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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. Quadrats were used to survey the algal communities in summer 2012 and spring 2013. Of the 49 taxa we recorded, *Cladophora nigrescens* and *Laurencia caduciramulosa* (a non-native species) are new records for Cyprus. Brown algae of the genus *Cystoseira* formed dense forests covering rocky substrata on shorelines with limited human access. *Cystoseira* 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 with lower biomass, such as nitrophilous *Ulva* and *Chaetomorpha* species, proliferated in spring. The Ecological Evaluation Index we used was a robust indicator of shoreline environmental quality. 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*, *Cystoseira*, ecological assessment, Marine Strategy Framework Directive.

1. 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 and Little, 1978). Their responsiveness to anthropogenic disturbances makes macroalgae a key group used to assess the ecological status of coastal waters. There have been no previous studies of seaweed communities and human impacts in Cyprus, and this setting is interesting since it is so highly oligotrophic (Kletou and 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 (EEIc) has been adopted in the central and eastern Mediterranean to assess the status of coastal waters using benthic primary producers (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 impacted as they have multiple pressure stressors acting synergistically (Firth et al., 2017). 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 and 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 using a biological indicator that has been developed for Mediterranean waters.

2. Methods

2.1 Study Area

Some areas to the west of Vasiliko Bay (south Cyprus) 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 stations were selected along *ca* 10 km of coastline extending from Agios Georgios westwards to Zygi eastwards and encompassing Vasiliko Bay (Figure 1). Conglomerate is the dominant substratum at stations 14-16. All other stations were limestone bedrock, the dominant intertidal and shallow sublittoral substratum. Stations 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 stations 9, 10 but sampling here occurred on natural submerged hard substrata.

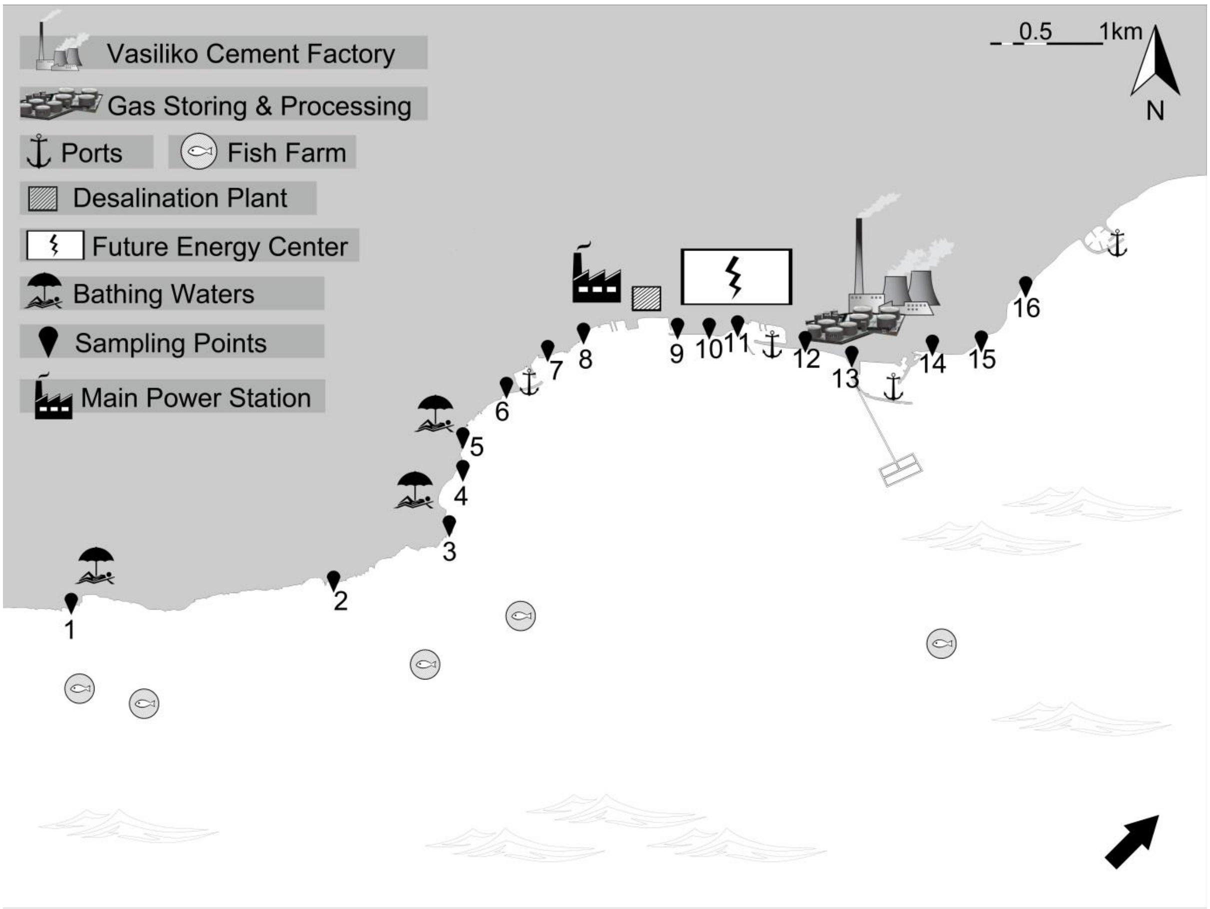

Figure 1. Coastal developments in Vasiliko bay, south Cyprus. There are bathing waters in the west with small natural beaches surrounded by limestone bedrock. The coastline in central-eastern parts of the bay is heavily industrialised, whereas east of the bay there is little coastal modification. The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

2.2 Sampling and analysis

At each sampling station, four replicate macroalgal samples 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, the holdfast and the lower parts of the thallus 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 and Guiry, 2018). To assess ecological quality, the abundance of the two Ecological State Groups (ESG I and ESG II), the Ecological Evaluation Index continuous formula (EEIc) and the Ecological Quality Ratio (EQR) were calculated for each station following Orfanidis et al. (2011).

2.3 Statistical Analysis

Preliminary analysis indicated that where macroalgae formed dense canopies, calculations of percent cover based on *in situ* vertical photographs underestimated short, encrusting and sciophilic macroalgae species that develop under the dense canopy of taller photophilic species. 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 cover (%) 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 stations and time, a 2-way analysis of variance (ANOVA) was computed. The fixed factor stations comprised 16 station 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 stations. In addition to this, a 1-way and a 2-way analysis of similarity (ANOSIM) were performed as complementary analysis based on stations, time and the crossing of the two.

The level of anthropogenic stress at each sampling station was calculated using the MALUSI index (Papathanasiou and Orfanidis 2017). 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. The area covered by each pressure around each sampling site was calculated with satellite images processed in photoQuad software (Trygonis and Sini 2012). Sampling stations were then grouped into three categories based on the MALUSI index scores (low impact 2-4, medium impact >4-8 and high impact >8-10). Sampling stations were also grouped based on the substratum type (natural limestone, natural conglomerate and ‘modified’). The ‘modified’ sampling stations were those on the external side of port breakwaters or where there was coastal hardening. Comparisons of the macroalgal community structure were conducted using 1-way ANOSIM followed by similarity percentage procedure (SIMPER) analysis 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 stations, 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 impact status (Dinno, 2016). A Mann-Whitney test was used for seasonal comparisons. Main and interaction effects between stations and time were identified using a 2-way ANOVA and to see how EEIc score matched with the MALUSI index scores, a Pearson's Correlation was computed.

For all 2-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. When assumptions were not met, the data were square-root transformed and the analysis was repeated. 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. Graphical material was generated with R-studio v3.4.2 package: ggplot2 (Wickham, 2016).

3. Results

3.1 Macroalgal abundance and biomass

A diverse range of macroalgal taxa were sampled from the upper subtidal, including 21 Ochrophyta spp., 15 Rhodophyta spp. and 11 Chlorophyta spp. (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.

Table 1. Taxa recorded, and % cover 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 State Group I) and opportunistic species (Ecological State 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 State Group**	**Cover %**
CHLOROPHYTA		
Acetabularia acetabulum (Linnaeus) P.C.Silva	*IC*	0.10
Anadyomene stellata (Wulfen) C.Agardh	*IC*	0.69
Caulerpa cylindracea Sonder	*IIA*	0.47
Chaetomorpha aerea (Dillwyn) Kützing	*IIB*	1.81
Chaetomorpha linum (O.F.Müller) Kützing	*IIB*	
Cladophora laetevirens (Dillwyn) Kützing	*IIB*	3.81
***Cladophora nigrescens* Zanardini ex Frauenfeld**	*IIB*	
Dasycladus vermicularis (Scopoli) Krasser	*IIA*	4.19

Flabellia petiolata (Turra) Nizamuddin	*IC*	0.00
Ulva intestinalis Linnaeus	*IIB*	5.82
Ulva linza Linnaeus	*IIB*	
OCHROPHYTA		
Cladostephus spongiosum (Hudson) C.Agardh	*IIA*	4.89
Cystoseira barbata (Stackhouse) C. Agardh	*IB*	1.58
Cystoseira barbatula Kützing	*IA*	27.66
Cystoseira cf. *elegans* Sauvageau	*IA*	0.04
Cystoseira compressa (Esper) Gerloff & Nizamuddin	*IB*	1.37
Cystoseira crinitophylla Ercegovic	*IA*	1.09
Cystoseira foeniculacea (Linnaeus) Greville f. *foeniculacea*	*IA*	4.81
Dictyopteris polypodioides (A.P.De Candolle) J.V.Lamouroux	*IIA*	1.64
Dictyota dichotoma (Hudson) Lamouroux	*IIA*	2.92
Dictyota implexa (Desfontaines) J.V.Lamouroux	*IIA*	0.35
Dictyota mediterranea (Schiffner) G. Furnari	*IIA*	11.21
Feldmannia irregularis (Kützing) Hamel	*IIB*	0.46
Feldmannia simplex (P. Crouan & H. Crouan) Hamel	*IIB*	0.06
Halopteris scoparia (Linnaeus) Sauvageau	*IIA*	14.36
Hydroclathrus clathratus (C. Agardh) M. Howe	*IIA*	0.03
Padina pavonica (Linnaeus) Thivy	*IB*	8.45
Sargassum vulgare C. Agardh	*IB*	0.82
Scytosiphon lomentaria (Lyngbye) Link	*IIB*	0.03
Sphacelaria cirrosa (Roth) C. Agardh	*IIA*	4.23
**Stypopodium schimperi* (Kützing) M.Verlaque & Boudouresque	*IIA*	0.21
Taonia atomaria (Woodward) J. Agardh	*IB*	0.05
RHODOPHYTA		
Botryocladia botryoides (Wulfen) Feldmann	*IIA*	0.01
Chondria dasyphylla (Woodward) C. Agardh	*IIA*	0.05
***Chondrophycus* cf. *glandulifera* (Kützing) Lipkin & P.C Silva**	*IIA*	0.07
Dasya corymbifera J. Agardh	*IIB*	1.54
Herposiphonia secunda (C.Agardh) Ambronn	*IIB*	0.02
Jania rubens (Linnaeus) J.V.Lamouroux	*IC*	12.31
Jania virgata (Zanardini) Montagne	*IC*	
****Laurencia caduciramulosa* Masuda & Kawaguchi**	*IIA*	0.08
Laurencia obtusa (Hudson) Lamouroux	*IIA*	0.10
Corallinaceae	*IC*	0.86
Peyssonnelia sp.	*IC*	0.09
Polysiphonia sp.	*IIB*	0.05
Rytiphlaea tinctoria (Clemente) C. Agardh	*IB*	0.66
Spermothamnion flabellatum Bornet	*IIB*	0.01
Wrangelia penicillata (C. Agardh) C. Agardh	*IIB*	0.41
TRACHEOPHYTA		
Cymodocea nodosa (Ucria) Ascherson	*IB*	0.35
Posidonia oceanica (Linnaeus) Delile	*IA*	0.37

There were around 4-10 macroalgal taxa per sampling station with the lowest diversity recorded at station 10, a heavily industrialized area (MALUSI stress index score = 10). At all stations, there was >100% algal cover due to multiple layers of vegetation, except at station 7 near the naval port and on conglomerate substrata (stations 14 – 16, Figure 2). Canopy-forming *Cystoseira* and other ESG I species dominated on undeveloped shores, but their abundance was low in industrialised areas. The total cover of opportunistic ESG II species on industrialised coasts (e.g. station 13) matched the cover of *Cystoseira*-dominated stations but their biomass was lower (Figure 2). The highest biomass was found at station 2, which also had the highest cover of *Cystoseira*. Abundance and biomass were significantly correlated (R = 0.943, p = 0.001), as expected.

There was a significant interaction between the effects of the stations and time on the ESG I cover and biomass. The effect of time was observed in most stations, and although ESG I coverage and biomass was reduced at some stations (i.e. 2, 6, and 9) between samplings, it increased in other stations (Table 2). The interactive effects between stations and time were also significant on the ESG II coverage, whereby all stations showed a change in cover from one sampling period to the other. Significant interaction between the effects of stations and time were notable in biomass of ESG II species as well. Although the biomass of opportunistic macroalgae was different between the stations, it only changed at a few stations between the two sampling periods and overall time did not affect the biomass of ESG II species (Table 2).

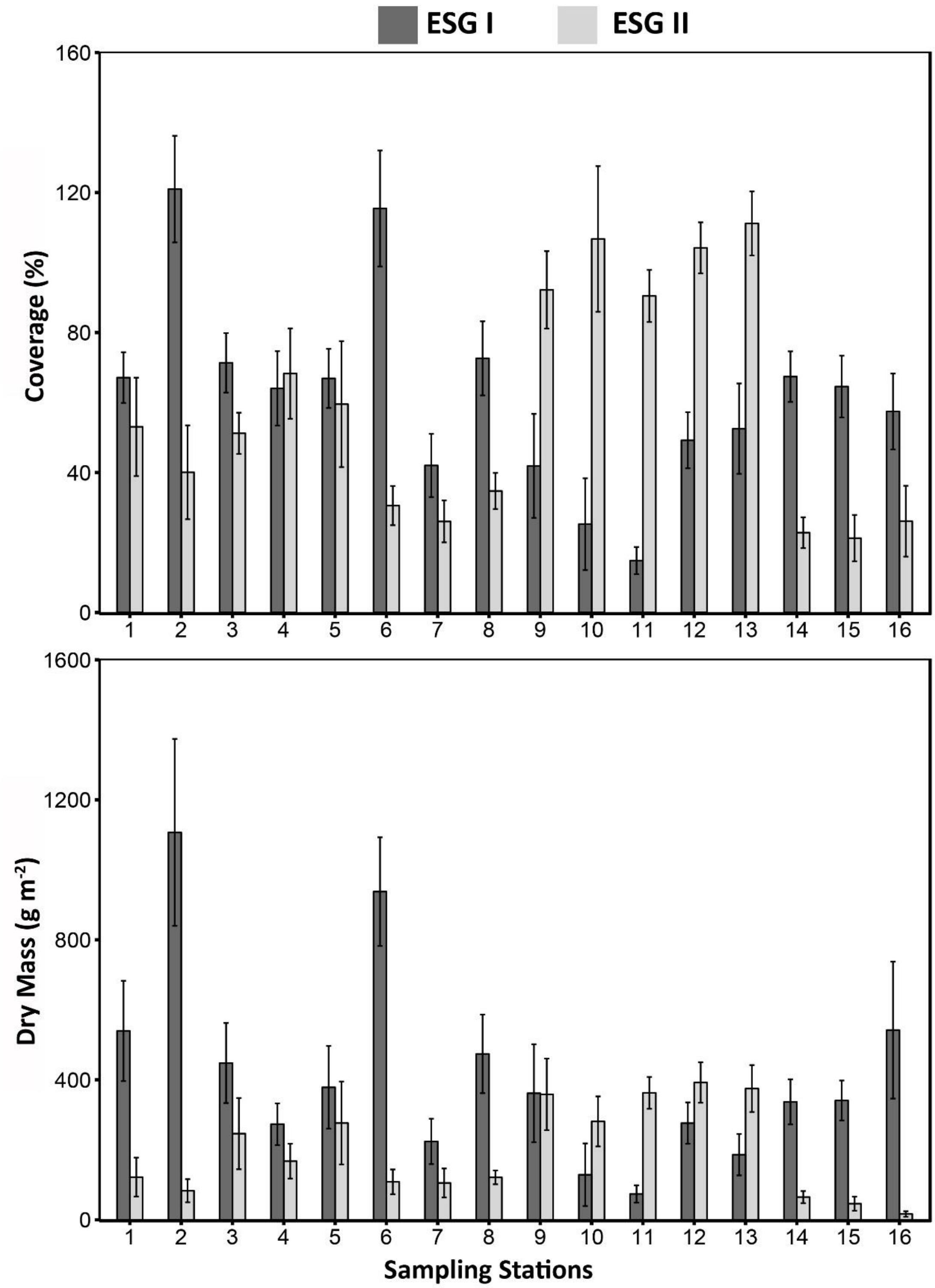

Figure 2. Contribution to average total cover (top panel) and average dry biomass (bottom panel) of macroalgae separated into Ecological State Group I (dark grey) and Ecological State Group II (light grey) (error bars = SE, n= 8-10), for sampling stations across Vasiliko Bay, south Cyprus in 2012-2013.

Table 2. Two-way ANOVA of Cover (%) and Dry mass (g m^{-2}) for two ecological macroalgal groups: ESG I and ESG II, based on stations, 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						
Cover (%)	Station	15	91511	6101	7.016	0.0000 sd
	Season	1	4797	4797	5.517	0.0208 sd
	Station x Season	15	23281	1552	1.785	0.0468 sd
Dry mass (g m^{-2})	Station	15	5416	361	6.508	0.0000 sd
	Season	1	1189	1189	21.427	0.0000 sd
	Station x Season	15	1792	120	2.153	0.0127 sd
ESG II						
Cover (%)	Station	15	661	44	11.636	0.0000 sd
	Season	1	49	49	12.87	0.0005 sd
	Station x Season	15	183	12	3.229	0.0002 sd
Dry mass (g m^{-2})	Station	15	3300	220	7.309	0.0000 sd
	Season	1	5	5	0.182	0.6704 ns
	Station x Season	15	1042	69	2.307	0.0072 sd

3.2 Community structure

Shifts in macroalgal community structure were observed across a gradient of impact (Figure 3; Table 3). Late-successional species, particularly *Cystoseira barbatula* and to a lesser extent *Cystoseira foeniculacea* f. *foeniculacea*, formed dense aggregations at the stations 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 (*Halopteris scoparia*, *Cladostephus spongiosus*, *Dictyopteris polypodioides*, *Dictyota dichotoma* and others) and nitrophilous green algae (*Ulva* spp. and *Cladophora* spp.) dominated.

Figure 3. Macroalgal community shifts across Vasiliko Bay, southern Cyprus in 2013. A climax community with *Cystoseira* spp. and seagrass 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).

Macroalgal community structure differed across stations (1-way ANOSIM, R = 0.6, $p < 0.05$) depending largely on levels of impact and substratum type and to a lesser extent on the time of sampling (Figure 4; Table 3). The macroalgal community at high impact stations was different compared to medium and low impact stations (Table 3). 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 stations (2-way ANOSIM, R = 0.4, $p < 0.05$; Figure 4). Spring blooms of green algae were recorded at some stations; for example, *Ulva* spp. increased from 0% to 54% cover at the industrial station 9 and *Chaetomorpha* spp. increased from 0-2% to 11-52% cover on conglomerate substrata (Table 4).

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 cover. The "sd" denotes significant different and "ns" denotes not significant.

Pairwise groups	**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
			Impact status		
Low Impact, Medium Impact	-0.063	0.899 ns	63.71	*D. mediterranea*	33.5, 8.5
Low Impact, High Impact	0.557	0.001 sd	83.85	*C. barbatula*	58.8, 6.2
Medium Impact, High Impact	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

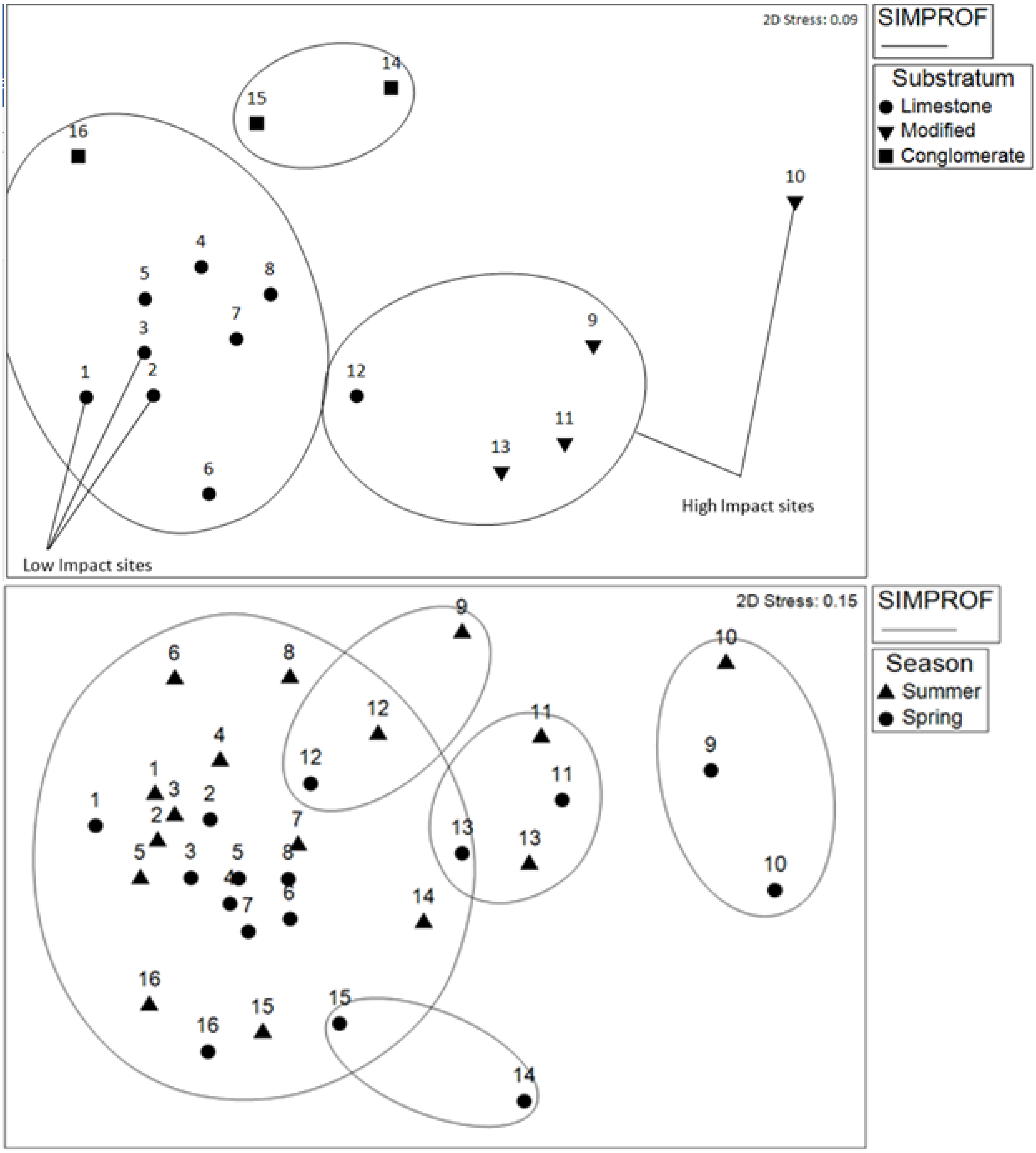

Figure 4. Macroalgal community similarities tested with a SIMPROF test (significant different groups are assigned with a SIMPROF line) and displayed as a non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis similarities. The top panel was run with the average macroalgal % cover at each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

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

Category	**Summer**			**Spring**		
Impact status	Top 3 indicator species for similarity	Contribution %	Station with the highest contr.	Top 3 indicator species for similarity	Contribution %	Station with the highest contr.
Low impact	*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 impact	*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 impact	*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

3.3 Ecological quality status

Shifts in macroalgal communities across the study area were well reflected by the EEIc biotic index and further supported by the MALUSI stress index (Figure 5, MALUSI data Table 2 in supplementary material). The two indices had a significant negative correlation (Pearson's correlation, $\rho = -0.647$, $p < 0.05$). Overall, there was significant inter-station variability on EQR reflected on both sampling periods (2-way ANOVA, df = 15, F = 8.808, $p < 0.05$). Low ecological quality was recorded at stations 10 – 13 in both seasons. Good-High ecological status was assessed at the other stations but in most cases, spring ecological assessments produced lower EQR values due to the increase in the abundance of opportunistic species (Figure 5). The highest ecological status scores were assessed at stations 2 and 6 which also had the highest macroalgal biomass whereas the lowest ecological status score was assessed at stations 10 and 11, which had among the lowest species diversity 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 station levels, showing significant differences in stations 1, 2, 5, 7, 9 and 16 (2-way ANOVA, df = 1, F = 8.035, $p < 0.05$). No interaction effect was observed between stations and time (2-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 impact as well as between modified and natural substrata (Table 4). No differences in the EQR scores were detected between natural substrata limestone and conglomerate and between low impact and medium impact sampling stations.

Table 4. The pairwise comparisons based on the EQR score calculated with the EEIc index (Orfanidis et al., 2011), and statistical differences between different seasons, substrata and impact status 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
Impact status		Kruskal-Wallis	2	$\chi^2 = 53.7$	0 sd
High Impact, Medium Impact	0.19, 0.70		-	z = -6.9	0 sd
High Impact, Low Impact	0.19, 0.71	Dunn's test	-	z = -5.3	0 sd
Low Impact, Medium Impact	0.71, 0.70		-	z = 0.4	1 ns

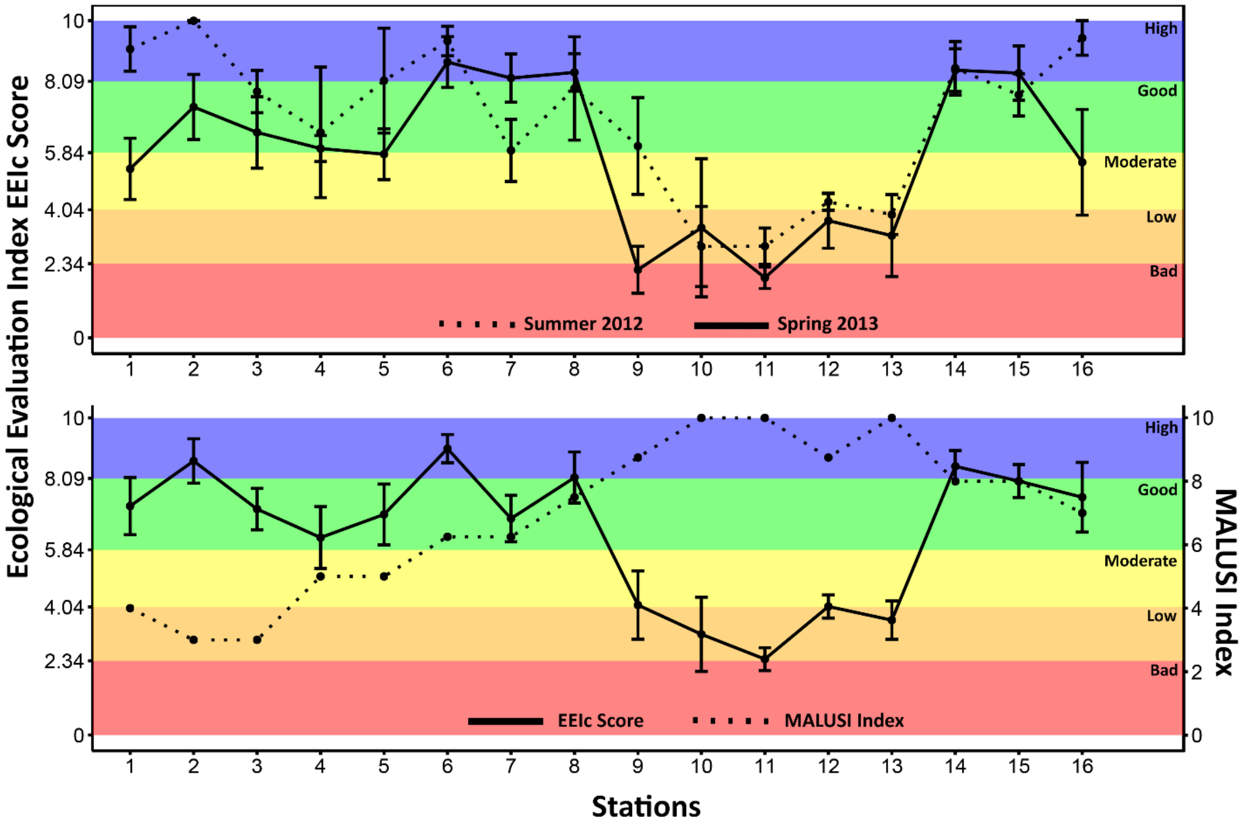

Figure 5. Top panel: ecological status across Vasiliko Bay, Cyprus, in summer 2012 and spring 2013 (error bars = SE, n= 4-6). In spring 2013, the status was lower in most cases lower due to an increased abundance of opportunistic species. Lower panel: average EEIc (error bars = SE, n= 8-10) and MALUSI indices. Low ecological status and high MALUSI scores were recorded at stations 9-13 but macroalgal communities revealed good ecological status elsewhere. *Note:* Ecological status colour ranges correspond only to EEIc scores.

4. Discussion

Our surveys on the south coast of Cyprus identified 49 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 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 (Sala et al., 2012). A 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 (Benedetti-Cecchi et al., 2001; Thibaut et al., 2005, 2015; Arévalo et al., 2007; Mangialajo et al., 2008; Pinedo et al., 2013; Tsiamis et al., 2013; Badreddine et al., 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 impacted 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 impacts, 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 and 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 the similar ecological status, as assessed with the Ecological Evaluation Index continuous formula (EEIc), mainly because macroalgal community structure was dominated by species of the same ecological group. The ecological evaluation scores were strongly negatively correlated with the MALUSI stress index, which demonstrates that the EEIc 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 reflects macroalgal community shifts from perennial species to opportunistic species as anthropogenic stress increases. The macroalgal index of ecological quality differed at some stations between the two survey periods, which confirms the need to sample two or more seasons a year to accurately assess the ecological quality of coastal waters using macroalgal-based indicators (Orfanidis et al., 2011).

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 on the marine ecosystem impacts of these developments. High ecological status was assessed in other coastlines of Cyprus monitored for WFD and MSFD (Stavrou and Orfanidis, 2012). In this study, low ecological status 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 in Cyprus will allow future comparisons and ecological assessments in the region. The bad ecological status 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 Marine Strategy Framework Directive.

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. In the Vasiliko Bay area, there are approved government plans to construct a port to serve fish farmers and construction has begun for a booming hydrocarbon industry, now that large gas reserves have been located. As pressures continue to mount it remains to be seen whether the Marine Strategy Framework Directive will be applied to ensure that marine resources are managed sustainably in Cyprus.

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Supplementary Material

Table 1. Taxa recorded, and % cover in 8-10 quadrats at each station, 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 State Group I) and opportunistic species (Ecological State Group II) are separated in five categories based on their sensitivity to pressures (Orfanidis et al., 2011).

Species/Taxa	**Functional Group**	**Sampling Station**															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Cystoseira cf elegans Sauvageau	IA	0.6															
Cystoseira barbatula Kützing emend. Cormaci, Furnari & Giaccone	IA	47.1	83.5	45.8	29.4	51.0	42.4	26.3	26.4			1.3	23.8	2.4	2.6	3.1	44.0
Cystoseira crinitophylla Ercegovic	IA									11.9	6.4						
Cystoseira foeniculacea (Linnaeus) Greville f. *foeniculacea*	IA				12.3		7.8		28.0	12.1	1.5	0.7	5.1		9.1	2.8	
Posidonia oceanica (Linnaeus) Delile	IA				5.8				3.4							0.3	
Cymodocea nodosa (Ucria) Ascherson	IB				0.5												5.4
Cystoseira barbata (Stackhouse) C. Agardh var. *barbata*	IB										2.9		3.1	0.9	1.0	7.6	1.0
Cystoseira compressa (Esper) Gerloff & Nizamuddin f. *compressa*	IB	1.6					8.9			3.1	0.5	2.8					
Padina pavonica (Linnaeus) Thivy	IB		0.7	2.6	1.4	8.8	8.6	4.9	7.2	0.7	1.5	2.5	1.6	21.4	37.5	2.9	5.4
Rytiphlaea tinctoria (Clemente) C. Agardh	IB	9.7	1.3			0.1											
Sargassum vulgare C. Agardh	IB									0.5	12.5	0.4		0.3			
Acetabularia mediterranea J.V.Lamouroux	IC																1.6
Anadyomene stellata (Wulfen) C.Agardh	IC	0.3	1.5	3.1	3.4			0.2	0.2						1.9	1.2	0.6
Flabellia petiolata (Turra) Nizamuddin	IC								0.6								
Jania spp. (*J. rubens* (Linnaeus) J.V.Lamouroux + *J. virgata* (Zanardini) Montagne)	IC	6.2	3.3	16.7	1.7	3.6	46.4	1.7	7.4	13.6		7.2	15.6	25.8	6.3	1.8	
Lithophyllum sp.	IC	1.9	3.8	3.3	0.6	3.4	1.5										
Peyssonnelia sp.	IC				0.6				0.1	0.3				1.1			
Taonia atomaria (Woodward) J. Agardh	IC													0.7			
Botryocladia botryoides (Wulfen) Feldmann	IIA			0.1													
Caulerpa racemosa var. cylindracea (Sonder) Verlaque, Huisman & Boudouresque	IIA	0.6		0.4	2.7	0.4			1.9			0.2		1.3	0.1		
Cladostephus spongiosus (Hudson) C.Agardh	IIA							4.6	1.6	16.4	7.0	0.7	4.5				

Dasycladus vermicularis (Scopoli) Krasser	IIA	1.1	4.1	1.9	13.5	22.1	7.3	3.5	1.4	0.5		0.9	2.9	1.0			
Dictyopteris polypodioides (A.P.De Candolle) J.V.Lamouroux	IIA								0.4	4.6	0.4	2.8	0.8	15.1	0.6		0.6
Dictyota dichotoma (Hudson) Lamouroux var. *dichotoma*	IIA	1.1			1.3	0.8	5.9	5.3	0.1	5.7	19.0			3.8	2.1	1.5	
Dictyota mediterranea (Schiffner) Furnari	IIA	44.1	25.2	31.6	26.8	13.6		4.8	15.5			0.3	1.4	3.4	0.1	0.5	8.1
Dictyota linearis (C. Agardh) Greville	IIA					5.8											
Dictyota sp.	IIA			0.1													
Halopteris scoparia (Linnaeus) Sauvageau	IIA		2.7						1.4	26.8	12.6	74.1	21.2	75.8	1.7		
Hydroclathrus clathratus (C.Agardh) M.A.Howe	IIA		0.3		0.1									0.4			
Laurencia caduciramulosa Masuda & Kawaguchi	IIA		0.8	0.4													0.1
Laurencia obtusa (Hudson) Lamouroux	IIA	1.0		0.5			0.1										
Sphacelaria cirrosa (Roth) C. Agardh	IIA	2.3	2.4	1.4	9.7	9.6	1.3	2.2	4.6	6.6			24.5			0.6	
Stypopodium schimperi (Kützing) M.Verlaque & Boudouresque	IIA			1.1					0.2	1.3				0.6		0.1	
Cladophora spp. (*C. laetevirens* (Dillwyn) Kützing + *C.nigrescens* Zanardini ex Frauenfeld)	IIB	0.1	2.2	4.2	9.6	3.6		3.5	5.5	1.7	0.2	5.1	0.7	0.5	8.6	12.9	1.0
Chaetomorpha spp. (*C. aerea* (Dillwyn) Kützing + *C. crassa* (C.Agardh) Kützing)	IIB		0.1	0.3		3.9									5.9	4.3	16.7
Chondria dasyphylla (Woodward) C.Agardh	IIB	0.5					0.3	0.3									
Chondrophycus cf. *glandulifer* (Kützing) Lipkin & Silva	IIB							0.8				0.1					
Chrysophyte sp.	IIB		0.4		0.8		0.3	0.2									
Cyanobacteria	IIB		0.3				0.3					1.3		0.1			
Dasya corymbifera J. Agardh	IIB	2.6	2.0	0.7	1.2	0.8	4.8	1.1	1.0	0.9		1.0	1.0	6.9	0.1	0.3	
Feldmannia irregularis (Kützing) Hamel	IIB				0.1		6.8		0.8		0.6						
Feldmannia simplex (P.L.Crouan & H.M.Crouan) G.Hamel	IIB						0.2						0.7				
Herposiphonia secunda (C.Agardh) Ambronn	IIB	0.1					0.3										
Polysiphonia sp.	IIB						0.9										
Scytosiphon lomentaria (Lyngbye) Link	IIB										0.6						
Spermothamnion flabellatum Bornet	IIB												0.2				
Ulva spp.(*U. intestinalis* Linnaeus + *U. linza* Linnaeus)	IIB				0.3			0.3		27.9	66.4	3.1					
Wrangelia penicillata (C. Agardh) C. Agardh	IIB				2.6		2.8							1.2			

Table 2. MALUSI index score for each sampling station indicating anthropogenic impact assessed using various stressors.

Station	Urban (codes 11)	Commercial & Industrial (codes 12, 13)	Agriculture (codes 21-24)	Mariculture	Sediment nutrient release	Sewage outfall	Irregular Fresh Water inputs	Harbour	**SUM**	Background trophic status	Stability of water column	Confinement	**MALUSI**
Site 1	0	0	1	1	0	2	0	0	4	1	1	1	4
Site 2	0	0	1	1	0	0	0	1	3	1	1	1	3
Site 3	0	0	1	1	0	0	0	1	3	1	1	1	3
Site 4	0	0	1	1	0	0	0	2	4	1	1	1.25	5
Site 5	0	0	1	1	0	0	0	2	4	1	1	1.25	5
Site 6	0	1	1	1	0	0	0	2	5	1	1	1.25	6.25
Site 7	0	1	1	1	0	0	0	2	5	1	1	1.25	6.25
Site 8	0	1	2	1	0	0	0	2	6	1	1	1.25	7.5
Site 9	0	1	2	1	0	0	0	3	7	1	1	1.25	8.75
Site 10	0	1	3	1	0	0	0	3	8	1	1	1.25	10
Site 11	0	1	3	1	0	0	0	3	8	1	1	1.25	10
Site 12	0	1	3	0	0	0	0	3	7	1	1	1.25	8.75
Site 13	0	1	3	1	0	0	0	3	8	1	1	1.25	10
Site 14	0	1	3	1	0	0	0	3	8	1	1	1	8
Site 15	0	1	3	1	0	0	0	3	8	1	1	1	8
Site 16	0	1	3	0	0	0	0	3	7	1	1	1	7