

# Tracing Localised Nutrient Pollution Events to *Posidonia oceanica* Epiphyte Community Assemblages, in the Eastern Aegean Sea

Gian Luca Naccarato

[gianluca.naccarato@htw-dresden.de](mailto:gianluca.naccarato@htw-dresden.de)

Dresden University of Applied Sciences

Arne Cierjacks

Dresden University of Applied Sciences

Alice Malcolm-McKay

University of Essex

María Moran García

Archipelagos Institute of Marine Conservation

Laura Macrina

King Abdullah University of Science and Technology

Anastasia Miliou

Archipelagos Institute of Marine Conservation

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## Research Article

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# Abstract

The occurrence of localised eutrophication events in small coastal communities of the Mediterranean threatens seagrass ecosystem functionality, necessitating the reliable identification of the onset and magnitude of these processes. This study examined the suitability of epiphyte assemblages on *Posidonia oceanica* leaves as an indicator of anthropogenic nutrient enrichment, for the first time in the eastern Aegean Sea. Seagrass biometric and meadow parameters were studied at control and potentially disturbed sites (n=3), in addition to epiphyte community composition and structure at a depth of 5 m during winter 2022. Sites were classified using a modified version of the Land Use Simplified Index. Potentially disturbed meadows exhibited decreased leaf length and shoot density, and increased epiphyte cover and biomass. Epiphyte community composition shifted significantly, with clear differences observed in red coralline algae, filamentous brown algae, bryozoans, and annelids. Results highlighted that reduced water quality, which is discontinuous and therefore difficult to tackle with a single direct measurement in the water column, from point and diffuse sources including a sewage outlet, agricultural activity and tourism, strongly affected seagrass bed condition. Here, substantial epiphyte cover was observed, causing direct (shading) and indirect (intensified grazing; physical leaf instability) effects. The study revealed that epiphyte communities, in combination with the index, serve as accurate and cost-effective indicators of coastal water pollution. Regular monitoring could aid in detecting higher nutrient loading at an early stage and potentially prevent long-term meadow decline.

## Introduction

In recent decades, globally intensified anthropogenic coastal activity has led to a drastic increase in terrestrial nutrient inputs to the marine environment (Reichelt-Brushett 2023). Nutrients, in particular nitrogen and phosphate, largely originate from point sources such as direct discharges from sewage treatment plants and diffuse sources like agricultural runoff. These enter downstream inshore waters via rivers, thus enhancing the risk of eutrophication (Malone and Newton 2020; Reichelt-Brushett 2023). The decrease in water quality can cause changes in primary production, species composition, and general ecosystem processes (Lepoint et al. 2007). The decay of algae blooms stimulated by nutrient pollution, along with hydro-climatic factors such as rising temperatures, can trigger hypoxic and anoxic conditions, leading to the collapse of aquatic communities (Derolez et al. 2020). Due to future climate trends, i.e. warmer waters, altered current conditions, and increased freshwater inflows, a more frequent occurrence of hypoxia and anoxia in coastal aquatic ecosystems is expected (Jager et al. 2018). Consequently, eutrophication is described as one of the major human-induced changes in shoreline waters and the greatest threat to the functionality and biodiversity of marine ecosystems (Field and Barros 2014; Malone and Newton 2020).

The Mediterranean Sea is among the most oligotrophic seas worldwide and, compared to other European waters, has a relatively low reported eutrophication problem (Andersen et al. 2019). Several policies, such as the Nitrates Directive (91/676/EEC) and the Urban Wastewater Treatment Directive (91/271/EEC) have been developed to standardise the protection of the marine environment against nutrient inputs. Their implementation has, thus far, successfully mitigated large-scale eutrophication events in cities and the estuaries of major rivers (Malagó et al. 2019). However, smaller communities have not fully met these objectives, partly due to the lack of appropriate training in sustainable agricultural practices (Komnitsas and Doula 2017; Vardopoulos et al. 2018). Additionally, communities with fewer than 2,000 inhabitants are not required to meet minimum wastewater treatment standards (Paraskevas et al. 2002), and inconsistent data collection hinders the assessment of their true significance as sources of pollution for the receiving water bodies. Spatial models by Pistocchi et al. (2022) show high risks of localised nutrient pollution in towns with poor wastewater management, caused by direct discharge of untreated or inadequately treated municipal and industrial wastewater and agricultural effluents (Powley et al. 2016). The constant growth of tourism in Mediterranean coastal towns leads to higher production of domestic waste and sewage, further increasing their impact on marine ecosystems (Paraskevas et al. 2002). The Greek Aegean Sea, located in the eastern part of the Mediterranean Basin, exemplifies this issue, as it consists of many small islands and rural areas with municipalities that are often not connected to a centralised wastewater collection system (Powley et al. 2016; Mela et al. 2022). As an attractive travel destination, the exploitation of the marine environment remains a key economic sector (Biszel and Biszel 2015). The expansion of these industries increasingly threatens ecologically relevant marine habitats, such as seagrass meadows, which are prolific along Mediterranean coastlines (Powley et al. 2016; Pastor et al. 2023).

Seagrasses are among the most important resources in coastal environments and provide fundamental ecosystem services, including filtering nutrients, providing food sources and habitats for herbivores, and capturing significant amounts of carbon (Green and Short 2003). Being particularly sensitive to nutrient enrichment, eutrophication is considered one of the main threats to seagrasses (Howarth et al. 2000; Boesch 2002; Howarth and Marino 2006). Excessive nutrient loads can reduce meadow productivity, leading to deterioration through decreased photosynthetic activity and assimilate production, due to biomass accumulation in epiphytic macroalgae and phytoplankton blooms (Bulthuis and Woelkerling 1983; van Montfrans et al. 1984; Cebrian et al. 1999; Balata et al. 2010). Simultaneously, increased epiphytic growth can encourage herbivore grazing, leading to direct damage of the plant (Ruiz et al. 2001).

In the Mediterranean, the endemic *Posidonia oceanica* (L.) Delile, 1813, one of four native seagrass species (Borum et al. 2004), is the most significant in terms of extent and productivity (Gobert et al. 2006). Over the past 60 years, anthropogenic pressures, namely coastal modification, decreasing water quality, mechanical damage, and invasive species, have led to a widespread regression of its original distribution in the Mediterranean Sea (Marbà et al. 2014; Telesca et al. 2015; de los Santos et al. 2019). Current projections by Chefaoui et al. (2018) indicate that dramatic declines of this protected species will likely continue in the coming decades, with their 'worst-case' model predicting functional extinction by 2100, as a result of synergic interactions between human disturbances and climate change. Nutrient overload is particularly detrimental, as eutrophic conditions increase *P. oceanica*'s sensitivity to other pressures like rising sea surface temperatures (Pazzaglia et al. 2020). However, due to their high spatial and temporal variability, diffuse pollution and their impacts on ecosystems are difficult to identify and monitor with single direct measurements (Arhonditsis et al. 2000; Aldaya et al. 2020; Reichelt-Brushett 2023). *P. oceanica* is already a well-established indicator of coastal ecosystem health (Pergent et al. 1995a) and, with its longevity and relatively long response time, can reveal cumulative pollution effects (Balata et al. 2010). Nonetheless, there is a risk of overlooking local nutrient inputs and the resulting gradual decline over years or decades (Unsworth et al. 2014). Thus, early detection of such disturbances is crucial for effective management strategies and avoiding long-term consequences (Giovannetti et al. 2010).

In this context, the response of epiphytes colonising the blades of *P. oceanica* came into focus during the last three decades (Piazzi et al. 2016). The leaves' long lifespan of over 30 weeks (Ott 1980) and large surface area provide a suitable substrate for complex epiphyte communities, often characterised by a high taxonomic diversity (Casola et al. 1987; Mazzella et al. 1989). Epiphytic organisms contribute to the meadow's primary production (Borowitzka et al. 2007), but also react more rapidly to alterations in water quality than their host, and may serve as an appropriate early warning indicator (Martínez-Crego et al. 2010; Giovannetti et al. 2010). Changes in macro-epiphytic biomass, spatial heterogeneity, and species composition, coupled with declines in *P. oceanica* morphological parameters, have been widely observed under nutrient-enriched regimes in both observational (Brahim et al. 2010; Mabrouk et al. 2013) and experimental studies (Leoni et al. 2006; Lepoint et al. 2007; Prado et al. 2008; Balata et al. 2010). While numerous publications addressed this issue in the western and southern Mediterranean, there is sparse information available from the Aegean Sea (Piazzi et al. 2016). Research in this region focuses largely on the response of specific epiphyte species groups to nutrients (Koçak et al. 2002; Kocak and Aydin-Onen 2014; Guresen et al. 2020) rather than the entire epiphyte assemblage. Previous studies around Lipsi island mainly describe *P. oceanica*'s phenology and distribution, overlooking potential sources of terrestrial eutrophication (Gütte and Miliou 2019).

To test the suitability of *P. oceanica* epiphyte communities as eutrophication indicators, local seagrass beds and leaf macro-epiphyte diversity were explored in association with areas of potential local water pollution through point and diffuse sources on one Aegean island, Lipsi. By comparing meadow structure, leaf epiphyte biomass and epiphytic community composition between potentially disturbed and control sites, this study aims to draw conclusions about the degree of anthropogenic pressure from surrounding land use to local seagrass beds by testing the following hypotheses:

1. Biometric and meadow parameters of *P. oceanica* decrease in potentially disturbed sites compared to control sites.
2. Differences in the condition of the meadows can be linked to anthropogenic influence through land use.
3. There are differences in the composition and abundance of epiphytes colonising the leaves of *P. oceanica* between potentially disturbed sites and control sites.

## Methodology

# Study area and Evaluation of Human Pressure

Epiphytic communities of *P. oceanica* were studied at sites around the island of Lipsi, Greece, in the eastern Aegean Sea (37° 18' 0" N, 26° 45' 0" E) (Fig. 1). Lipsi is located in the northern part of the Dodecanese complex, covers approximately 16 km<sup>2</sup> with an estimated coastline of 35 km. The island typically hosts ~ 800 residents, though tourism during summer months can increase this five-fold (Bardis et al. 2020). Touristic and local pressure on coastal waters is distributed unevenly, being largely concentrated around the main village of Lipsi Town in the southwest.

Six bays were sampled, each assigned with a unique environmental quality rating to classify them into two conditions: (1) *control sites (CS)* (Kimisi, Christos and Vroulia control or 'VrouliaCS') and (2) *potentially disturbed sites (PDS)* (Katsadia, Urban Bay and Vroulia). Classification was based on a modified version of the Land Use Simplified Index (LUSI), originally presented by Flo et al. (2019), which enables the quantitative assessment of terrestrial anthropogenic disturbances on coastal waters by indirectly estimating nutrient loads from various origins and their impact on aquatic systems. The estimates would typically be based on the percentage of land coverage (from the coastline to 1.5 km inland) of pressure sources. However, due to Lipsi's small land mass, this study based area assessments on the presence and intensity of each respective pressure source around the bays (see Table 1). Since Lipsi has no rivers, the factor of riverine pressure was excluded from the calculation and replaced by the factor *tourism*, as it had been linked to local eutrophication (Lepoint et al. 2007; Giovannetti et al. 2010).

Table 1

Modified pressure categories of anthropogenic origin and their pressure scores used to calculate the Land Uses Simplified Index (LUSI) value for Lipsi island, following Flo et al. (2019). h/d = hours per day

| Land use pressures                                                                                                                                                                                       |                           |                  |           | Pressure score | Coastline morphology correction factor |      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------|-----------|----------------|----------------------------------------|------|
| Urban                                                                                                                                                                                                    | Agricultural              | Industrial       | Touristic |                |                                        |      |
| Not present                                                                                                                                                                                              | Not present               | Not present      | < 4 h/d   | 0              | Convex                                 | 0,75 |
| Previously inhabited                                                                                                                                                                                     | Animal husbandry          | Present          | 4–10 h/d  | 1              | Concave                                | 1,25 |
| Inhabited                                                                                                                                                                                                | Annual crops/ fruit trees | Direct discharge | > 10 h/d  | 2              | Straight                               | 1    |
| Land use pressures consisted of four categories: urban, agricultural, industrial, and tourism, which, along with coastal morphology, resulted in the LUSI value calculated using the following equation: |                           |                  |           |                |                                        |      |

$$LUSI = (urban\ score + agricultural\ score + industrial\ score + touristic\ score) \times coastline\ correction\ factor$$

To account for past human influence, such as diffuse pollution from pollutant runoff (Reichelt-Brushett 2023), the urban pressure was differentiated between 'inhabited' and 'previously inhabited' areas. To evaluate industrial pressure, on-site assessments additionally determined whether there was a direct discharge of wastewater. Agricultural use was distinguished between animal husbandry, widespread on Lipsi (mainly goat farming), and crop cultivation. Thereby, the risk of eutrophication from increased erosion of upper soil particles into the sea, attributed to reduced vegetative cover due to grazing (Arhonditsis et al. 2000; Karydis 2005), was assumed to be lower than that associated with synthetic fertilisers. Touristic pressure was categorised by the average hours per day of vessel presence in the studied bays, using 2018 vessel presence data from Global Fishing Watch (2022), as it was considered more representative of tourism than data collected during the time of the Covid-19 pandemic.

As defined by Flo et al. (2019), a low LUSI value indicates low land use pressure, while a high LUSI score indicates that coastal waters are 'highly influenced by continental pressures, or that these pressures are not diluted'. Given this, and extensive knowledge of the local areas, a LUSI value of 3 was defined as the threshold for classifying sites as either CS (< 3) or PDS (> 3). CS meadows were predominantly isolated from intensive anthropogenic land use. Urban Bay meadows were located near a sewage pipe discharging wastewater directly into the sea. The areas surrounding Katsadia and Vroulia are marked by settlement and agriculture, with Katsadia being a tourist hotspot with an adjacent industrial landfill.

Table 2  
Calculation of the LUSI value of different sites on Lipsi Island. CCF = Coastline correction factor, PDS = Potentially Disturbed Sites, CS = Control Sites.

| Site       | Urban | Agricultural | Industrial | Touristic | CCF  | LUSI Value  | Condition |
|------------|-------|--------------|------------|-----------|------|-------------|-----------|
| Vroulia CS | 0     | 1            | 0          | 0         | 1,25 | <b>1,25</b> | CS        |
| Christos   | 0     | 1            | 0          | 1         | 1    | <b>2</b>    | CS        |
| Kimisi     | 0     | 1            | 0          | 1         | 1,25 | <b>2,5</b>  | CS        |
| Vroulia    | 2     | 1            | 0          | 0         | 1,25 | <b>3,75</b> | PDS       |
| Urban Bay  | 1     | 1            | 2          | 0         | 1,25 | <b>5</b>    | PDS       |
| Katsadia   | 2     | 2            | 1          | 2         | 1,25 | <b>8,75</b> | PDS       |

## Data Collection and Experimental Design

Sample collection was conducted by free diving during a four-week period between January and February 2022. To minimise natural variability caused by hydrodynamics and light intensity, and to obtain comparable results with similar studies (e.g., Brahim et al. 2010, 2014; Mabrouk et al. 2013), all samples were collected at a depth of 5 m. Since a spatially heterogeneous epiphyte community structure was observed during pilot surveys, a hierarchical sampling design (Supplementary Fig. 1) was chosen to minimise the risk of random effects of other variables (Logan 2010). At each site, three plots of a 5 x 5 m homogeneous *P. oceanica* meadow were randomly selected, with a minimum distance of 10 m. Within each plot, seven randomly chosen leaves were sampled and the exact GPS coordinates of the plot were saved. Only the external leaves of the shoots were collected, as these are the oldest and most likely to contain mature epiphyte communities (Vanderklift and Lavery 2000; Buia et al. 2003). Leaf samples were preserved in 70% ethanol (Williams and Van Syoc 2007) until laboratory analysis. To assess meadow condition, seagrass biometrics according to Pergent-Martini et al. (2005) were collected including: shoot density, number of leaves per shoot, and meadow percent cover. The aforementioned metrics were evaluated by counting the shoots within one 0.25 x 0.25 m quadrat (n = 7) and counting the leaves of a randomly chosen shoot within the quadrat. Meadow percent cover was estimated by visual inspection within each 5 x 5 m plot. Environmental parameters of light intensity and temperature were measured on each plot using a HOBO Pendant® MX Temperature/Light Data Logger (MX2202). The logger recorded data every ten seconds for five minutes during sample collection, and the data was averaged for each plot. Additionally, sediment type was recorded, and turbidity was visually determined using a white dive slate as a substitute for a Secchi disk and a transect, representing the distance in metres beyond which the slate was no longer visible.

## Laboratory Analysis

For each *P. oceanica* leaf sample, the length and width were measured to calculate the Leaf Area Index (LAI) (Buia et al. 2003). Both sides of the apical part of the leaves (first 9 cm from the tip) of each blade were observed for epiphyte identification and coverage (Giovannetti et al. 2010) using a stereoscope (Zuzi, Series 250) and a compound-microscope (Motic, B1 Series, magnifications: 4x, 10x). Epiphyte taxa were grouped at family level and into morphological units, with algae sorted according to Steneck & Dethier (1994). Cnidaria were sorted by class, and remaining animals and Chromista by phylum. Epiphyte coverage of each family and morphological group was expressed as a percentage of the apical surface, estimated using an Optika® microscope camera (Model C-B1) together with the Optika® software LiteView for macOS (Supplementary Fig. 2). Epiphyte and leaf biomass were evaluated as dry weight (DW) to determine epiphyte load (EL) ( $\text{g epiphytes} \times \text{g}^{-1} \text{ leaf DW}$ ). Epiphytes were gently scraped off the leaf using a scalpel (Libes 1986) (Supplementary Fig. 3) and both leaves and epiphytes were dried at 60°C for 48 hours using a dehydrator (Excalibur 3900) (Bessey and Heithaus 2015).

## Data Analysis

The effect of meadow condition (PDS; CS) on epiphytic functional groups, plant, and meadow features (LAI, leaf length and width, shoot density), EL, and total coverage was assessed using a nested analysis of variance (ANOVA). The model consisted of the factors Condition (2 levels, fixed), Site (3 levels, random and nested within Condition), and Plot (3 levels, random and nested within Site). To meet the assumptions of a linear model, all metric variables were checked for normality using histograms, while normality of residuals and homoscedasticity were evaluated through visual inspections of residuals, quantile plots, and residuals versus fitted values plots (Zuur et al. 2007). Log or square-root transformations were applied if heterogeneous variance was detected. All analyses were carried out in R (R Core Team 2024, version 4.3.3).

For multivariate analyses, taxonomic data were log transformed to increase the influence of rare taxa by down-weighting dominant ones (Clarke et al. 2006; Anderson et al. 2008; Šmilauer and Lepš 2014). To visualise patterns of epiphytic assemblages among sampling units and underlying environmental variables (meadow coverage, EL, LAI, LUSI, sediment type, turbidity, light intensity, temperature), a nonmetric multidimensional scaling (NMDS) was performed. The Bray-Curtis dissimilarity matrix was used as distance measure, as it is suitable for representing gradients in quantitative species composition (Šmilauer and Lepš 2014). The final model with the lowest stress had three dimensions with a maximum of 20 random starts. Environmental data was fitted onto the ordination to portray the correlation of the variables with the epiphyte assemblages, using the *envfit* function (Oksanen et al. 2015), which applied a Monte-Carlo Test with 1000 permutations to evaluate the significance of each variable. Only variables with a significant *p*-value ( $p < 0.05$ ) were included in the presented results. To test for different patterns of epiphyte assemblages between the two conditions while accounting for the hierarchical structure of the design, a nested non-parametric multivariate analysis of variance based on permutations (PERMANOVA) (Anderson 2001) was used. Factors included in the model were uniform to the ANOVA. Bray-Curtis similarity index was used for similar reasons as for the NMDS, with *p*-values obtained by permutation of residuals under the full model. For validation of the PERMANOVA results, a post-hoc test for homogeneity of multivariate dispersions (PERMDISP) was applied (Anderson 2017).

Ultimately, to identify taxa responsible for the differences between conditions, an analysis of similarity percentage (SIMPER) (Clarke 1993) was conducted. By pairwise comparison between the groups, it calculates the average percent contribution of each taxon to the overall dissimilarity, producing a ranking of taxa from high to low contribution. PERMANOVA, PERMDISP, and SIMPER were calculated using the PRIMER-e software (Anderson et al. 2008, version 7).

## Results

### Biometric and Condition Parameters

The nested ANOVA analysis indicated that all biometric parameters of *P. oceanica* differed between PDS and CS except leaf width: shoot density (MS = 134.13,  $F_{(1,4)} = 8.02$ ,  $p < 0.05$ ), leaf length (MS = 84.37,  $F_{(1,4)} = 12.42$ ,  $p < 0.05$ ), and LAI (MS = 23.66,  $F_{(1,4)} = 11.83$ ,  $p < 0.05$ ) (Table 4). Control meadows were characterised by a higher average shoot density (CS:  $203.17 \pm 4.6$ ; PDS:  $170.16 \pm 5.26$  shoots/m<sup>2</sup>) and longer leaves (CS:  $40.91 \pm 1.01$ ; PDS:  $22.88 \pm 0.88$  cm), resulting in a significantly higher LAI (CS:  $3.84 \pm 0.17$ ;  $1.75 \pm 0.13$ ) (Table 3).

An inverse pattern was observed in the condition parameters related to epiphytes, as both the mean epiphyte coverage and EL in the PDS substantially increased compared to CS (MS = 178.51,  $F_{(1,4)} = 14.82$ ,  $p < 0.05$  and MS = 5.17,  $F_{(1,4)} = 12.29$ ,  $p < 0.05$ , respectively). Specifically, the mean EL in PDS ( $0.86 \pm 0.06$  gg<sup>-1</sup> DW) was fourfold higher than that of the CS ( $0.21 \pm 0.02$  gg<sup>-1</sup> DW) (Fig. 2), while the mean epiphyte coverage was more than three times higher (CS:  $6.41 \pm 0.62\%$ ; PDS:  $24.46 \pm 1.63\%$ ) (Table 3).

### Epiflora and Epifauna

A total of 34 taxa of macro-epiphytes, belonging to 25 different families, were detected on the leaves of *P. oceanica* at all sites investigated, with four identified at species level and the remainder at genus level (31 in CS and 34 in PDS): 17 Macroalgae (3 Encrusting algae, 14 Filamentous algae), 8 Bryozoa, 5 Hydrozoa, 2 Foraminifera and 2 Annelida (Table 6). Assemblages at all sites were dominated by red encrusting algae of the genera *Hydrolithon* (Foslie) Foslie, 1909 and *Fosliella* M.A.Howe, 1920 (Fig. 3). Disparities in their cover values were significant between the two conditions (MS = 61.97,  $F_{(1,4)} = 28.95$ ,  $p < 0.01$ ), with PDS exhibiting a nearly fivefold increase in mean coverage ( $18.22 \pm 1.38\%$ ) compared to CS ( $3.81 \pm 0.49\%$ ). Similarly, *P. oceanica*

leaves in PDS were significantly more colonised by filamentous forms ( $MS = 11.92$ ,  $F_{(1,4)} = 12.13$ ,  $p < 0.05$ ), being particularly noticeable at Urban Bay (Fig. 3). In contrast to the crustose algae, filamentous algae were represented by a larger number of taxa, of which the brown algae *Sphacelaria cirrosa* (Roth) C.Agardh, 1824 (Fig. 5) was the predominant species in both conditions, followed by various genera of the red algae family Rhodomelanceae.

Among animals, differences in percent cover values were only detected for annelids ( $MS = 0.78$ ,  $F_{(1,4)} = 11.09$ ,  $p < 0.05$ ). The group consisted of the segmented worms *Janua heterostropha* (Montagu, 1803) and *Hydroides sp.*, which were equally abundant across PDS ( $0.13 \pm 0.02\%$ ) whilst coverage significantly decreased in CS ( $0.04 \pm 0.01\%$ ). The highest coverage recorded was for the group of bryozoans, whose values were subject to a high variability both at the level of sites and the level of plots (Fig. 3). For instance, within plots located in Katsadia, values ranged from 33.9% to < 3%, while in Christos plots, moss animals were nearly absent. In total, eight different bryozoans were identified across all sampling points, of which *Electra posidoniae* Gautier, 1954 and *Aetea truncata* (Landsborough, 1852) were the only taxa found in meadows at all sites (Table 6), while *Lichenopora sp.* exclusively occurred in PDS. Hydrozoa exhibited a comparable level of variability between sites and plots, similar to that of bryozoans. Taxa of the genera *Obelia sp.*, *Campanularia sp.*, *Laomedea sp.*, and *Plumularia sp.* had higher coverage values in PDS, whereas *Sertularia sp.* was more frequently encountered in CS. The group of Foraminifera comprised two taxa belonging to the genera *Planorbulina* d'Orbigny, 1826, and *Elphidium* Montfort, 1808; the latter was less abundant in control sites (Table 6). Collectively, foraminifera showed homogenous cover across all sites, with moderately higher values in PDS. In general, cover values rarely exceeded 1%.

Table 3

Descriptive statistics of *P. oceanica* biometric and meadow parameters, and the epiphyte functional groups in the two conditions.

| Percentage cover of functional groups    | PDS           |       |      |       |      | CS            |       |      |      |      |
|------------------------------------------|---------------|-------|------|-------|------|---------------|-------|------|------|------|
|                                          | mean $\pm$ SE |       |      | min   | max  | mean $\pm$ SE |       |      | min  | max  |
| Encrusting algae                         | 18.22         | $\pm$ | 1.38 | 1.27  | 46.6 | 3.81          | $\pm$ | 0.49 | 0.62 | 21.7 |
| Filamentous algae                        | 1.81          | $\pm$ | 0.17 | 0.1   | 6.73 | 0.41          | $\pm$ | 0.05 | 0.01 | 2.30 |
| Bryozoans                                | 3.88          | $\pm$ | 0.83 | 0.07  | 33.9 | 1.83          | $\pm$ | 0.33 | 0.01 | 11.5 |
| Hydrozoans                               | 0.12          | $\pm$ | 0.02 | 0     | 0.76 | 0.08          | $\pm$ | 0.03 | 0    | 1.29 |
| Annelida                                 | 0.13          | $\pm$ | 0.02 | 0     | 0.74 | 0.04          | $\pm$ | 0.01 | 0    | 0.32 |
| Foraminifera                             | 0.31          | $\pm$ | 0.03 | 0     | 1.11 | 0.23          | $\pm$ | 0.03 | 0.00 | 1.30 |
| Biometric and meadow parameters          | PDS           |       |      |       |      | CS            |       |      |      |      |
|                                          | mean $\pm$ SE |       |      | min   | max  | mean $\pm$ SE |       |      | min  | max  |
| Leaf area index                          | 3.50          | $\pm$ | 0.25 | 0.449 | 4.81 | 7.68          | $\pm$ | 0.35 | 1.05 | 10.4 |
| Epiphyte load (g / g DW)                 | 0.86          | $\pm$ | 0.06 | 0.06  | 2.5  | 0.21          | $\pm$ | 0.02 | 0.04 | 0.77 |
| Total coverage (%)                       | 24.46         | $\pm$ | 1.63 | 1.82  | 57.5 | 6.41          | $\pm$ | 0.62 | 1.34 | 23.5 |
| Leaf length (cm)                         | 22.88         | $\pm$ | 0.88 | 8.5   | 42   | 40.91         | $\pm$ | 1.01 | 22.4 | 60.9 |
| Leaf width (cm)                          | 0.78          | $\pm$ | 0.01 | 0.5   | 0.95 | 0.80          | $\pm$ | 0.01 | 0.65 | 1    |
| Shoot density (shoots / m <sup>2</sup> ) | 170.16        | $\pm$ | 5.26 | 96    | 288  | 203.17        | $\pm$ | 4.6  | 128  | 320  |

Table 4

Results of nested analysis of variance (ANOVA) on biometric and meadow parameters of *P. oceanica* between potentially disturbed and control sites.

| Biometric and meadow parameters |     |       |          |              |               |          |              |                |          |              |
|---------------------------------|-----|-------|----------|--------------|---------------|----------|--------------|----------------|----------|--------------|
| Leaf area index                 |     |       |          |              | Epiphyte load |          |              | Total coverage |          |              |
| Source of Variation             | df  | MS    | <i>F</i> | <i>P</i>     | MS            | <i>F</i> | <i>P</i>     | MS             | <i>F</i> | <i>P</i>     |
| Condition (= C)                 | 1   | 23.66 | 11.84    | <b>0.026</b> | 6.60          | 13.17    | <b>0.022</b> | 63.44          | 12.26    | <b>0.025</b> |
| Sites (C) (= St)                | 4   | 2     | 11.89    | <b>0.000</b> | 0.50          | 11.79    | <b>0.000</b> | 5.17           | 6.91     | <b>0.004</b> |
| Block (St(C)) (= B)             | 12  | 0.17  | 1.26     | 0.253        | 0.04          | 1.05     | 0.410        | 0.75           | 2.80     | <b>0.002</b> |
| Residual                        | 108 | 0.13  |          |              | 0.04          |          |              | 0.27           |          |              |
| Total                           | 125 |       |          |              |               |          |              |                |          |              |
| Log(x + 1)                      |     |       |          |              | Sqrt(x)       |          |              | Log(x + 1)     |          |              |
| Leaf length                     |     |       |          |              | Leaf width    |          |              | Shoot density  |          |              |
| Source of Variation             | df  | MS    | <i>F</i> | <i>P</i>     | MS            | <i>F</i> | <i>P</i>     | MS             | <i>F</i> | <i>P</i>     |
| Condition (= C)                 | 1   | 10.82 | 11.67    | <b>0.027</b> | 0.02          | 0.585    | 0.487        | 34337          | 8.02     | <b>0.047</b> |
| Sites (C) (= St)                | 4   | 0.93  | 11.96    | <b>0.000</b> | 0.04          | 1.98     | 0.162        | 4283           | 1.48     | 0.269        |
| Block (St(C)) (= B)             | 12  | 0.08  | 2.53     | <b>0.006</b> | 0.02          | 3.66     | <b>0.000</b> | 2885           | 2.24     | <b>0.014</b> |
| Residual                        | 108 | 0.03  |          |              | 0.01          |          |              | 1289           |          |              |
| Total                           | 125 |       |          |              |               |          |              |                |          |              |
| Log(x + 1)                      |     |       |          |              | None          |          |              | None           |          |              |

Table 5  
Results of nested analysis of variance (ANOVA) on epiphytic functional groups of *P. oceanica* leaves between potentially disturbed and control sites.

| Epiphytic functional groups |     |       |          |              |                   |          |              |              |          |              |
|-----------------------------|-----|-------|----------|--------------|-------------------|----------|--------------|--------------|----------|--------------|
| Encrusting algae            |     |       |          |              | Filamentous algae |          |              | Bryozoans    |          |              |
| Source of Variation         | df  | MS    | <i>F</i> | <i>P</i>     | MS                | <i>F</i> | <i>P</i>     | MS           | <i>F</i> | <i>P</i>     |
| Condition (= C)             | 1   | 61.97 | 28.95    | <b>0.006</b> | 11.92             | 12.13    | <b>0.025</b> | 3.07         | 0.31     | 0.605        |
| Sites (C) (= St)            | 4   | 2.14  | 2.96     | 0.065        | 0.98              | 5.01     | <b>0.013</b> | 9.75         | 29.40    | <b>0.000</b> |
| Block (St(C)) (= B)         | 12  | 0.72  | 2.40     | <b>0.009</b> | 0.2               | 1.97     | <b>0.034</b> | 0.33         | 1.02     | 0.436        |
| Residual                    | 108 | 0.3   |          |              | 0.1               |          |              | 0.33         |          |              |
| Total                       | 125 |       |          |              |                   |          |              |              |          |              |
| Log(x + 1)                  |     |       |          |              | Log(x + 1)        |          |              | Log(x + 1)   |          |              |
| Hydrozoans                  |     |       |          |              | Annelida          |          |              | Foraminifera |          |              |
| Source of Variation         | df  | MS    | <i>F</i> | <i>P</i>     | MS                | <i>F</i> | <i>P</i>     | MS           | <i>F</i> | <i>P</i>     |
| Condition (= C)             | 1   | 0.23  | 1.77     | 0.254        | 0.78              | 11.09    | <b>0.029</b> | 0.26         | 1.73     | 0.259        |
| Sites (C) (= St)            | 4   | 0.13  | 1.18     | 0.368        | 0.07              | 0.90     | 0.494        | 0.15         | 1.71     | 0.212        |
| Block (St(C)) (= B)         | 12  | 0.11  | 1.94     | <b>0.037</b> | 0.08              | 3.24     | <b>0.001</b> | 0.09         | 1.75     | 0.066        |
| Residual                    | 108 | 0.06  |          |              | 0.02              |          |              | 0.05         |          |              |
| Total                       | 125 |       |          |              |                   |          |              |              |          |              |
| Sqrt(x)                     |     |       |          |              | Sqrt(x)           |          |              | Sqrt(x)      |          |              |

## Epiphyte Community Structure

The NMDS ordination of leaf epiphyte assemblages illustrated a clear separation between the two conditions, at a stress of 0.096 (Fig. 4). PDS were subjected to less variation in species composition than CS. PERMANOVA detected significant differences between the two conditions, as well as between the random factors in the model. The majority of variability in the data could be explained by the fixed factor Condition, according to the estimated components of variation (EMS) (Table 7). Subsequent PERMDISP did not find evidence that these differences were due to dispersion and therefore confirmed location effects (Supplementary Table 1). Species composition was related to differences in the meadow descriptors: LAI, meadow coverage and EL. In addition, the level of disturbance expressed as LUSI, turbidity, and temperature influenced the epiphyte assemblages. Among all, the EL and LUSI showed the strongest correlation with the species data, while being negatively correlated with the other explanatory variables mentioned (Supplementary Table 2). SIMPER analyses conducted on epiphyte coverage on *P. oceanica* leaves revealed a notable dissimilarity between PDS and CS (54.44%). Taxa mainly responsible for the differences were the encrusting rhodophytes *Hydrolithon sp.* and *Fosliella sp.*, the bryozoans *E. posidoniae*, *Lichenopora sp.* and *Walkeria sp.*, the ochrophyte *S. cirrosa*, and filamentous rhodophytes belonging to the family Rhodomelaceae (*Laurencia sp.*, *Polysiphonia sp.*, *Herposiphonia sp.*, *Dasya sp.*). All of these taxa showed higher abundances in PDS and had a cumulative contribution of 76.95% to the differences (Table 6).

Table 6

Results of analysis of similarity percentages (SIMPER) on percentage cover of epiphyte families on the apical 9 cm of *P. oceanica* leaves, analysed on log (x + 1) transformed data. X CS (%) and X PDS (%) represent the mean percentage cover of the respective family. B = Bryozoa, F = Foraminifera, A = Annelida, H = Hydrozoa, EA = Encrusting algae, FA = Filamentous algae, FG = Functional Group, Av. Diss. = Average dissimilarity, Diss. /sd = Dissimilarity /standard deviation, Con. (%) = Contribution of each family to the Bray-Curtis dissimilarity between conditions, Cum. (%) = Cumulative contribution. Average dissimilarity = 54.44.

| Family           | Genus and Species                        | FG | X (PDS) | X (CS) | Av. Diss. | Diss. /sd | Con. (%) | Cum. (%) |
|------------------|------------------------------------------|----|---------|--------|-----------|-----------|----------|----------|
| Hydrolithaceae   | <i>Hydrolithon sp.</i>                   | EA | 18.21   | 3.81   | 17.97     | 1.82      | 33.01    | 33.01    |
|                  | <i>Fosliella sp.</i>                     |    |         |        |           |           |          |          |
| Electridae       | <i>Electra posidoniae</i>                | B  | 2.80    | 1.72   | 9.99      | 1.14      | 18.35    | 51.36    |
|                  | Gautier, 1954                            |    |         |        |           |           |          |          |
| Sphacelariaceae  | <i>Sphacelaria cirrosa</i>               | FA | 0.78    | 0.08   | 4.99      | 0.82      | 9.17     | 60.53    |
|                  | (Roth) C.Agardh, 1824                    |    |         |        |           |           |          |          |
| Rhodomelaceae    | <i>Laurencia sp.</i>                     | FA | 0.72    | 0.22   | 4.22      | 1.13      | 7.76     | 68.29    |
|                  | <i>Polysiphonia sp.</i>                  |    |         |        |           |           |          |          |
|                  | <i>Herposiphonia sp.</i>                 |    |         |        |           |           |          |          |
|                  | <i>Dasya sp.</i>                         |    |         |        |           |           |          |          |
| Lichenoporidae   | <i>Lichenopora sp.</i>                   | B  | 0.29    | 0.00   | 2.53      | 0.79      | 4.65     | 72.93    |
| Walkeridae       | <i>Walkeria sp.</i>                      | B  | 0.42    | 0.01   | 2.19      | 0.50      | 4.02     | 76.95    |
| Planorbulinidae  | <i>Planorbulina sp.</i>                  | F  | 0.19    | 0.23   | 1.99      | 1.09      | 3.66     | 80.61    |
| Ceramiaceae      | <i>Antithamnion sp.</i>                  | FA | 0.22    | 0.08   | 1.64      | 0.72      | 3.01     | 83.63    |
|                  | <i>Ceramium sp.</i>                      |    |         |        |           |           |          |          |
| Fenestrulinidae  | <i>Fenestrulina sp.</i>                  | B  | 0.16    | 0.04   | 1.60      | 0.65      | 2.95     | 86.57    |
| Serpulidae       | <i>Janua heterostropha</i> Montagu, 1803 | A  | 0.13    | 0.04   | 1.27      | 1.06      | 2.34     | 88.92    |
|                  | <i>Hydroides sp.</i>                     |    |         |        |           |           |          |          |
| Elphidiidae      | <i>Elphidium sp.</i>                     | F  | 0.12    | 0.00   | 1.15      | 0.68      | 2.11     | 91.02    |
| Campanulariidae  | <i>Obelia sp.</i>                        | H  | 0.08    | 0.04   | 0.99      | 0.66      | 1.81     | 92.84    |
|                  | <i>Campanularia sp.</i>                  |    |         |        |           |           |          |          |
|                  | <i>Laomedea sp.</i>                      |    |         |        |           |           |          |          |
| Aeteidae         | <i>Aetea truncata</i>                    | B  | 0.10    | 0.04   | 0.95      | 0.64      | 1.74     | 94.58    |
|                  | Landsborough, 1852                       |    |         |        |           |           |          |          |
| Tubuliporidae    | <i>Tubulipora sp.</i>                    | B  | 0.11    | 0.01   | 0.93      | 0.51      | 1.72     | 96.30    |
| Sertulariidae    | <i>Sertularia sp.</i>                    | H  | 0.03    | 0.05   | 0.65      | 0.39      | 1.19     | 97.49    |
| Callithamniaceae | <i>Crouania sp.</i>                      | FA | 0.02    | 0.00   | 0.31      | 0.50      | 0.57     | 98.06    |
|                  | <i>Aglaothamnion sp.</i>                 |    |         |        |           |           |          |          |
| Chordariaceae    | <i>Giraudya sp.</i>                      | FA | 0.01    | 0.02   | 0.30      | 0.74      | 0.55     | 98.61    |
| Dictyotaceae     | <i>Dictyota sp.</i>                      | FA | 0.02    | 0.00   | 0.22      | 0.29      | 0.41     | 99.02    |
| Spyridiaceae     | <i>Spyridia sp.</i>                      | FA | 0.01    | 0.00   | 0.15      | 0.61      | 0.27     | 99.29    |

| Family          | Genus and Species     | FG | $\bar{X}$<br>(PDS) | $\bar{X}$<br>(CS) | Av.<br>Diss. | Diss.<br>/sd | Con.<br>(%) | Cum.<br>(%) |
|-----------------|-----------------------|----|--------------------|-------------------|--------------|--------------|-------------|-------------|
| Plumulariidae   | <i>Plumularia sp.</i> | H  | 0.01               | 0.00              | 0.12         | 0.19         | 0.21        | 99.50       |
| Crisiidae       | <i>Crisia sp.</i>     | B  | 0.00               | 0.01              | 0.11         | 0.72         | 0.20        | 99.69       |
| Cladophoraceae  | <i>Cladophora sp.</i> | FA | 0.01               | 0.00              | 0.07         | 0.32         | 0.12        | 99.82       |
| Chlidoniidae    | <i>Chlidonia sp.</i>  | B  | 0.00               | 0.00              | 0.05         | 0.45         | 0.10        | 99.91       |
| Lithophyllaceae | <i>Amphiroa sp.</i>   | EA | 0.00               | 0.00              | 0.04         | 0.20         | 0.07        | 99.99       |
| Ectocarpaceae   | <i>Ectocarpus sp.</i> | FA | 0.00               | 0.00              | 0.01         | 0.13         | 0.01        | 100.00      |

Table 7

Results of nested non-parametric multivariate analysis of variance based on permutations (PERMANOVA) performed on epiphyte assemblages on leaves of *P. oceanica*. *P*-values were obtained by permutations under the full model. Bold numbers indicate significant effects. EMS = Estimates of components of variation, d.f. = degrees of freedom, MS = mean squares.

| Source of Variation | d.f. | MS     | Pseudo- <i>F</i> | <i>P</i> (perm) | EMS    |
|---------------------|------|--------|------------------|-----------------|--------|
| Condition (= C)     | 1    | 39509  | 39286            | <b>0.001</b>    | 467.49 |
| Sites (C) (= St)    | 4    | 10057  | 87963            | <b>0.001</b>    | 424.45 |
| Block (St(C)) (= B) | 12   | 1143.3 | 18996            | <b>0.001</b>    | 77.35  |
| Residual            | 108  | 601.84 |                  |                 | 601.84 |
| Total               | 125  |        |                  |                 |        |

## Discussion

### Leaf Area Index as a Metric for Seagrass Meadow Condition

The LAI is a widely used parameter to describe *P. oceanica* meadows (Pergent-Martini et al. 2005), with higher values indicating a larger photosynthetic area, allowing the plant to produce more substrates for growth and storage (Ott 1980). Simultaneously, the index provides insight into the gross and net primary production of seagrass systems (McComb et al. 1981). Since average LAI values were approximately 50% lower in PDS than those for CS, it can be assumed that the meadows are not only in a lower overall condition but their ecosystem functioning will also be affected. Given the findings reported by Champenois and Borges (2012), which demonstrated a strong correlation between the LAI and the gross primary production of *P. oceanica* meadows, it can be further inferred that the poor condition of the PDS meadows results in a significantly lower total amount of organic carbon assimilated through photosynthesis per given unit of time and area.

Comparisons with studies conducted under similar conditions (season, depth, and sediment type) suggest that the PDS meadows are indeed in poor condition. Their values resemble those reported by Helber et al. (2021) ( $3.32 \pm 0.24$ ), who collected samples near a sewage discharge and defined the meadows as disturbed. The LAI of CS, although higher, cannot be described as optimal. Noticeably higher values can be found in other studies, such as those from a National Marine Park in northwestern Greece (Tsirika et al. 2007) ( $\sim 12.0$ ) or off the coast of Tunisia (Mabrouk et al. 2013) ( $14.79 \pm 1.40$ ). Responsible for the overall comparatively low LAI is the factor of shoot density, which in the latter two studies considerably exceeded observations from Lipsi.

### Shoot Density as an Indicator of Long-Term Physical Damage and Water Pollution

Shoot density is considered an effective metric to describe the direct human impact on *P. oceanica* meadows, with many studies referring to the scale of Giraud (1977), revised by Pergent et al. (1995b), which aims to define a vitality density of meadows in relation to the underlying depth gradient (Pergent-Martini et al. 2005). According to this, both meadows of PDS and CS are to be

interpreted as disturbed since they fall, on average, into the penultimate category of *very sparse beds* (150-300 shoots/m<sup>2</sup>). The overall low density of the meadows is most likely attributable to anchor damage caused by sailing tourists who often anchor within the otherwise hard-to-access bays, which, with the exception of the harbour, lack permanent mooring buoys. With an average loss of 34 shoots per anchor cycle (Francour et al. 1999), it is considered one of the main threats to Mediterranean seagrasses (Borum et al. 2004; Pergent-Martini et al. 2005). Due to its low horizontal growth rate (0.01-0.06 m y<sup>-1</sup>, (Marbà and Duarte 1998)), *P. oceanica* requires extended periods to recover from significant physical damage (Kendrick et al. 2005), as presented by Helber et al. (2021), who observed no significant signs of recovery in a protected meadow within a decade.

Interestingly, meadows in Urban Bay also exhibit low shoot density, despite the bay being generally avoided by tourists due to the wastewater treatment plant. Instead, the decrease may be linked to regular sewage discharge, as observed in related studies (Brahim et al. 2010; Mabrouk et al. 2013). Wastewater particles reduce water clarity (Supplementary Figure 6), leading to reduced light penetration and photosynthetic activity, which results in lower carbon assimilation rates, consequently causing imbalances in the plant's carbon budget (Ruiz et al. 2001). In an *in situ* experiment, Ruiz and Romero (2001) showed that this stress can cause severe mortality of shoots, estimating a 20–30% loss within four months at a shading rate of 40%. Further support for this assumption can be found from the NMDS plot, where Urban Bay samples negatively correlate with factor turbidity. Higher turbidity can also occur without active discharge, as fine particles can be deposited on the seafloor and are more likely to resuspend during stronger wind events or current activity (Moore et al. 1997). This may also explain a similar pattern observed in the epiphyte communities in Vroulia. In contrast to the other sites, the bay is characterised by fine sand and is protected from strong currents by its fjord-like morphology, slowing wave action and facilitating fine sediment deposition. The grazed land around the bay may have additionally influenced the accumulation of fine particles due to land erosion.

#### Epiphyte Cover and Load as an Indicator for Eutrophication and Proxy for Shading Rates

The EL represents a key metric for *P. oceanica* meadow condition (Pergent-Martini et al. 2005). This extends to other seagrass species (Frankovich and Fourqurean 1997), making it useful for comparisons at a spatial scale and between species. "Critical threshold ranges" by Nelson (2017) reveal that PDS meadows can be classified in the second highest range "high epiphyte load", linked to 50% light attenuation and seagrass decline. Elevated rates in Katsadia and Urban Bay, even signify "very high epiphyte load", leading to over 60% light reduction. These figures evoke heightened concern considering the consequences of shading, as discussed earlier. Conversely, CS meadows exhibit significantly lower values, averaging below 0.4 g g<sup>-1</sup> DW, which is not associated with seagrass decline. However, the threshold ranges should be treated with caution, since only six of the 36 data sources Nelson (2017) evaluated dealt with *P. oceanica* and responses may vary between species. Nevertheless, here they seem to give plausible reasoning to the observed results and are consistent with the other descriptors, defining PDS meadows as disturbed.

Nelson (2017) agrees with many others (e. g. Borum 1985; Peterson et al. 2007) that a high EL is primarily a consequence of increased nutrient levels in the water column. More specifically, Lepoint et al. (2007), identified that crustose coralline algae in particular, on *P. oceanica* leaves absorb quantitatively less N, but do so quicker than their host, leading to rapid coverage expansion when nutrients are available. The authors additionally argue that the short life cycle of epiphytic algae makes storage strategies of little importance and causes biomass to increase during nutrient loading. In the present study, the group of encrusting algae is most likely responsible for differences in the metric, given their significantly greater cover values in PDS meadows. These algae exhibit distinctive cell walls containing a calcite form of calcium carbonate (Dawes 2016), a characteristic trait that accounts for the primary contribution of the taxa to the EL, estimated by Borowitzka et al. (1990) at 40-60%. Consequently, it can be assumed that PDS meadows are indeed exposed to elevated nutrient concentrations, likely originating from point sources such as the sewage treatment plant, as well as non-point sources from adjacent agricultural areas.

#### Decreased Leaf Length as a Consequence of Increased Epiphyte Loads

The leaf length in PDS decreased significantly compared to CS. Following the annual growth cycle of *P. oceanica* provided by Ott (1980), the average leaf length during sampling time is estimated to be 40-50 cm. While CS meadows are on average within the given interval, PDS meadows fall short of it by half, indicating disturbance. Wave exposure, a natural stressor affecting leaf length (Pace et al. 2017), is minimised by the careful selection of sites with similar hydrodynamics for paired PDS and CS. Kimisi

and Urban Bay for instance, share a southwestern orientation and geomorphological characteristics, yet leaves in Kimisi averaged more than twice the length of those in Urban Bay, with lower EL. Here, the reduction can more likely be linked to direct and indirect effects of local eutrophication events caused by direct wastewater discharge, similar to observations in fertilisation experiments (Leoni et al. 2006), near fish farms (Ruiz et al. 2001), and in close proximity of industrial and urban wastewater discharges (Brahim et al. 2010; Mabrouk et al. 2013; Helber et al. 2021). Accordingly, samples from Urban Bay may be affected by ammonium poisoning from nutrient deposits altering sediment biogeochemistry, potentially leading to imbalances in the plant's nutrient budget (Burkholder et al. 1992). However, more frequently the decline is associated with an elevated EL and its shading effect (Short et al. 1995), corroborating our findings. Indirect effects of epiphytic overgrowth may additionally intensify grazing pressure by herbivores (Apostolaki et al. 2012), with leaf epiphytes contributing greatly to the upper trophic level (Morgan and Kitting 1984). Certain epiphytic taxa anchor deeply within the tissue, resulting in physical instability of the leaf apices, potentially causing them to break more easily (Harlin 1980), as noted during laboratory analysis (Supplementary Figure 7). A higher leaf production as an adaptive response to this stress to compensate for reduced photosynthetic rates, as proposed by Helber et al. (2021) and Leoni et al. (2006) could not be observed in Lipsi. In contrast, Guidetti (2001) reported an immediate reduction in leaf numbers following shading disturbances, indicating unresolved aspects of this relationship.

It is worth noting that other research on wastewater impacts, for instance by Balestri et al. (2004), did not identify any reduction and critique leaf length as a sensitive parameter to human disturbance, citing the findings of Guidetti and Fabiano (2000). While Balestri et al. (2004) investigated wastewater from a chemical factory, primarily composed of calcium carbonate and heavy metals, Guidetti and Fabiano (2000) examined terrigenous discharges from coastal areas, implying that the composition of the wastewater is significant. It further supports the hypothesis that specific nutrients, particularly phosphate and nitrogen (Powley et al. 2016) within the effluent affect the response of the plant. This suggests that meadows around Urban Bay are impacted by poorly or not properly treated discharges, which have already been proven to be enriched in high levels of nitrogen (Lepoint et al. 2004; Helber et al. 2021).

#### Abundance Patterns of Epiphytic Functional Groups as a Function of *P. oceanica* Leaf Age

Epiphyte assemblages in both PDS and CS meadows were dominated by encrusting algae, followed by bryozoans and filamentous algae, consistent with other studies conducted in winter (Lepoint et al. 2007; Tsirika et al. 2007; Prado et al. 2010). The algae-to-animal ratio aligns with expectations given the sampling depth and leaf part observed, as more light at the leaf apex allows the epiflora to out-compete the epibiota (Borowitzka et al. 2007), especially in shallow waters, with higher light intensity (Casola et al. 1987; Lepoint et al. 1999; Tsirika et al. 2007). Zooepiphytes, in contrast, tend to dominate the lower part of the leaf, resulting in an apical-basal pattern observed on most seagrasses with ribbon-shaped leaves, like *P. oceanica* (Borowitzka et al. 2007).

For the more specific epiphyte structure, the leaf age plays a crucial role (Casola et al. 1987), as the assemblages are a consequence of successive colonisation of the different functional groups (Van der Ben 1971). Thus, the observed dominance of encrusting algae at all sites is not surprising, since the studied part of the leaves is the oldest and the organisms are among the pioneers in colonising young leaves (Novak 1984; Trautman and Borowitzka 1999). Following the pioneering species, other epiphytes can settle. One of the first is the bryozoan *E. posidoniae* (Van der Ben 1971), which often reaches the highest cover values among the epifauna, as in the present study (Lepoint et al. 2007). The further colonisation of hydrozoans, filamentous algae, annelids, and foraminifera is determined by numerous biological (e.g., herbivory pressure) and microclimatic factors (e.g., light, temperature), leading not only to large differences in cover between leaves of the same shoot but also between leaf sides and axes (Casola et al. 1987) which explains the large variability observed at the plot level.

#### Overall Shift in Epiphyte Composition and Coverage Indicate Nutrient Enrichment

The epiphyte community composition in PDS changed significantly compared to CS. Numerous related studies from the Western Mediterranean (Martínez-Crego et al. 2010; Mabrouk et al. 2013; Brahim et al. 2014) indicate that these shifts are the effect of elevated nutrient levels in disturbed areas. In this study, the hypothesis finds support in two observations. Firstly, PDS samples showed a strong positive correlation (Quinn and Keough 2002) with eutrophication-indicating parameters, namely the EL and the

LUSI, while being negatively correlated with the factor of turbidity. Secondly, the variability in species communities largely corresponded to environmental conditions, suggesting a reasonable classification of the sites, and supporting LUSI efficiency.

Yet, it must be considered that most similar studies were conducted during late summer months, when the growth of epiphytic algae, strongly tied to the solar cycle, reaches its peak (Ott 1980; Lepoint et al. 2007). Prado et al. (2008) even argue that nutrient enrichments affect epiphyte assemblages exclusively during these months, as resources such as light and temperature are too limited in winter (Dez. – Apr.), drawing from a year-long experiment involving monthly water column nutrient additions. If these resources are not at their optimum, epiphyte communities are assumed to follow the seasonal succession described by Ballesteros (1987). However, Prado et al. (2008) examined the changes from month to month. The disparities found in our study may be attributed to the age of the communities. Based on the annual growth cycle given by Ott (1980), leaves studied emerged around August. Pete et al. (2015), in their investigation into the initial colonisation of epiphytes revealed that macroalgae could establish themselves on *P. oceanica* leaves rapidly within a brief period of 3-7 days. Hence, it is plausible that nutrient enrichment during the warm and sunny weeks of late summer and early autumn may have influenced the community structure. This inference aligns with the conclusions of Prado et al. (2008), who noted significant shifts in epiphyte communities extending until November. Nevertheless, it is important to consider that direct measurement of water quality was not conducted, and the analysis relies solely on the correlation between two disparate indicators: *P. oceanica* condition parameters and the epiphyte communities. Therefore, it is highly advisable to gather samples at the designated locations and directly measure water quality and nutrient concentrations to corroborate the hypotheses put forth in this study.

#### Suitability of Individual Epiphytic Flora and Fauna as Indicator-Species

##### Epiflora

The taxa most responsible for differences between PDS and CS belonged to the functional groups of encrusting and filamentous algae, with red coralline algae contributing mostly to the dissimilarity by a sharp increase of coverage in PDS, as widely observed in nutrient-rich conditions (Cambridge and McComb 1984; Borum 1985; Wear et al. 1999). Represented by *Hydrolithon* sp. and *Fosliella* sp., their cover values in CS coincide with those within an MPA investigated in winter by Mabrouk et al. (2015), while values in PDS are on average five times higher. Beyond the earlier described general responses of epiphytic algae to nutrient enrichment, these taxa possess supplementary benefits compared to filamentous algae. First, they demonstrate the capacity to assimilate nitrate and ammonium throughout the year. Secondly, they have lower nutrient and light requirements (Steneck and Dethier 1994) elucidating the notable increase in coverage during winter. Nevertheless, a significant increase of filamentous algae was also evident, predominantly attributed to red algae species within the genera *Laurencia* J.V.Lamouroux, 1813, *Polysiphonia* Greville, 1823, *Herposiphonia* Nägeli, 1846, and *Dasya* C.Agardh, 1824, along with the brown algae *S. cirrosa*, accounting for the observed disparities in composition. Compared to other studies (e. g. Martínez-Crego et al. 2010), the cover values here were lower overall, likely because erect algae reach their peak growth during late summer (Ballesteros 1987). Despite this, they can persist in shallow depths on the apical leaf sections during winter, as described by Tsirika et al. (2007) in Northern Greece, whose identified taxa largely coincide with those presented here.

Among those observed, we propose *S. cirrosa* as an indicator species for eutrophic conditions. The phaeophyte reached the highest cover values among the group and showed particularly high abundances near the wastewater discharge (Appendix 6). Large development of brown epiphytic algae was also reported in other studies dealing with the effects of discharged residential sewage (Giovannetti et al. 2010; Helber et al. 2021) and was observed in fertilised plots (Wear et al. 1999; Lepoint et al. 2007; Prado et al. 2010). Furthermore, Prado et al. (2008) experimentally demonstrated that structural alterations in leaf-epiphyte communities were largely attributed to a strong stimulation of *S. cirrosa*, corroborating our results. Filamentous algae in general and particularly photophilic brown algae have been shown by Cebrian et al. (1999) to have a higher growth rate, in terms of biomass increase, than crustose algae and are therefore considered to indicate nutrient enrichment effectively (Lepoint et al. 2007). In conclusion, our results indicate that, when seasonality is considered, the epifloral structure emerges as a suitable tool for detecting water pollution caused by nutrient overload in Lipsi.

##### Epifauna

Among animals, cover values in PDS increased significantly within the phylum Annelida, with other functional groups also exhibiting heightened values. Despite the fact that annelids, along with other epiphytic suspension feeders, are not directly influenced by nutrient levels, they may thrive in environments with enhanced food availability (Prado et al. 2010). They mainly feed on plankton and organic particles, which are increasingly found at nutrient-rich sites (Fourqurean et al. 1993). Increased animal abundance has also been found on *P. oceanica* leaves by Kocak and Aydin-Onen (2014) in the Aegean Sea near fish farms, while Egea et al. (2020) observed elevated cover values of annelids in fertilised plots in a *Cymodocea nodosa* (Ucria) Ascherson, 1870 meadow in summer. The biomass increase-induced shift in epiphytic fauna in PDS meadows therefore may be related to higher nutrient concentrations.

However, when considering the entire animal assemblage, our findings are only partially coherent with similar studies. The main contributor to the differences between conditions was the bryozoan *E. posidoniae*, which was often observed in Italy (Balata et al. 2008, 2010) and Tunisia (Brahim et al. 2010; Mabrouk et al. 2013). Similarly, other bryozoans for instance *Lichenopora* sp., *Fenestrulina* sp., and hydrozoans belonging to the family Campanulariidae Johnston, 1836 contributed to dissimilarities in studies mentioned. Different contribution values for others, such as the bryozoan *Walkeria* sp., and the foraminifera *Planorbulina* sp. may be related to both the high spatial variability of epiphyte communities (Balata et al. 2007) and the choice of statistical methods. Warton et al. (2012) critique SIMPER analysis, arguing it confounds mean differences between groups and variation within groups, resulting in the risk that variable species are filtered out instead of characteristic ones. This can indeed be assumed for *E. posidoniae*, which received high values in most similar studies, while being subject to high variability. Considering this, proposing an indicator species within the zoepiphytes is not feasible, especially since the response of invertebrates to nutrients is not as clearly described as for epiphytic algae.

## Ecological Conclusion

This research on epiphyte communities on *P. oceanica* leaves around Lipsi Island has provided comprehensive insights into epiphytic and plant-specific responses to various types of anthropogenic disturbances and their correlation with the LUSI. Using common *P. oceanica* meadow descriptors, it was shown that PDS meadows defined by the LUSI are indeed in a disturbed state compared to CS meadows. They are characterised by reduced meadow coverage, lower shoot density, shorter leaf canopy, and increased EL and coverage, potentially leading to significant impairments in both photosynthetic performance and carbohydrate allocation for storage and growth.

While the decreased shoot density in control meadows, likely stems from direct physical anchor damage, our findings suggest that the deteriorated parameters of PDS meadows are attributable to water pollution from point sources, such as the discharge of untreated or inadequately treated wastewater, and diffuse sources like adjacent agricultural activities. Our study confirms that leaf overgrowth by epiphytes, along with their community structure, in combination with the LUSI, serve as sensitive and easy-to-implement indicators of ecological water quality changes in the shallow depths around Lipsi. Their rapid response time could facilitate the early detection of localised eutrophication events, with the brown algae *S. cirrosa* proposed as an indicator species for nutrient-rich conditions. This approach presents a cost-effective alternative to continuous measurements using standardised monitoring stations.

Given the slow regeneration and recolonization process of *P. oceanica* and its sensitivity to such stressors, early detection is highly important for implementing effective conservation measures. We advocate for regular monitoring of *P. oceanica* meadows and their leaf epiphyte assemblages around Lipsi Island, as well as other small islands under similar anthropogenic pressure. Additionally, we recommend the installation of permanent mooring buoys and investment in educational outreach targeting tourists and locals to ensure the long-term preservation of this iconic keystone species along Lipsi's coastline.

## Declarations

## Competing Interests

The authors do not have any pertinent financial or non-financial interests to declare.

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# Author Contribution

GLN: Conceptualization, Methodology, Investigation, Data curation, analysis and visualization, Writing – original draft preparation, review and editing. AC: Data analysis, Writing – review and editing, Supervision. AMM: Conceptualization, Methodology, Writing – review and editing, Supervision, Project administration. MMG: Supervision. LM: Supervision, Project administration, Writing – review and editing. AM: Resources, Project administration, Funding acquisition. All authors approved the final version of the manuscript. Gian Luca Naccarato (GLN); Arne Cierjacks (AC); Alice Malcom-McKayb(AMM); Maria Moran Garcia (MMG); Laura Macrina (LM); Anastasia Miliou (AM)

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# Data Availability

All data utilized and analysed in this study is available in the Github repository, <https://github.com/glnaccarato/Epiphyte-com-strpos-ocea>.

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## Appendix

Appendix 6 is available with this version.

## Figures

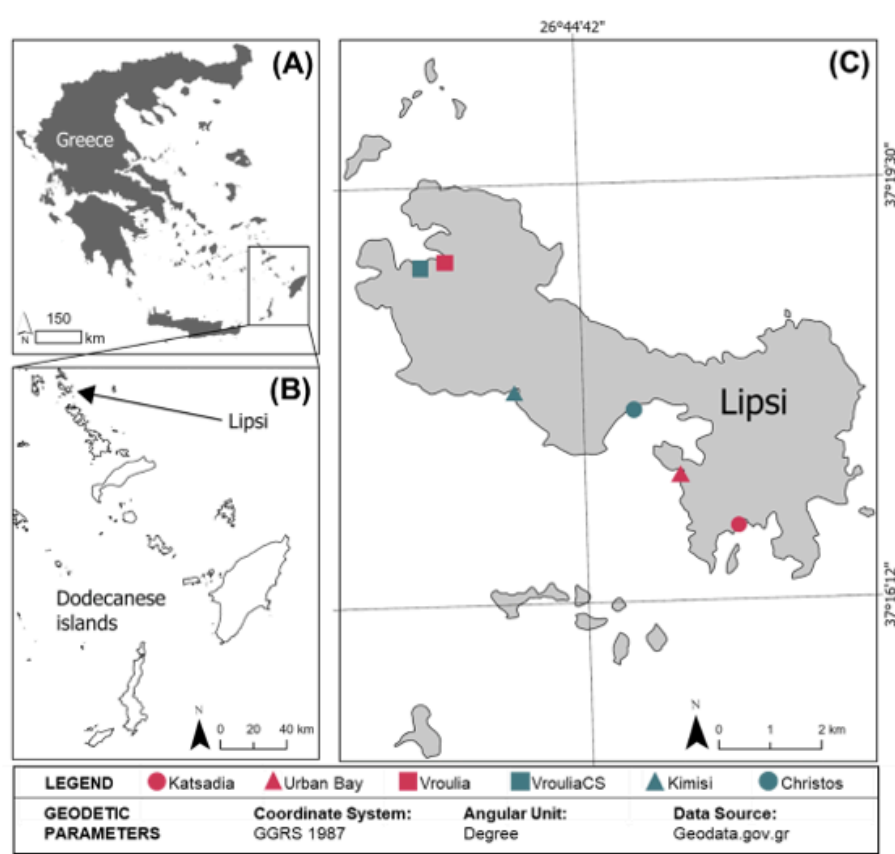


Figure 1

Geographical location map of the study area. Panel (A) presents the geographical location of the Dodecanese islands in the Aegean Sea. Panel (B) shows the location of Lipsi island within the Dodecanese complex. Panel (C) displays the six sampling stations of this study on Lipsi island. Red symbols = potentially disturbed sites; Green symbols = control sites.

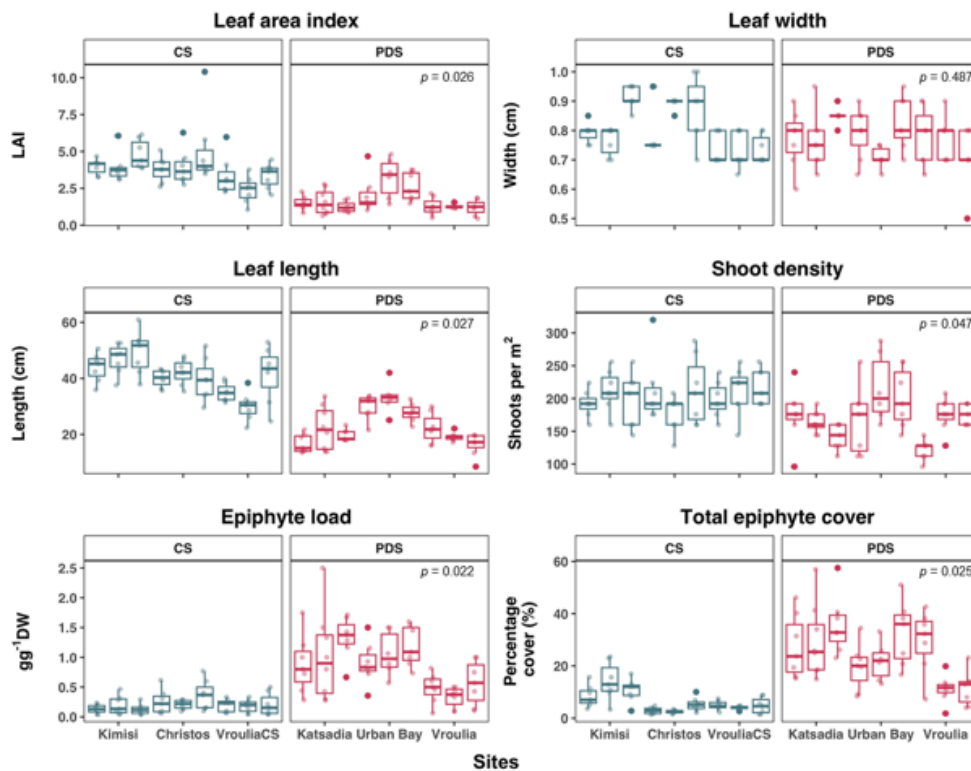


Figure 2

Leaf area index, leaf width, leaf length, shoot density, epiphyte load, and total epiphyte cover at control sites (CS) and potentially disturbed sites (PDS). The provided  $p$ -value is the result of the nested ANOVA conducted between PDS and CS. Data presented as boxplots separated by plot for each site. Box represents the range between the first and third quartile, and the horizontal line within the box the median. Whiskers show the minimum and maximum values. Observations that lay either over the third quartile + 1.5 x IQR or below the first quartile - 1.5 x IQR are shown as filled circles.

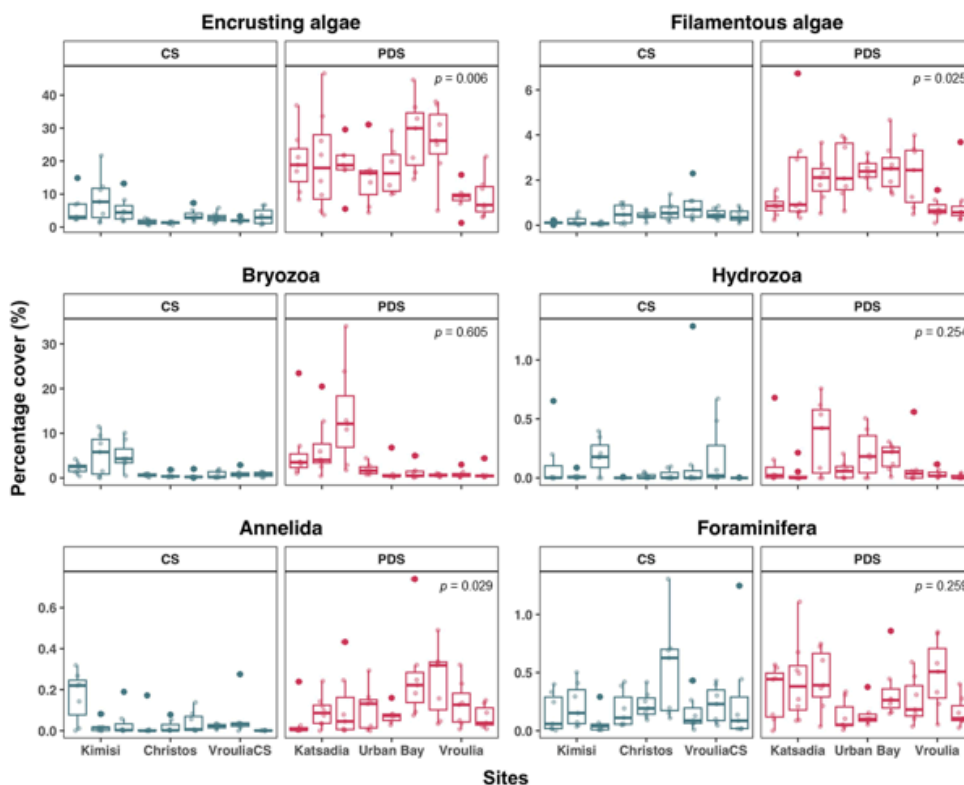


**Figure 3**

Percentage cover (%) of the main epiphyte functional groups on the apical 9 cm of *P. oceanica* leaves at control sites (CS) and potentially disturbed sites (PDS). The provided  $p$ -value is the result of the nested ANOVA conducted between PDS and CS. Data presented as boxplots, separated by plot for each site. Box represents the range between the first and third quartile, and the horizontal line within the box the median. Whiskers show the minimum and maximum values. Observations that lay either over the third quartile + 1.5 x IQR or below the first quartile - 1.5 x IQR are shown as filled circles.

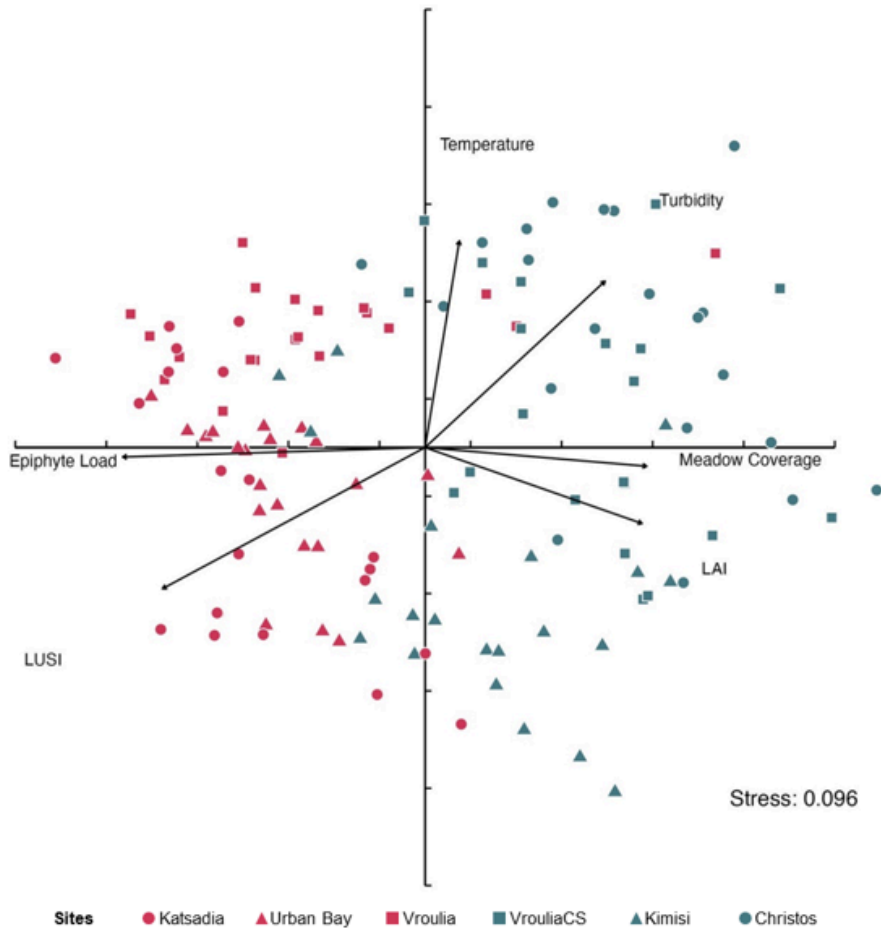


**Figure 4**

Non-metric multidimensional scaling (NMDS) plot of samples obtained from Bray-Curtis dissimilarities, calculated on log (x+1) transformed data showing differences in epiphyte assemblages in potentially disturbed sites (PDS, red symbols) and control sites (CS, green symbols). Vectors show correlations between epiphyte composition and environmental variables. Only significant correlations ( $p < 0.05$ ) are presented.

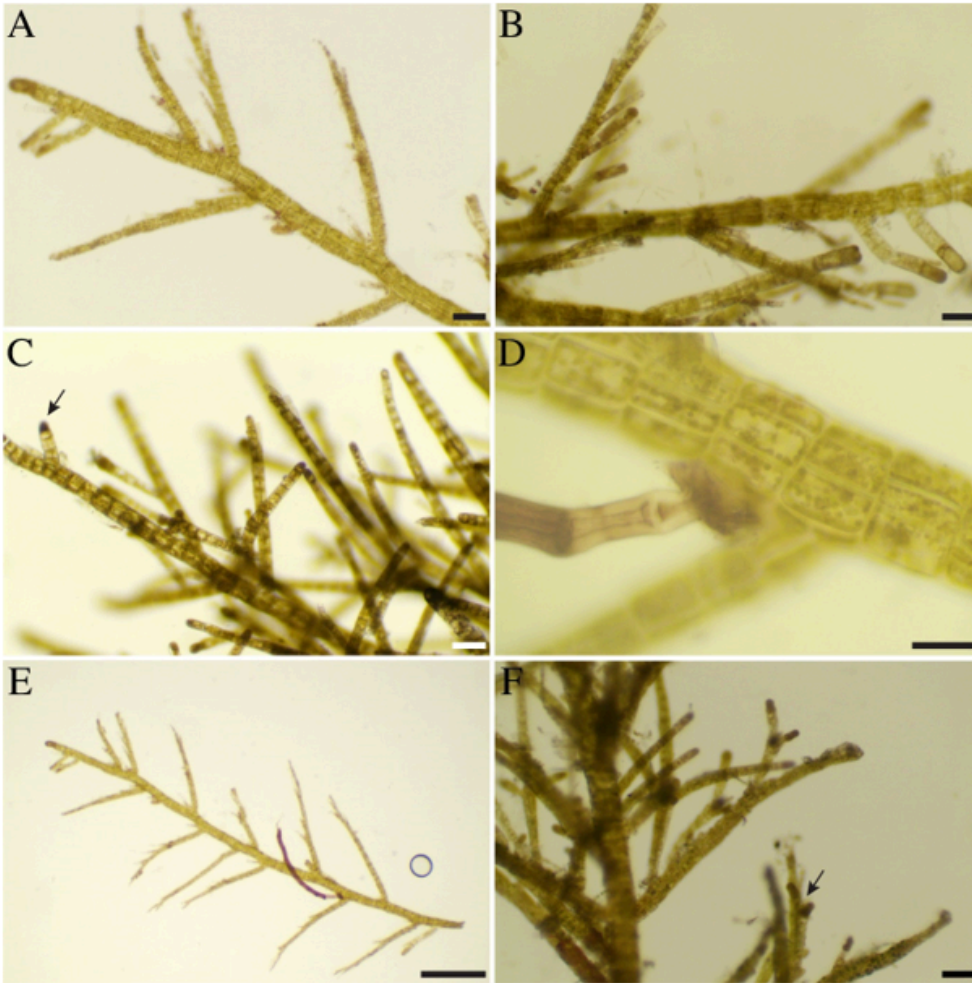


**Figure 5**

Morphological characteristics of *Sphacelaria cirrosa* (Roth) C. Agardh, 1824 (images by author). A and B: Central stem 100 µm wide. C and F: Arrows indicate large, dark apical cells. D: Central cells 88-103 µm long. E: Filiform thallus with transverse and longitudinal cell divisions. [Scale bars: A, B, C, F: 0.1 mm; D: 70 µm; E: 0.5 mm.] Species identification followed the key provided by García-Redondo et al. (2019).

## Supplementary Files

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