected by the EEI-c biotic index and further supported by the MALUSI stress index (Fig. 5, MALUSI data Table 2 in supplementary material). The two indices had a significant negative correlation on averaged seasonal values (Pearson’s correlation, $\rho = -0.647$, $p < 0.01$) and in the summer (Pearson’s correlation, $\rho = -0.729$, $p < 0.01$), but not in the spring (Pearson’s correlation, $\rho = -0.487$, $p = 0.056$). Overall, there was significant inter-site variability on EQR reflected on both sampling periods (two-way ANOVA, df = 15, F = 8.808, $p < 0.05$). Low ESC was recorded at sites 10 – 13 in both seasons. Good-High ESC was assessed at the other sites but in most cases, spring ecological assessments produced lower EQR values due to the increase in the abundance of opportunistic species (Fig. 5). The highest ESC scores were assessed at sites 2 and 6, which also had the highest macroalgal biomass whereas the lowest was assessed at sites 10 and 11, which had among the lowest species di-

Table 4. The three species with maximum contribution (%) to site similarity, their contribution % to the similarity, and the site with their highest abundance within each category for summer and spring, generated via SIMPER analysis of similarity.

Category	**Summer**			**Spring**		
MALUSI stress categories	Top 3 species with max. contribution (%) for site similarity	Contribution (%)	Site with the highest contr.	Top 3 species with max. contribution (%) for site similarity	Contribution (%)	Site with the highest contr.
Low stressed sites	*C. barbatula*	47.1	2	*C. barbatula*	40.6	2
	Jania spp.	24.6	2	*D. mediterranea*	34.9	1
	D. mediterranea	18.9	3	*D. vermicularis*	5.9	3
Medium stressed sites	*C. barbatula*	37.2	16	*C. barbatula*	35.3	8
	Cladophora spp.	22.1	15	*P. pavonica*	21.3	15
	Jania spp.	8.2	6	*Jania* spp.	12.2	6
High stressed sites	*H. scoparia*	44.6	13	*H. scoparia*	53.0	11
	Jania spp.	17.7	9	*Ulva* spp.	12.3	9
	Cladophora spp.	4.5	11	*Jania* spp.	7.2	12
Substratum						
Limestone	*C. barbatula*	37.1	2	*C. barbatula*	43.7	2
	Jania spp.	18.1	6	*D. mediterranea*	20.2	1
	D. mediterranea	12.9	3	*Jania* spp.	11.2	6
Modified	*H. scoparia*	51.1	13	*H. scoparia*	54.7	11
	Jania spp.	17.5	9	*Ulva* spp.	18.6	9
	Cladophora spp.	5.0	11	*D. dichotoma*	9.4	10
Conglomerate	*C. barbatula*	38.2	16	*P. pavonica*	34.2	15
	Cladophora spp.	25.0	15	*Chaetomorpha* spp.	27.0	16
	P. pavonica	19.9	14	*C. barbata*	16.6	15

versity and biomass. The overall EQR of the Vasiliko Bay was similar in spring and summer (Man-Whitney test, W = 5106, p = 0.09), although the effect of time on EQR was prominent on some site levels, showing significant differences in sites 1, 2, 5, 7, 9 and 16 (two-way ANOVA, df = 1, F = 8.035, $p < 0.05$). No interaction effect was observed between sites and time (two-way ANOVA, df = 15, F = 1.559, $p > 0.05$). Significant differences of the EQR score were also observed between the different levels of coastal stress as well as between modified and natural substrata (Table 5). No differences in the EQR scores were detected between natural substrata limestone and conglomerate and between low and medium stressed sampling sites.

Discussion

Our surveys on the southern coast of Cyprus identified 51 taxa of macrophytes. Three species are reported for the first time from Cypriot waters, expanding the existing checklist of seaweed species (Tsiamis *et al.*, 2014). One of these, *Laurencia caduciramulosa*, is native to SE Asia and was described for the first time from the Mediterranean Sea by Furnari *et al.* (2001).

Our results are consistent with global observations that human impacts combine to cause the loss of perennial canopy-forming brown seaweeds and a proliferation of opportunistic macroalgae (Scherner *et al.*, 2013; Strain *et al.*, 2014). In our surveys, canopy-forming *Cystoseira* dominated shallow subtidal hard substrata showing

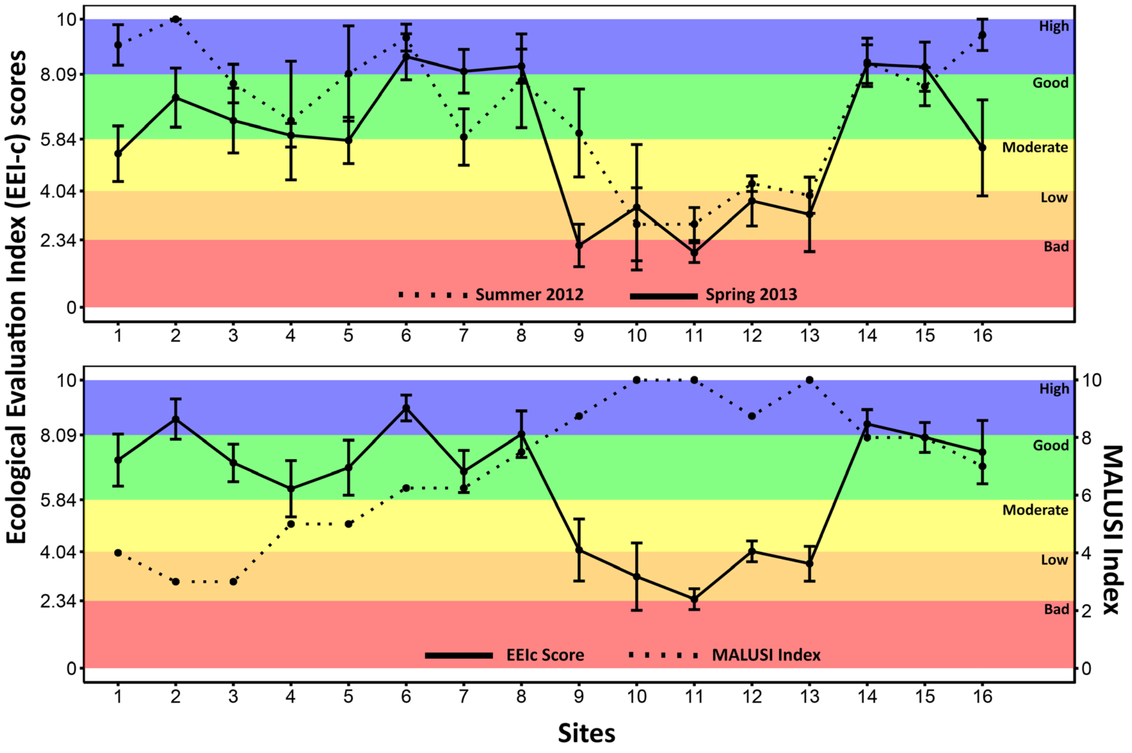

Fig. 5: Top panel: Mean values (error bars = SE, n = 4-6) variation of Ecological Evaluation Index (EEI-c) across Vasiliko Bay, Cyprus, in summer 2012 and spring 2013. Lower panel: Mean values (error bars = SE, n = 8-10) variation of EEI-c and MALUSI index across Vasiliko Bay, Cyprus, for the period summer 2012 to spring 2013. Ecological status classes colour categorisation corresponds to EEI-c scores.

Table 5. The pairwise comparisons based on the EQR score calculated with the EEI-c index (Orfanidis et al., 2011), and statistical differences between different seasons, substrata and stress category in Vasiliko Bay, Cyprus. The "sd" denotes significant different and "ns" denotes not significant.

Groups		Average EQR	Statistical test	df	test statistic	*p*-value
Season (Summer, Spring)		0.59, 0.48	Mann-Whitney	-	W = 5106.0	0.09 ns
Substratum			Kruskal-Wallis	2	$\chi^2 = 42.3$	0 sd
	Limestone, Modified	0.63, 0.17		-	z = 5.7	0 sd
	Limestone, Conglomerate	0.63, 0.75	Dunn's test	-	z = 1.5	0.21 ns
	Conglomerate, Modified	0.75, 0.17		-	z = 5.7	0 sd
Stress category			Kruskal-Wallis	2	$\chi^2 = 53.7$	0 sd
	High, Medium	0.19, 0.70		-	z = -6.9	0 sd
	High, Low	0.19, 0.71	Dunn's test	-	z = -5.3	0 sd
	Low, Medium	0.71, 0.70		-	z = 0.4	1 ns

the good environmental quality of waters, in which human access was limited to recreation. Algal biomass was considerably higher than at impacted sites as there were more perennial species present, an indication of a healthy shallow rocky reef ecosystem (Panayotidis *et al.*, 2004; Sala *et al.*, 2012). The canopy of *Cystoseira barbatula* diminished near industrialised areas and got replaced by simpler communities, dominated by stress-resistant and ephemeral species such as *Halopteris scoparia* and *Ulva* spp. Similar community shifts from canopy-forming fucoids to bushy, turf or fleshy opportunistic species have been widely reported across gradients of impact around the Mediterranean Sea (Benedetti-Cecchi *et al.*, 2001; Thibaut *et al.*, 2005, 2015; Arévalo *et al.*, 2007; Mangialajo *et al.*, 2008; Orlando-Bonaca *et al.*, 2008; Perkol-Finkel & Airoldi, 2010; Pinedo *et al.*, 2013; Tsiamis *et al.*, 2013; Iveša *et al.,* 2016; Badreddine *et al.*, 2018; Orlando-Bonaca & Rotter, 2018), but this is the first time it is reported from the oligotrophic waters of Cyprus.

Opportunistic algae dominated in spring at some impacted sites, but they did not approach the high levels of biomass found in unimpacted *Cystoseira* forests. Blooms of green algae (*Ulva* and *Chaetomorpha* spp.) occurred on highly stressed shores during spring, which may be due to eutrophication, whereas a bloom of *Dictyota mediterranea* was recorded in spring on the western side of the study area reflecting the typical annual cycle of Dictyotales (Tronholm *et al.*, 2008).

The most significant factors that affected shallow subtidal communities were human stress level, calculated with the MALUSI index, and the type of substratum available for seaweed growth. On breakwaters and coastline defences *Cystoseira* spp. were almost absent, even though these were constructed using natural limestone boulders several decades ago. This emphasises the fact that man-made structures do not function as surrogates of natural rocky reefs (Bulleri & Chapman, 2010), as they are composed of different assemblages of species and can have significantly lower abundances of large perennial algae (Ferrario *et al.*, 2016). Despite differences in macroalgal community structure, the two natural substrata studied (limestone and conglomerate), had similar ESC, as assessed with the EEI-c, mainly because macroalgal community structure was dominated by species of the same ESG. The averaged and the summer ESC scores were negatively correlated with the MALUSI stress index, which demonstrates that the EEI-c is a robust way of assessing the environmental quality of coastal waters as it is unaffected by natural variability of communities due to different type of substratum and in the summer, macroalgal community shifts from perennial species to opportunistic species reflect an increasing gradient of anthropogenic stress. The macroalgal biotic index (EEI-c) differed at some sites between the two survey periods as opportunistic seaweed increased abundance in spring, even at low stressed sites. This confirms the need to sample in summer to accurately assess the ESC of coastal waters using macroalgal-based indicators (Orfanidis *et al.*, 2011). Spring assessments can produce the ‘worst’ ESC scores, but unlike summer assessments, they may not be always representative of anthropogenic stress.

As in many places around the world, a single human generation has transformed the coastline of Cyprus creating a heavily industrialised foreshore in Vasiliko Bay. Despite major alterations to the area, there had been no assessments off the marine ecosystem impacts of these developments. High ESC was reported on other coastlines of Cyprus monitored for WFD and MSFD (Stavrou & Orfanidis, 2012). In this study, low ESC was assessed along industrialised coastlines where artificial breakwaters and coastal hardening had modified the shores. There was likely a combination of several impacts such as contamination from ports, cement dust deposition, litter, warm water from a power station, brine from a desalination unit and possibly waste effluents from fish farms operations. Major industrial developments are still underway in Vasiliko Bay, in 2017 land reclamation killed the last remnant of *Cystoseira* habitat in the eastern side of the bay. We recommend that *Cystoseira* forests receive more attention when coastal developments are evaluated in Cyprus. Our baseline data on macroalgal communities will allow future comparisons and ecological assessments in the region. The bad ESC scored along the modified, industrial coastline should alert those responsible for managing the use of coastal marine resources in Cyprus as attempts may be needed to meet the obligations of the European MSFD.

In summary, it is not too late to conserve *Cystoseira* forests by raising public awareness and mitigating human impacts on coastal ecosystems (Gianni *et al.*, 2013). The disappearance of these fucoid forests leads to systems with lower biodiversity and reduced ecosystem services to humanity (Chapin *et al.*, 2000; Cardinale *et al.*, 2012). Shallow reefs around parts of Cyprus are still covered in luxuriant *Cystoseira* forests, but this habitat is threatened by coastal developments. At the industrialised and modified forefront of Vasiliko Bay, the *Cystoseira* canopy and was replaced by opportunistic algae that had lower biomass per area. Ecological assessments showed that the coastal waters in this side of the bay did not meet the quality targets of European Directives. Despite the ecological degradation, industrialisation is expanding, and construction has begun for a booming hydrocarbon industry, now that large gas reserves have been located. Furthermore, there are approved government plans to construct a new port to serve fish farmers, near the dense *Cystoseira* forests west of Vasiliko Bay. As pressures continue to mount, it remains to be seen whether the MSFD will be applied to ensure that marine resources are managed sustainably in Cyprus.

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