Microcalcareous seaweeds as sentinels of trophic changes and CO_2 trapping in transitional water systems

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ABSTRACT

Microcalcareous epiphytic seaweeds (MES) are macroalgae more sensitive than aquatic angiosperms to environmental degradation and, with their presence/absence, these species act like sentinels providing useful information on the ecological status of environments. In this study, we analyzed the environmental parameters in water column and surface sediments in relation to macrophyte variables from 257 sites, distributed in the main Italian transitional water systems (TWS). The results showed that MES are strongly correlated to pH changes, the main parameter that regulates their presence/absence. The optimal growth range is between pH 7.80 and 8.35; out of these values their growth is reduced or hampered. In oxidized sediments the carbonate crusts, composed by Mg-Calcite (an unstable compound that in the sediments quickly turns into calcite), can permanently trap up to 2.47 tonnes ha^{-1} yr^{-1} of CO_2, increasing sediment thickness of approx. 0.06–0.21 mm yr^{-1}.

1. Introduction

In transitional water systems (TWS), one of the main effects of eutrophication processes is the abnormal development of nuisance macroalgae (Morand and Briand, 1996). During the fast growth period, they trap significant loads of carbon, nitrogen and phosphorus, which return to the environment when the biomass exceeds the carrying capacity of the system (Sfriso et al., 1987) and collapse. In eutrophic environments, production and decomposition processes alternate quickly every year, with more or less intense phenomena, rapidly changing most of the parameters of water column and surface sediments and affecting benthic communities and macrophyte assemblages. An important consequence is the disappearance of aquatic angiosperms (seagrasses and aquatic plants) and many sensitive macroalgae, which are replaced by tionitrophilic taxa or, in the worst conditions, by phytoplankton and cyanobacteria (Orfanidis et al., 2003; Sfriso et al., 2007; Viaroli et al., 2008; Sorokin and Zakuskina, 2010; Munari and Mistri, 2012).

Among the most sensitive macroalgae, there are crustose coralline algae. These organisms are microcalcareous epiphytic seaweeds (MES), a few microns thick and 50–200 μm in width, mainly composed by the genera *Hydrolithon*, *Pneophyllum* and *Melobesia*, which are strongly affected by these environmental changes. The frequent fluctuations of environmental parameters in the water column, especially of pH, can prevent the presence of these MES and hamper their growth and carbon fixation, as previously reported for many Corallinales taxa (Fabricius et al., 2015; Hofmann and Bischof, 2014; Mccoy, 2013). Their disappearance/appearance is the fastest response to the deterioration/improvement of the environment. These microscopic macroalgae, which grow as epiphytes on leaves of aquatic angiosperms and thalli of larger seaweeds, respond faster than seagrasses and aquatic plants to environmental changes. In fact, they appear or disappear within a few months, or even in a few days, during hypo-anoxic conditions that lower the pH below the average values (8.10–8.30, Sfriso et al., 2019a) normally recorded in good-high quality TWS.

MES species are present in all the transitional environments of the Mediterranean Sea, resulting in an excellent bioindicator of trophic changes (Orfanidis et al., 2003; Sfriso et al., 2009, 2014). Unlike aquatic plants that once disappeared take several years to recolonize the environment with small millimetric seeds, MES produce gametes (2–6 μm) or spores (20–60 μm) that are easily removable by tides and able to quickly recolonize substrata. Therefore, surveying their presence/absence could be a fast and cheap method to monitor the trophic status of transitional areas and to foresee the environmental quality trends with months or years in advance.

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This paper aims to: 1) assess the sensitivity of MES to environmental changes, analyzing sets of data collected by our team in the main TWS of the Italian coasts and reproducing field conditions in laboratory; 2) evaluate the carbonate type and quantity from MES and surface sediments in order to understand the contribute of MES and their associated community to the sediment accretion and composition.

2. Materials and methods

2.1. Data set analyses

During the last decade, several sampling campaigns were carried out in the main Italian TWS in order to study the composition and characteristics of the biological communities, in agreement with the requirements of the European Water Directive (2000/60/EC). The aim was to assess the main biotic components (phytoplankton, macroalgae, aquatic angiosperms, benthic fauna, fish fauna) as indicators of water quality, since they temporally integrate the effects of anthropogenic pressures, providing more reliable results than punctiform sampling of single physico-chemical parameters, nutrients and pollutants.

Among the biotic components, macroalgae and aquatic angiosperms, which in TWS are considered together because of their morphofunctional similarities and habitat overlapping (Orfanidis et al., 2003, 2011; Sfriso et al., 2009, 2014; Viaroli et al., 2008), are the organisms with a faster response to environmental changes. Therefore, we examined the number of macroalgal taxa and the number of sensitive taxa, following Sfriso et al. (2007) and the "Istituto Superiore per la Protezione e Ricerca Ambientale" (ISPRA) lists (ISPRA, 2011); the cover and biomass of total macroalgae; the biological quality ratio (BQR), obtained applying the Macrophyte Quality Index (MaQI, Sfriso et al. 2014); the cover of aquatic angiosperms (*Cymodocea nodosa* (Ucria) Ascherson, *Zostera marina* Linnaeus, *Zostera noltei* Hornemann, *Ruppia cirrhosa* (Petagna) Grande) and the number of MES (*Pneophyllum fragile* Kützing, *Hydrolithon boreale* (Foslie) Y.M. Chamberlain, *H. cruciatum* (Bressan) Y.M. Chamberlain, *H. farinosum* (J.V. Lamouroux) Penrose & Y.M. Chamberlain, and *Melobesia membranacea* (Esper) J. V. Lamouroux).

The following common parameters in the water column (dissolved oxygen (%DO); water transparency; salinity; pH; ammonium; nitrites; nitrates; reactive phosphorus (RP); chlorophyll-*a* (Chl-*a*)) and surface sediment (sediment fraction < 63 μm (Fines); total phosphorus (Ptot); inorganic phosphorus (Pinorg); organic phosphorus (Porg); total carbon (Ctot); inorganic carbon (Cinorg); organic carbon (Corg) and total nitrogen (Ntot)) were evaluated.

Dissolved oxygen was determined with a portable Oxi 196 oximeter (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Oxygen concentrations were reported as percentage of saturation (%DO) taking into account water temperature and salinity. Water transparency was measured with a Secchi disk. Measurements were reported as a percentage of water column visibility due to the shallow bottoms and tidal excursion that in some lagoons is relatively high (0.5–1 m). A value corresponding to 100% means that the bottom was visible, 50% means that the disk disappeared half way to the bottom (Sfriso et al., 2017). pH values were measured with a portable pH-meter (pH 25) of the CRISON Instruments (Barcelona, Spain).

Water samples were obtained by mixing six water column samples collected with a home-made cylindrical sampler (length: 1.50 cm, diameter: 4 cm) which was repeatedly plunged into the water and poured to a tank. Sub-samples of 0.1–1.0 L were filtered through GF/F Whatman glass fiber filters (porosity: 0.7 mm). Water samples and filters were stored frozen at −18 °C for reactive phosphorus (RP), dissolved inorganic nitrogen (DIN as the sum of ammonium, nitrite and nitrate) determination according to Strickland and Parsons (1984), and chlorophyll-*a* (Chl-*a*) analyses according to Lorenzen (1967). Other water sub-samples were collected for salinity, which was determined in the laboratory by chlorine titration according to Oxner (1962). All nutrient analyses were carried out in triplicate.

Sediments were collected with a Plexiglas corer (i.d. 10 cm). Two sub-samples of the first 5 cm top layer of three cores carefully mixed together were retained, one to determine sediment density and grain-size and the other to analyse total nitrogen (Ntot), total carbon (Ctot), inorganic carbon (Cinorg), organic carbon (Corg), total phosphorus (Ptot), inorganic phosphorus (Pinorg), organic phosphorus Porg). Density was obtained as g cm^{-3} of wet and dry sediment according to Sfriso et al. (2003).

The amount of dry sediment per volume unit (dry density = g DW cm^{-3}) was measured by drying ca. 30 g of homogenised sediment at 110 °C in volumetric containers up to constant weight, whereas Fines (fraction < 63 μm) were determined by wet sieving ca. 50 g of dry sediment through 63 μm mesh sieves by the Endecotts LTD, London, England. (Sfriso et al., 2005).

Total nitrogen and Ctot concentrations were obtained by CHNS Analyzer (Vario-MICRO, Elementar CHNS, Thermo Fisher Scientific Inc., Italy), after sediment freezing, lyophilization and pulverization. Inorganic carbon was determined after removing Corg by burning samples at 440 °C for two hours. Organic carbon was determined by difference. Inorganic and total phosphorus were, respectively, determined before and after combustion at 550 °C for two hrs, with dissolution in 1 N HCl and spectrophotometric measurements according to Aspila et al. (1976). Organic phosphorus was obtained by difference.

All sediment analyses were carried out in duplicate on two different days (four replicates) and values were retained when the difference was < 5%. To compare the nutrient concentrations of the surface sediments of each site, values were normalized with reference to the amount of dry sediment per volume unit (dry density).

Samples were collected in late spring-early summer, since 2005 to 2014, from 257 sites, distributed in the most wide Italian TWS (Venice: 550 Km^2 in 2011 and 2014, Grado and Marano: 158 km^2 in 2007, Valli di Comacchio: 110 Km^2, Po delta: 178 Km^2, Pialassa della Baiona: 11 Km^2 and Lesina: 51 km^2 in 2009 and Orbetello: 27 km^2 in 2005), with a water surface accounting for ca. 78% of the total one (Sfriso et al., 2017).

2.2. Carbon and carbonate analysis

The carbonate concentration and the species composition of epiphytic MES were studied in the macrophytes more densely colonized by MES: the seaweed filaments of *Chaetomorpha linum* (O.F. Müller) Kützing and the seagrass leaves of *Cymodocea nodosa,* and in sediment samples bearing dense populations of macroalgae. Samples of *C. linum* filaments, massively covered by MES crusts, were collected in Dogà fishing valley. The macroalgae were freeze-dried in order to determine the dry/wet weight ratio and stored for analysis. Dried *C. linum* and MES samples were analyzed together because the carbonate crusts were not separable from the *Chatomorpha* filaments.

MES were also sampled from old leaves of *C. nodosa*. In the Venice Lagoon, this subtropical species starts to grow in April-May and the old leaves are completely covered by MES till October, when they fall for winter rest (Sfriso and Ghetti, 1998). The number of shoots and leaves per square meter, their height and the surface area of both sides were determined every month, considering the older leaves covered by MES that settled on the sediments inside the prairie. Ten old leaves were collected, weighted and measured; then MES were carefully scraped with a razor blade. The resulting sample was freeze dried, homogenized and analyzed to determine the carbon concentrations for surface unit.

The dried samples were ground in a mortar and analyzed by CHNS Analyzer (Vario-MICRO, Elementar CHNS, Thermo Fisher Scientific Inc., Italy) to measure Inorganic Carbon content after loss-on-ignition of organic matter at 440 °C for 2 h (Schumacher, 2002). The $CaCO_3$, CO_3^{2-} and CO_2 contents were estimated starting from the concentration of Inorganic Carbon: Cinorg/12*100, C/12*60 and Cinorg/12*44; the percentage of organic matter (OM) was determined from the

Table 1
Non-parametric Spearman's coefficients between environmental parameters of the water column, surface sediments and macrophytes variables recorded in 257 sites of the Italian Transitional Environments. In bold significant values at p < 0.05; in bold and underlined significant values at p < 0.01.

	Parameters	Macroalgae						Angiosperms					p < 0.05	p < 0.01
		MES	Sensitive	MaQI	Taxa	Cover	Biomass	C. nodosa	Z. marina	Z. noltei	R. cirrhosa	Total Angiosperms		
WATER	%DO	0.15	0.160	0.15	−0.07	0.17	0.11	0.24	0.08	0.03	−0.07	0.157	6	1
	Transparency	0.37	0.35	0.34	0.20	0.21	0.11	0.23	0.23	0.18	0.11	0.35	9	9
	Salinity	0.19	0.28	0.17	0.37	−0.03	−0.22	0.09	0.124	−0.01	−0.17	0.09	7	6
	pH	0.49	0.53	0.44	0.161	0.21	0.04	0.36	0.20	0.21	0.09	0.41	9	9
	NH_4^+	−0.07	−0.01	−0.155	0.21	−0.13	−0.18	−0.03	−0.15	−0.118	−0.06	−0.15	5	2
	NO_2^-	−0.20	−0.20	−0.21	−0.10	−0.15	−0.01	−0.06	−0.17	−0.14	−0.09	−0.21	7	5
	NO_3^-	−0.21	−0.25	−0.20	−0.25	−0.13	0.07	−0.07	−0.121	−0.01	−0.01	−0.11	5	4
	DIN	−0.164	−0.15	−0.25	0.03	−0.17	−0.09	−0.06	−0.20	−0.10	−0.06	−0.20	6	5
	RP	−0.17	−0.19	−0.120	−0.18	−0.02	0.124	0.00	−0.13	0.02	0.124	−0.08	6	3
	Chl-*a* tot	−0.39	−0.44	−0.36	−0.42	0.19	0.19	−0.27	−0.20	−0.18	−0.01	−0.37	9	9
	Fines	−0.26	−0.24	−0.23	−0.17	0.00	0.06	−0.19	−0.20	−0.04	0.00	−0.27	7	7
SURFACE SEDIMENTS	Ptot	−0.25	−0.31	−0.23	−0.23	0.22	0.33	−0.26	−0.10	−0.124	−0.03	−0.24	9	8
	Pinorg	−0.19	−0.26	−0.157	−0.17	0.17	0.29	−0.20	0.02	−0.17	−0.157	−0.17	10	8
	Porg	−0.30	−0.30	−0.29	−0.23	0.13	0.17	−0.25	−0.27	−0.02	0.163	−0.28	10	9
	Ctot	0.14	0.14	0.17	0.21	−0.20	−0.32	0.10	0.09	0.116	−0.05	0.14	7	5
	Cinorg	0.13	0.14	0.18	0.157	−0.27	−0.34	0.13	0.17	0.07	−0.19	0.17	10	6
	Corg	0.00	−0.01	0.00	0.05	0.20	0.10	−0.119	−0.08	0.02	0.21	−0.06	2	2
	Ntot	−0.17	−0.22	−0.13	−0.25	0.162	0.25	−0.10	−0.15	0.08	0.18	−0.10	8	6
p < 0.05 per r ≥ |0.124|		16	16	16	14	14	10	9	12	6	7	13		
p < 0.01 per r ≥ |0.161|		13	12	12	13	10	9	8	8	4	5	10		

concentration of Organic Carbon: Corg * 1.724 according to Nelson and Sommers (1982) based on the assumption that, commonly, in soils organic matter contains approximately 58% of carbon.

The composition of the different carbonate minerals was determined by X-ray analysis. The samples were ground and analyzed by X-ray powder diffraction using a Panalytical X'Pert 3 Powder with CuKa radiation between 20° and 65° 2θ. Peak positions of the carbonate minerals were identified using UNI EN 13925-2: 2006.

The obtained results allowed to determine the amount and type of carbonates that settled on surface sediments, to estimate the annual sediment thickness increase and to evaluate the amount of CO_2 permanently trapped on the bottom.

2.3. Experimental viability

Leaves of *C. nodosa* covered by MES were collected in September and October 2019 in the Venice Lagoon (Italy), and more precisely near the internal area of the Malamocco sea inlet, a station with high ecological conditions. The leaves were acclimated overnight in seawater at 22 °C and were employed in the experimental setup. The first experiment was performed to evaluate the effect of pH on the global coverage and viability of MES, by incubating seagrass leave fragments, 5 cm long, at four different pH values (7.6, 7.8, 8.1, 8.4) for two weeks. For each pH value, a 250 mL flask was prepared containing 100 mL of filtered seawater (GF-F fiberglass filters, 0.7 µm) buffered with TRIS-HCl 10 mM. The pH was adjusted with few drops of diluted NaOH to obtain the different tested values. Before being incubated at the tested conditions, each seagrass leaf fragment was marked with a diagonal cut to recognize the two sides and photographed, centimeter by centimeter, with a steromicroscope (Optika stero zoom SZM-LED2) both in visible light and fluorescence. The stereomicroscope was equipped with a 10 W green LED, an orange photo filter and a digital image acquisition system. The leave fragments were kept under a continuous light of 30 µmol photons m^{-2} s^{-1} (red and blue LED ratio 2:1) at 22 °C in mild agitation (50 rpm) and were photographed again after 5 days, 1 week and 2 weeks. The photographs (5 per leaf, i.e. one for each centimeter) were processed by ImageJ (additional information on the steps used for image processing is provided in supplementary material) to measure the epifluorescent living cover/total cover (LC/TC) ratio.

2.4. Light microscopy

For light microscopy, fragments of the seagrass leaves from the viability experiment at 1 week were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.9 at 4 °C for at least 24 h. Successively, the samples were dehydrated in a graded ethanol series followed by propylene oxide and embedded in Araldite resin. Semi-thin sections (1 µm) were cut with an Ultracut S, Reichert ultramicrotome (New York, USA) stained with basic toluidine blue (O'Brien and McCully, 1981), and placed in slides. The slides were then rinsed with distilled water, dried, and mounted with Eukitt (Sigma-Aldrich) and examined at DMR Leica light microscope equipped with a digital image acquisition system. The images were processed by photoshopCS4 to highlight the pixels with deep blue values in red (additional information on the steps used for image processing and the original images (Fig. S1, supplementary material) without highlights are provided in Supplementary material).

2.5. Statistical analyses

A matrix composed by 18 environmental parameters was analyzed to determine the non-parametric Spearman's coefficients. The considered parameters concerned the water column (%DO; transparency; salinity; pH; ammonium; nitrite; nitrate; dissolved inorganic nitrogen (DIN); RP; Chl-*a*), the surface sediments (Fines; Ptot; Pinorg; Porg; Ctot; Cinorg; Corg; Ntot) and 11 macrophyte variables (total macroalgal taxa;

Table 2

Non-parametric Spearman's coefficients between Microcalcareous Epiphytic Seaweeds (MES), Sensitive macroalgae, the Macrophyte Quality Index (MaQI) and macrophyte variables. In bold and underlined significant values at p < 0.01.

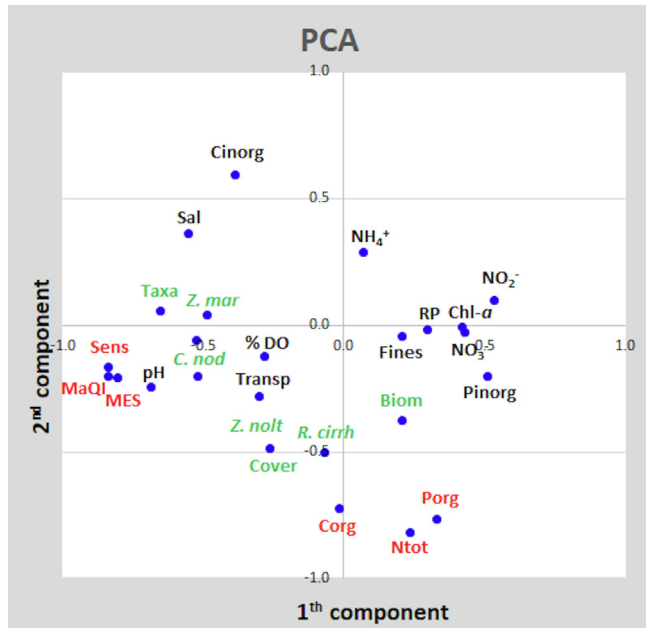| | Macroalgae | | | Aquatic angiosperms | | | | |
	Taxa	Cover	Biomass	*Cymodocea nodosa*	*Zostera marina*	*Zostera noltei*	*Ruppia cirrhosa*	Total Angiosperms
MES	<u>**0.53**</u>	0.06	− 0.07	<u>**0.49**</u>	<u>**0.47**</u>	<u>**0.33**</u>	<u>**0.20**</u>	<u>**0.73**</u>
Sens	<u>**0.62**</u>	0.07	− 0.11	<u>**0.47**</u>	<u>**0.44**</u>	<u>**0.32**</u>	<u>**0.21**</u>	<u>**0.68**</u>
MaQI	<u>**0.44**</u>	0.10	0.01	<u>**0.50**</u>	<u>**0.51**</u>	<u>**0.38**</u>	<u>**0.19**</u>	<u>**0.78**</u>
p < 0.01 per r ≥ |0.161|								

Fig. 1. PCA biplot between the first two components. In red variables/parameters with a significant loading > 0.7. In green macrophyte variables.

macroalgal biomass; percent macroalgal cover; number of sensitive macroalgae; number of small calcareous macroalgae; percent cover of *C. nodosa, Z. marina, Z. noltei, R. cirrhosa*; percent cover of aquatic angiosperms; the Macrophyte Quality Index). Data were considered significant at p < 0.05 and p < 0.01.

After removing the redundant parameters, the Principal Component Analysis (PCA) was used to highlight the associations among all the considered parameters and macrophyte variables.

Both correlation and PCA analyses were performed using the STA-TISTICA software, Release 10 (StatSoft Inc., Tulsa, USA).

The data obtained from the image analysis on the MES cover were checked for normality by Shapiro-Wilk test; both *t*-test and Wilcoxon test were applied to compare the cover before and after treatment and to identify significant variations over time at different pH values.

3. Results

3.1. Environmental data analysis

Spearman's coefficients between macrophyte variables, the environmental parameters of water column, surface sediments and MES are reported in Table 1.

The highest number of significant correlations (p < 0.05 = 16) between macrophyte variables and environmental parameters was shown by MES, sensitive macroalgae and MaQI, followed by macroalgal cover and the number of total macroalgal taxa (p < 0.05 = 14). Total aquatic angiosperms showed 13 significant correlations (p < 0.05) and, among them, *Z. marina* was the most correlated (p < 0.05 = 12), followed by *C. nodosa* (9), *R. cirrhosa* (7) and *Z. noltei* (6). Eventually for

a p < 0.01 the MES displayed the highest number of significant correlations (14).

The environmental parameters that showed the highest number of correlations (p < 0.01 = 9) with macroalgae and aquatic angiosperms were water transparency, pH, Chl-*a*, and Porg. However, the highest r values were recorded for pH correlated with MES, sensitive macroalgae and MaQI.

These three variables (MES, Sensitive macroalgae, MaQI) were also highly positively correlated (p < 0.01) with the other macrophyte variables (Table 2), especially with the total aquatic angiosperm cover.

The output of the PCA showed that 36% of the total site variation was explained by two components and 52.5% by four components. The loadings of the first two components have been plotted in a plane (Fig. 1) to highlight their aggregation and the variables with a higher loading (> 0.7): MES, sensitive species and MaQI along the 1st component; organic carbon, organic phosphorus and total nitrogen along the 2nd component. Moreover, the biplot highlighted the close association of MES (Fig. 2) with the sensitive species and MaQI and, less closely, with the different aquatic angiosperms.

In particular, MES had a decreasing correlation starting from *C. nodosa* to *Z. marina, Z. noltei* and *R. cirrhosa*. The number of total macroalgal taxa was also well correlated with these variables. The environmental parameters strongly associated with the presence of calcareous taxa were pH and water transparency, followed by salinity and %DO. Conversely, the parameters that hindered the presence of calcareous and sensitive taxa and that were responsible of the environmental degradation were: nutrient concentrations, both in the water column and surface sediments; the presence of high macroalgal or phytoplankton biomass (based on the Chl-*a* values); and the percentage of Fines (sediment fraction < 63 μm).

3.2. Calcareous taxa and dataset analysis

The presence of at least one MES was recorded for 107 sites (41.6% of the total). For 53 sites (20.6%), two MES taxa and, for 19 sites (7.4%), three MES taxa were detected. Only one site had four taxa. The remaining 150 sites did not show calcareous macroalgae. The relationship between calcareous macroalgae and field-measured water pH is displayed by Fig. 3.

The presence of, at least, one calcareous taxon was reported at pH 7.93, whereas at lower pH values MES where never recorded. The maximum number of MES was recorded starting from pH 8.30, with the highest number at pH 8.35, showing a polynomial trend.

In addition, at pH values higher than 8.30, in stations colonized also by aquatic angiosperms, where anoxic conditions do not occur and the pH hardly drops below 8.00, MES formed dense crusts both on angiosperm leaves (Fig. 4d, e) and on the other macroalgae of bigger size (Fig. 4a, b, c) and colonized any hard substratum present on the bottoms.

3.3. Experimental viability

The parameter that was most significantly associated with the presence and abundance of MES was the pH in the water column. Therefore, different values of pH were tested on old *C. nodosa* leaves
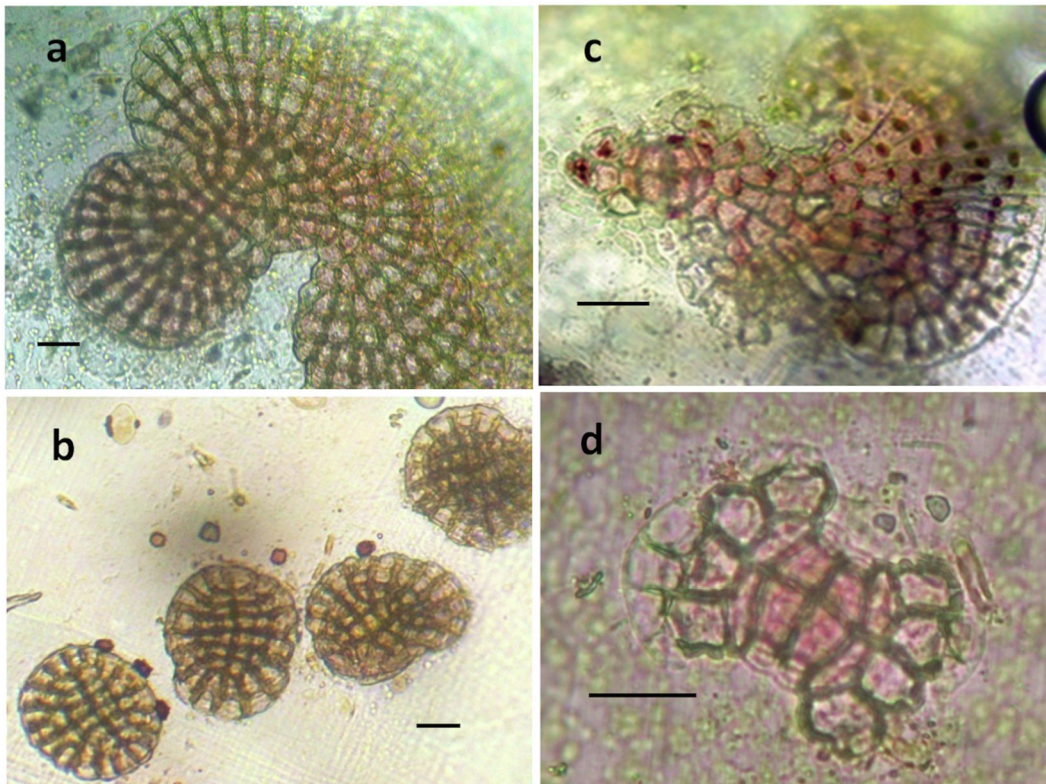
Fig. 2. Pictures of the most abundant MES in the Venice Lagoon: a, b) *Pneophyllum fragile*; c, d) *Hydrolithon boreale*. Bar = 25 μm.
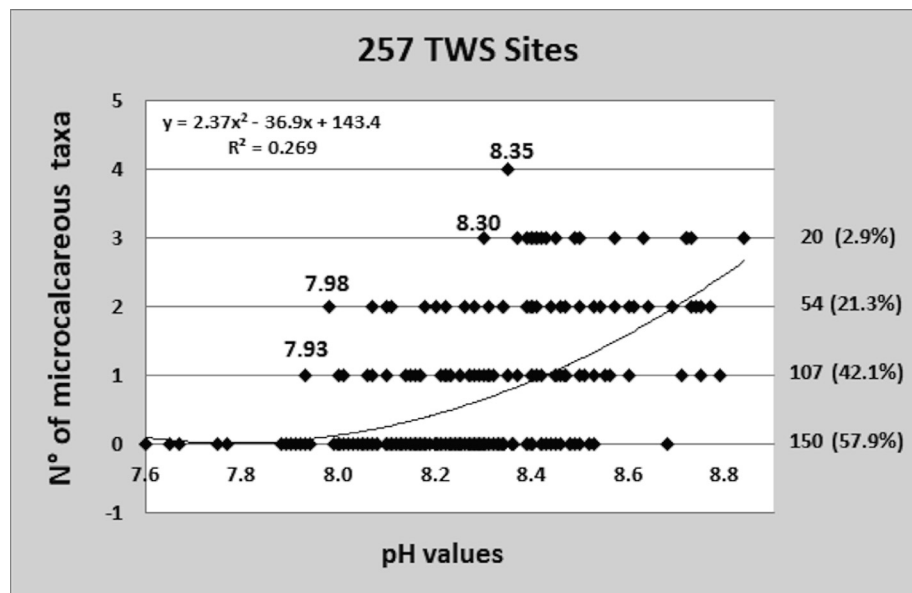

Fig. 3. Relationship between pH values and the number of MES.

bearing MES to verify the impact of this parameter on MES cover and viability over a period of 2 weeks. The experimentally obtained results displayed a global mean cover of 69 ± 14%, without significant variations in the pH range: 7.6–8.4, from time 0 (t_0) to 2 weeks. However, some significant changes in algal viability occurred at pH 7.6, 7.8 and 8.4, with a visible bleaching (dead crusts without phycoerythrin) that increased during the experimental time (2 weeks). MES viability was measured by phycoerythrin epifluorescence that quenches in dead tissues. The Fluorescent Living Cover/Total Cover (LC/TC) ratio of MES displayed different values at t_0, due to the different viability of field samples. Despite this, it was possible to appreciate trends of viability

loss or recovery within a time range of two weeks. As reported in the boxplot of Fig. 5 a progressive decrease in the fluorescent LC/TC ratio was found for both pH 7.6 (0.30) and 7.8 (0.36). Conversely, at pH 8.1 a stable subsistence of alive tissues was found and an increase in the fluorescent LC/TC ratio (of about 0.66–0.69) in 7 days revealed the optimal pH condition for MES growth and recovery. At pH 8.4 a dramatic loss of viability, with a drastic reduction in fluorescent LC/TC cover from 0.60 to 0.01 in only 5 days, was observed.

At time 0 and after one week at the different pH values (7.6, 7.8, 8.1, 8.4), the differences in the carbonate structure of MES on *C. nodosa* leaves were observed in cross section (Fig. 6).
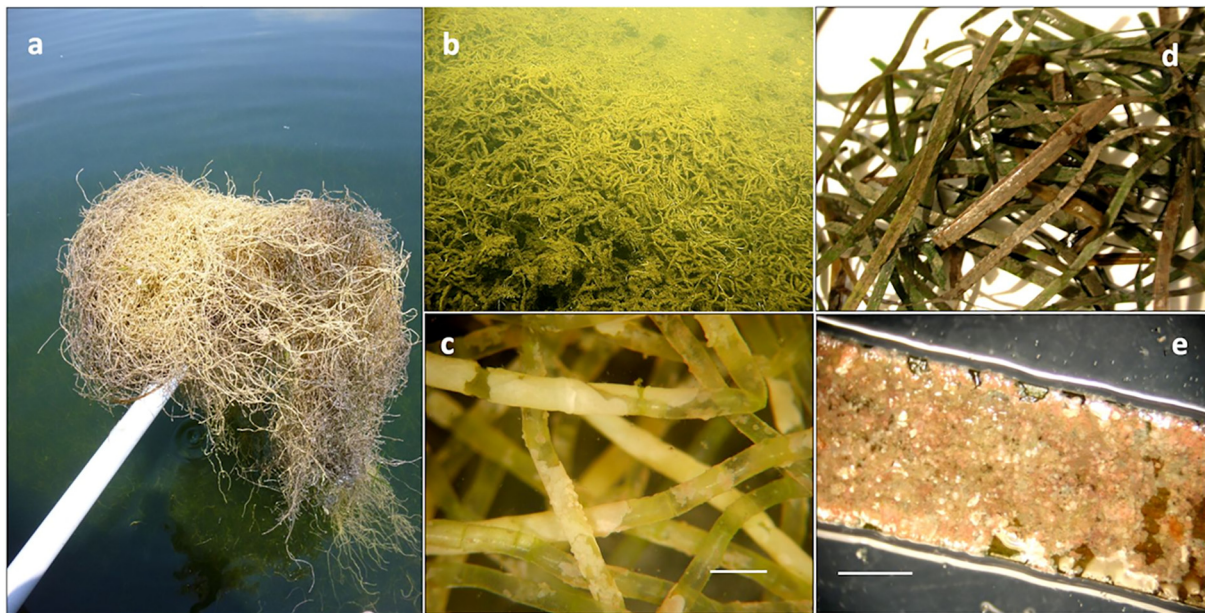
Fig. 4. a) Filaments of *Chaetomorpha linum* completely colonized by MES; b) Sedimentation of died *Chaetomorpha* covered by MES onto the surface sediment; c) Particular of MES crusts on *Chaetomorpha* filaments. Bar = 1 mm; d*) Cymodocea nodo*sa leaves covered by MES; e) Particular of MES crusts in a leaf stretch of *C. nodosa.* Bar = 2 mm.
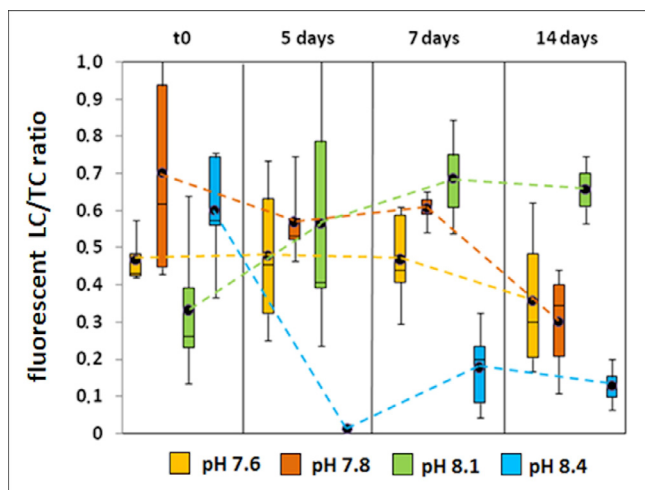

Fig. 5. Boxplot of the fluorescent fraction of MES on the total cover representing the fraction of vital unbleached MES at t_0, 5 days, 7 days and 14 days. The experimental design was carried out at pH 7.6, 7.8, 8.1 and 8.4. Every box represents 5 replicates, with the black circles representing the mean values. The dash lines highlight the LC/TC trends.

At time 0, MES were undamaged, well structured, with chloroplasts and vital cells visible inside them (Fig. 6: a1, a2). After one week of incubation at pH 7.6 (Fig. 6: b1, b2), MES structures appeared disorganized and live cells were no longer visible. Furthermore, the thickness of the calcareous layer was extremely reduced by dissolution of the carbonate crusts. At pH 7.8 (Fig. 6: c1, c2), the carbonate skeleton was intact but no chloroplasts, nor live tissues were visible inside, and a deeply dyed biomass, probably composed of negatively charged exopolysaccharides, was found enveloping the MES cells. At pH 8.1 (Fig. 6: d1, d2), the cells were viable with thick cell wall. Conversely, at pH 8.4 (Fig. 6: e1, e2), the carbonate structure was well organized, but vital cells disappeared almost completely. This was confirmed by the fluorescent LC/TC ratio analysis showing that at pH 8.4 MES were present but dead.

3.4. Carbonate precipitation and CO_2 trapping

MES colonize both macroalgae, such as *C. linum*, and aquatic angiosperms (mostly *C. nodosa*) forming dense crusts on their surface. Carbonate analysis of MES collected from *C. linum* and from the surface sediments, on which the macroalgal biomass was found, are reported in Table 3.

Chaetomorpha linum samples massively colonized by MES had an Inorganic Carbon (Cinorg) content of 5.83–7.63%, accounting for a 29.1–38.2% of carbonates (CO_3^{2-}) represented by Mg-Calcite. On average, the amounts of carbon compounds produced per kg of *C. linum* dry weight (dw) were 182, 115, 561 g kg^{-1} dw of Ctot, Corg and $CaCO_3$, respectively. They corresponded to approx. 3 kg m^{-2} of fresh biomass, the mean value recorded per square meter in Dogà fishing valley.

The carbonate analysis showed that MES produced high Mg-Calcite (> 4 mol % $MgCO_3$), a highly unstable compound that quickly loses Mg and is converted into low-Mg Calcite and Calcite, the least soluble form of calcium carbonate ($CaCO_3$) with a small compact structure. In surface sediments, Mg-Calcite was absent, whereas Calcite was very variable based on the pH of surface sediments. In July 2011, the study area was completely colonized by *C. linum* bearing MES and, on the bottom, a dense layer of carbonate fragments with a pH close to 7.8 was observed, allowing the presence of high amounts of Calcite (96%). However, in September macroalgae partially collapsed, lowering pH to 7.3, and the presence of Calcite reduced to only the 2%. Similarly, in June 2014 and 2018, the presence of Calcite was regulated by pH in surface sediments that ranged from 7.4 to 7.8. In the sediment sampled in 2018, a high amount of Aragonite, a more compact rhombic phase of calcium carbonate, was also recorded. However, Aragonite originates mainly from gastropod shells (*Bittium scabrum* (Olivi, 1792), *Gibbula adriatica* (Philippi, 1844), *Ceritium vulgatum* (Bruguière, 1792)) living inside the angiosperm prairies and the mats of *Chaetomorpha*. Aragonite also tends to dissolve at low pH values; in fact, in the canals of the historical center of Venice, where pH is close to 6, no shells of gastropods or bivalves are present. Eventually, Dolomite ($MgCa(CO_3)_2$), a calcium-magnesium carbonate, was recorded in the sediments ranging from 12 to 40% of the carbonate fraction. Dolomite probably originated from the erosion of the Dolomites, the mountains of the Veneto Region,
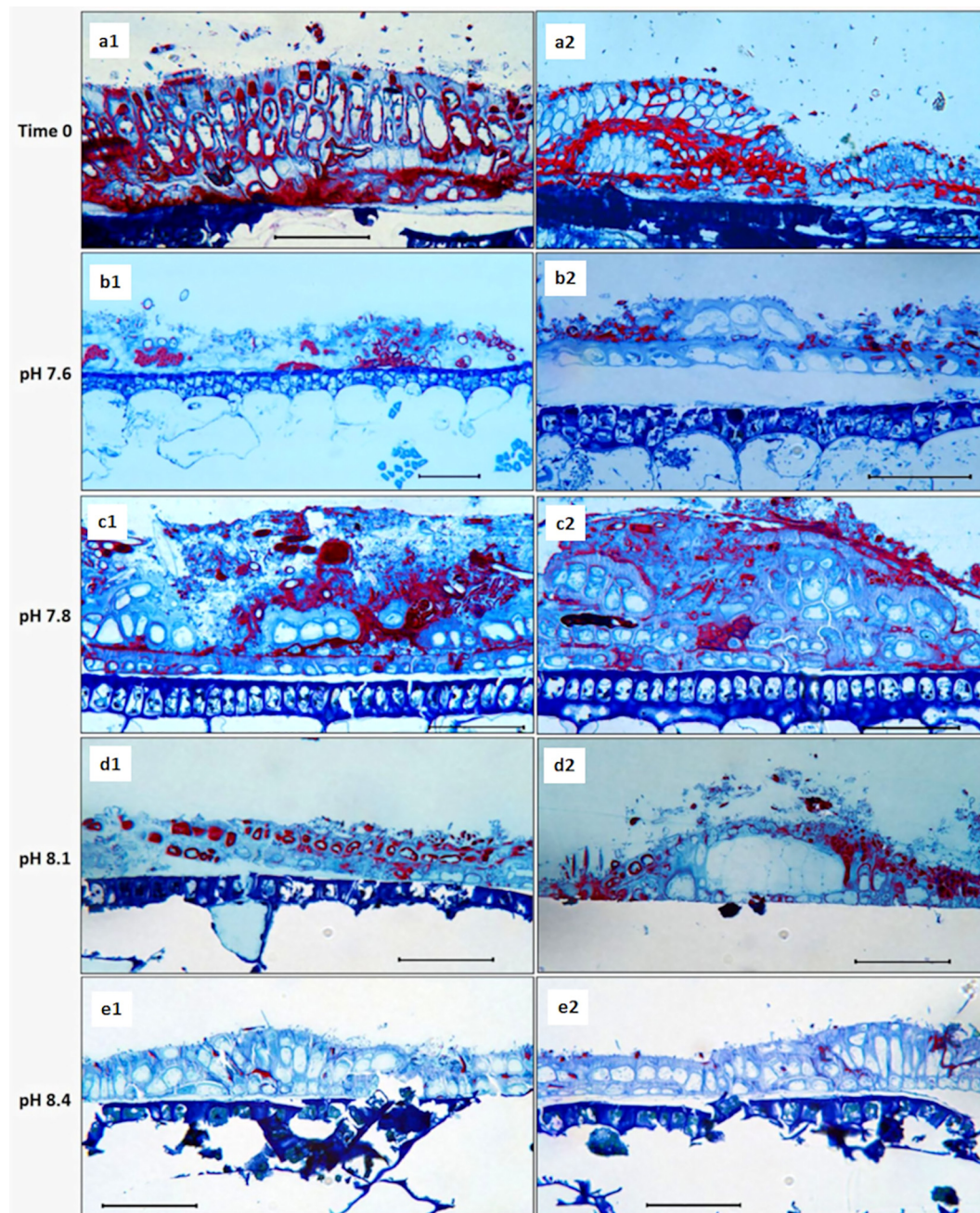
Fig. 6. Cross sections of MES on *C. nodosa* at the time 0 (a1, a2) and after 1 week at pH 7.6 (b1, b2), pH 7.8 (c1, c2), pH 8.1 (d1, d2) and pH 8.4 (e1, e2). In red the details of MES with the highest toluidine blue color density were highlighted. The size bars represent 50 μm.

and was transported by rivers, such as the Piave, which in the past flowed in the lagoon close to this area (before its diversion to the sea).

The amounts of inorganic carbon and carbonates trapped by MES on the leaves of aquatic angiosperms were also estimated on a prairie of *C. nodosa,* the species more affected by these epiphytes, in the area of Santa Maria del Mare (SMM) near the Malamocco sea inlet (Table 4). MES colonized the leaves of this species from July to November, when they reached the maximum cover; then *C. nodosa* completely lost its leaves up to the following late spring. Taking into account the number of leaves per square meter and the amount of old leaves completely covered by crusts of MES, it was calculated that the leaves fallen on surface sediment accounted for an equivalent leaf area surface (two leaf faces) of approx. 27.5 m^2.

MES scraped from the old leaves collected in October and November showed a dw of 1.18–1.55 mg cm^{-2}, which trapped 0.05–0.08 mg cm^{-2} yr^{-1} of Cinorg, accounting for 0.46–0.69 mg cm^{-2} yr^{-1} of CaCO$_3$. On average, the amount of carbon compounds that settled on surface sediments were 52.5, 33.5 and 158 g m^{-2} yr^{-1} of Ctot, Corg and CaCO$_3$, respectively (Table 4).

The estimation of the mean amount of carbonates trapped in surface sediments as Calcite by *C. linum* and *C. nodosa* is reported in Table 5, where the permanently and labile carbonates and CO$_2$ trapping are reported.

MES highest efficiency to permanently accumulate carbonates in surface sediments was shown by *C. linum* with 561 g dw m^{-2} yr^{-1}, respect to the 158 g dw m^{-2} yr^{-1} accumulated by *C. nodosa* (ratio 3.5) (Table 5). This can be attributed to the filamentous shape and higher specific surface of *C. linum* (Fig. 4a, b, c), which accounted for about 0.208 and 0.059 mm m^{-2} yr^{-1}, respectively. The amounts of CO$_2$ permanently trapped by MES in oxidized surface sediments, was

Table 3
Carbon and Carbonate concentrations in the MES on *Chaetomorpha linum* and surface sediments.

			Carbon				Carbonates							Amorphic substance
		Date	Ctot	Cinorg	Corg	Cinorg/Ctot	CO$_3^{2-}$	CaCO$_3$	Calcite	Mg-Calcite	Aragonite	Dolomite	Total	Total
			%					%						%
Dogà Fishing Valley	*Chaetomorpha* with MES	11/06/2014	18.0	5.83	12.2	32.4	29.1	48.6	–	37	–	–	37	54
		11/06/2014 bis	18.4	7.63	10.8	41.4	38.2	63.6	–	40	–	–	40	56
		mean	18.2	6.73	11.5	37.0	33.6	56.1						55
	Sediment	04/07/2011	7.86	4.03	3.8	51.3	20.1	33.6	96	–	–	–	96	–
		08/09/2011	7.26	4.02	3.2	55.4	20.1	33.5	2	–	3	35	40	–
		11/06/2014	8.81	6.83	2.0	77.5	34.1	56.9	19	–	–	40	59	–
		28/06/2018	8.60	4.72	3.9	54.9	23.6	39.3	3	–	54	12	69	–
		mean	8.13	4.90	3.23	60.3	24.5	40.8						–
		g kg^{-1} dw of *Chaetomorpha*												
		mean	182	67	115	370	336	561						

Table 4
Carbon and Carbonate concentrations in the MES on *Cymodocea nodosa* leaves.

MES in *C. nodosa* leaves

Station	Seagrass	Date	MES cm^{-2}		Ctot	Cinorg	Corg	Cinorg/ Ctot	CO$_3^{2-}$	CaCO$_3$	Ctot	Cinorg	Corg	CO$_3^{2-}$	CaCO$_3$
			mg fw	mg dw	%						mg cm^{-2} yr^{-1}				
SMM	*C. nodosa*	20-ott-19	5.30	1.18	14.0	4.64	9.39	33.1	23.2	38.7	0.17	0.05	0.11	0.27	0.46
SMM	*C. nodosa*	18-nov-19	4.98	1.55	13.9	5.37	8.57	38.5	26.9	44.8	0.22	0.08	0.13	0.42	0.69
		mean	5.14	1.37	14.0	5.01	8.98	35.8	25.0	41.7	0.19	0.07	0.12	0.35	0.58
		std	0.23	0.26	0.06	0.52	0.58	3.85	2.58	4.30	0.04	0.02	0.02	0.10	0.17
										g m^{-2} leaves yr^{-1}					
										mean	1.91	0.69	1.22	3.45	5.75
										std	0.35	0.20	0.15	1.00	1.66
										m^2 m^{-2} of leaves	27.5				
										g m^{-2} sediment yr^{-1}					
										mean	52.5	19.0	33.5	94.9	158

estimated to be approx. 0.70 and 2.47 tonnes ha^{-1} yr^{-1}, respectively. The labile Organic Matter (OM) deposition in surface sediments by MES accounted for an additional 0.058–0.198 mm m^{-2} yr^{-1} and 1.23–4.21 tonnes ha^{-1} yr^{-1}, but this deposition is usually only temporary and a large part of the loosely fixed carbon returns into the atmosphere.

4. Discussion

The microcalcareous epiphytic seaweeds (MES) that colonize thalli of bigger macroalgae and leaves of aquatic angiosperms are excellent bioindicators of the trophic status in transitional water systems (TWS). Indeed, they are more sensitive to environmental changes than aquatic angiosperms, being able to appear or disappear in few months quickly responding to changes of the ecological status. This is due to the small size (few microns) of their spores and gametes, which are easily carried by tides and currents. Conversely, aquatic angiosperms are not able to re-colonize the environment after their disappearance because of limitations in seed dispersal. In fact, the size of their seeds is greater than that of macroalgal spores and gametes, measuring some millimetres, and thus they sink quickly (Sfriso et al., 2019b). For this reason, the European Union funded the project *LIFE12 NAT/IT/000331 SERESTO* "Habitat 1150* (Coastal Lagoon) recovery by seagrass restoration. A new strategic approach to meet HD & WFD objectives" (web site www.

lifeseresto.eu). The aim of the project was to establish new angiosperm meadows in the northern part of the Venice Lagoon by means of transplantation of small sods or single rhizomes (Sfriso et al., 2019b).

MES are present in all the studied TWS, starting from those with moderate ecological conditions. When bad-poor environments are improved, MES spontaneously re-appear, through spores and gametes produced elsewhere and transported by tides and currents, and quickly re-colonize larger macroalgae, shells and stones present on the bottom. Moreover, long-distance dispersal was already suggested for small-sized taxa, such as *Hydrolithon* spp., *Pneophyllum fragile* and *Melobesia membranacea*, which grow as epiphytes on drifting leaves (Rindi et al., 2019). The presence of these MES was favoured by low nutrient concentrations, clear waters and high pH values in the water column. The optimal pH range for the viability of these macroalgae was between 7.9 and 8.3. At medium-high pH values (pH > 8.30–8.35) all different species were found to be present in field forming thick calcareous crusts that covered the substrates. However, critical conditions developed for slightly higher pH values. Already after one week, the experimental results indicated that at pH 8.4 there was a severe impairment of cell viability, with a visible crust bleaching. This could be due to the inhibition, at high pH values (8.4–9.3), of the Carbonic Anhydrase, the enzyme responsible for HCO$_3^-$ utilization via CO$_2$ conversion. Carbonic Anhydrase inhibition was reported to severely decrease photosynthetic rates in many macroalgal species (Middelboe et al., 2007; Semesi et al.,

Table 5
Permanent and labile $CaCO_3$ and organic matter deposition, CO_2 trapping and sediment thickness increase. In bold the most significant results.

MES permanently $CaCO_3$ deposition		
	Cymodocea nodosa	*Chaetomorpha linum*
kg dw m^{-2}	–	1.0
dw/fw	–	0.37
m^2 m^{-2} of leaves yr^{-1}	27.5	–
g dw m^{-2} of leaves yr^{-1}	5.75	–
g dw m^{-2} yr^{-1}	**158**	**561**
Calcite density	2.7	2.7
cm^3 m^{-2} yr^{-1}	59	208
mm m^{-2} yr^{-1} as CaCO$_3$	**0.059**	**0.208**
Ratio	**3.5**	
MES labile Organic matter deposition		
g Corg dw m^{-2} yr^{-1}	**33.5**	**115**
g OM dw m^{-2} yr^{-1}	**58**	**198**
mm m^{-2} yr^{-1} as OM	**0.058**	**0.198**
MES Permanently CO$_2$ trapping		
g m^{-2} yr^{-1}	**70**	**247**
Kg m^{-2} yr^{-1}	0.070	0.247
Kg ha^{-1} yr^{-1}	696	2468
T ha^{-1} yr^{-1}	**0.70**	**2.47**
MES labile CO$_2$ trapping		
g m^{-2} yr^{-1}	**123**	**421**
Kg m^{-2} yr^{-1}	0.123	0.421
Kg ha^{-1} yr^{-1}	1228	4210
T ha^{-1} yr^{-1}	**1.23**	**4.21**

2009; Williamson et al., 2014). Conversely, at pH values lower than 7.8, MES viability was reduced in laboratory conditions and no MES were recorded in the field. Additionally, a steady decrease in the number of MES species was recorded from pH 8.3 to 7.4, as previously reported for crustose coralline algae (Fabricius et al., 2015), with a steep transition of MES presence/absence at pH 7.8. A similar threshold was identified near pH 7.7 by Cox et al. (2015) and Martin et al. (2008) for Mediterranean crustose coralline species. In artificial cultures, a pH decrease was reported to reduce competitivness, reproduction, recruitment and to increase mortality in the germination of MES spores (Cumani et al., 2015; Kroeker et al., 2012; Porzio et al., 2011; Rindi et al., 2019).

When *C. linum* and the oldest angiosperm leaves completely covered with MES die, the calcareous crusts are buried in the bottom in the form of refractory detritus and, under oxidized conditions, progressively form new sediment that raises the bottom level. The vertical accretion rates of sediment due only to MES presence in seagrass meadows is usually poorly considered, while in literature there is information on sediment vertical accretion rates due to seagrasses (especially *Posidonia* sp.). Serrano et al. (2016) and Duarte et al. (2013) reported that seagrasses produced approx. 2.0 mm of new sediment per year, and Saderne et al. (2019) calculated about 1 mm yr^{-1} of vertical accretion rates based only on $CaCO_3$. These estimates for the whole seagrass meadows differ by one order of magnitude from the vertical $CaCO_3$ accretion rates found for MES in this study (0.059–0.208 mm m^{-2} yr^{-1}). However, the contribution of MES only in terms of carbonate production per square meter (158–561 g $CaCO_3$ m^{-2} yr^{-1}) is comparable to that of *Posidonia oceanica* seagrass meadows. Canals and Ballesteros (1997) reported a carbonate production ranging from 60 to 70 g m^{-2} yr^{-1} in *P. oceanica* meadows, with an average production in the Mallorca-Menorca shelf of 100 g $CaCO_3$ m^{-2} yr^{-1} from the north-western Mediterranean Sea. Barron et al. (2006) and Romero (1988) reported similar values (51.4 g and 82–158 g $CaCO_3$ m^{-2} yr^{-1}, respectively) for seagrasses from the same area. MES carbonate production was comparable with values from coral reefs (75 to 250 g m^{-2} yr^{-1}; Stockman et al., 1967), rhodolith beads (210 g $CaCO_3$ m^{-2} yr^{-1}; Canals and Ballesteros, 1997), rocky bottoms dominated by coralligenous algae (30–240 g $CaCO_3$ m^{-2} yr^{-1} to 464 g

$CaCO_3$ m^{-2} yr^{-1}; Wefer, 1980; Canals and Ballesteros, 1997) and with values from the California shelf benthic communities (400 g $CaCO_3$ m^{-2} yr^{-1}; Smith, 1972). Therefore, in TWS characterized by good-high ecological conditions and by the presence of macroalgae and aquatic angiosperms covered by MES, the CO_2 permanently trapped in surface sediments by MES ranges between 0.70 and 2.47 Tonnes ha^{-1} yr^{-1}.

Some sediments, such as those analyzed in June 2018 in the Dogà fishing valley, can contain also high amounts of aragonite, which originates mainly by shells of gastropods living inside the macrophyte beds. These organisms are particularly abundant in good-high environmental conditions and their death significantly contribute to the CO_2 trapping and to carbonate accumulation in sediments, where their shells represent 54% of the sediment matrix.

5. Conclusions

The lines of evidence provided in this study converge to describe MES as a solid indicator of stable water and sediment chemical-physical conditions and of TWS good-high ecological status. In laboratory conditions, the exposure of MES to pH < 7.80 and to pH > 8.35 compromised their viability already after two weeks and this is in agreement with the absence of species at pH lower than 7.93 in the environment. Stable water transparency and reduced trophy were closely related to the appearance and survival of these calcareous organisms. Moreover, the contribution of macroalgae and seagrasses covered by MES to the sediment accretion is very significant, especially considering the permanent contribute in oxidized sediments in areas of good-high ecological value. Therefore, MES presence and viability in aquatic habitats can be used as a practical Litmus Test Paper Strip to evaluate the environmental quality facilitating the ecological assessment of transitional areas and providing the environmental Agencies with rapid information on environmental trends.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106692.

References

Aspila, K.I., Agemian, H., Chau, A.S.J., 1976. A semi-automated method for the determination of inorganic, organic and total phosphorus in sediments. Analyst 101, 187–197. https://doi.org/10.1039/an9760100187.

Barron, C., Duarte, C.M., Frankignoulle, M., Borges, A.V., 2006. Organic Carbon Metabolism and Carbonate Dynamics in a Mediterranean Seagrass (*Posidonia oceanica*) Meadow. Estuar. Coast. 29, 417–426. https://doi.org/10.1007/BF02784990.

Canals, M., Ballesteros, E., 1997. Production of carbonate particles by phytobenthic communities on the Mallorca-Menorca shelf, northwestern Mediterranean Sea. Deep-Sea Res. Pt. II 44, 611–629.

Cox, T.E., Schenone, S., Delille, J., Díaz-Castañeda, V., Alliouane, S., Gattuso, J.-P., Gazeau, F., 2015. Effects of ocean acidification on *Posidonia oceanica* epiphytic community and shoot productivity. J. Ecol. 103, 1594–1609. https://doi.org/10.1111/1365-2745.12477.

Cumani, F., Bradassi, F., DiPascoli, A., Bressan, G., 2015. Marine acidification effects on reproduction and growth rates of Corallinaceae spores (Rhodophyta). Rapp. Comm. Int. Mer Medit. 39, 735.

Duarte, C.M., Losada, I.J., Hendriks, I.E., Mazarrasa, I., Marbà, N., 2013. The role of coastal plant communities for climate change mitigation and adaptation. Nat. Clim. Chang. 3, 961–968. https://doi.org/10.1038/nclimate1970.

Fabricius, K.E., Kluibenschedl, A., Harrington, L., Noonan, S., De'ath, G., 2015. *In situ* changes of tropical crustose coralline algae along carbon dioxide gradients. Sci. Rep. 5, 9537. https://doi.org/10.1038/srep09537.

Hofmann, L.C., Bischof, K., 2014. Ocean acidification effects on calcifying macroalgae. Aquat. Biol. 22, 261–279. https://doi.org/10.3354/ab00581.

ISPRA, 2011. Protocolli per il campionamento e la determinazione degli elementi di qualità biologica e fisico-chimica nell'ambito dei programmi di monitoraggio ex 2000/60/CE delle acque di transizione, in: El-Pr-TW-Protocolli Monitoraggio - 03. 06., ISPRA.

Kroeker, K.J., Micheli, F., Gambi, M.C., 2012. Ocean acidification causes ecosystem shifts via altered competitive interactions. Nat. Clim. Chang. 2, 1–4. https://doi.org/10.1038/nclimate1680.

Lorenzen, C.J., 1967. Determination of chlorophyll in sea water. UNESCO Techn. Papers Marine Sci. 35–120.

Martin, S., Rodolfo-Metalpa, R., Ransome, S., Buia, M.C., Gattuso, J.P., Hall-Spencer, J., 2008. Effects of naturally acidified seawater on seagrass calcareous epibionts. Biol. Lett. 4, 689–692. https://doi.org/10.1098/rsbl.2008.0412.

Mccoy, S.J., 2013. Morphology of the crustose coralline alga *Pseudolithophyllum muricatum* (Corallinales, Rhodophyta) responds to 30 years of ocean acidification in the North East Pacific. J. Phycol. 1837, 830–837. https://doi.org/10.1111/jpy.12095.

Middelboe, A.L., Hansen, P.J., Middelboe, A.L., Hansen, P.E.R.J., 2007. Direct effects of pH and inorganic carbon on macroalgal photosynthesis and growth. Mar. Biol. Res. 3, 134–144. https://doi.org/10.1080/17451000701320556.

Morand, P., Briand, X., 1996. Excessive growth of macroalgae. A symptom of environmental disturbance. Bot. Mar. 39, 491–516. https://doi.org/10.1515/botm.1996.39.1-6.491.

Munari, C., Mistri, M., 2012. Ecological status assessment and response of benthic communities to environmental variability: the Valli di Comacchio (Italy) as a study case. Mar. Environ. Res. 81, 53–61. https://doi.org/10.1016/j.marenvres.2012.08.008.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. in: Page, A.L. (ed.) Methods" of Soil Analysis, Part 2. Agronomy No. 9, Mono- graph Series, American Society of Agronomy, Madison, Wis., pp. 539-79.

O'Brien, T.P., McCully, M.E., 1981. The Study of Plant Structure. Termarcarphi, Melbourne.

Orfanidis, S., Panayotidis, P., Stamatis, N., 2003. An insight to the ecological evaluation index (EEI). Ecol. Indic. 3, 27–33. https://doi.org/10.1016/S1470-160X(03)00008-6.

Orfanidis, S., Panayotidis, P., Ugland, K.I., 2011. Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values. Mediterr. Mar. Sci. 12 (1), 199–231. https://doi.org/10.12681/mms.60.

Oxner, M., 1962. The Determination of Chlorinity by the Knudsen Method and Hydrographical Tables. Manufacturing Co, New York, G.M, pp. 63.

Porzio, L., Buia, M.C., Hall-Spencer, Jason M., 2011. Effects of ocean acidification on macroalgal communities. J. Exp. Mar. Biol. Ecol. 400, 278–287. http://hdl.handle.net/10026.1/1331.

Rindi, F., Braga, J.C., Martin, S., Peña, V., Gall, L.L., Caragnano, A., Aguirre, J., 2019. Coralline Algae in a Changing Mediterranean Sea: how can we predict their future, if we do not know their present? Front. Mar. Sci. 6, 723. https://doi.org/10.3389/fmars.2019.00723.

Romero, J., 1988. Epifitos de las hojas de Posidonia oceanic: variaciones estacionales y batimetricas de biomasa en la pradera de las islas Medes (Girona). Oecologia Aquatica 9, 19–25.

Saderne, V., Geraldi, N.R., Macreadie, P.I., Maher, D.T., Middelburg, J.J., Serrano, O., Almahasheer, H., Arias-Ortiz, A., Cusack, M., Eyre, B.D., Fourqurean, J.W., Kennedy, H., Krause-Jensen, D., Kuwae, T., Lavery, P.S., Lovelock, C.E., Marba, N., Masqué, P., Mateo, M.A., Mazarrasa, I., McGlathery, K.J., Oreska, M.P.J., Sanders, C.J., Santos, I.R., Smoak, J.M., Tanaya, T., Watanabe, K., Duarte, C.M., 2019. Role of carbonate burial in Blue Carbon budgets. Nat. Commun. 10, 1106. https://doi.org/10.1038/s41467-019-08842-6.

Schumacher, B.A., 2002. Method for the determination of total organic carbon (TOC) in soil and sediment. Ecological Risk Assessment Support Center, Office of Research and Development. US Environmental Protection Agency. Las Vegas, NV, pp. 23.

Semesi, I.S., Beer, S., Björk, M., 2009. Seagrass photosynthesis controls rates of calcification and photosynthesis of calcareous macroalgae in a tropical seagrass meadow. Mar. Ecol. Prog.

Serrano, O., Ricart, A.M., Lavery, P.S., Mateo, M., Arias-Ortiz, A., Masque, P., Rozaimi, M., Steven, A.D., Duarte, C., 2016. Key biogeochemical factors affecting soil carbon storage in Posidonia meadows. Biogeosciences 13, 4581–4594. https://doi.org/10.5194/bg-13-4581-2016.

Sfriso, A., Marcomini, A., Pavoni, B., 1987. Relationship between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice lagoon. Mar. Environ. Res. 22, 297–312. https://doi.org/10.1016/0141-1136(87)90005-5.

Sfriso, A., Facca, C., Ceoldo, S., Silvestri, S., Ghetti, P.F., 2003. Role of macroalgal biomass and clam fishing on spatial and temporal changes in N and P sedimentary pools in the central part of the Venice lagoon. Oceanol. Acta 26 (1), 3–13.

Sfriso, A., Facca, C., Marcomini, A., 2005. Sedimentation rates and erosion processes in the lagoon of Venice. Environ. Int. 31 (7), 983–992.

Sfriso, A., Facca, C., Ghetti, P.F., 2007. Rapid Quality Index (R-MaQI), based mainly on macrophyte associations, to assess the ecological status of Mediterranean transitional environments. Chem. Ecol. 23 (6), 1–11. https://doi.org/10.1080/02757540701702918.

Sfriso, A., Facca, C., Ghetti, P.F., 2009. Validation of the Macrophyte Quality Index (MaQI) set up to assess the ecological status of Italian marine transitional environments. Hydrobiologia 617, 117–141.

Sfriso, A., Ghetti, P.F., 1998. Seasonal variation in the biomass, morphometric parameters and production of rhizophytes in the lagoon of Venice. Aquat. Bot. 61, 207–223.

Sfriso, A., Facca, C., Bonometto, A., Boscolo, R., 2014. Compliance of the Macrophyte Quality index (MaQI) with the WFD (2000/60/EC) and ecological status assessment in transitional areas: the Venice Lagoon as study case. Ecol. Indic. 46, 536–547. https://doi.org/10.1016/j.ecolind.2014.07.012.

Sfriso, A., Buosi, A., Facca, C., Sfriso, A.A., 2017. Role of environmental factors in affecting macrophyte dominance in transitional environments: the Italian Lagoons as a study case. Mar. Ecol. 38 (2), e12414. https://doi.org/10.1111/maec.12414.

Sfriso, A., Buosi, A., Mistri, M., Munari, C., Franzoi, P., Sfriso, A.A., 2019a. Long-term changes of the trophic status in transitional ecosystems of the northern Adriatic Sea, key parameters and future expectations: The lagoon of Venice as a study case. In: Mazzocchi, M.G., Capotondi, L., Freppaz, M., Lugliè, A, Campanaro, A. (Eds.) Italian Long-Term Ecological Research for understanding ecosystem diversity and functioning. Case studies from aquatic, terrestrial and transitional domains. Nature Conservation 34: 193-215. DOI:10.3897/natureconservation.34.30473.

Sfriso, A., Buosi, A., Tomio, Y., Juhmani, A.S., Facca, C., Sfriso, A.A., Franzoi, P., Scapin, L., Bonometto, A., Ponis, E., Rampazzo, F., Berto, D., Gion, C., Oselladore, F., Cacciatore, F., Boscolo Brusà, R., 2019b. Aquatic angiosperm transplantations: a tool for environmental management and restoring in transitional water systems. Water 11, 2135. https://doi.org/10.3390/w11102135.

Smith, S.V., 1972. Production of calcium carbonate on the mainland shelf of southern California. Limnol. Oceanogr. 17, 28–41.

Sorokin, Y.I., Zakuskina, O.Y., 2010. Features of the Comacchio ecosystem transformed during persistent bloom of picocyanobacteria. J. Oceanogr. 66, 373–387. https://doi.org/10.1007/s10872-010-0033-9.

Stockman, K.W., Ginsburg, R.N., Shinn, E.A., 1967. The production of lime mud by algae in south Florida. J. Sediment. Petrol. 41, 798–808.

Strickland, J.D.H., Parsons, T.R., 1984. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can. 167, 310.

Viaroli, P., Bartoli, M., Giordani, G., Naldi, M., Orfanidis, S., Zaldívar, J.M., 2008. Community shifts, alternative stable states, biogeochemical controls and feed-backs in eutrophic coastal lagoons: a brief overview. Aquat. Conserv. 18, S105–S117. https://doi.org/10.1002/aqc.956.

Wefer, G., 1980. Carbonate production by algae *Halimeda, Penicillus,* and *Padina.* Nature 285, 323–324.

Williamson, C.J., Brodie, J., Museum, N.H., Goss, B., Yallop, M., 2014. *Corallina* and *Ellisolandia* (Corallinales, Rhodophyta) photophysiology over daylight tidal emersion: interactions with irradiance, temperature and carbonate chemistry. Mar. Biol. 161, 2051–2068. https://doi.org/10.1007/s00227-014-2485-8.