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School of the Environment

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MSc Integrated Coastal Management

MSc Thesis

The effect of grazing on macrophytic communities of shallow rocky reefs in Lesvos

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Ευχαριστίες

Εν αρχή ην ο επιβλέπων! Θα ήθελα να ευχαριστήσω θερμά τον επιβλέποντα της διπλωματικής μου εργασίας, Αναπληρωτή καθηγητή κ. Στέλιο Κατσανεβάκη, για την ανάθεση του θέματος, την εμπιστοσύνη που έδειξε στο προσωπό μου, τόσο για την διαχείριση του θέματος όσο και του καταδυτικού εξοπλισμού και τις συνεχείς εποικοδομητικές συμβουλές που μου έδεινε. Ο χώρος που μου αφήσατε ώστε να εκφράσω τις απορίες μου και να καταθέσω τις σκέψεις μου, μου έδωσε αυτοπεποίθηση για την εξέλιξη της διπλωματικής αλλά και του εαυτού μου.

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Abstract

Intense grazing activity on macrobenthic communities is considered a driver, that can result to altered ecosystem functioning. Along the Mediterranean coasts, algal forests with complex structure are considered key habitats that form a fixed three-dimensional structure with high biodiversity and productivity. Overgrazing (i.e. the phenomenon where grazing rate prevails over algal growth rate) poses a serious threat and can lead to the reduction of habitat structure complexity and composition and the creation of rocky barren areas.

A herbivore exclusion experiment was conducted at three stations along the southeastern part of Lesvos Island, in order to assess the potential grazing effects of sea urchin and fish herbivory on rocky substrate habitats. During the seven-month fieldwork (April to October 2017), PP-R frame cages (40×60×25cm) were installed on horizontal or slightly sloped surfaces and a plastic mesh was used for covering the cages (2×2cm opening). The selected stations had rocky substrate. At each site three replicates of two different treatment types and a control were placed between 1-5m depth. The two different treatment cages were: (1) fully-closed cages, which excluded both herbivorous fish and sea urchins from grazing activity and (2) open-top cages, which excluded only sea urchins. Moreover, no-cage surfaces with distinct markings, served as controls, where no restrictions were applied to herbivores. Monitoring with photoquadrats was conductedevery two weeks, in order to monitor the possible algal growth on the quadrats

The results of the monitoring revealed that algal growth was higher inside both types of cages in contrast to the respective control surfaces. No significant differences were found between the two cage types in terms of total algal biomass. This suggests that herbivore fish grazing had minor effect compared to sea urchin grazing, which was found to be the most important grazing factor. Grazing activity has a strong impact on macroalgal communities, almost depleting canopy and erect algae, leading to turf dominated habitats that ultimately alter the composition to less complex and flattened in structure algal communities.

Περίληψη

Η εντατική βόσκηση στις μακροβενθικές βοικοινωνίες θεωρείται στρεσσογόνος παράγοντας που τελικά οδηγεί στην διαφοροποιήση της φυσιολογικής λειτουργίας ενός οικοσυστήματος. Κατά μήκος των ακτών της Μεσογείου, τα δάση μακροφυκών που είναι σύνθετα δομημένα, θεωρούνται ως ενδιαιτήματα υψηλής σημασίας, τα οποία διαθέτουν τρισδιάστατη δομή σταθερού υποστρώματος και χαρακτηρίζονται από υψηλή βιοποικιλότητα. Η υπερβόσκηση (το φαινόμενο όπου ο ρυθμός βόσκησης υπερτερεί του ρυθμού ανάπτυξης φυκών), αποτελεί σοβαρή απειλή και μπορεί να οδηγήσει στην μείωση της δομικής ποικιλότητας και της βιοποικιλότητας ενός οικοσυστήματος, με την δημιουργία γυμνών βραχωδών περιοχών.

Ένα πείραμα αποκλεισμού βοσκητών διεξήχθη σε τρεις σταθμούς στο νοτιοανατολικό τμήμα της νήσου Λέσβου, προκειμένου να εκτιμηθούν οι δυνητικές επιπτώσεις της βόσκησης των αχινών και των φυτοφάγων ψαριών, σε ενδιαιτήματα σκληρού υποστρώματος. Κατά την επτάμηνη παρακολούθηση (Απρίλιος-Οκτώβριος 2017), τοποθετήθηκαν κλουβιά υλικού PP-R (διαστάσεων 40×60×25cm) σε οριζόντιες ή ελαφρώς κεκλιμένες επιφάνειες και χρησιμοποιήθηκε πλαστικό πλέγμα για την κάλυψή τους (διαστάσεις ανοίγματος 2×2cm). Οι επιλεγμένοι σταθμοί, διέθεταν βραχώδες υπόστρωμα και σε κάθε σταθμό εφαρμόστηκαν τρεις επαναλήψεις, δύο διαφορετικών πειραματικών κλουβιών και μίας απλά οριοθετημένης επιφάνειας σύγκρισης, σε βάθος 1-5m. Οι δύο διαφορετικοί κλωβοί ήταν: (1) πλήρως κλειστά κλουβιά, όπου απέκλειαν τόσο τα φυτοφάγα ψάρια, όσο και τους αχινούς (2) ανοιχτής οροφής κλουβιά, όπου απέκλειαν μόνο τους αχινούς. Τέλος, επιφάνειες χωρίς κλουβί, με διακριτά σημάδια, λειτούργησαν ως συγκριτικές επιφάνειες ελέγχου (control). Ανά δύο εβδομάδες πραγματοποιούνταν φωτογραφική απεικόνιση, για την παρακολούθηση της πιθανής ανάπτυξηςτων μακροφυκών στις πειραματικές επιφάνειες.

Τα αποτελέσματα της μελέτης αποκάλυψαν ότι ηανάπτυξητων μακροφυκών ήταν υψηλότερη εντός και των δύο τύπων κλωβών συγκριτικά με τις αντίστοιχες επιφάνειες ελέγχου. Δεν διαπιστώθηκαν σημαντικές διαφορές ανάμεσα στους διαφορετικούς τύπους κλουβιών, όσον αφορά τη συνολική βιομάζα, γεγονός που υποδηλώνει πως τα φυτοφάγα ψάρια έχουν χαμηλότερη επίδραση σε σύγκριση με τους αχινούς που αποτέλεσαν τον σημαντικότερο παράγοντα βοσκητικής πίεσης. Η υπερβόσκηση προκαλεί έντονες επιπτώσεις στις μακροβενθικές κοινωνίες, σχεδόν εξαφανίζοντας τα τρισδιάσταταδάση μακροφυκών. Με αυτό τον τρόπο, το ενδιάιτημα οδηγείται σε ένα καθεστώς όπου κυριαρχούν εφήμερα φύκη περιορισμένης ανάπτυξης (turf), που τελικά δημιουργούν φυκοκοινωνίες με χαμηλότερη δομική ποικολότητα.

Contents

1. Introduction

Certain factors, such as climate change, habitat fragmentation and loss of biodiversity can influence the function of an ecosystem (Perkol-Finkel & Airoldi, 2010). These factors though, are not always stable and the state of an ecosystem can be altered, because of certain environmental drivers, such as hydrodynamic forces (i.e. currents, storms, waves, tides) (Perkol-Finkel & Airoldi, 2010). Cumulative stressors (pollution, invasion, nutrient inputs, etc.) together with the loss of keystone predators (Paine, 1966), have severely modified the food web. These changes are obvious in the top-down interactions between (1) predators and herbivores and (2) herbivores and macroalgae, resulting to the increase of grazing activity and the alteration of the ecological status in a marine ecosystem.

Intense grazing activity on macrobenthic communities is considered a stressor that ultimately results to the alteration of the ecosystem's functioning (Eklöf *et al.*, 2008). This type of pressure is a serious threat for rocky habitats. After the loss of keystone predators, herbivore population increases and overgrazing occurs (i.e. the phenomenon where grazing rate prevails over algal growth rate)(Eklöf *et al.*, 2008). This pattern is common in temperate coastal areas and leads to simplified food webs, with loss of habitat for many macrobenthic organisms since algal forests that provide a more stable habitat and biogenic structure, are degraded. Studies have shown that both sea urchins (Lawrence, 1975; Carpenter, 1984; Scheffer *et al.*, 2001) as well as herbivorous fish (Zenetos *et al.*, 2012) are responsible for these ecosystem alterations, which are commonly described as 'phase-shifts' and ultimately lead to the creation of wide 'barren' rocky areas, which are deprived of erect, canopy formed algae and in their place encrusting, calcareous algae are now dominant (Benedetti-Cecchi *et al.*, 1998;Bulleri *et al.*, 2002; Zenetos *et* al., 2012; Scheffer*et al.*, 2001).

The need for algal forests is of primary importance in shallow sublittoral rocky habitats. Along with other descriptors, algal forests can also characterize an ecosystem (Boudouresque *et al*. 2014). Many biochemical and geophysical processes, such as nutrient cycling, carbon sequestration and complex food-webs, are attributed to the function of algal forests and seagrass meadows (Burnell *et al.*, 2013; Duarte, 2002; Orth *et al.*, 2006). Along the Mediterranean coasts, algal forests with complex structure are considered key habitats (Salomidi *et al.*, 2012). Literature records mention *Cystoseira* species (order Fucales) as the main canopy forming component in the shallow sublittoral rocky Aegean habitats (Montesanto & Panayotidis, 2001). These canopy-forming species are in a dominant state across the Mediterranean rocky reefs (Cheminée et al. 2013), creating a complex tri-dimensional structure and harboring high biodiversity and productivity (Ballesteros, 1992; Ballesteros *et al.*, 1998; Hoffmann*et al.*, 1992). These upright, well-developed formations, though, are very susceptible to grazing from herbivorous native or alien species (Salomidi *et al.*, 2016). *Cystoseira* spp. and their biota are considered sensitive to anthropogenic induced impacts, hence they are characterized as reliable ecological indicators (Ballesteros *et al.*, 2007; Mangialajo *et al.*, 2007). As a result, records show a reduction in algal species richness, biomass and the area coverage across the Mediterranean coasts (Thibaut *et al.*, 2015;Thibaut *et al.*, 2005). According to Perkol-Finkel and Airoldi (2010), anthropogenic effects are also a factor that contributes drastically to the strong decline of algal forests and the shift to ‘barren’ regime platform. Perkol-Finkel and Airoldi (2010) also note that due to the fact that macroalgal forests are under continuous pressure, six Mediterranean species of the genus *Cystoseira* are included in the Bern Convention (Council of Europe, 1998) and the Mediterranean Action Plan lists (also under the framework of the Barcelona Convention), and are thus considered as a priority for conservation and protection. Piazzi *et al.*(2018) suggest an assessment of the ecological quality of *Cystoseira* communities under the European Water Framework Directive and the European Marine Strategy Directive (EC, 2000, 2008).

Herbivore exclusion is a widely applied method for the ecological monitoring of phytobenthos and the effects of herbivorous organisms. Grazer exclusion creates a platform for comparing two different states. The initial state, where the grazing activity is still in progress, and the experimental state, where grazers have no or restricted access to a defined area. Herbivore exclusion experiments have been applied in both marine (Alves *et al.*, 2003; Sala *et al.*, 2011; Thacker *et al.*, 2001) and terrestrial habitats (Young *et al.*, 1998). Not many caging experiments have been carried out in the Aegean Sea and the present study is among few research projects conducted along with Baggini *et al.*(2015), Tsirintanis *et al.*(2018) and Sala *et al.*(2011).

The present study investigated the potential impact of grazing activity from herbivorous fish and sea urchins on phytobenthic communities in shallow sublittoral rocky habitats and gathered information on the mechanisms that shape the structure of macroalgal communities. For this reason, a caging experiment that excluded the herbivorous organisms was conducted to assess the shifts in structure and composition, in area cover and biomass of the photophilous macroalgal communities through the experimental time.

2. Materials and Methods

2.1 Study area

The study area is located in the SE part of Lesvos Island (NE Aegean Sea, Mediterranean Sea (Fig. 1). The location of the sampling stations and the sampling period for the caging experiment are summarized in Table 1.

Table 1: Sampling areas, station code name, geographic coordinates of stations and sampling period

Sampling areas	Code name	Coordinates	Sampling period
Charamida beach	CHA	39.0138778˚ N, 26.5571500˚ E	4/2017- 10/2017
Agios Ermogenis beach	ER	39.0133861˚ N, 26.5430611˚ E	
Gera	GE	39.0631139˚ N, 26.5276500˚ E	

The coasts of Charamida and Ermogenis face south, to the open sea and are exposed to south, south-east winds. In both sites, there are extended and dense *Posidonia oceanica* meadows.

Gera station, is situated in the gulf of Gera, which is a semi-enclosed gulf, with a mean depth of about 10 m (max. 15-17 m at the center of the gulf). The gulf is characterized by intense seasonal variability, with different physical and chemical characteristics, compared to the open Aegean Sea. Specifically in the winter, the gulfs' whole water column temperature ranges between 9-11 ^{o}C and surface salinity is lower than deeper water layers. Vice versa, in the summer the water column is characterized by a warmer and saltier pattern and stratification is noticeable (Kolovoyiannis & Tsirtsis, 2005).

All three stations consist of rocky reefs and Gera station additionally has a soft-bottom substrate. The caging experiment was launched at the rocky part of the stations.

Fig. 1: Map of the sampling stations for the caging experiment in Lesvos Island at the NE Aegean Sea.

2.2 Experimental design

For the purpose of the experiment, it was decided to apply two different treatments:

- A fully-closed cage, which excluded both herbivorous fish and sea urchins from grazing activity, and
- An open-top cage mode, which excluded sea urchins only. Fish grazing activity was possible in this type of treatment.

Also, no-cage surfaces were predefined, with noticeable markings, to serve as controls. These surfaces were not covered by any type of cage and were used as indicators of the current state of grazing activity at each studying area. For each of the two treatments and for the control, three replicates were placed at each station.

The constructing material for the cages was Polypropylene Random type (PP-R) (40×60×25 cm) and a plastic mesh was used for covering the cages (2×2 cm opening). The cages were secured with thick, plastic tire ups on climbing hangers, at horizontal or gently sloping rocky surfaces at depths between 1-5 m. The positioning of the cages was predetermined in a previous experimental setup (Tsirintanis *et al*., 2018).

Regular site monitoring took place during the experimental period. Cleaning and maintaining the cages was essential, to improve field errors, fix mechanical damages and reduce potential biases (by mesh colonization from marine organisms). Isolated incidents of misplaced cages have occurred due to intense hydrodynamic conditions, such as waves, currents or sporadic storms. In that case, the cages were replaced as soon as weather conditions were favorable.

2.3 Photosampling

During the seven-month experimental period (April to October), sampling took place twice per month in order to monitor the experimental surfaces. Photographic samples of all experimental and control surfaces were taken, with a Canon PowerShot S110 compact digital camera (35 mm lens, 4000×2248 resolution), by placing a PVC frame (40×60 cm) at the base of each cage. For the control samples, the frame was placed between the corresponding markings.

Due to the geometric distortion that was observed, the photographic samples had to be pre-processed via the Camera Calibration Toolbox for Matlab (Bouguet, 2015) to be corrected prior to any type of analysis. The calibration process that was followed is described in detail by Wehkamp & Fischer (2014) and was also used by Tsirintanis *et al.* (2018) (photographic proof on the Supplementary material).

The resulting undistorted photosamples, were analyzed via the photoQuad software (Trygonis & Sini, 2012), using 100 stratified random points per image sample. Each point was assigned to a set of predetermined benthic categories (Table 2), to determine the percentage coverage of algal species and other benthic groups. Algal juvenile stages of low coverage were considered as turf in the processing. Chrysophyte mucillagenous algae were also reported, thought due to their seasonal, short circled pattern were not taken into consideration to the statistical analysis.

Table 2: The benthic categories used for the analysis of photosamples. Modified from Littler *et al.* (1983),Orfanidis *et al.* (2001), Salomidi *et al.*(2012) and Tsirintanis *et al.* (2018).

Benthic categories	Description	Species examples
Bare rock	Barren rocky areas, with zero algal or animal cover	
Animals	Benthic invertebrates	*Reptadeonella violacea*
Erect algae (Canopy form)[1]	Upright well-developed thalli, with thick blades and branches. Large sized perennial, slow growing algae.	*Cystoseria, Sargassum spp.*
Erect algae (Bushy form)[1]	Upright well-developed thalli, with coarse branches, moderate-height. Fleshy medium sized, typically occupy the open space between and underneath the canopy.	*Padina, Dictyota spp.*

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[1] Canopy and Bushy forming algal species were pooled to one benthic category (‘Erect algae’), due to weak growth of the former in all three sampling stations

Turf algae	Consisting of minute, typically seasonal and fast growing species. Forming algal carpets.	Various low-lying opportunistic species and juvenile macroalgal stages. *Sphacelaria, Cladophora sp.* Cyanophyte, Chrysophyte mats.
Encrusting calcareous algae	Prostrate development, with calcified and stony texture and low-lying height, forming flat epilithic crusts.	*Lithophyllum, Mesophyllum sp.*

2.4 Data analysis

Algal coverage was converted to biomass, with conversion factors from coverage measurements provided by Ballesteros (1992) and Tsirintanis *et al.*(2018). There were no reported conversion factors for a specific alga, *Halimeda tuna*, a calcified chlorophyte, so a sample was collected from Gera (species only reported at that site) site with scraping, using a PVC frame (20×20 cm), dried at 120^{o}C for 24 hours and then the dry weight was measured in a high precision scale (0.01 g precision), in order to convert the dry mass into a biomass value. All reported biomasses, refer to dry mass.

A two-way ANOVA was conducted, to investigate potential effect of site, treatment, as well as the combined effect of site×treatment in biomass values of each algal category (individually and combined). A Tukey and LSD post-hoc tests were carried out to determine the homogenous groups between the different factor levels. The statistical analysis was undertaken using IBM SPPS Statistics 23 software package.

3. Results

3.1 Algal biomass

In terms of mean biomass (all treatments pooled) in the start of the experiment, encrusting algae dominated in Charamida station with 32.7 ± 8.4 gm^{-2} (mean value ± SE), followed by Gera with 17.2 ± 7.9 gm^{-2} and Ermogenis with 16.2 ± 7.9 gm^{-2} (Fig. 2a). The second group was turf algae with values of 30.5 ± 7.0 gm^{-2} in Charamida station, followed by Ermogenis with 24.2 ± 6.5 gm^{-2}, and Gera with 22.1 ± 6.5 gm^{-2}. Erect algae had the lowest biomass values of all algal groups, being highest in Gera (13.6 ± 4.8 gm^{-2}), followed by Charamida (9.305 ± 5.1 gm^{-2}), and Ermogenis (6.8 ± 4.8 gm^{-2}). During the peak of algal growth, turf algae dominated in Gera with 49.4 ± 5.5 gm^{-2}, followed by Charamida with 36.3 ± 5.9 gm^{-2} and Ermogenis with 41.7 ± 5.5 gm^{-2}. The second group was encrusting algae with values of 45.6 ± 9.8 gm^{-2} in Charamida, followed by Gera with 31.3 ± 9.2 gm^{-2} and Ermogenis 18.4 ± 9.2 gm^{-2}. Erect algae had the lowest biomass values, being highest in Gera (32.9 ± 7.9 gm^{-2}), followed by Charamida (28.0 ± 8.5 gm^{-2}), and Ermogenis (22.7 ± 7.9 gm^{-2}) (Fig. 2b).

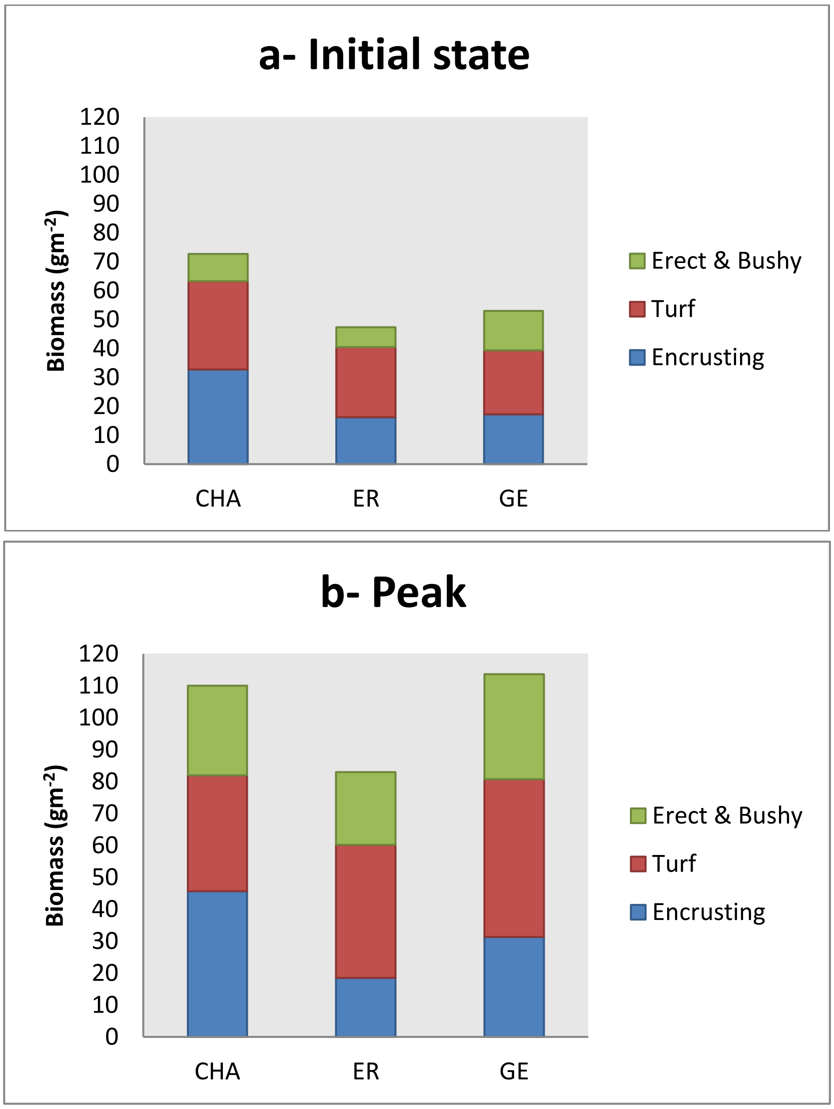
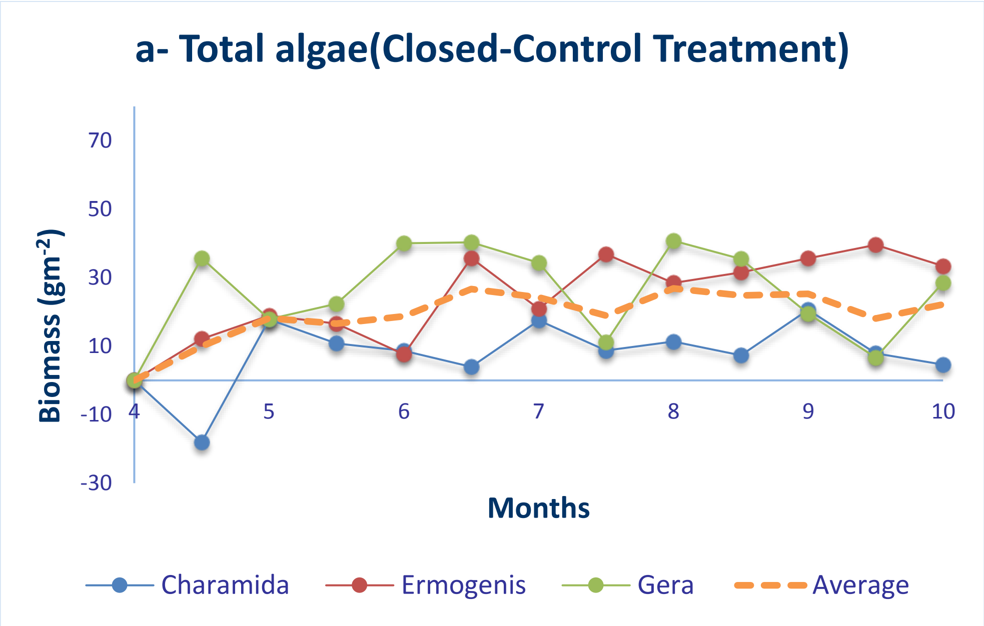
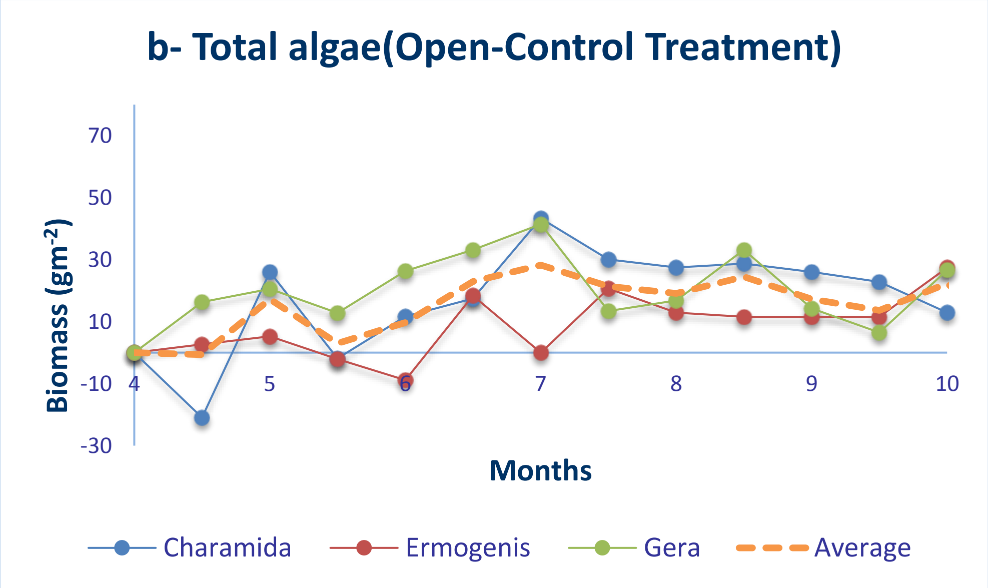


Fig. 2: Mean values of biomass on the three main algal categories per sampling stations at the start of the experiment (April-a) and during the peak of algal growth (June-b).

When examining the average algal biomass difference between closed-control (Fig. 3a) and open-control (Fig. 3b) treatments, the difference in the closed cages from the controls showed a peak in the middle of June at 26.6 gm^{-2} which was followed by small fluctuations until the end of the experiment (Fig. 3a). The biomass difference in the open cages from the controls, recorded a peak in July at 28.24 gm^{-2} and a slow gradual decrease in the values has followed (Fig. 3b). An additional comparison was made between the two different treatment types, closed-open cages, (Fig. 3c), in order to estimate the potential difference in algal biomass under no grazing pressure and partial herbivore activity. The results showed that between partial and no herbivory respectively, very low to null growth rates as well as low biomass values were recorded throughout the experiment.

According to the two-way ANOVA test, significant difference was found in Erect, Turf and Total algae groups in response to treatment (Table 3). The post-hoc Tukey test specified that the significant difference occurs between Closed-Control treatments only, whereas LSD test resulted significant differences in Open-Control treatments as well (Table 4).





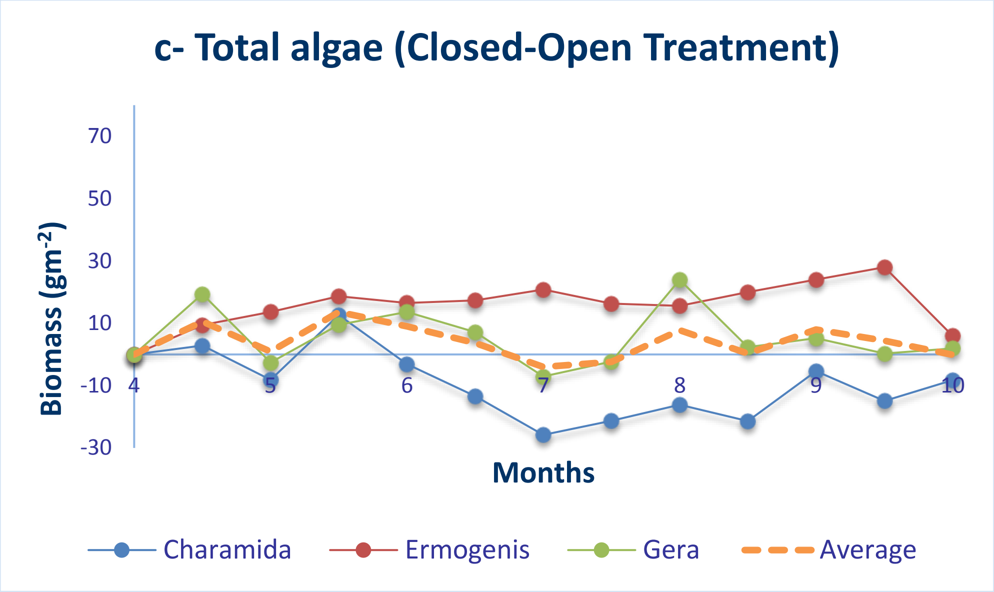


Fig. 3: Differences in the average total algal biomass (including erect, turf and encrusting algae), between treatment and control surfaces per sampling station throughout the experimental period: a) Closed cages-control, b) Open cages-control and c) Closed cages-Open cages. Orange dashed line represents average differences. Two points in each month represent twice per month visitations.

Table 3: Two-way ANOVA test results. Mean differences in biomass of the three main algal categories. Significant differences are indicated in bold, where p<0.05.

Factors	Erect			Turf			Encrusting			Total		
	Df	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
Site	2	1.629	0.225	2	1.592	0.232	2	0.99	0.906	2	0.074	0.190
Treatment	2	4.168	**0.020**	2	5.317	**0.013**	2	1.175	0.333	2	3.484	0.195
Site × Treatment	4	0.446	0.774	4	1.756	0.184	4	0.549	0.702	4	0.579	0.682

Table 4: Post-hoc Tukey and LSD test results. Main differences between sampling stations, in biomass growth for the three main algal categories.

Factor	Erect		Turf		Encrusting		Total	
Post-hoc test	Tukey	LSD	Tukey	LSD	Tukey	LSD	Tukey	LSD
Treatment	*p*		*p*		*p*		*p*	
Closed-Control	**<0.05**	**<0.05**	**<0.05**	**<0.05**	-	-	-	-
Open-Closed	-	-	-	-	-	-	-	-
Control-Open	-	**<0.05**	-	**<0.05**	-	-	-	-

4. Discussion

The present study constitutes a follow up to the research of Tsirintanis *et al.* (2018), offering further temporal and quantitative data in order to better describe and comprehend the effect of grazing activity on macroalgal communities. Therefore the selection criteria for the sampling stations are evident to be predefined by the before mentioned research study. Both studies present an insight into the possible drivers that contributed on the overgrazing regime and lead to the current conditions on the three sites.

The two different types of treatments allowed the quantification of grazing effects on adjacent macroalgal benthic communities. As seen in the results, both cage treatments, compared to the control plots, contributed to macroalgal growth. In terms of biomass, significant differences in algal growth between cage treatment and control areas were revealed in turf, erect and total algae groups. As seen in Fig.3, at the beginning of the experiment, the difference of the total average biomass between the treatments and the control initiated from zero (Fig. 3a-fully closed cages) or slightly negative values (Fig.3b-open top cages) that gradually increased. In mid-June, closed cage treatments peaked on algal development, followed by small fluctuations, until the end of the experiment (Fig. 3a). The biomass in the open cages differed from the controls, since it showed a peak in July, followed by a slow gradual decrease in the values (Fig. 3b). A similar pattern is described from Tsirintanis *et al.* (2018) (peak values in mid-August), confirming the results of our study. Both patterns agree with the typical seasonal growth cycle of Mediterranean phytocommunities (biomass peak during late spring-summer) (Hereu *et al.*, 2008; Martí *et al.*, 2005; Sala & Boudouresque, 1997; Tsirintanis *et al.*, 2018).

From the perspective of site factor, both studies have reported that in mid- summer (algal peak) erect algae had developed in terms of biomass, (Fig.2). *Padina pavonica* was the most frequent algae, followed by *Dictyota* sp. and *Halopteris* sp. (latter not recorded at Gera site), representing Phaeophyta and Chlorophyta *Acetabularia acetabulum* following next for all three sites. Corallines *Corallina* sp. coming first and *Jania* sp. following, were reported only at Gera site. The two Corallinaceae species as well as Ochrophyta *Padina pavonica* are preferred for grazing from sea urchin species *Paracentrotus lividus* and *Arbacia lixula* (Baggini *et al*., 2015; Flukes *et al*., 2012; Privitera *et al*., 2008).

No substantial difference was observed between closed and open cages treatments, regarding algal biomass growth (Fig. 3c). This indicates that fish herbivory has minor influence in the shaping of the algal communities, compared to sea urchin herbivory. The study of Tsirintanis *et al*. (2018) came to the same conclusion and a relation was made between the different grazing patterns of the two herbivore categories with the low fish biomass that was observed in the study site. Fish tend to consume algal biomass more slowly by biting pieces out of algal leaves, therefore leaving room for further algal development whilst sea urchins remove the algal thalli, functioning in a ‘scraping’ fashion (Hereu, 2006). Similar results in temperate study regions support these findings (Hereu *et al*., 2008). Sea urchin grazing pressure may lead to the creation of rocky barren areas and low structure complexity habitats with also low primary production that lack erect algal communities. This affects the population of coastal fish that use macroalgal assemblages for food and shelter (Gianguzza *et al*., 2011) and leads to the domination of encrusting red algae on the barren rocks (Guidetti & Sala, 2007; Sala, *et al*., 1998). In the study of Poore *et al.*(2012), the herbivorous activity was more focused in Phaeophyceae and Chlorophyta rather than seagrasses, cyanobacteria and/or Rhorophyta.

Algal turf along with encrusting algae were the dominant benthic categories at the beginning of the experiment (Fig. 2). Algal turf is consisted of minute, typically seasonal and fast growing species, which form algal carpets or mats (Littler*et al*., 1983; Salomidi *et al*., 2012). Although in the juvenile stage, many algae form turfs, various low-lying opportunistic species are typical indicators of disturbed environmental conditions, fragmented with low structural complexity, degraded ecosystemic habitats (Airoldi, 1998; Balata*et al*., 2015; Orfanidis*et al*., 2001). Turf algae dominated in Gera station in terms of biomass values, during the peak algal growth whereas in Charamida station the group recorded the lowest values (also seen in Fig.2). Encrusting algae followed with the highest values in Charamida and the lowest in Ermogenis. Relieved of grazing pressure, inside the cages turf algae occupied the available rocky bottom at a higher rate in Gera rather than Charamida. This indicates a more disturbed habitat in means of environmental conditions since the prevalence of turf algae is associated with degraded and fragmented environments (Airoldi, 1998; Balata *et* al.,2015). Due to their opportunistic nature, turf phytocommunities are able to withstand the herbivorous activities of fishes. Resistance also applies for encrusting algae due to their hard and rigid thall (Salomidi *et al.*, 2016).

There was no record of canopy-forming macroalgae (e.g. *Cystoseira* spp.) inside the cages. Canopy-forming species, are widely identified as the climax stage in a succession of photophilous algal communities (Pérès & Picard, 1964) and the main component in pristine ecosystems (Salomidi *et al.*, 2016). As Poore *et al.*(2012) suggests, at a global scale, the most affected groups from grazing pressure are the up-right, well developed in structure, canopy and bushy form algae (e.g. Fucales, Laminariales, Dictyotales). Numerous studies emphasize the importance of these species, as they structure complex algal communities and contribute to the creation of algal forests not only around Lesvos Island, but also in the Aegean Sea and the Mediterranean Sea as well (Montesanto & Panayotidis, 2001; Panayotidis*et al.*, 1999; Sales *et al.*, 2012; Tsiamis *et al.*, 2006). In the study of Tsirintanis *et al.*(2018), canopy forming algae were only spotted at the upper infralittoral limit (e.g. 0-1m depth), in the shape of small patches. The restriction of these macroalgal species can support the argument of overgrazing activity (Salomidi *et al.*, 2016) in the deeper waters and one assumption can be that the upper inflalittoral limit is exposed to other risks, such as seabird predation or water movement and so herbivorous fish would not risk grazing. That is because canopy and bushy algae are more attractive to herbivorous fish, as their tasty and voluminous leaves tend to make them prone to overgrazing (Salomidi *et al.*, 2016).

An interesting observation was the chlorophyte alga *Halimeda tuna*, which was present in one control plot at Gera site, but throughout the experiment was not consumed by any herbivore group. This can be attributed to the fact that this is a calcified seaweed which is able to produce chemical based feeding deterrents (metabolites are halimedatrial and diterpenoid tetraacetate) (Hay, 1984; Paul & Fenical, 1983) that act as a defense mechanism against herbivory (Paul &Van Alstyne, 1992). Where high levels of herbivory are noticed, *Halimeda* spp. are the typical algae type that can persist in the area (Hillis-Colinvaux, 1980), from their ability to produce chemical and morphological defenses. The latter being their capability of high levels of calcification (Hillis-Colinvaux, 1980; Paul & van Alstyne, 1988; Paul & Van Alstyne, 1992).

Direct or indirect anthropogenic-induced physical modifications in a habitat can also be taken into account as possible drivers for the decline and the prolonged scarcity in the natural recovery of algal forests. Perkol-Finkel and Airoldi (2010), conducted their research at Monte Conero, an area with a naturally physically unstable rocky substrate that apart from cliff erosion is also burdened by rock mining and sediment additions for tourist cobble beaches nourishment. In a regime of unstable rocky habitats, canopies showed to be severely damaged, suffered mortality and exclusion due to boulder displacement and possible overturn in case of a severe hydrodynamic phenomenon (storm, current, tide). Anthropogenic effects, such as destruction of habitat, eutrophication, overfishing, are also responsible for triggering the decline of marine algal forests (Perkol-Finkel & Airoldi, 2010). Areas of low carnivorous fish and apex predator biomass, accompanied by the prevalence of herbivorous species are perceived as overfished areas (Giakoumi *et al.*, 2012; Salomidi *et al.*, 2016). In the study of Tsirintanis *et al.*(2018), a possible connection is made between the large size of the recorded sea urchins and the low biomass of *Diplodus* spp. individuals that were counted through visual census surveys, followed by recordings of smaller-sized wrasses (*Coris julis* and *Thalassoma pavo*). *Diplodus* spp. are the main predators of adult sea urchins (*Diplodus sargus* followed by *Diplodus vulgaris*) (Sala, 1997; Salomidi *et al*., 2016). Intense fishing pressure is an obstacle to the development of carnivorous fish that function as predators and can control the growth rate of herbivorous populations.

The decision not to record just the final state of the macroalgal communities (common approach in such studies, i.e. Baggini *et al*., 2015), but to synthesize a time-series of in-between states in algal-growth, proved to be more informative. Possible bias may surface from using conversion factors for coverage and biomass, although it is not anticipated to affect the comparisons between treatments and controls, potentially just the absolute biomass values, since conversion factors have a variability.

Misplaced, broken cages or cut tire ups, have occurred due to intense hydrodynamic conditions in our study area. Cages and tire ups were fixed or replaced as soon as weather conditions improved. There were also indications of human interference, concerning the integrity of the cages (i.e. slashed meshes from local fishermen, considered as traps for marine organisms, especially at Gera site), although signs were attached to the cages, stating the nature of the cages presence. In this cage the whole mesh was replaced. Severe meteorological conditions posed as a postponing factor for several sampling visits, but site visits were rescheduled as soon as weather conditions allowed so.Even after the alteration in the caging material to a more durable one (PVC to PP-R), it seems that a more resilient material, would provide even better results, as well as different securing methods. Tire ups were more effective than ropes, but still they were able to brake at intense hydrodynamic conditions.

In conclusion, the present study provides further evidence that intense grazing pressure is responsible for the diminished macroalgal growth and low complexity structure of the shallow sublittoral rocky habitats in the southeastern part of Lesvos Island. Erect algae was observed to be the most affected group by the grazing pressure via herbivore exclusion. Anthropogenic stressors, such as overfishing, may be the possible driving forces that led to the current conditions, creating increasingly flatter coastal marine seascapes and algal communities with a less complex structure and sporadic vegetation.

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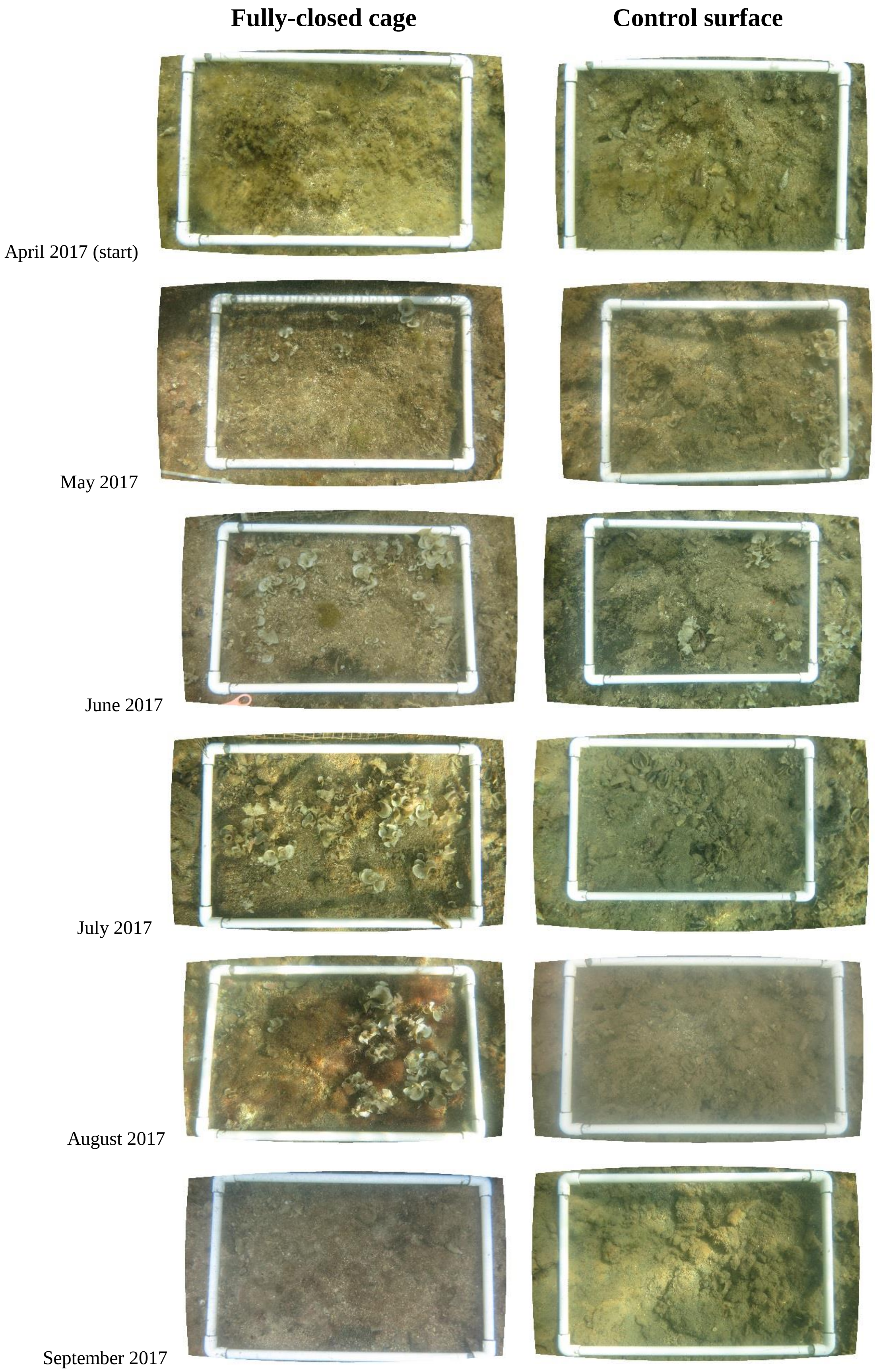
APPENDIX

Example of photographic samples- *Supplementary material*



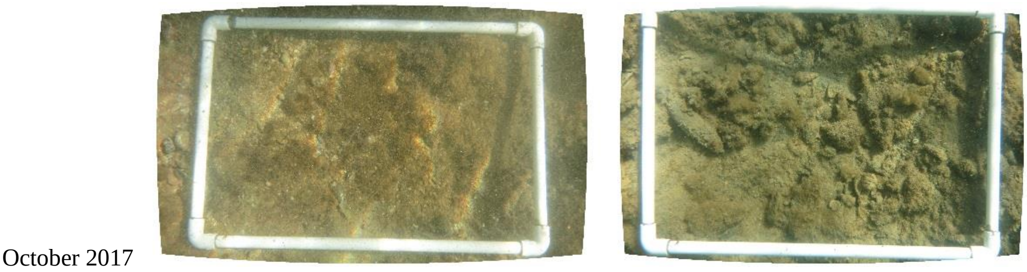
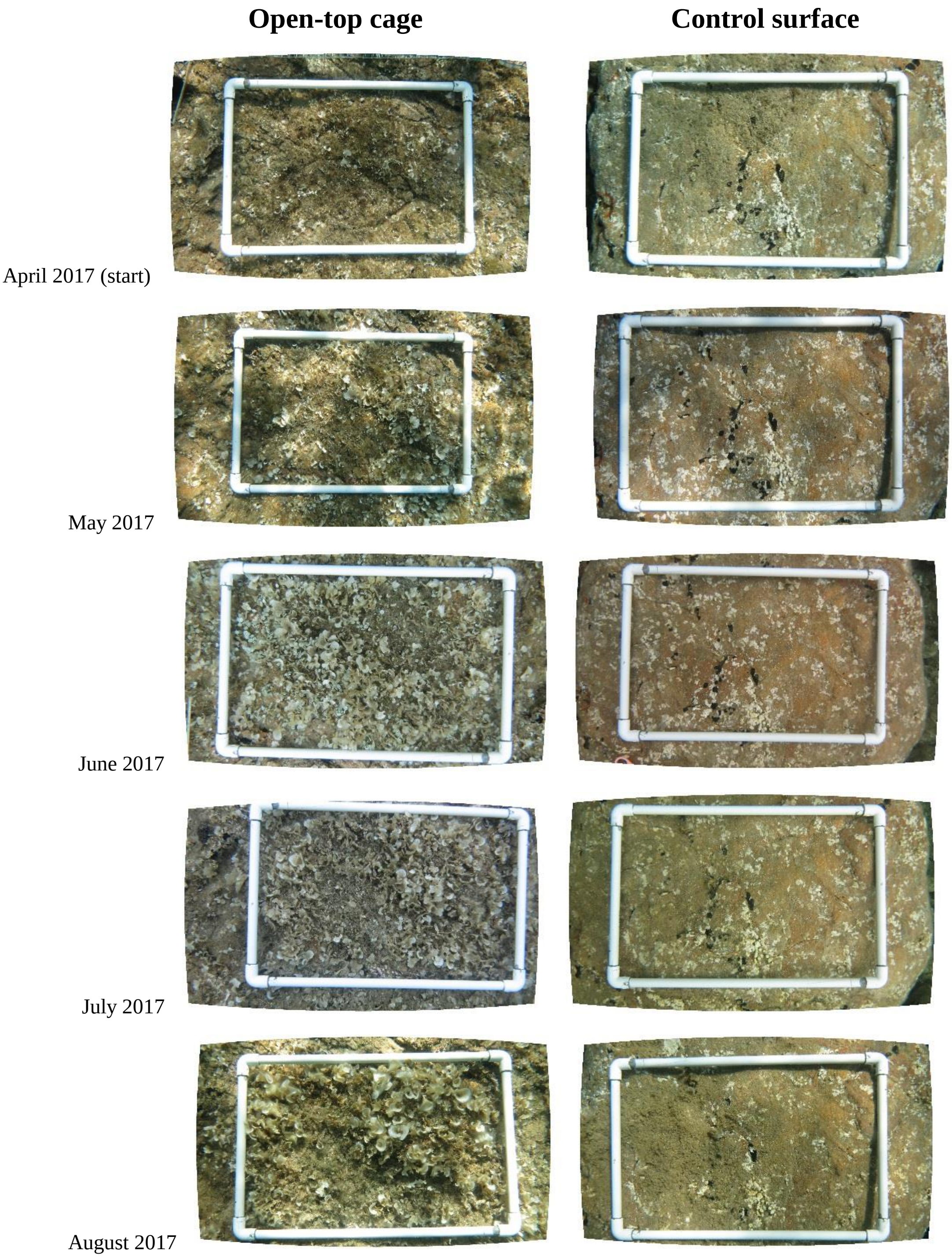


Fig. S1: Example of photographic samples: Undistorted photographic samples time series that depict algal growth monitored inside a fully-closed cage (left column) in contrast to one of the control surfaces of the same site (right column).



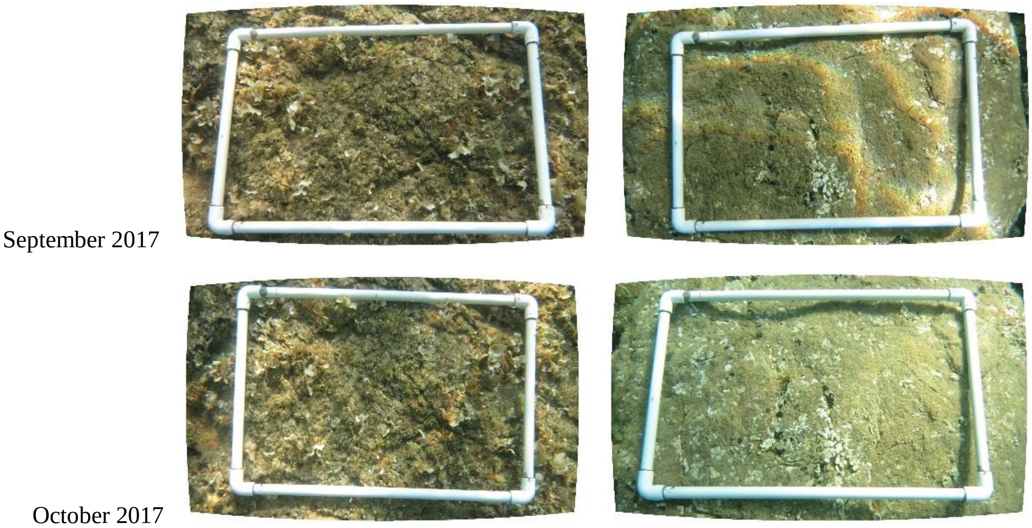
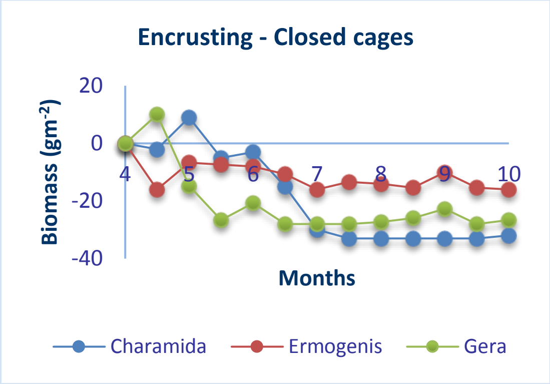
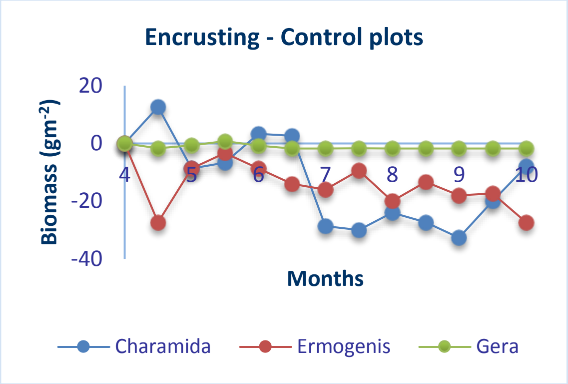
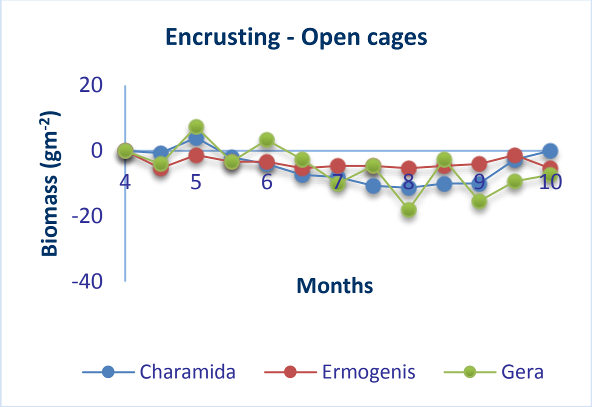
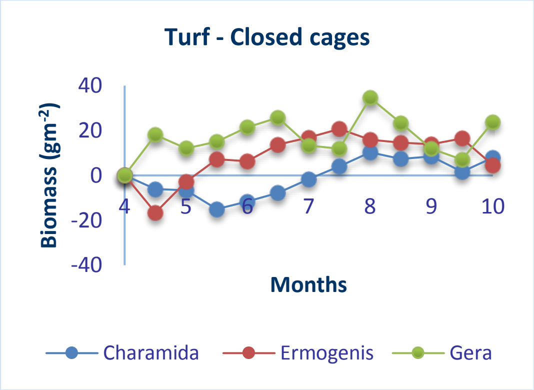


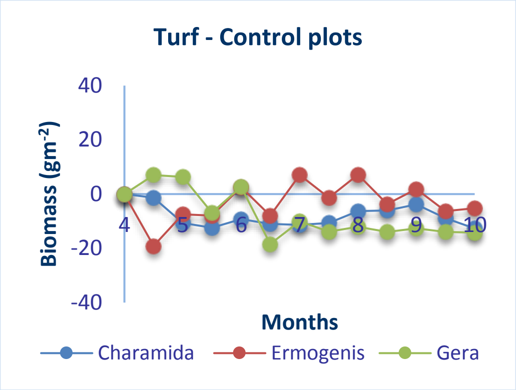
Fig. S2: Example of photographic samples: Undistorted photographic samples time series that depict algal growth monitored inside an open-top cage (left column) in contrast to one of the control surfaces of the same site (right column).


Encrusting - Closed cages
Biomass (gm-2)
20
0
-20
-40
4
5
6
7
8
9
10
Months
Charamida
Ermogenis
Gera


Encrusting - Control plots
Biomass (gm-2)
20
0
-20
-40
4
5
6
7
8
9
10
Months
Charamida
Ermogenis
Gera


Encrusting - Open cages
Biomass (gm-2)
20
0
-20
-40
4
5
6
7
8
9
10
Months
Charamida
Ermogenis
Gera


Turf - Closed cages
Biomass (gm-2)
40
20
0
-20
-40
4
5
6
7
8
9
10
Months
Charamida
Ermogenis
Gera


Turf - Control plots
Biomass (gm-2)
40
20
0
-20
-40
4
5
6
7
8
9
10
Months
Charamida
Ermogenis
Gera

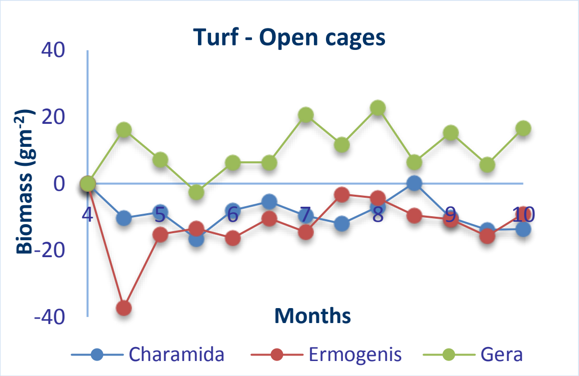

Turf - Open cages
Biomass (gm-2)
40
20
0
-20
-40
4
5
6
7
8
9
10
Months
Charamida
Ermogenis
Gera

Fig. S3: The exhibited algal growth for all algal morphological groups that were used as the main algal categories (Table 2) at each experimental treatment.