



Indirect effects of fish cage aquaculture on shallow *Posidonia oceanica* seagrass patches in coastal Greek waters

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ABSTRACT: Over the last 3 decades fish cage aquaculture has increased exponentially in the eastern Mediterranean Sea and has induced various levels of environmental change in coastal waters. The most apparent negative changes involve the degradation of the native seagrass *Posidonia oceanica* (L.) Delile. Our study examined the effects of fish cage aquaculture on seagrass health, sea urchin density and herbivore feeding pressure in shallow seagrass patches in the coastal waters of 2 Greek islands (Evia and Crete) between February and May of 2008. Evia and Crete represent a wide range of fish farming intensities, from small-scale (75 t yr⁻¹) to large-scale (1000 t yr⁻¹) fish production, respectively. On both islands, the seagrass variables, shoot productivity, standing crop and leaf morphometrics (length and width) were significantly lower ($p < 0.0001$) in impacted seagrass patches adjacent to fish farms compared with control patches. In addition, significantly higher sea urchin densities and herbivore feeding pressure (percentage of shoots with herbivore grazing scars) were found in impacted patches on both islands. Higher leaf tissue nitrogen and epiphyte loads were also found in impacted patches, but these increases could not be attributed to fish farming alone. Our results show that negative effects on seagrass patches can occur as a result of fish farming at both small-scale and large-scale intensities and that increased sea urchin densities and feeding pressures are important indirect effects of coastal aquaculture on these islands.

KEY WORDS: Aquaculture · Seagrass · *Posidonia oceanica* · *Paracentrotus lividus* · Nitrogen · Herbivory · Indirect effects

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INTRODUCTION

Seagrass ecosystems are among the most productive (Duarte & Chiscano 1999) and economically valuable (Costanza et al. 1997) on Earth and are found along the coasts of every continent except Antarctica. In the Mediterranean Sea, *Posidonia oceanica* (L.) Delile is the dominant seagrass species and is commonly found at depths shallower than 40 m (Boudouresque & Meinesz 1982). This slow-growing monocot forms dense meadows or patches, which provide a myriad of ecosystem services (Duarte 2002). Although *P. oceanica* meadows once covered an area

between 25 000 and 45 000 km² throughout the Mediterranean Sea (Pasqualini et al. 1998), basin-wide declines of 5 to 20% have been estimated (Boudouresque et al. 2009). Furthermore, there is evidence of rapid declines in *P. oceanica* meadows at the regional scale (Marbà et al. 1996, 2005, Delgado et al. 1999, Duarte 2002). This loss has been largely a result of the cumulative effects of various anthropogenic influences, including increased aquaculture practices (Marbà et al. 2005, Pergent-Martini et al. 2006, Gonzalez-Correa et al. 2007).

Aquaculture has increased rapidly in the Mediterranean Sea over the last 3 decades (Karakassis et al.

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2000, Belias et al. 2003), and some of the largest expansions have occurred in the eastern portion. Production in Greece has increased by 2 orders of magnitude during that time to $114\,888\text{ t yr}^{-1}$ in 2008 (FAO 2009a). Since 2003, aquaculture production has surpassed wild fisheries production in Greece (FAO 2009b), and this trend will probably continue as wild fish populations decline and food demand increases (FAO 2010). Currently, Greece is the largest producer of cultured gilthead seabream *Sparus aurata* and seabass *Dicentrarchus labrax* in the European Union, with nearly 270 active fish farming operations (Belias et al. 2003). Although public sentiment remains ambivalent about aquaculture expansion in many coastal regions in Greece, economic benefits seem to overshadow environmental considerations, thus driving public acceptance of the practice (Katravidis et al. 2003).

Coastal fish farming practices use floating net pens stocked with predominantly carnivorous species that feed on dry pellet feeds. Uneaten food and fecal material pass directly through the nets into the surrounding environment, supplying organic matter and nutrients to the water column (Dalsgaard & Krause-Jensen 2006, Holmer et al. 2008) and surficial sediments (Wu 1995, Karakassis et al. 2000, Ruiz et al. 2001, Holmer et al. 2003, Apostolaki et al. 2007, Díaz-Almela et al. 2008). Increased nutrient concentrations in the water column surrounding these fish farms are thought to be of less concern than their effects on sediments (Karakassis et al. 2005, Pitta et al. 2006), as they are rapidly assimilated into phytoplankton biomass and grazed by planktivores (Pitta

et al. 2009). In the surrounding sediments, however, nutrients and organic matter can accumulate (Pusceddu et al. 2007), which can stimulate bacterial growth and increase benthic respiration (La Rosa et al. 2001, Vezzulli et al. 2002). This may lead to anoxic events and toxic porewater conditions for benthic organisms (Belias et al. 2003, Frederiksen et al. 2007, Holmer et al. 2008, Papageorgiou et al. 2009). These conditions can persist even after fish farming practices have ended (Delgado et al. 1999, Karakassis et al. 1999).

Coastal fish farming has been considered one of the largest sources of stress on seagrass ecosystems at the local scale in the Mediterranean Sea (Duarte 2002, Holmer et al. 2008). Direct effects of coastal fish farming on *Posidonia oceanica* meadows include altered sediment chemistry (Pusceddu et al. 2007), increased porewater toxicity (Frederiksen et al. 2007) and bacterially induced anoxic events (Lopez et al. 1998, Ruiz et al. 2001, Hyland et al. 2005, Pérez et al. 2007). Similarly, ambient light reduced through shading from net structures (Delgado et al. 1999, Pergent et al. 1999), effluent discharge (Ruiz et al. 2001) or nutrient-mediated epiphytic overgrowth (Delgado et al. 1999, Ruiz & Romero 2001, Ruiz et al. 2001, Cancemi et al. 2003) also stress seagrasses.

Another factor affecting seagrass ecosystems near coastal fish farms in the Mediterranean Sea is intense herbivore grazing pressure (Tomas et al. 2005a, Prado et al. 2007a), which is enhanced around fish farms (Ruiz et al. 2001, 2009, Holmer et al. 2003). High densities of the sea urchin *Paracentrotus lividus* and the sparid fish *Sarpa salpa* can directly con-

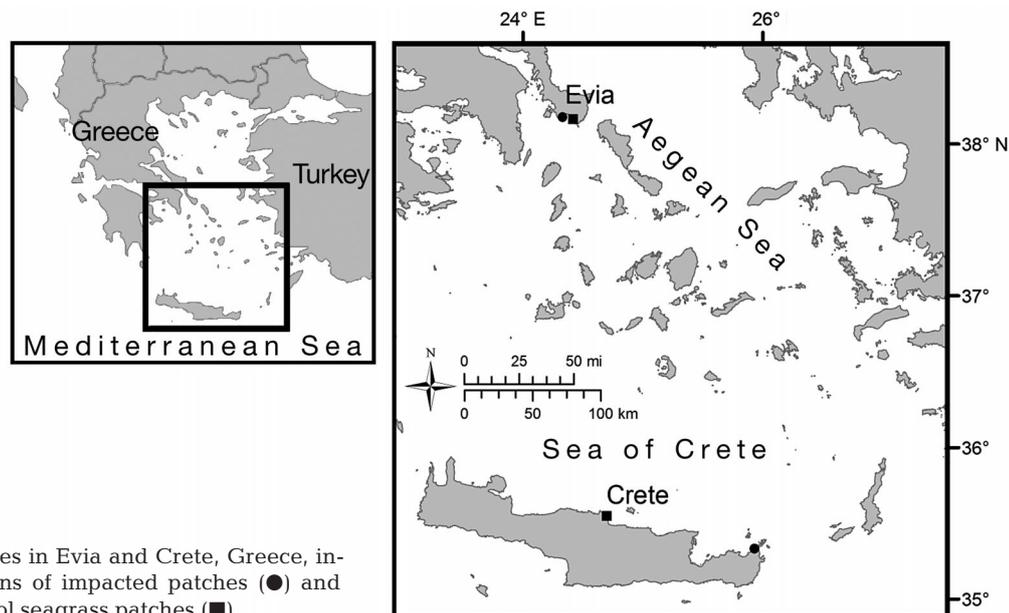


Fig. 1. Study sites in Evia and Crete, Greece, indicating locations of impacted patches (●) and control seagrass patches (■)

tribute to seagrass meadow decline and reduced productivity in meadows affected by cage culture (Ruiz et al. 2009). Understanding the various effects of coastal aquaculture on surrounding seagrass ecosystems is therefore vital in managing the continued practice and future expansion of this industry.

The effects of relatively large-scale fish farming operations on *Posidonia oceanica* meadows at depths ≥ 5 m have received considerable attention in the Mediterranean Sea (Pergent-Martini et al. 2006, Holmer et al. 2008). Much less research has evaluated the effects of smaller-scale operations on shallower (2 to 3 m) seagrass patches found closer to the shore. Shallow patches are ecologically important as they are more productive (leaf and rhizome) and have higher shoot density per square meter (Pergent-Martini et al. 1994) than conspecifics in deeper meadows, while also providing substantial forage for herbivores (Tomas et al. 2005b). The objectives of this study were to evaluate the effects of both small-scale and large-scale fish farm production on shoot morphometrics, productivity, sea urchin grazing pressure and nutrient content in shallow *P. oceanica* seagrass ecosystems. This research (1) examined seagrass shoot morphometrics and productivity between control seagrass patches and those affected by fish farming (impacted) on 2 Greek islands, (2) quantified sea urchin density and herbivore feeding pressure, (3) quantified epiphyte load across all patches and (4) evaluated nitrogen and carbon concentrations in seagrass and epiphyte tissues. These parameters were selected as they are commonly used (Pergent-Martini et al. 2005) and provide reliable means to evaluate the effects of aquaculture on the Mediterranean Sea.

MATERIALS AND METHODS

Experimental design

This study examined 4 monotypic and isolated patches of the seagrass *Posidonia oceanica* twice during February to May in 2008. Sampled seagrass patches were located in the coastal waters of 2 Greek islands, Evia and Crete (Fig. 1). On each island, one impacted seagrass patch and one control seagrass patch were sampled. Seagrass patches were isolated and no other seagrasses were in the visible vicinity (~ 50 m), as assessed by SCUBA divers. Impacted seagrass patches were located near fish farms (100 m southwest of cages in Evia and 200 m southeast of cages in Crete), while control seagrass patches were located far away (7 km east of cages in Evia and

100 km west of cages in Crete). All 4 sampled patches were selected based on their similarity in area (~ 12 m²), depth (~ 2 to 3 m), distance from land (~ 4 to 5 m) and isolation from other patches or meadows. Although the control patch in Crete was much farther away from the impacted patch, it fulfilled the above requirements, as no other appropriate control patch was found in the same bay as the fish farm. Water temperature and salinity were measured and recorded during each sampling event by using SCUBA diver computers (Sherwood Profile Dive Computer) and hand-held refractometers (A. Krüss HR27-100).

Study sites

Seagrass patches were selected to ensure they were located in bays of similar coastal morphology and human development, in which there was no riverine input and seasonal tourism was modest. Aquaculture effluent was believed to be the only significant source of nutrient pollution to the impacted seagrass patches on both islands, where no major urban development was present within 1 km. The Sitia fish farm (Crete) at the time of this study operated approximately 45 to 50 net cages and produced about 1000 t yr⁻¹ of sea bass and gilthead seabream (Pitta et al. 2009). Cages were suspended above depths that ranged from 15 to 30 m over coarse to fine grain sand (Papageorgiou et al. 2009). In total, approximately 2000 t of feed were released to farmed fish annually at this site (Pitta et al. 2009), which led to enriched water concentrations of dissolved inorganic nitrogen and phosphorus in the surrounding area (Pitta et al. 2006). In contrast, the fish farm in Karystos (Evia) was much smaller and had only 4 to 5 seasonally active net pens that produce about 75 t yr⁻¹ of mostly gilthead seabream and some sea bass. Cages were located over a depth of 15 m.

Granulometry and sediment organic matter

For each sampling event, February/March and April/May, 4 sediment cores (4.5 cm inner diameter) were collected by SCUBA divers at each seagrass patch in Crete. One core was taken for analysis of the sediment grain size (0 to 5 cm depth), while the other cores were used for analysis of loss on ignition (LOI) organic matter at 0 to 1, 1 to 3, and 3 to 5 cm depths. Cores were taken < 0.5 m outside of the seagrass patch, at a depth of ≥ 15 cm. Although we first

attempted to obtain core samples inside seagrass patches, the core samplers could not penetrate to the proper depth. Collected sediment was extruded, sectioned and frozen. For granulometry samples, sediment was freeze-dried (Chemlab SB4) and passed through a series of sieves (Gray 1981). In April/May, only 2 cores were taken for LOI analysis in impacted and control patches. Samples for LOI analysis from each respective depth and from both sampling events were freeze-dried, massed for initial weight, combusted in ceramic crucibles in a muffle furnace (Nabertherm B170) at 500°C for 48 h and reweighed. The organic content of the sediment was recorded as the percent difference in mass between the initial dried sample and the combusted sample (Kristensen & Andersen 1987).

Seagrass productivity, morphometrics and epiphyte load

Seagrass productivity was evaluated in all seagrass patches during both February/March and April/May sample periods, which corresponded to periods of low and high fish farm feeding rates, respectively (Papageorgiou et al. 2009). In each seagrass patch, 3 PVC rings (16.5 cm diameter) were haphazardly thrown by SCUBA divers. Rings that landed <1 m from the seagrass patch's edge were excluded to eliminate potential edge effects. All shoots originating from inside the rings were marked for productivity analysis by using a modified seagrass marking technique (Alcoverro et al. 2000). Marked shoots were allowed to grow for at least 27 to 48 d before they were harvested. All shoots within each respective PVC ring were then placed into separate dive bags and returned to the lab for processing. Marked seagrass shoots were randomly selected from each dive bag for productivity measurements. Approximately 30 shoots (3 replicates of 10 per sampling date) were collected from each seagrass patch to estimate productivity in each patch.

For all shoots, after blades were rinsed and epiphytes were removed by gently scraping with a razor blade, total leaf length and width were measured to the nearest millimeter. All leaf material from the shoot base to the needle mark and newly germinated seagrass blades with no needle mark were considered to be new growth, while the remaining material was considered to be old growth. All new and old leaf biomasses were separated and freeze-dried before weighing. Epiphyte loads (mg epiphyte g⁻¹ seagrass) were calculated by using shoots gathered indepen-

dently from those collected for productivity measurements. During February/March sampling, SCUBA divers haphazardly collected 100 shoots from each seagrass patch (control and impacted) at both islands. Similarly, in April/May, 30 shoots were haphazardly collected from all patches. Shoots and epiphytes were processed and weighed following the procedures described above; however, epiphyte loads were not quantified for shoots collected during April/May as seagrass biomass was not weighed.

Nutrient content in leaves and epiphytes

Total nitrogen and carbon content of seagrass blades and epiphytes were quantified during February/March for seagrass patches in Evia and Crete, corresponding with the time at which the highest N content is observed in *Posidonia oceanica* (Alcoverro et al. 2000). Dried seagrass biomass and epiphytes were pulverized into a fine powder, and total nitrogen and carbon were determined by oxidation in a Flash EA 1112 elemental analyzer (CE Instruments) following the methods of Atkinson & Smith (1983).

Sea urchin density and herbivore feeding pressure

Seagrass shoot density (shoots m⁻²) and sea urchin density (ind. m⁻²) were estimated by means of benthic surveys, in which all shoots and urchins were counted within 5 replicate and haphazardly tossed 0.25 m² quadrats. Quadrats were rejected if they landed within 0.5 m of the patch edge. Benthic surveys were conducted in all seagrass patches during February/March and April/May.

Herbivore feeding pressure was quantified by using shoots harvested for epiphyte load quantification. In the lab, harvested shoots were cut from rhizomes at the ligula and carefully cleaned of all epiphytes. Because both sea urchin *Paracentrotus lividus* and fish *Sarpa salpa* make species-specific grazing scars (Boudouresque & Meinesz 1982), shoots could be scored into one of the following categories: no grazing scars (NG), sea urchin scars only (U), fish scars only (F) and both sea urchin and fish scars (B). Herbivore feeding pressure was quantified on a per shoot basis by means of a modified grazing scar frequency method developed by Alcoverro et al. (1997), in which the total number in each scar category was divided by the total number of shoots examined.

Table 1. Results of a 2-level nested ANOVA with 'Farming intensity/Island' (small-scale, Evia vs. large-scale, Crete) as a fixed factor nested within another fixed factor, 'Fish farming' (impacted patch versus control patch); $p < 0.05$ was considered significant. LOI: loss on ignition; SS: seagrass shoot. Transformation functions applied to data that did not meet assumptions of normality (Shapiro-Wilks test; level of significance, α , in parentheses) are shown

Variable	— Farming intensity/Island —				— Fish farming —				Transformation (α)
	df	MS	<i>F</i>	<i>p</i>	df	MS	<i>F</i>	<i>p</i>	
LOI (0–1 cm)					1	0.000	0.225	0.648	x^2 (0.08)
LOI (1–3 cm)					1	0.000	1.657	0.234	x (0.19)
LOI (3–5 cm)					1	0.005	1.064	0.333	$-x^2$ (0.24)
Leaf length (mm)	1	11.150	1.117	0.304	2	405.570	40.622	<0.0001	Ranks ^a
Leaf width (mm)	1	0.302	1.537	0.230	2	3.108	15.805	<0.0001	x (0.95)
Standing crop (g m ⁻²)	1	0.001	0.049	0.828	2	0.747	56.030	<0.0001	$\log_{10}x$ (0.10)
Shoot productivity (mg SS ⁻¹ d ⁻¹)	1	0.001	0.047	0.832	2	0.331	25.011	<0.0001	$\log_{10}x$ (0.30)
Epiphyte load (mg epiphyte g ⁻¹ grass)	1	1963890.000	225.954	<0.0001	2	479528.000	55.172	<0.0001	Ranks ^a
Total N (%N), leaf	1	2.357	203.823	<0.0001	2	0.335	28.997	0.0002	x (0.42)
Total C (%C), leaf	1	0.070	0.282	0.610	2	4.326	17.505	0.001	x (0.89)
Total N (%N), epiphyte	1	0.053	22.420	0.001	2	0.017	7.253	0.016	$-x^{-1}$ (0.38)
Total C (%C), epiphyte	1	0.000	29.198	0.001	2	<0.0001	2.414	0.151	$-x^2$ (0.60)
Seagrass density (shoots m ⁻²)	1	0.673	7.141	0.011	2	1.744	18.511	<0.0001	$x^{0.25}$ (0.36)
Sea urchin density (ind. m ⁻²)	1	90.000	1.709	0.199	2	1581.430	30.025	<0.0001	Ranks ^a

^aFollowing Conover & Iman (1981)

Statistical analysis

A 2-level nested analysis of variance (ANOVA) with 'Farming intensity/Island' (small-scale, Evia vs. large-scale, Crete) used as a fixed factor nested within another fixed factor, 'Fish farming' (impacted patch vs. control patch). Statistical analyses were conducted using R statistical software (CRAN R v. 2011.1, package stats, www.R-project.org/) at an α of 0.05. All data was analyzed for normality using a Shapiro-Wilk normality test prior to analysis. Data that did not meet assumptions of normality were transformed prior to analysis by using functions in Table 1. Data for leaf length (mm), urchin density (ind. m⁻²) and epiphyte load (mg g⁻¹ seagrass) could not be transformed to pass normality tests and analyses were run on ranked data following Conover & Iman (1981). Leaf length and epiphyte load failed to have equal variance ($p < 0.05$), while sea urchin density data passed tests of equal variance ($p = 0.121$). We acknowledge that the sampling design had limitations, as a result of having only sampled one control

and one impacted patch on each island. As intra-island variability was not assessed in this study, significant differences between impacted and control patches and among islands were carefully interpreted. Table 1 contains a summary of our statistical analysis and significance levels for all variables.

RESULTS

Study site parameters

Water temperature and salinity measurements were similar between impacted and control patches on each island. While water temperatures at Evia and Crete ranged from 15 to 16°C in February/March, they rose to 19 and 20°C, respectively, in April/May. Salinity values ranged from 38 to 40 in Evia and 40 to 41 in Crete during February/March, and decreased to 37/38 and 38/39 psu, respectively, during April/May, presumably owing to increased precipitation.

Table 2. Sediment variables (mean \pm SD) for control and impacted *Posidonia oceania* patches at Crete. ϕ : Krumbein (1934) ϕ scale for grain size analysis

Seagrass	Loss on ignition (LOI)			Granulometry	
	0–1 cm depth (mg)	1–3 cm depth (mg)	3–5 cm depth (mg)	% coarse to fine sand (4–2 ϕ)	% silt–clay
Control	0.012 \pm 0.001	0.011 \pm 0.001	0.011 \pm 0.001	99.801 \pm 0.004	0.199 \pm 0.004
Impacted	0.011 \pm 0.003	0.012 \pm 0.000	0.055 \pm 0.096	99.867 \pm 0.135	0.133 \pm 0.135

Granulometry and LOI data collected and analyzed for patches in Crete were similar between control and impacted seagrass patches. Both Cretan seagrass patches sampled had sediments composed almost entirely of fine sand ($4 > \phi > 2$ fraction, Table 2; Papa-georgiou et al. 2009). Sediment organic content in the impacted seagrass patch was not significantly different (Table 1) compared with the control seagrass patch at any sediment depth analyzed (Table 2).

Seagrass morphometrics, productivity and epiphyte load

Seagrass shoots in impacted patches were significantly shorter and narrower (Table 1) than control patches at both islands (Fig. 2). Specifically, seagrass leaves were 2.8 and 4 times shorter and 0.8 and 1.25 mm narrower in impacted seagrass patches compared with control patches at Evia and Crete, respectively. Shoot productivity ($\text{mg shoot}^{-1} \text{d}^{-1}$) was also significantly reduced in impacted patches (Table 1), and control patches exhibited 1.94 and 2.45 times greater productivity than impacted patches at Evia and Crete, respectively (Fig. 2). Epiphyte loads ($\text{mg epiphyte g}^{-1}$ seagrass) were significantly greater

(Table 1) in impacted seagrass patches than in control patches on both islands during February/March, but this difference could not be attributed to fish farming as significant differences in epiphyte loads between islands were also found (Fig. 3).

Nutrient content in leaves and epiphytes

Nitrogen content of seagrass tissues were reduced in Evian seagrass patches compared with that in Cretan patches (Fig. 4). At both islands N content of seagrass tissues in the impacted patch were significantly greater than those in respective control seagrass tissues (Fig. 4), but this could not be specifically attributed to fish farming, as a significant island effect was found (Table 1). Leaf tissue carbon content, however, was significantly greater (Table 1) in impacted patches at both islands (Fig. 4). Ratios of carbon to nitrogen (C:N) were higher in control seagrass tissues examined compared with those from impacted seagrass patches at both islands. C:N ratios for seagrass tissues from Evian patches were higher than the respective Cretan seagrass patches (Table 3).

Nitrogen content in epiphytes tissues was significantly different between islands (Table 1), and this overshadowed the differences seen between seagrass patches (Fig. 4). Carbon content in epiphytes did not differ significantly between control and impacted seagrass patches at either island, but did differ significantly between islands (Table 1), and Evian epiphytes had lower carbon content than those in Cretan patches.

Seagrass and sea urchin survey

Seagrass shoot density among all patches, islands and sampling events ranged from 272 ± 72 (mean \pm SD) to 799 ± 39 shoots m^{-2} . In Evia the control seagrass patch was denser than the impacted patch (Table 3) while in Crete, shoot density in the impacted patch was denser than the control patch. A significant difference in shoot density between islands was also found, suggesting that other factors besides fish farming may also be affecting seagrass density.

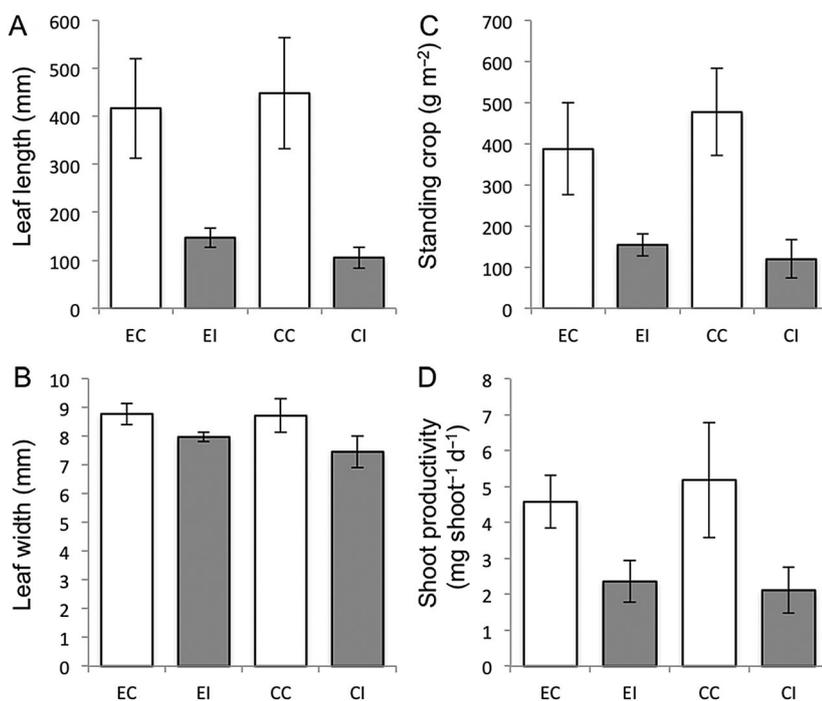


Fig. 2. *Posidonia oceanica*. Seagrass variables (mean \pm SD) in control and impacted seagrass patches in Evia and Crete: (A) leaf length (mm), (B) leaf width (mm), (C) standing crop (g shoot m^{-2}) and (D) shoot productivity ($\text{mg shoot}^{-1} \text{d}^{-1}$). EC: Evia control; EI: Evia impacted; CC: Crete control; CI: Crete impacted

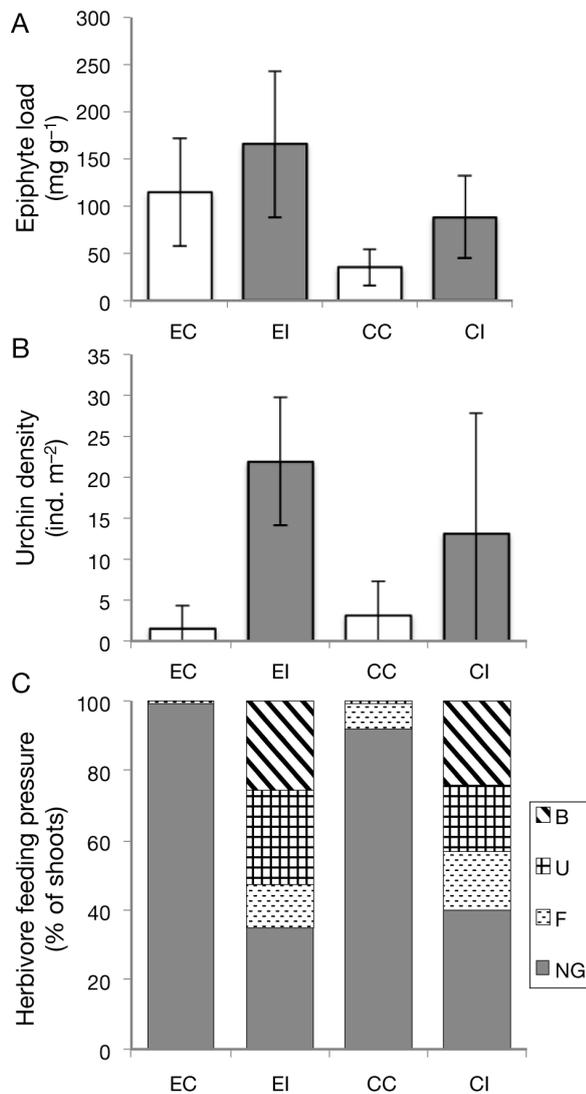


Fig. 3. *Posidonia oceanica*. (A) Mean \pm SD epiphyte load (mg dry weight g^{-1}) in seagrass patches sampled in February/March. (B) Mean sea urchin *Paracentrotus lividus* density \pm SD (ind. m^{-2}). (C) Herbivore feeding pressure on seagrass shoots across seagrass patches. B: % seagrass shoots with both fish and urchin grazing scars; U: % seagrass shoots with at least one urchin grazing scar; F: % seagrass shoots with at least one fish grazing scar; NG: % shoots with no grazing scars. EC: Evia control; EI: Evia impacted; CC: Crete control; CI: Crete impacted

Paracentrotus lividus counts were consistently greater in impacted seagrass patches at both islands. Impacted patches at both Evia and Crete had significantly higher (Table 1) sea urchin densities (mean = 18 ± 12 ind. m^{-2} , mode = 12 ind. m^{-2} , $n = 20$) than those in control patches (mean = 2 ± 4 ind. m^{-2} , mode = 0 ind. m^{-2} , $n = 20$) (Fig. 2). In control patches at both islands, 60% of surveyed quadrats had no sea urchins, while at least 1

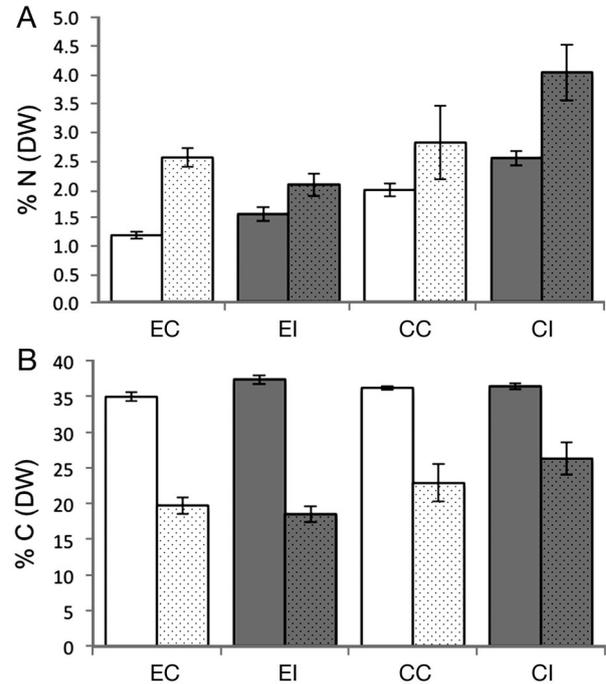


Fig. 4. *Posidonia oceanica*. Mean \pm SD tissue (A) N content (% dry weight [DW]) and (B) C content (% DW) for leaf (plain bars) and epiphyte (dotted bars) in control and impacted seagrass patches during February/March. EC: Evia control; EI: Evia impacted; CC: Crete control; CI: Crete impacted

Table 3. *Posidonia oceanica*. Seagrass shoot density (mean \pm SD) and carbon to nitrogen (C:N) nutrient ratios (mean % DW) for leaf and epiphyte tissues in control and impacted seagrass patches at Evia and Crete

Site	Seagrass shoot density (shoots m^{-2})	C:N ratio for leaf tissue	C:N ratio for epiphyte tissue
Evia			
Control	666 ± 169	30.1	7.8
Impacted	407 ± 76	24.3	9.0
Crete			
Control	319 ± 88	18.5	8.2
Impacted	549 ± 145	14.8	6.5

urchin was found in every replicated quadrat in impacted patches. Sea urchins were found at all strata of the seagrass ecosystem, and ranged from the upper canopy to the exposed rhizosphere.

Herbivore feeding pressure

Total herbivore feeding pressure (percentage of shoots with herbivore grazing scars) was low in control patches (0.5% at Evia and 8% at Crete) while

impacted patches at Evia and Crete had much higher values (65.5 and 60%, respectively) (Fig. 2). Differences among specific categorical groups (sea urchin scar only, fish scar only, and both urchin and fish scar) were also observed. Sea urchin feeding pressure was greater than fish feeding pressure in impacted patches but not in control patches for both islands. Urchin feeding pressure in impacted patches accounted for 42 and 32% of the grazing scars found on shoots at Evia and Crete sites, respectively. When shoots that had scars from both sea urchins and fish were included, urchin scars were found on 53 and 43% of the shoots examined in impacted patches at Evia and Crete, respectively.

DISCUSSION

Coastal aquaculture is considered to be a significant threat to *Posidonia oceanica* conservation in the Mediterranean Sea (Pergent-Martini et al. 2006, Holmer et al. 2008). While research to elucidate the full effects of these operations is still in its infancy, previous efforts have focused on elucidating effects on seagrass meadows at depths ≥ 5 m in the vicinity of fish farm cages. Only a handful of studies have examined the potential effects on shallower seagrass meadows and patch communities (Delgado et al. 1997, 1999, Pergent et al. 1999, Ruiz et al. 2001), which are more productive (Pergent-Martini et al. 1994) and support higher herbivore densities (Tomas et al. 2005b, Prado et al. 2007a) than those at deeper depths. This study provides descriptive and correlative data to increase our understanding of the multiple effects that fish farms have on shallow seagrass patches. Our results demonstrate that substantial direct and indirect negative effects of aquaculture can be found in shallow seagrass patches adjacent to both large (Crete) and smaller (Evia) fish farming operations.

Seagrass patches sampled adjacent to fish farms on both islands were substantially degraded compared with control seagrass patches, as indicated by significantly reduced mean leaf length, width and productivity. Differences in leaf length between control and impacted patches on both islands in this study were comparable to those found in previous studies (Delgado et al. 1999, Dimech et al. 2002, Apostolaki et al. 2009), and control shoots were approximately 2 to 4 times longer than those in impacted patches. Shoot productivity was significantly lower in impacted patches compared with control patches at both islands (Fig. 2) and within or near ranges found by other studies in the Mediterranean Sea (2 to 4 mg

shoot⁻¹ d⁻¹ in impacted seagrasses and 6 to 7.25 mg shoot⁻¹ d⁻¹ in control seagrasses (Ruiz et al. 2001, 2009). Shoot productivity was not significantly different between the islands, although true comparisons were not possible in the present study as the distance of impacted patches from the respective fish farms was not uniform, and bottom current velocity and water circulation were not monitored.

Sediment organic matter from LOI revealed no differences in sediments between control and impacted sites in the largest fish farm in this study (Crete). Previous studies that have examined sediments under and in close proximity to fish farm cages (0 to 40 m) have revealed 2- to 4-fold increases in organic matter (Karakassis et al. 2000, Ruiz et al. 2001), and enrichment of sediments decreased as distance from the fish farms increased. In our study, it was not surprising that organic enrichment of sediments was not found, as the impacted seagrass meadow in Crete was over 200 m away from fish cages. Although organic matter enrichment could not be detected in these sediments and increased epiphyte loads in impacted patches could not be attributed to fish farming, seagrasses seemed responsive to nutrient enrichment from fish farming activities, as C:N ratios in leaf tissues from affected sites were lower than control tissues at both islands. Negative effects in impacted patches in this study seem to be more attributable to indirect factors such as increased herbivory compared with direct factors, although other stressors (e.g. porewater sulfide toxicity) cannot be definitively ruled out.

Impacted seagrass patches in this study had significantly higher densities of sea urchins compared with the respective control patches. Previous studies conducted at greater depths found similarly high sea urchin densities ranging from 11 to 50 ind. m⁻² (Holmer et al. 2008, Ruiz et al. 2009) in impacted seagrass meadows. Sea urchin densities in our control patches were comparable with densities found in pristine seagrass patches and ranged from 0 to 6 ind. m⁻² (Boudouresque & Verlaque 2001). Not only were higher densities of urchins found in impacted patches, but urchin feeding pressure was considerably greater, and 53 and 43% of all shoots had at least one urchin scar at Evia and Crete, respectively. In control patches the feeding pressure was almost exclusively exerted by fish, and 0% of shoots had an urchin scar at Evia and only 1% at Crete.

Sea urchin populations in Mediterranean seagrasses are recruitment limited as evidenced by the lack of certain sized cohorts of the population (Tomas et al. 2004). Tomas et al. (2004) further suggested that

urchin populations in healthy seagrass meadows are maintained by the migration of larger individuals from adjacent rocky areas. While the factors leading to urchin aggregations in impacted seagrass meadows are not fully understood, they may partially be due to urchin attraction or migration toward increased organic matter in the vicinity of a cage or more abundant and palatable epiphyte food sources (Holmer et al. 2003, Balata et al. 2010). This is especially likely as 90% of the nitrogen assimilated by *Paracentrotus lividus* is derived from epiphyte food (Tomas et al. 2006) and sea urchins preferentially graze epiphytized seagrass leaves before consuming unepiphytized leaves (Boudouresque & Verlaque 2001). In our study, we cannot definitively attribute observed higher epiphyte loads and enriched leaf tissue nitrogen content to fish farming, but it is likely a major contributor. Combining these factors may partially explain the significantly greater urchin densities and feeding pressures observed in impacted patches on both islands. Similar preferential grazing by herbivores on more palatable or nitrogen-rich plant material is not uncommon and has been documented for a variety of marine fauna (Bjorndal 1980, Boyer et al. 2004, Goecker et al. 2005, Tewfik et al. 2005).

Since intra-island variability in seagrass patches was not assessed in the present study, oceanographic conditions between waters surrounding the 2 islands or unknown natural or anthropogenic inputs in these regions may also be responsible for the increases in nitrogen content in seagrass and epiphyte tissues observed in these impacted sites. Additionally, taxonomic assessment of epiphyte communities in control and impacted patches was not undertaken in this study. Taxonomic differences in epiphyte communities also probably contribute to the observed increases in nitrogen content and palatability of epibiota (Balata et al. 2010) and may possibly explain the differences in nitrogen content of epiphyte tissues across patches and islands.

Sea urchins are a major structuring force in seagrass meadows of the Mediterranean Sea (Prado et al. 2007b); however, few studies have directly quantified urchin herbivory in seagrass patches affected by aquaculture. Ruiz et al. (2009) demonstrated that urchin grazing of aquaculture-influenced seagrasses effectively reduced areal coverage by 50% relative to caged exclusions. Although we did not perform a similar experiment to that of Ruiz et al. (2009), sea urchins in this study were commonly observed grazing in seagrass canopies of impacted seagrass patches at both islands, while this behavior was

never observed in the taller seagrass canopies of control patches. Mechanical breakage due to increased epiphyte loading in nutrient enriched meadows (Leoni et al. 2006, 2008) and increased grazing (Ruiz et al. 2001, 2009) probably caused the reductions of leaf length observed in this study. Consequently, these shorter seagrass shoots had lower C:N ratios and had greater epiphyte loads than control conspecifics. These factors may provide an explanation for the increased sea urchin density and increased feeding pressures observed in impacted patches, as the food material present is more dense, palatable and accessible.

Holmer et al. (2008) recommended a 400 m minimum operating distance for fish farming operations to avoid adversely affecting surrounding *Posidonia oceanica* ecosystems. These recommendations were based on relatively productive (260 to 1150 t yr⁻¹) fish farms located far (500 to 2600 m) from the shore (Holmer et al. 2008). The fish farms examined in the present study were substantially closer to shore (<100 m) and represented both large (Crete) and small (Evia) annual productions. Our results demonstrate that substantial degradation of *Posidonia oceanica* patches can occur regardless of the scale of fish farming intensity, and large declines were observed both 200 m from the Crete fish farm and 100 m from the Evia fish farm. Although a definitive safe operating distance cannot be proposed from our results, we emphasize the importance of proper zoning regardless of fish farming intensity. Further evaluation of the effects of small intensity aquaculture operations is warranted, especially as this has not been studied in great detail in the Mediterranean Sea.

In conclusion, this study presents evidence of the negative effects of fish farming on patches of the seagrass *Posidonia oceanica* in shallow water by demonstrating significant reductions in seagrass morphology and productivity in response to both large (1000 t yr⁻¹) and small (75 t yr⁻¹) scale fish farming intensities. Aside from the direct negative effects of aquaculture on these seagrasses, we suggest that indirect effects can be equally as substantial to seagrass ecosystem health. Increased herbivore densities and feeding pressures in impacted patches at both islands suggest that grazing by sea urchins is probably enhanced indirectly by aquaculture effluent in Greece. Our results support the application of scientifically recommended zoning policies (Holmer et al. 2008) for coastal aquaculture regardless of production intensity to mitigate potential direct and indirect threats to surrounding seagrass ecosystems.

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