`

University of the Aegean

School of the Environment

Department of Marine Sciences

MSc Integrated Coastal Management

Assessment of grazing effects on phytobenthic community structure at shallow rocky reefs: an experimental field study in the NE Aegean Sea

Tsirintanis Konstantinos

Mytilene 2017

`

Ευχαριστίες

`

Abstract

Intense grazing pressure on Mediterranean macroalgal photophilous communities poses a serious threat on the benthic assemblages of shallow sublittoral rocky habitats. Overgrazing can deplete flourished macroalgal communities and lead to reduction of habitat complexity and species diversity. To quantify the potential effects of sea urchin and fish herbivory on the rocky reefs of the north Aegean Sea, a grazer exclusion caging experiment was conducted at the southeastern part of Lesvos Island over a six-month period (May to October 2016). Custom–made cages were constructed using PVC frames (40×60×25 cm) and a plastic mesh (2×2 cm opening). The cages were mounted on horizontal or gently slope substrate surfaces at three rocky reef sites, between 1–5 m depth. At each site, three replicates of two different treatments and control surfaces were applied: (1) fully–closed cages that excluded both sea urchins and large herbivorous fish, (2) open–top cages that excluded sea urchins only, and (3) control surfaces with no restrictions on herbivores. Bimonthly photoquadrat sampling was used to monitor the experimental surfaces. Underwater surveys for the estimation of sea urchin population density (by quadrat sampling) and the total fish biomass (by strip transects) were also conducted. The main grazers observed were the sea urchins *Arbacia lixula* and *Paracentrotus lividus*, and the herbivorous fish *Sarpa salpa*. Experimental monitoring of benthic cover and algal biomass showed significant algal growth inside the cages compared to control surfaces. The results showed that grazing activity had a strong impact on algal communities, eradicating erect algae, hampering the development of turfs and thus altering the structure of macroalgal communities towards a less complex and sparse vegetation.

`

Περίληψη

Η εντατική βόσκηση στις φωτόφιλες βιοκοινότητες μακροφυκών της Μεσογείου αποτελεί σοβαρή απειλή για τις βενθικές οργανισμικές συνευρέσεις των ρηχών ενδιαιτημάτων σκληρού υποστρώματος της υποπαραλιακής ζώνης. Η υπερβόσκηση μπορεί να εξαφανίσει βιοκοινότητες μακροφυκών που ευδοκιμούν και να οδηγήσει σε επιπτώσεις στην πολυπλοκότητα του ενδιαιτήματος και την ποικιλότητα ειδών. Προκειμένου να ποσοτικοποιηθεί η δυνητική επιρροή της φυτοφαγικής δραστηριότητας των αχινών και των φυτοφάγων ψαριών στους βραχώδεις υφάλους του Βορείου Αιγαίου, έλαβε χώρα ένα πείραμα αποκλεισμού βοσκητών μέσω κλωβών στο νοτιοανατολικό κομμάτι της νήσου Λέσβου για μια περίοδο 6 μηνών (Μάιος-Οκτώβριος 2016). Τα αυτοσχέδια κλουβιά κατασκευάστηκαν από σωλήνες PVC (διαστάσεων 40×60×25 εκ.) και πλαστικό πλέγμα (ανοίγματος 2×2 εκ.). Τα κλουβιά εγκαταστάθηκαν σε επιφάνειες οριζόντιας ή ήπιας κλίσης, σε βραχώδεις υφάλους που εντοπίστηκαν στους τρείς διαφορετικούς σταθμούς, σε βάθη 1-5 μέτρων. Σε κάθε σταθμό εφαρμόστηκαν τρείς ρέπλικες, δύο διαφορετικών πειραματικών φάσεων αλλά και οριοθετημένες επιφάνειες σύγκρισης. Συγκεκριμένα αυτές ήταν: 1) πλήρως κλειστά κλουβιά που απέκλειαν ψάρια και αχινούς, 2) κλουβιά ανοιχτής άνω επιφανείας που απέκλειαν μόνο τη βόσκηση των αχινών, 3) περιοχές του πυθμένα χωρίς κλουβιά με διακριτά σημάδια. Δύο φορές το μήνα, πραγματοποιούνταν οπτική καταγραφή σε πλαίσιο για την παρακολούθηση της εξέλιξης των πειραματικών επιφανειών. Επίσης πραγματοποιήθηκαν οπτικές μέθοδοι καταγραφής για την εκτίμηση της πληθυσμιακής πυκνότητας των αχινών και της βιομάζας των ψαριών. Οι βασικοί βοσκητές που παρατηρήθηκαν στην περιοχή ήταν οι αχινοί *Arbacia lixula* και *Paracentrotus lividus* και το φυτοφάγο ψάρι *Sarpa salpa*. Η πειραματική παρακολούθηση της βενθικής επιφανειακής κάλυψης και της βιομάζας μακροφυκών εντός των κλωβών, έδειξαν σημαντική ανάπτυξη συγκριτικά με τις δειγματοληπτικές επιφάνειες των επιφανειών σύγκρισης. Τα αποτελέσματα δείχνουν πως η διαδικασία της βόσκησης έχει έντονο αντίκτυπο στις βιοκοινότητες φυκών μέσω της πλήρους εξαφάνισης ανορθωμένων ειδών και του περιορισμού επέκτασης του χλοοτάπητα. Έτσι, η βόσκηση στην περιοχή αλλάζει τη δομή των βιοκοινοτήτων των μακροφυκών προς μια βλάστηση πιο απλοϊκή και αραιή.

Contents

`

1. Introduction

Algal forests represent a fundamental feature of the shallow sublittoral rocky substrates. They contribute to the regulation of essential biophysical processes, such as primary production and nutrient cycling (Boudouresque et al., 2014), provide refugee for fish and invertebrates, and have a vital role for the conservation of benthic communities (Ballesteros, 1990; Gianni et al., 2012 and references therein; Cheminee et al., 2013). Healthy phytobenthic communities are among the most important European seabed biotopes for the provision of ecosystem services (Salomidi et al., 2012). In the Mediterranean Sea, well-developed macroalgal forests, mainly consisting of *Cystoseira* spp (order Fucales), signify a "pristine" state of the environment. These canopy-forming species are particularly susceptible to multiple stressors that affect coastal ecosystems, and have suffered major losses in species diversity, area cover, and biomass (Thibaut et al., 2005; 2015). Anthropogenic pressures, such as illegal fishing practices, pollution, habitat fragmentation and climate change have led to the degradation of macroalgal forests (Guidetti et al., 2003; Airoldi et al., 2009; Verges et al., 2016) and have stimulated drastic shifts in community structure and functioning (Airoldi & Beck, 2007; Bianchi et al., 2014). For this reason, rocky habitats that are predominated by macroalgal forests have been classified as endangered under the European Red list of habitats (Gubbay et al., 2016).

Intense grazing pressure on macroalgal photophilous communities poses a significant threat on hard substrate benthic assemblages. In the absence of predators, an uncontrolled increase in the abundance of herbivores may affect the ratio between grazing activity and plant growth, and lead to overgrazed conditions (Burnell et al., 2013). A severe depletion of erect algal cover due to overgrazing can eventually lead to the creation of unproductive areas of low complexity, the so called "rocky-barrens" that are characterized by extended bare rock and the dominance of encrusting algae (Benedetti-Cecchi et al., 1998; Bulleri et al., 2002; Sala et al., 2011). The transition from diverse phytobenthic communities to encrusting algae-dominated barrens is more common along north temperate coastal regions in comparison to tropical and south temperate shores (Filbee-Dexter & Scheibling, 2014). Existing evidence suggests that rocky barrens represent an alternative stable ecosystem state, as the depletion of algal buds and seeds protracts the time needed for recovery, thus rendering restoration to the initial state very difficult (Filbee-Dexter & Scheibling, 2014).

`

Herbivore exclusion is a common experimental approach that provides insights on ecological processes and the biological interactions between plants and grazers (Lotze & Worm, 2001; Gagnon et al., 2004; Baggini et al., 2015). The experimental exclusion of herbivores offers a direct comparison between two different dynamic states: one exposed to herbivory (control), and one that can rest from grazing pressure (experimental modification). The wide application of this approach at a global scale (Poore et al., 2012), suggests that the algal groups that are most heavily affected by grazing pressure are fleshy algae, especially species with an up-right structure, usually belonging to the orders of Fucales and Laminariales. In the eastern basin of the Mediterranean Sea, there is a scarcity of experimental studies that evaluate the impact of grazers on primary producers. while no such study has ever been conducted in the North Aegean Sea.

The aim of this study was to investigate the potential effects of fish and sea urchin grazing activity on the photophilous macroalgal communities of shallow sublittoral rocky reefs, and provide insight on the processes that shape the current state of macroalgal assemblages. To this end, a herbivore-exclusion caging experiment, alongside complementary fish and sea urchin surveys were conducted in order to quantify grazing pressure, and assess the changes in composition, area cover and biomass of macroalgal communities over time.

`

2. Materials and Methods

2.1 Study area

The study took place in the southeastern part of Lesvos Island, located in the North Aegean Sea, Greece, Mediterranean Sea (Fig. 1). A herbivore exclusion caging experiment was conducted at the rocky reefs of three sites: Charamida (39.0138778° N, 26.5571500° E), Ermogenis (39.0133861° N, 26.5430611° E) and Gera (39.0631139° N, 26.5276500° E). Charamida and Ermogenis are southward facing coastal areas, exposed to open sea and primarily affected by south and south-eastern winds. In both sites, rocky reefs form part of the coastline and are adjacent to extended *Posidonia oceanica* meadows. The site of Gera is located in a semi-enclosed, shallow-water gulf (10 m) that is characterized by high seasonal variability in terms of physico-chemical and hydrodynamic conditions (Kolovogiannis & Tsirtsis, 2005). During winter (November–March), the seawater inside the gulf is characterized by low temperatures (9–11°C) and a slow water circulation pattern. Rocky reefs are primarily found in the south-eastern coasts of the gulf, and border with soft-bottom substrates.
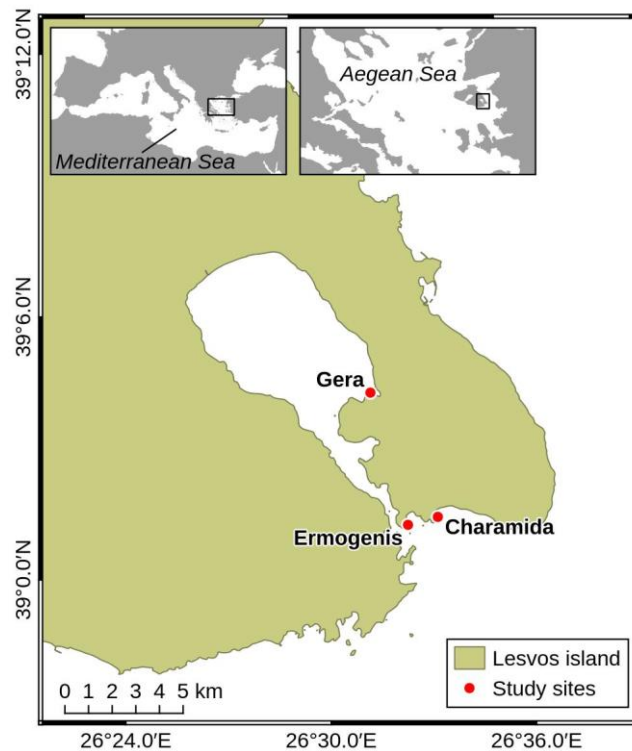

Fig. 1: Location of the study sites on the southeastern part of Lesvos island of the north Aegean Sea.

`

2.2 Experimental design

The herbivore exclusion experiment included two types of treatment and one type of control surfaces: (1) fully-closed cages that excluded both sea urchins and large herbivorous fish, (2) open-top cages that excluded sea urchins but were exposed to fish grazing, and (3) control surfaces with no cages, permanently defined by discreet markings. Three replicates of each treatment were applied at each site. The cages were constructed using PVC frames ($40\times60\times25$ cm) and a plastic mesh (2×2 cm opening). Cages were installed on horizontal or gently slope rocky surfaces, between 1–5 m depth. Manual climbing drills were used to make holes on the rocky surfaces (Fig. S1a, Supplementary material), stainless steel bolt anchors were clamped on climbing hangers that were placed in these holes (Fig. S1b&c), and the cages were secured using ropes tied to the hangers (Fig S1d). Regular visits to the cages were required for cleaning and maintenance purposes throughout the experimental period, in order to avoid potential biases caused by the mechanical damage of cages or the fouling of the mesh by macroinvertebrates and algae.

2.3 Photographic sampling

Bimonthly photoquadrat sampling was conducted for six months to monitor the progress of the experiment. Photoquadrat samples were collected using a Canon PowerShot S110 digital camera (35 mm lens, 4000×2248 resolution) and a 40×60 cm PVC frame that was positioned at the base of each cage or between the markings of the control surfaces. Prior to any further analysis, the raw photoquadrat samples were pre-processed with the Camera Calibration Toolbox for Matlab (Bouguet, 2015) in order to correct the geometric distortion introduced by the optics. The calibration data set consisted of 18 images of a waterproof checkerboard (7×10 cells, cell size: 40×40 mm) that was mounted on a rigid planar surface and photographed underwater in different orientations and positions (Fig. S2, Supplementary material), using the identical digital camera and zoom settings used to acquire the actual photoquadrat samples (for a practical guide on this procedure, see Wehkamp & Fischer, 2014). The undistorted images were then analyzed with the photoQuad software (Trygonis & Sini, 2012) using the stratified random points method with N = 100 points superimposed per image. In order to quantify the percentage cover of algal species and other benthic groups,

`

points were assigned to one of the following five categories: bare rock, benthic animals, encrusting calcareous algae, turf algae, and erect algae (Table 1). Note that canopy-forming and bush-forming macroalgae were eventually combined under the unified category "erect algae", due to the weak growth of canopy-forming algae in all three sampling sites.

Table 1: Benthic categories used for the analyses of photographic samples. The categorization and description of the different algal morphological groups were adopted and modified from Littler & Littler (1983), Orfanidis et al. (2001), and Salomidi et al. (2009).

Benthic categories	Description	Examples
Bare rock	Areas of rock bearing no algal or animal cover.	
Animals	Benthic invertebrates, mainly referring to sessile species.	*Reptadeonella violacea*
Erect algae (Canopy form)	Upright well-developed thalli, with thick blades and branches, forming canopy forests.	*Cystoseria spp.*
Erect algae (Bushy form)	Upright well-developed thalli, with coarse branches, moderate-height, forming bushy aggregations.	*Halopteris sp.*
Turf algae	Thin and soft thalli, that are delicately branched, of low-lying height, forming algal carpets.	*Acetabularia acetubulum*, various low-lying opportunistic species and juvenile macroalgal stages.
Encrusting, calcareous algae	Prostrate development, with calcified and stony texture and low-lying height, forming flat epilithic crusts.	*Lithophyllum sp.*

To convert coverage to biomass estimates, the main benthic categories (as in Table 1) were sampled to estimate the average biomass per unit area of each subcategory. Samples of fresh algae were collected with scraping, using a plastic frame of 20×20 cm with an attached net in order to avoid loss of sample material. Samples were sorted into distinct species or to the lowest taxonomic level discernible, and any sediment or dirt was removed. All samples were dried at 120 ˚C for 24 hours, and their dry weight was measured using a high-precision scale (0.01 g precision). In the case of species for which no fresh samples were available, the conversion factors from coverage measurements provided by Ballesteros (1992) were used. All reported biomasses refer to dry mass.

2.4 Fish and sea urchin communities

Fish communities and sea urchin populations were studied by visual surveys at each site during two different seasons (summer and autumn). At each site, fish were recorded along three replicate strip transects (25×5 m) at a mean depth of 2.5 m. The diver moved along the line transect and recorded the species and size (visually estimated total length) of each individual fish spotted. Fish biomass was estimated using the allometric length-weight relationship $W=a L^b$, where W is weight (in g), L is the recorded length (in cm), while the allometric parameters a and b were obtained from Moutopoulos and Stergiou (2002), and if not reported therein from FISHBASE (Froese and Pauly, 2011). Moreover, each fish taxon was classified to a functional group based on published data on feeding habits (Stergiou and Karpouzi, 2002). The trophic categories used were: apex predators, carnivores, herbivores, detrivores and planktivores. The abundance of sea urchins was estimated along the same transects using a 1×1 m quadrat frame placed every 5 m (i.e. a total of six quadrats at every strip transect). The test diameter (excluding the spines) of each individual was measured with plastic calipers.

2.5 Data analysis

To investigate the effect of the cages on algal biomass, the differences between the biomass at each sampling period and the initial biomass at time zero were estimated for every case. Three-way ANOVA was applied to these differences in biomass, to investigate differences in biomass growth (of each macroalgal category separately and all combined) by time, site and treatment. Tukey-test was applied to determine homogenous groups among the different factor levels. All statistical analyses were performed using R v3.3.2 (R Development Core Team, 2017).

3. Results

3.1 Fish and sea urchin communities

A total of 29 fish species were recorded that belonged to 12 families. Sparids and Labrids were the families with the highest number of species. Fish biomass ranged between 0.50 and 3.12 g m^{-2} (Fig. 2). Highest biomass levels were recorded at the site of Ermogenis, where summer and autumn surveys produced similar values (3.12 g m^{-2} and 3.08 g m^{-2} respectively). On the contrary, biomass values in the sites of Charamida and Gera were generally lower than Ermogenis and displayed a high level of seasonal variability.
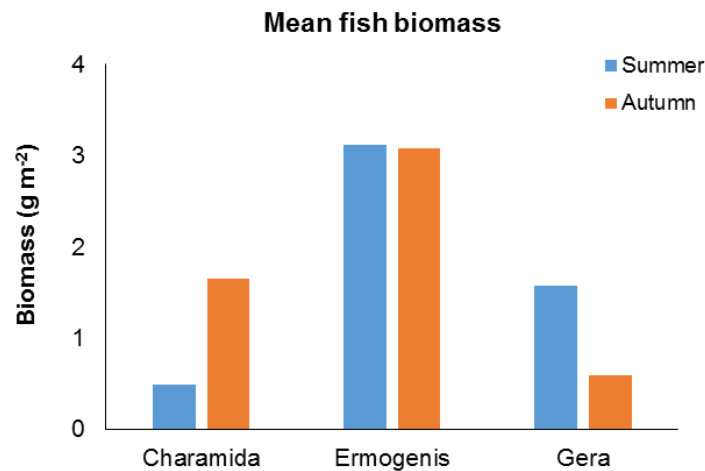
Mean fish biomass

Fig. 2: Mean biomass values recorded among the three study sites.

Overall, the average biomass density for herbivorous fish was 0.85 g m^{-2}, accounting for the largest trophic group proportion of the total fish biomass (47.6%, Table 2). This is almost entirely attributed to *Sarpa salpa*, which had a total 0.84 g m^{-2} biomass, while *Sparisoma cretense* was rarely recorded, representing only 0.3% of the herbivores. Sea urchin potential predators (*Diplodus sargus, Diplodus vulgaris, Coris julis and Thalassoma pavo*) accounted for 14.4% of total fish biomass (0.27 g m^{-2}), with *D. vulgaris* being the most abundant at a mean biomass value of 0.15 g m^{-2} (8.3% of total fish biomass), and *D. sargus* being the least abundant with a mean value of only 0.01g m^{-2} (0.6% of total fish biomass).

Table 2: Proportion of fish biomass per trophic group (N = 2024, total biomass= 1316.70 g).

Biomass of fish trophic groups (%)				
Site	Apex predators	Carnivores	Detrivores	Herbivores	Planktivores
Charamida	-	38.7	-	14.9	46.4
Ermogenis	1.3	24.4	6.9	60.7	6.7
Gera	-	45.6	-	43.0	11.4
Average	0.8	31.9	4.0	47.6	15.8

`

Three sea urchin species were encountered in our surveys: *Arbacia lixula, Paracentrotus lividus* and *Sphaerechinus granularis.* Sea urchin density ranged between 1.73 ± 1.25 (mean ± SE) and 5.53 ± 1.12 individuals/m^2, but their numbers varied considerably across sites (Fig. 3). *Arbacia lixula* was the most abundant species in Charamida site with an average abundance of 1.87 ± 0.33 individuals/m^2, while *P. lividus* abundance was much lower with below one individual/m^2 during both seasonal surveys (Fig. 3). In the site of Ermogenis, *A. Lixula* and *P. lividus* had similar densities but values were overall lower compared to Charamida. At Gera site, *P. lividus* was the only sea urchin recorded, reaching a maximum value of 5.53 ± 1.12 individuals/m^2 in June. *S. granularis* was only recorded at the site of Charamida with an abundance of 0.73 ± 0.47 in June.
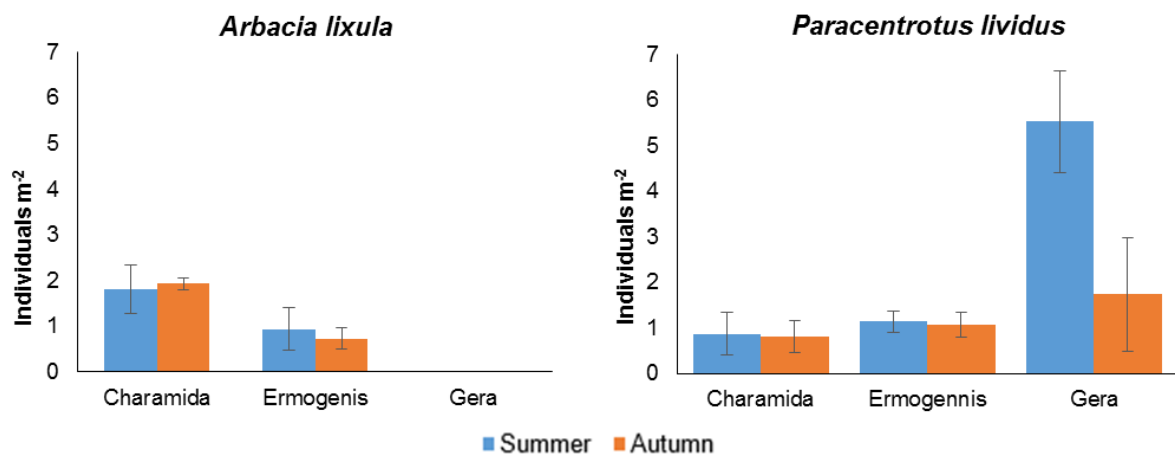
Fig. 3: Mean density of the two dominant sea urchin species in the sampling sites, tabulated by season; error bars indicate 95% confidence intervals.

With regards to size frequency, the majority of the *A. lixula* individuals (77.5%, N=40) belonged to the 2–4 cm and 4–6 cm diameter classes, indicative of medium to large sizes (Fig. 4). The most abundant size class for *Paracentrotus lividus* individuals was that of 2–4 cm (45.3%, N=53).
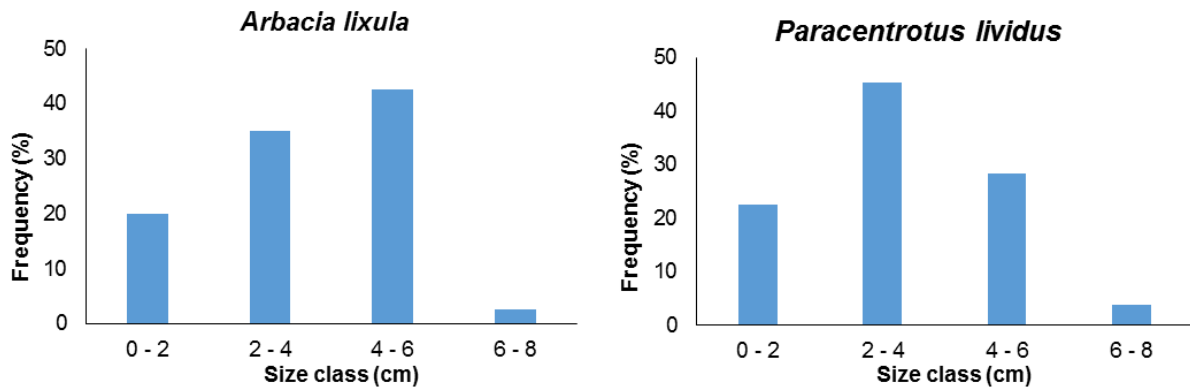
`

Fig. 4: Size frequency distribution of sea urchins (test diameter without spines).

3.2 Benthic cover

Turf algae, followed by bare rock, were the dominant benthic categories at the start of the experiment (May 2016), together occupying $90.67 \pm 2.46\%$ (mean value $\pm$ SE) of the total cover of the experimental surfaces in Charamida, $93.34 \pm 1.94\%$ in Ermogenis, and $95.78 \pm 0.96\%$ in Gera (Fig. 5). Percentage cover of encrusting calcareous algae ranged between $1.67 \pm 0.38\%$ in Gera and $7.78 \pm 2.15\%$ in Charamida, while the erect algae and benthic animal categories displayed extremely low initial cover values (i.e. <3% and <1%, respectively).
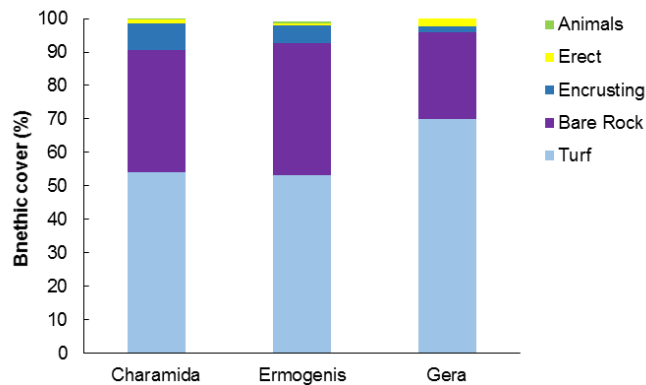
Fig. 5: Mean percentage cover of the different benthic categories in the three study sites at the start of the experiment.

Erect algae in fully-closed cages showed an overall increase compared to controls until the middle of August, and maintained a 5–10% increased coverage in comparison to controls until October (Fig. 6a). Despite different temporal patterns among sites, in all three cases the difference in erect algal coverage between fully-closed cages and control surfaces was positive (Fig. 6a). Open top cages average cover differentiations to controls followed a similar increase pattern until June but then decrease until no cover alteration is exhibited on July (Fig.

`

6b). By the end of August positive differentiations have been recovered, and the open top cage treatment retains a 4-6% increase in relation to control areas (Fig. 6b). According to the ANOVA results (Table 3), erect algal cover was significantly different among distinct treatments ($p<0.001$) and sites ($p<0.01$). Erect algal cover was significantly higher in the fully-closed cages in relation to the control, however no significant difference was found in erect algal cover between fully-closed and open-top cages (Table 4).
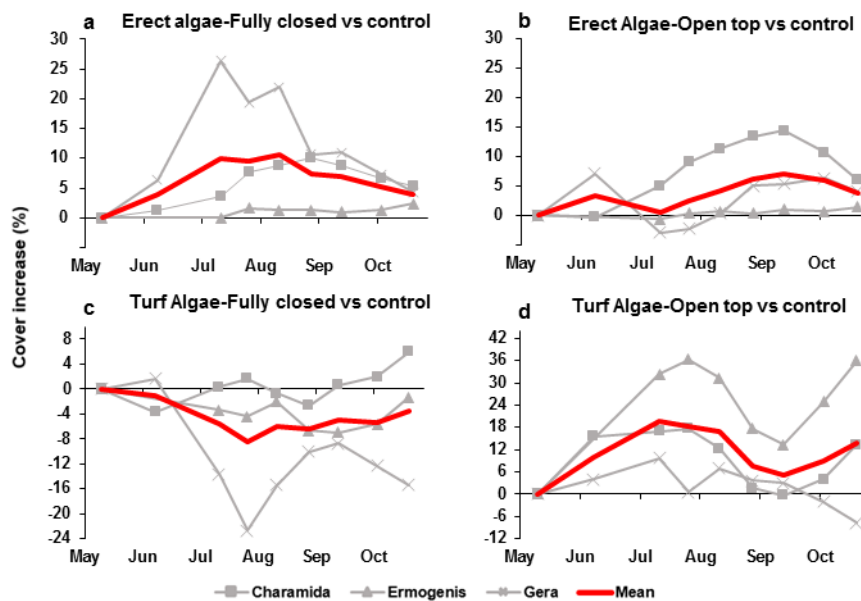
Fig. 6: Differences in the area cover of erect (a, b) and turf (c, d) algae between different types of treatment across time. Left pannel: differences between fully-closed cages and control; Right pannel: differences between open-top cages and control. Grey lines with different symbols indicate distinct sites. Red line denotes mean differences across sites.

Turf algal cover in fully-closed cages scheme a negative average alteration compared to control areas with various fluctuating patterns among sites (Fig. 6c). On the other hand turfs showed an overall increase in cover at open-top cages compared to control surfaces through experimental time (Fig 6d). This positive differentiation fluctuates as well, but retains differences from 5-20% in coverage (Fig. 6d). The ANOVA results for turf algal cover (Table 3) indicated a significant difference among distinct treatments and sites ($p<0.001$ in both cases). Turf algal cover in open-top cages differentiated more significantly to controls than in fully-closed treatment and furthermore that a difference occurs even in between them (Table 4).
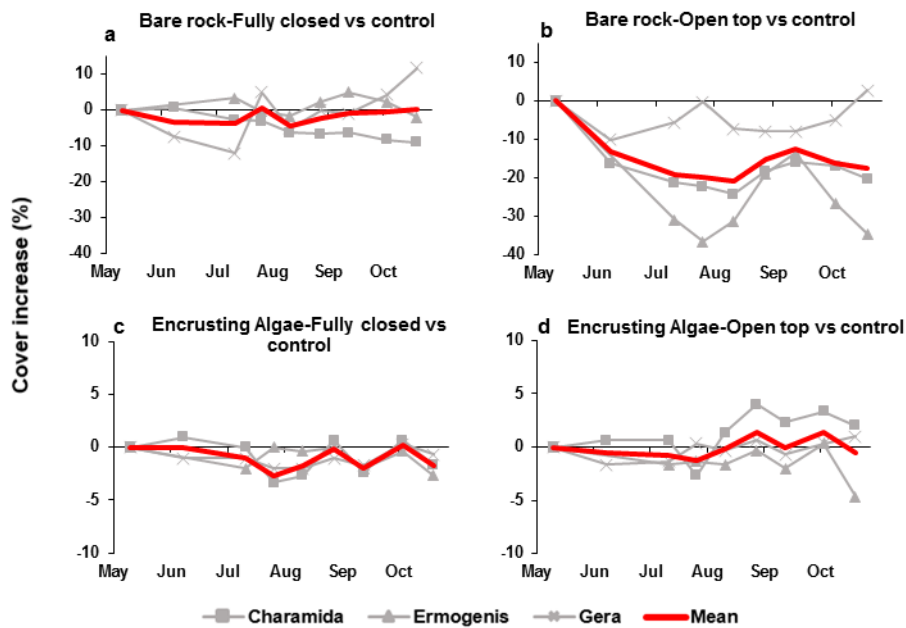
Fig. 7: Differences in the area cover of bare rock (a, b) and encrusting calcareous algae (c, d) between different types of treatment across time. Left hand side: differences between fully-closed cages and control treatments, Right hand side: differences between open-top cages and control treatment. Grey lines with different symbols indicate distinct sites. Red line denotes mean differences.

No significant difference was found in the bare rock coverage between fully-closed and control surfaces (Table 4). On the contrary, bare rock cover in open-top cages displayed a much more abrupt decrease compared to control treatments (Fig. 7b), and was found to significantly decrease in comparison to both controls and fully-closed cages. Encrusting calcareous algae, exhibited small, though non-significant, cover fluctuations (Fig. 7c,d).

Table 3: Results of the ANOVA test regarding mean differences in benthic cover for distinct benthic categories across time, site and experimental treatment.

Factors	Animals			Bare rock			Erect			Encrusting			Turf		
	Df	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
Time	6	0.33	0.919	6	1.20	0.319	6	0.28	0.943	6	2.84	<0.05	6	0.87	0.523
Site	2	14.40	<0.001	2	15.84	<0.001	2	6.21	<0.01	2	10.04	<0.001	2	32.00	<0.001
Treatment	2	18.35	<0.001	2	44.23	<0.001	2	14.23	<0.001	2	6.25	<0.01	2	35.63	<0.001

Table 4: Results of the Tuckey's post-hoc test regarding pairwise differences in benthic cover for distinct benthic categories across site and experimental treatment factor levels.

Factor levels	Animals	Bare rock	Erect	Encrusting	Turf
Site					
Ermogenis - Charamida	p<0.001	-	p<0.01	-	p<0.001
Gera - Charamida	p<0.01	p<0.001	-	p<0.001	p<0.001
Gera - Ermogenis	-	p<0.001	p<0.05	p<0.01	p<0.001
Experimental treatment					
Fully-closed cages vs Control	-	-	p<0.001	p<0.01	p<0.05
Fully-closed cages vs Open-top	p<0.001	p<0.001	-	p<0.05	p<0.001

11

`

Open-top cages vs Control	$p<0.001$	$p<0.001$	$p<0.05$	-	$p<0.001$

3.3 Algal Biomass

Similar to cover values, turf algae dominated in all sites in terms of total biomass (Fig. 8). Charamida site presented the highest algal biomass levels at the initial state of the experiment among the experimental surfaces with 75.1936 ± 8.78 g m^{-2} (mean value $\pm$ SE) , followed by Gera (39.93 ± 5.21 g m^{-2}) and Ermogenis (35.76 ± 3.57 g m^{-2}). Encrusting calcareous algae was the second group in terms of initial biomass, while erect algae biomass values were overall very low at this initial stage of the experiment, with a mean initial biomass contribution by only 1.49 ± 0.55 g $\times$ m^{-2}.
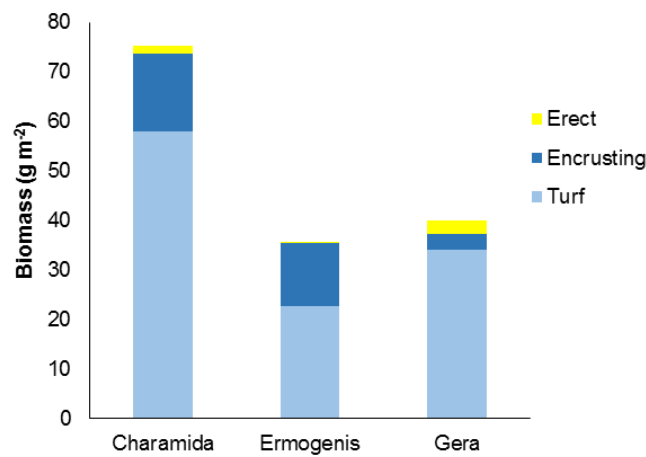

Fig. 8: Mean biomass values of different algal categories in the three study sites at the start of the experiment.

Average biomass increase of all algal morphological groups (Total algae) for the fully–closed cages, reached a difference peak to the controls at 16.01 g m^{-2} by the middle of August and then the alteration displayed a gradual decrease until the end of the experiment (Fig. 9a). Biomass difference between open–top cages and control areas, remained relatively high until October presenting a peak differentiation at the beginning of this month at 18.13 g m^{-2}. Macroalgal community growth was found to be significantly different in response to site and treatment (Table 5). The post-hoc pairwise Tukey test indicated similar significant differences, between controls and the rest of the cage treatments, as well as between Charamida and the remaining of the sites (Table 6).
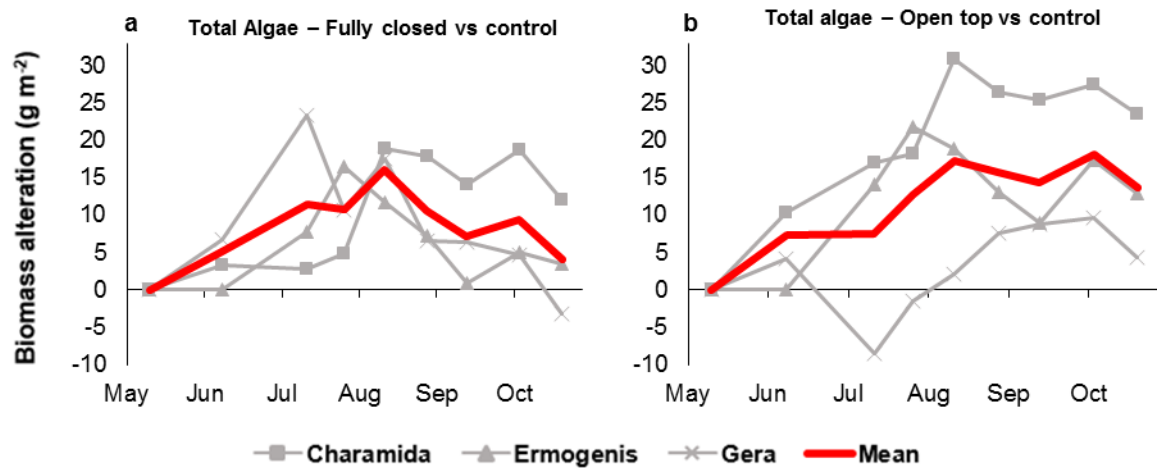
Fig. 9: Differences in the total algal biomass of erect, turf and encrusting algae (Total algae), between distinct treatements: a) Fully-closed cages versus control, and b) Open-top cages versus control, in the three study sites throughout the experimental period. Grey lines with different symbols indicate distinct sites. Red line denotes mean differences.

Table 5: Results of the ANOVA test regarding mean differences in biomass for distinct algal groups across time, site and experimental treatment.

Factors	Erect			Turf			Total algae		
	Df	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
Time	6	0.24	-	6	2.54	<0.05	6	1.65	0.151
Site	2	7.31	<0.01	2	34.08	<0.001	2	15.79	<0.001
Treatment	2	16.71	<0.001	2	23.58	<0.001	2	24.59	<0.001

Table 6: Results of the Tuckey's post-hoc test regarding pairwise differences in biomass growth for distinct algal groups across site and experimental treatment factor levels.

Factor levels	Erect	Turf	Total algae
Site			
Ermogenis - Charamida	p<0.01	p<0.01	p<0.001
Gera - Charamida	-	p<0.001	p<0.001
Gera - Ermogenis	p<0.01	p<0.001	-
Experimental treatment			
Fully-closed cages vs Control	p<0.001	p<0.05	p<0.001
Fully-closed cages vs Open-top	p<0.05	p<0.001	-
Open-top cages vs Control	p<0.01	p<0.001	p<0.001

`

4. Discussion

The herbivore exclusion experiment revealed significant differences in macroalgal growth between cage treatments and control areas. At the same time, distinct macroalgal groups presented variable responses to herbivory. Bushy erect algae was the mostly impacted algal group. Species, such as *Padina pavonica*, *Jania sp.* and *Halopteris* spp. were practically absent in our photoquadrat samples at the beginning of the experiment. By mid-summer this group presented a well-developed layer in the cage treatments, both in terms of area cover and biomass, while it remained relatively scarce in control areas. In the study area, canopy forming algae were only found in small patches at the upper infralittoral limit (0 – 0.5 m depth), and were generally absent from our treatments, the only exception being some thinly grown tufts of *Dictyota* spp. and *Dictyopterys* spp. in some cages. Several studies have underlined the importance of canopy forming algal species (e.g. of the genus *Cystoseira*) in structuring complex phytocommunities around Lesvos island, other parts of the Aegean Sea, and in the rest of the Mediterranean Sea (Panayotidis et al., 1999; Montesanto & Panayotidis, 2001; Tsiamis et al., 2006; Sales et al., 2012).The spatial confinement of dense canopy forming algal assemblages at the upper infralittoral zone in other parts of the Aegean Sea has been mainly associated to the presence of large schools of herbivorous fish (Salomidi et al. 2016).

The remarkable development of a bushy erect algal stratum within our cage treatments, which reached high values of cover and biomass (early July at the site of Gera), further supports the hypothesis that grazing activity may be of the primary inhibiting factors that prevent the growth of canopy forming species in the study area. Overgrazed conditions may considerably delay the regrowth of complex algal communities, and especially the development of perennial algal species (e.g. Capdevila et al., 2016; Filbee-Decter & Scheibling, 2014 and references therein). Ecological traits of different species, alongside various localized abiotic factors, determine the potential presence and growth rates of canopy forming algae in rocky sublittoral habitats (Benedetti-Cecchi et al., 1998; Sala et al. 2012). Benedetti-Cecchi et al. (1998) showed that the growth of canopy forming algae was not enhanced even after 18 months of sea-urchin exclusion in a Mediterranean sea-urchin barren. On the contrary a fish exclusion caging experiment in the eastern Mediterranean, allowed for a dense *Cystoseira* spp. canopy to develop after a three month-period (Sala et al., 2011).

`

Algal turf was the dominant benthic category during the start of the experiment. Even so, experimental progression of herbivore exclusion allowed for further development of these filamentous species. In the absence of grazing pressure inside the cages, turfs colonized the available rocky substrate while in control areas the assembly of the herbivore guild hampered their expansion. A prevalence of turf algae has been generally associated to disturbed environmental conditions and habitats of low complexity (Airoldi, 1998; Balata et al., 2015). Especially opportunistic turf algal forms, with thin sheet-like thalli and a rapid seasonal reproductive growth, are typical of degraded ecosystems (Orfanidis et al., 2001).

Our experimental design involved two types of cage treatments in order to quantify the effects of: a) the exclusion of sea urchins (i.e. in open-top cages), and b) the combined exclusion of both sea urchins and herbivorous fish (i.e. in fully-closed cages) on the local macroalgal communities. Erect and turf algae developed conversely inside the two cage treatments. Fully closed exclusion exhibited a more potent development of erect species, than open top cages. Open top cages on the other hand illustrated a more promising growth of algal turfs. Phytophagous fish are the most multitudinous trophic group in our study area. Total fish biomass analysis showed that the herbivore *Sarpa salpa* is by far the most dominant species of the community. Herbivorous fish preferably consume well-developed fronds of erect species while turf communities feature opportunistic characteristics that allow them to withstand this herbivorous impact (Salomidi et al., 2016). Nevertheless, these observations are schemed patterns. Overall, total algal biomass growth exhibited no significant differences between the two cage treatments. Sea urchins *Arbacia lixula* and *Paracentrotus lividus* appear at moderate densities among our sites. Nevertheless, even at these densities the two echinoids species have been shown to maintain habitats of low complexity devoid of erect algal assemblages with sparse rocky vegetation (Privitera et al., 2008 and references therein).

Fish assemblages in our study area exhibited extremely low biomass values, similar to those found by Giakoumi et al. (2012) in a baseline study of the shallow rocky reefs in the Cyclades Archipelago at the Southern part of the Aegean Sea. That is because the clear majority of our fish records are young specimens of low length (L<10cm). At the same time, medium to large sea urchin individuals appear as the most frequent class sizes of local populations. *Diplodus sargus* and *Diplodus vulgaris* are considered as major sea urchin predators and when at high adult densities (10-15 individuals/ 100 m2) they can achieve sea urchin population control (Guidetti 2006; Guidetti and Sala, 2007). The carnivores *Coris jolis* and *Thalassoma pavo* can have an impact on sea urchin populations by consuming juveniles (Hereu et al., 2005).

`

However, sea urchin predation rate is analogous to predator size and conversely analogous to prey size (Guidetti, 2004; Guidetti and Sala, 2007). Another aspect of the community that we have observed in our analysis is the almost absolute absence of apex predators and the prevalence of the phytophagous fish biomass among the community's trophic groups. Apex predators are strongly related to communities of high total fish biomass and habitats relieved of fishery pressure (Sala et al., 2012). These facts enhance the hypothesis of overexploited biological resources in this region and it is therefore possible that intense fishing prevents the development of adequate predator densities and sizes for the top down predation control on sea urchins.

In the study area, especially in the nearshore reefs of Gera, the destructive and illegal fishing of the "protected" date mussel *Lithophaga lithophaga* is still being practiced. This is an endolithic species living inside carbonate rocks and its harvesting is usually conducted by SCUBA divers equipped with special sledgehammers, who break the rock to collect the bivalves (Katsanevakis et al. 2011). The impact of this destructive practice has not yet been assessed in this area, nevertheless the destruction of the rocky habitats was clearly visible. This fishing practice causes the degradation of shallow rocky reefs by the creation of rocky barrens. The newly formed bare rock surfaces consist an ideal habitat for the thriving of sea urchins populations (Fanneli et al., 1994; Guidetti et al., 2003). Furthermore, overfishing enhances sea urchins survival and favors the persistence of barrens.

The evidence from this experimental study suggests that grazing pressure defines algal community growth and structure at the shallow rocky reefs of the northeastern Aegean Sea. Herbivore exclusion revealed that erect algae are substantially impacted by the grazing pressure of herbivores and they are almost completely eradicated from most of the rocky reefs, while algal turf development is also significantly impeded. Moderately abundant sea urchin communities of *Arbacia lixula* and *Paracentrotus lividus*, along with the additive impact of the herbivorous fish *Sarpa salpa* are responsible for the maintenance of this overgrazed and degraded state of rocky reefs in this region.

`

5. References

Airoldi, L., 1998. Roles of disturbance, sediment stress, and substratum retention on spatial dominance in algal turf. Ecology 79 (8), 2759–2770.

Airoldi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. Oceanogr. Mar. Biol. Annu. Rev. 45, 345–405.

Airoldi, L., Connell, S.D., Beck, M.W., 2009. The Loss of Natural Habitats and the Addition of Artificial Substrata. Marine Hard Bottom Communities. Ecol. Stud. 206, 269–280.

Baggini, C., Issaris, Y., Salomidi, M., Hall-Spencer, J., 2015. Herbivore diversity improves benthic community resilience to ocean acidification. J. Exp. Mar. Biol. Ecol. 469, 98–104.

Balata, D., Piazzi, L., Bulleri, F., 2015. Sediment deposition dampens positive effects of substratum complexity on the diversity of macroalgal assemblages. J. Exp. Mar. Biol. Ecol. 467, 45–51.

Ballesteros, E., 1990. Structure and dynamics of the *Cystoseira caespitosa* Sauvageau (Fucales, Phaeophyceae) community in the North-Western Mediterranean. Sci. Mar. 54 (2), 155–168.

Ballesteros, E., 1992. Els vegetals i la zonació litoral: espècies, comunitats i factors que influeixen en la seva distribució. Phd Thesis. Barcelona: Institut d'Estudis Catalans.

Benedetti-Cecchi, L., Bulleri, F., Cinelli, F., 1998. Density dependent foraging of sea urchins in shallow subtidal reefs on the west coast of Italy (western Mediterranean). Mar. Ecol. Prog. Ser. 163, 203–211.

`

Bianchi, C.N., Corsini-Foka, M., Morri, C., Zenetos A., 2014. Thirty years after: dramatic change in the coastal marine ecosystems of Kos Island (Greece), 1981-2013. Mediterr. Mar. Sci. 15 (3), 482–497.

Boudouresque, C.F., Ruitton, S., Bianchi, C.N., Chevaldonne, P., Fernadez, C., Harmelin-Vivien, M., Ourgaud, M., Pasqualini, V., Perez, T., Pergent, G., Thibaut, T., Verlaque, M., 2014. Terrestrial versus Marine diversity ecosystems. And the winner is: the Marine realm. Proceedings of the 5th Mediterranean Symposium on Marine Vegetation, 7–28 October 2014, Portorož, Slovenia. pp. 11–25.

Bouguet, J.Y., 2015. Camera calibration toolbox for Matlab. Available online at: http://www.vision.caltech.edu/bouguetj/calib_doc/ (accessed 16.05.17).

Bulleri, F., Bertocci, I., Micheli, F., 2002. Interplay of encrusting coralline algae and sea urchins in maintaining alternative habitats. Mar. Ecol. Prog. Ser. 243, 101–109.

Capdevila, P., Hereu, B., Riera, J.L., Linares, C., 2016. Unravelling the natural dynamics and resilience patterns of underwater Mediterranean forests: insights from the demography of the brown alga *Cystoseira zosteroides*. J. Ecol. 104, 1799–1808.

Cheminée, A., Sala, E., Pastor, J., Bodilis, P., Thiriet, P., Mangialajo, L., Cottalorda, J.M., Francour, P., 2013. Nursery value of *Cystoseira* forests for Mediterranean rocky reef fishes. J. Exp. Mar. Biol. Ecol. 442, 70–79.

Fanelli, G., Piraino, S., Belmonte, G., Geraci, S., Boero, F., 1994. Human predation along Apulian rocky coasts (SE Italy): desertification caused by *Lithophaga lithophaga* (Mollusca) fisheries. Mar. Ecol. Prog. Ser. 110, 1–8.

Filbee-Dexter, K., Scheibling R.E., 2014. Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. Mar. Ecol. Prog. Ser. 495, 1–25.

`

Froese, R., Pauly, D., 2011. FishBase World Wide. Web electronic publication. www.fishbase.org.

Gagnon, P., Himmelman, J.H., Johnson, L.E., 2004. Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. Mar. Biol. 144, 1191–1203.

Giakoumi, S., Cebrian, E., Kokkoris, G.D., Ballesteros, E., Sala, E., 2012. Relationships between fish, sea urchins and macroalgae: The structure of shallow rocky sublittoral communities in the Cyclades, Eastern Mediterranean. Estuar. Coast. Shelf Sci. 109, 1–10.

Gianni, F., Bartolini, F., Airoldi, L., Ballesteros, E., Francour, L., Guidetti, P., Meinesz, A., Thibaut, T., Mangialajo, L., 2013. Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. Adv. Oceanogr. Limnol. 4 (2), 83–101.

Gubbay, S., Sanders, N., Haynes, T., Janssen, J.A.M., Rodwell, J.R., Nieto, A., Criado, M.G., Beal, S., Borg, J., Kennedy, M., Micu, D., Otero, M., Saunders, G., Calix, M., 2016. European Red List of Habitats Part 1. Marine habitats. Publications office of the European Union.

Guidetti, P., Fraschetti, S., Terlizzi, A., Boero, F., 2003. Distribution patterns of sea urchins and barrens in shallow Mediterranean rocky reefs impacted by the illegal fishery of the rock-boring mollusc *Lithophaga lithophaga*. Mar. Biol. 143, 1135–1142.

Guidetti, P., 2004. Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in shallow Mediterranean rocky reefs. Helgoland Mar. Res. 58, 110–116.

Guidetti, P., 2006. Marine reserves reestablish lost predatory interactions and cause community changes in rocky reefs. Ecol. Appl. 16 (3), 963–976.

`

Guidetti, P., Sala, E., 2007. Community-wide effects of marine reserves in the Mediterranean Sea. Mar. Ecol. Prog. Ser. 335, 43–56.

Hereu, B., Zabala, M., Linares, C., 2005. The effects of predator abundance and habitat structural complexity on survival of juvenile sea urchins. Mar. Biol. 146, 293–299.

Katsanevakis, S., Poursanidis, D., Issaris, Y., Panou, A., Petza, D., Vassilopoulou, V., Chaldaiou, I., Sini, M., 2011. "Protected" marine shelled molluscs: thriving in Greek seafood restaurants. Mediterr. Mar. Sci. 12 (2), 429–438.

Kolovogiannis, V., Tsirtsis, G., 2005. Implementation of a high resolution, 3 dimensional, hydrodynamic model to a shallow, semi-enclosed water body, Gulf of Gera-Lesvos. Proceedings of the 9th Conference on Environmental Science and Technology, 1–3 September 2005, Rhodes island, Greece. pp. 791–797.

Littler, M.M., Littler, D.S., 1983. Evolutionary strategies in a tropical barrier reef system: Functional-form groups of marine macroalgae. J. Phycol. 19, 229–237.

Lotze, H., K., Worm, B., 2001. Strong bottom-up and top-down control of early life stages of macroalgae. Limnol. Oceanogr. 46 (4), 749–757.

Montesanto, B., Panayotidis, P., 2001. The *Cystoseira* spp. communities from the Aegean Sea (NE Mediterranean). Mediterr. Mar. Sci. 2 (1), 57–67.

Moutopoulos, D.K., Stergiou, K.I., 2002. Length-weight and length-length relationships of fish species from the Aegean Sea (Greece). J. Appl. Ichthyol. 18, 200–203.

Orfanidis, S., Panayotidis, P., Stamatis, N., 2001. Ecological evaluation of transitional and coastal waters: A marine benthic macrophytes-based model. Mediterr. Mar. Sci. 2 (2), 45–65.

`

Panayotidis, P., Feretopoulou, J., Montesanto, B., 1999. Benthic vegetation as an ecological quality descriptor in an eastern Mediterranean coastal area (Kalloni Bay, Aegean Sea, Greece). Estuar. Coast. Shelf Sci. 48 (2), 205–214.

Poore, A.G.B., Campbell, A.H., Coleman, R.A., Edgar, G.J., Jormalainen, V., Reynolds, P.L., Sotka, E.E., Stachowicz, J.J., Taylor, R.B., Vanderklift, M.A., Emmet Duffy, J., 2012. Global patterns in the impact of marine herbivores on benthic primary producers. Ecol. Lett. 15, 912–922.

Privitera, D., Chiantore, M., Mangialajo, L., Glavic, N., Kozul, W., Cattaneo-Vietti, R., 2008. Inter- and intra-specific competition between *Paracentrotus lividus* and *Arbacia lixula* in resource-limited barren areas. J. Sea Res. 60, 184–192.

R Development Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0.

Sala, E., Kizilkaya, Z., Yildirim, D., Ballesteros, E., 2011. Alien marine fishes deplete algal biomass in the eastern Mediterranean. PLoS ONE 6 (2), e17356.

Sala, E., Ballesteros, E., Dendrinos, P., Di Franco, A., Ferretti, F., Foley, D., Fraschetti, S., Friedlander, A., Garrabou, J., Guclusoy, H., Guidetti, P., Halpern, B.S., Hereu, B., Karamanlidis, A.A., Kizilkaya, Z., Macpherson, E., Mangialajo, L., Mariani, S., Micheli, F., Pais, A., Riser, K., Rosenberg, A.A., Sales, M., Selkoe, K.A., Starr, R., Tomas, F., Zabala, M., 2012. The structure of Mediterranean rocky reef ecosystems across environmental and human gradients, and conservation implications. PLoS ONE 7 (2): e32742. https://doi.org/10.1371/journal.pone.0032742

Sales, M., Ballesteros, E., Anderson, M.J., Iveša, L., Cardona, E., 2012. Biogeographical patterns of algal communities in the Mediterranean Sea: *Cystoseira crinita* -dominated assemblages as a case study. J. Biogeogr. 39, 140–152.

`

Salomidi, M., 2009. Rapid Assessment of the Coastal Ecological Status (RACES) by the use of visual sampling of the upper infralittoral phytobenthic communities: a step towards the implementation of the Water Framework Directive. PhD Thesis. University of the Aegean, Mytilene, Greece.

Salomidi, M., Katsanevakis, S., Borja, A., Braeckman, U., Damalas, D., Galparsoro, I., Mifsud, R., Mirto, S., Pascual, M., Pipitone, C., Rabaut, M., Todorova, V., Vassilopoulou, V., Vega Fernadez, T., 2012. Assessment of goods and services, vulnerability, and conservation status of European seabed biotopes: a stepping stone towards ecosystem-based marine spatial management. Mediterr. Mar. Sci. 13 (1), 49–88.

Salomidi, M., Giakoumi, S., Gerakaris, V., Issaris, Y., Sini, M., Tsiamis, K., 2016. Setting an ecological baseline prior to the bottom-up establishment of a marine protected area in Santorini Island, Aegean Sea. Mediterr. Mar. Sci. 17 (3), 720–737.

Stergiou, K.I., Karpouzi, V.S., 2002. Feeding habits and trophic levels of Mediterranean fish. Rev. Fish Biol. Fisher. 11, 217–254.

Thibaut, T., Pinedo, S., Torras, X., Ballesteros, E., 2005. Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Alberes coast (France, North-western Mediterranean). Mar. Pollut. Bull. 50, 1472–1489.

Thibaut, T., Blafune, A., Boudouresque, C.F., Verlaque, M., 2015. Decline and local extinction of Fucales in the French Riviera: the harbringer of future extinctions? Mediterr. Mar. Sci. 16 (1), 206–224.

Trygonis, V., Sini, M., 2012. photoQuad: A dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. J. Exp. Mar. Biol. Ecol. 424-425, 99–108.

`

Tsiamis, K., Orfanidis, S., Diapoulis, A., Panayotidis, P., Siakavara, A., Tsiagga, E., Vakirtzi, I., 2006. *Cystoseira crinita* community under reference conditions (wfd 2000/60/ee) in the Aegean Sea (eastern Mediterranean). Proceedings of the 8th Hellenic Symposium on Oceanography and Fisheries, 4–8 June 2006, Thessaloniki, Greece. pp. 319–323.

Vergés, A., Doropoulos, C., Malcolm, H.A., Skye, M., Garcia-Pizá, M., Marzinelli, E.M., Campbell, A.H., Ballesteros, E., Hoey, A.S., Vila-Concejo, A., Bozec, Y.M., Steinberg, P.D., 2016. Long-term empirical evidence of ocean warming leading to tropicalization of fish communities, increased herbivory, and loss of kelp. Proc. Natl. Acad. Sci. USA 113 (48), 13791–13796.

Wehkamp, A., Fischer, P., 2014. A practical guide to the use of consumer-level digital still cameras for precise stereogrammetric *in situ* assessments in aquatic environments. Underwater Technol. 32, 111–128.

`

APPENDIX

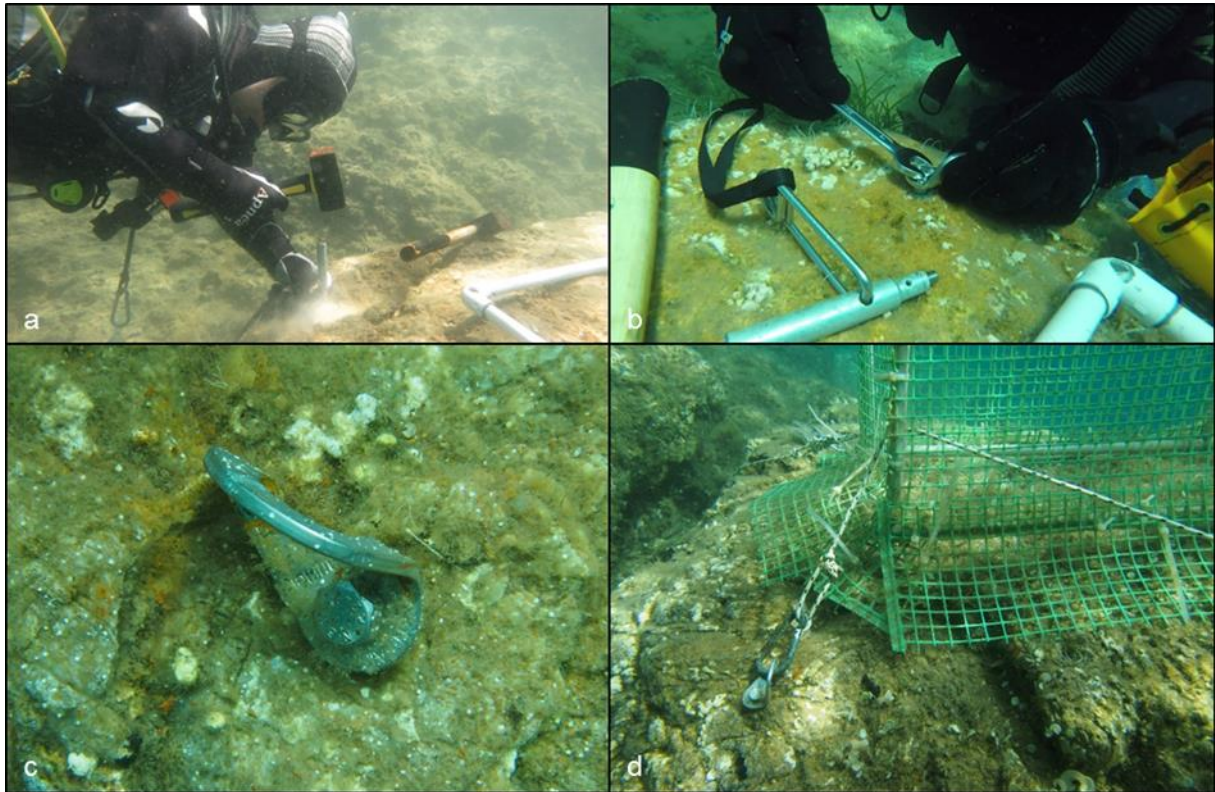Setting cages on the study sites rocky reefs-*Supplementary material*

Fig. S1: a) Use of manual climbing drills in order to make holes on the rocky surfaces, b&c) clamping of bolt anchors on stainless steel hangers, established in the opened holes, d) secured cage position with rope tied in the climbing hangers.

`

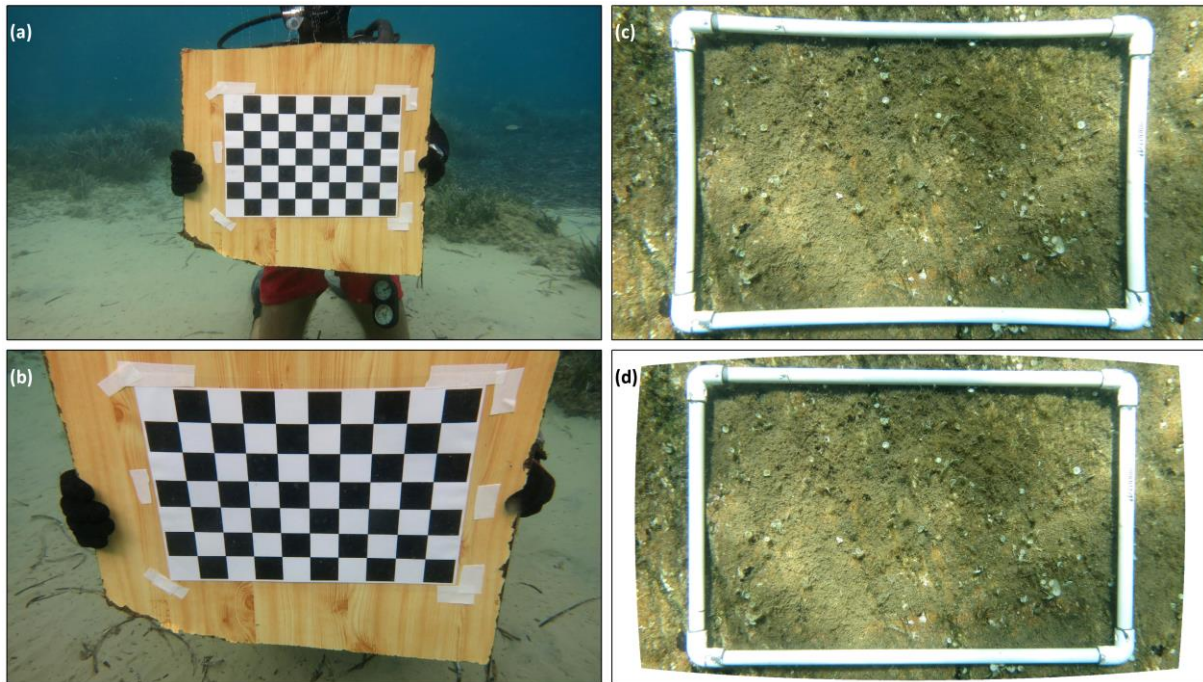Camera calibration and undistortion process-*Supplementary material*

Fig. S2: (a–b) Example images of the planar checkerboard that was photographed from various view angles in order to compute the camera's intrinsic calibration parameters; the latter encode the optical and geometric characteristics of the camera, including the geometric distortion introduced by the optics. Panels (c) and (d) show an actual photoquadrat sample before and after correction of lens distortion, respectively.

`