2018-09

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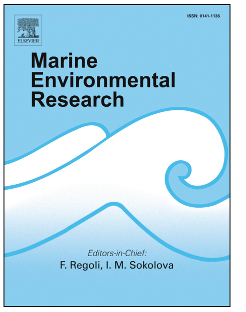
Kletou, D

http://hdl.handle.net/10026.1/11665

10.1016/j.marenvres.2018.06.007
Marine Environmental Research
Elsevier

Accepted Manuscript



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PII: S0141-1136(18)30183-1

DOI: 10.1016/j.marenvres.2018.06.007

Reference: MERE 4543

To appear in: *Marine Environmental Research*

Received Date: 7 March 2018

Revised Date: 9 June 2018

Accepted Date: 11 June 2018

Please cite this article as: Kletou, D., Kleitou, P., Savva, I., Attrill, M.J., Antoniou, C., Hall-Spencer, J.M., Seagrass recovery after fish farm relocation in the eastern Mediterranean, *Marine Environmental Research* (2018), doi: 10.1016/j.marenvres.2018.06.007.

Seagrass recovery after fish farm relocation in the eastern Mediterranean

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ABSTRACT

Finfish aquaculture has damaged seagrass meadows worldwide as wastes from the farms can kill these habitat-forming plants. In Cyprus, the Mediterranean endemic *Posidonia oceanica* is at its upper thermal limits yet forms extensive meadows all around the island. Understanding this under-studied isolated population may be important for the long-term survival of the species given that the region is warming rapidly. When fish farming began around Cyprus in the 90s, cages were moored above seagrass beds, but as production expanded they were moved into deeper water further away from the meadows. Here, we monitored the deepest edge of meadows near fish farms that had been moved into deeper waters as well as at a decommissioned farm site. Four *P. oceanica* monitoring systems were set up using methods developed by the Posidonia Monitoring Network. Seagrass % coverage, shoot density, % of plagiotropic rhizomes, shoot exposure, leaf morphometry, and sediment organic matter content and grain size were monitored at 11 fixed plots within each system, in 2012-2014 and in 2017. Expansion at the lower depth limit of seagrass meadows was recorded at all monitoring sites. Most other *P. oceanica* descriptors either did not change significantly or declined. Declines were most pronounced at a site that was far from mariculture activities but close to other anthropogenic pressures. The most important predictor affecting *P. oceanica* was depth. Monitoring using fixed plots allowed direct comparisons of descriptors over time, removes patchiness and intra-meadow variability increasing our understanding of seagrass dynamics and ecosystem integrity. It seems that moving fish farms away from *P. oceanica* has helped ensure meadow recovery at the deepest margins of their distribution, an important success story given that these meadows are at the upper thermal limits of the species.

Keywords: aquaculture; bioindicators; Cyprus; ecological monitoring; ecosystem change; eastern Mediterranean; seagrass.

1. INTRODUCTION

Seagrasses are major contributors to human well-being and the economies of coastal countries (Barbier et al., 2011; Campagne et al., 2015; Dewsbury et al., 2016). Their meadows are among the most productive ecosystems on Earth but are declining at unprecedented rates (Waycott et al., 2009; Costanza et al., 2014). They provide: coastal protection from erosion, by attenuating waves and stabilising sediments; water purification by assimilating nutrients and pollutants; transfer of matter and energy up trophic levels and sustaining fisheries; carbon sequestration to help mitigate climate change; and provision of complex habitat for enhanced biodiversity, which boosts tourism, recreation, education and research (Barbier et al., 2011; Campagne et al., 2015). However, there are multiple mounting pressures, including: sediment and nutrient runoff, physical disturbance, invasive species, disease, commercial fishing practices, aquaculture, overgrazing, algal blooms and global warming, which have caused major declines in seagrasses, raising awareness of the need to protect, monitor, manage and restore these habitats (Orth et al., 2006; Barbier et al., 2011).

The endemic seagrass *Posidonia oceanica* (Linnaeus) Delile, 1813, forms one of the most important coastal ecosystems in the Mediterranean Sea. Its rhizomes propagate vertically as well as horizontally, producing reefs called “matte” that can extend many meters down into the sediment and persist for millennia, resulting in the largest documented stores of organic carbon among seagrasses (Buia et al., 2004; Lo Iacono et al., 2008; Fourqurean et al., 2012; Lavery et al., 2013). The seagrass forms meadows that can extend up to 15 km wide and clone for thousands, possibly tens of thousands of years (Arnaud-Haond et al., 2012). The structurally complex meadows are the climax stage of many upper subtidal bottoms extending from the surface down to depths of 40-45 m in oligotrophic clear waters; supporting hundreds of associated species (Piazzi et al., 2016). An estimated 34% of *P. oceanica* meadows died in the last half century, classifying the *P. oceanica* habitat as an ‘endangered’ ecosystem (Telesca et al., 2015).

The Mediterranean coast is home to about 250 million people and supports about one third of all global tourism, which is anticipated to reach 0.5 billion arrivals per year by 2030 (Randone et al., 2017). Residents and tourists place a high demand on seafood. In the last two decades, there has been a dramatic growth in the Mediterranean aquaculture production expanding approximately 5% annually (Massa et al., 2017). The development of fish aquaculture along the Mediterranean coasts has caused localised losses of *P. oceanica* (Delgado et al., 1997; Pergent et al., 1999; Ruiz et al., 2001; Cancemi et al., 2003; Pergent Martini et al., 2006; Diaz-Almela et al., 2008; Holmer et al., 2008; Pérez et al., 2008; Apostolaki et al., 2009). Several factors cause this damage including reduction of light under the cages (Ruiz et al., 2001), an increase in particulate matter and nutrient concentrations in the water, which can cause an increase in epiphyte biomass (Delgado *et al*. 1997), enhanced herbivory (Holmer et al., 2003), expansion of competitive opportunistic seaweed (Holmer et al., 2009), sulphide invasion into the roots (Frederiksen et al., 2007), and high input of organic matter into the sediments (Cancemi et al., 2003; Apostolaki et al., 2007; Diaz-Almela et al., 2008). Organic enrichment may be the most important factor as this can lead to anoxic and toxic benthic conditions causing high *P. oceanica* mortality (Pérez et al., 2008). Seagrass loss can continue even after several years of fish farming cessation as the matte itself begins to rot (Delgado et al., 1999; Apostolaki et al., 2010). *Posidonia oceanica* losses are considered irreversible over human time-scales, because it grows slowly (only 3-4 cm per year) and has extremely low natural colonization rates (Boudouresque et al., 2012). Although sexual reproduction rates are speeded up by warming, seedlings usually settle at the shallow boundaries of seagrass meadows (Balestri et al., 2017). Hence, the deep and exposed seagrass meadows near fish farms have the lowest recruitment rates limited to vegetative propagation and horizontal growth under low light conditions.

Today, fish farm cages are moored 1-3 km off Cyprus in water depths of 22-75 m, cultivating mainly gilthead seabream (*Sparus aurata* Linnaeus, 1758) and European seabass (*Dicentrarchus labrax* Linnaeus, 1758). National mariculture production expanded from 210 tonnes in 1994 to 6625 tonnes in 2016, now exceeding 80% of the total fisheries production (data from Department of Fisheries and Marine Research, Cyprus). The number of licenced units has remained the same for many years and the production increase is due to existing units that have expanded production, especially during the last decade. Most fish farms are within the Vasiliko-Moni area in south Cyprus. Fish farming in the area started in the mid-1990s with small production units (100-300 tonnes per year), using floating cages starting at 22-28 m depth and over seagrass meadows. One of these units ceased operations soon after, the rest of the farms expanded and are now each licenced to produce 1000-1800 tonnes per year. A prerequisite to receive expansion permits by the national authorities was to relocate cages in deeper water and further away from the *P. oceanica* meadows. Currently, the shallowest cages in Vasiliko-Moni area are found at the depth of about 37 m, but seagrass meadows still exist within the impact zone (<400 m) of aquaculture effluents (Holmer et al., 2008).

Despite the temporal and spatial scale of this development, no studies have been conducted to evaluate the effects of the fish farm units to the adjacent *P. oceanica* meadows. In this study, four *P. oceanica* observatory systems were established near major fish farms that have just relocated and expanded deeper and at a reference (decommissioned fish farm) site in the Vasiliko-Moni area. The aims were: to assess progression or regression of *P. oceanica* meadow's edge, evaluate the changes in *P. oceanica* and sediment descriptors between the two sampling periods for each monitoring system and examine whether fish farm or environmental drivers are affecting these descriptors. Our study shows how fish farm impacts to seagrass beds can be monitored effectively, assisting integrated coastal management decisions.

2. MATERIALS AND METHODS

2.1 Study area

Four *P. oceanica* monitoring systems were set up using standardised methods developed by the Posidonia Monitoring Network (PMN) (Boudouresque et al., 2000; Pergent, 2007) at the warmest and easternmost geographic limits of *P. oceanica*. Three systems were deployed at the regressive lower limits of seagrass meadows near active fish farm sites (SW, BI, EM) that recently relocated and expanded production to deeper nearby waters (Figure 1, Table 1). During the first data collection, the three farms had a total production *ca* 2.5 kt yr^{-1} and operated shallow cages near the seagrass meadows investigated (EM) or had just relocated to deeper water as they expanded (SW and BI). At the second data collection three to five years later, the three farms had a total production larger than 4 kt yr^{-1} (Figure 1, Table 1). The fourth monitoring system (REF) was set up *ca* 300 m far from a licensed small (*ca* 100 t yr^{-1}) production unit, which operated for a few years in the nineties and ceased operations about 15 years ago. This monitoring system was set at stable lower limits of the seagrass meadow, over 3.5 km from the nearest fish farms and resembles the natural deeper boundaries of *P. oceanica* meadows in the coastal area studied (Table 1).

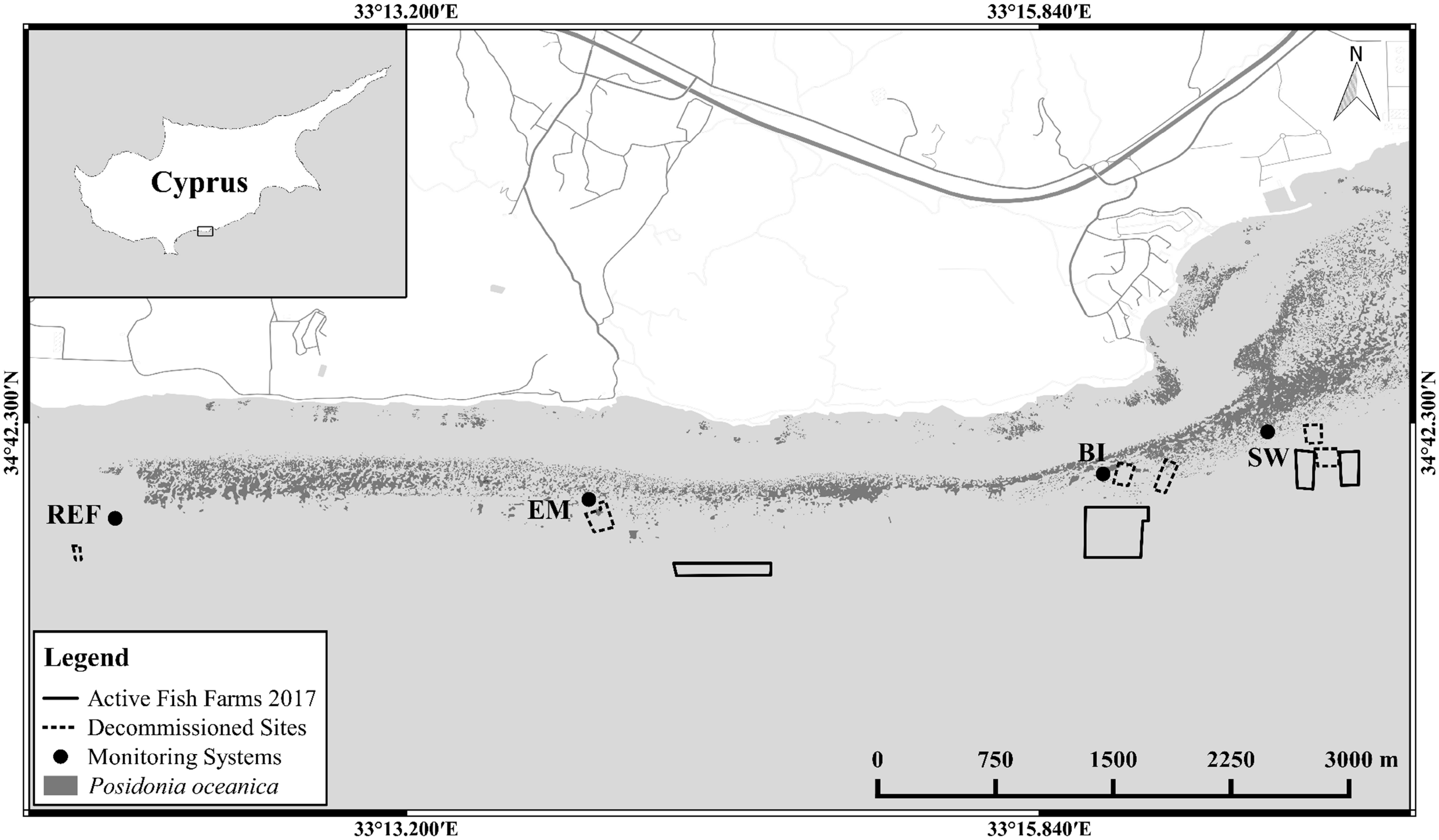


Figure 1. Locations of *P. oceanica* monitoring systems and coastal areas occupied by decommissioned and active fish farms. The surrounding meadows of *P. oceanica* are also shown.

Table 1. Details of *Posidonia oceanica* monitoring systems set up near farms operating in south Cyprus.

***Posidonia oceanica* Monitoring System – Fish farm information**	**Distance from the fish farm, depth and time of data collection.**	**Added Value**
<u>SW</u> Established in 1995 with licenced annual production 300 t yr^{-1}. Licenced production increased to 450 t yr^{-1} in 2008, 750 t yr^{-1} in 2010, 1000 t yr^{-1} in 2013, 1250 t yr^{-1} in 2014 and to 1500 t yr^{-1} after 2016. Shallow cages relocated in deeper water in 2011. # of finfish cages in 2017: 36	About 275 m northwest of the existing finfish cages and 240 m west of the previous position of cages, which were relocated deeper in 2011. The monitoring system was set up at regressive lower limits, at 25-26 m depth, in the summer of 2012. First set of data were collected the same period. Second set of data were collected in early autumn 2017.	Future monitoring and comparison with the data presented in this study, will be able to detect whether the relocation but expansion of the fish farm in deeper waters had any impacts on the adjacent *P. oceanica* meadows.
<u>BI</u> Established in 1993 with licenced annual production 300 t yr^{-1}. Licenced production increased to 500 t yr^{-1} in 2004, 900 t yr^{-1} in 2007, 1300 t yr^{-1} in 2009, 1500 t yr^{-1} in 2014 and to 1800 t yr^{-1} in 2017. Shallow cages relocated in deeper water in 2011. # of finfish cages in 2017: 66	About 250 m north of the existing finfish cages and less than 100 m west of the previous position of the cages, which were relocated deeper in 2011. The monitoring system was established at regressive lower limits, at 22-23 m depth, in the autumn of 2012. First set of data were collected two years later in autumn of 2014. Second set of data were collected in autumn 2017.	Future monitoring and comparison with the data presented in this study, will be able to detect whether the relocation but expansion of the fish farm in deeper waters had any impacts on the adjacent *P. oceanica* meadows.
<u>EM</u> Established in 1993 with a licence to produce 100 t yr^{-1}. In 2011 it received permit to produce 1000 t yr^{-1} at a new, deeper (50 m depth) site over 600 m further offshore. The old shallow mooring system was gradually decommissioned and went from 10 cages in 2012, to 6 in 2013 to 2 in 2014 to 1 in 2015. # of finfish cages in 2017: 0 in old and 22 in new mooring.	About 100 m north/northwest of the shallow mooring, which ceased operations gradually. Operations moved and expanded deeper about 750 m southeast from the monitoring system. The monitoring system was established at regressive lower limits, at 21-23 m depth, in the summer / autumn of 2012. First set of data were collected the same period. Second set of data were collected in autumn 2017.	Future monitoring and comparison with the data presented in this study will provide vital information about the recovery rates of the *P. oceanica* meadow following the cessation of mariculture operations in the near vicinity.
<u>REF</u> Farming started in mid-nineties and lasted about a decade (production *ca* 100 t yr^{-1}). It has remained inactive for *ca* fifteen years.	About 300 m northeast from a small production unit, which terminated operations a long time ago. The monitoring system was established at stable lower limits, at 28-29 m depth, in the summer of 2013. First set of data were collected the same period. Second set of data were collected in early autumn 2017.	Future monitoring and comparison with the data presented in this study will provide a point of reference for other monitoring systems and if fish farming initiates near this system it will provide baseline data and vital information about the direct effects of the fish farm on the adjacent *P. oceanica* meadows.

2.2 Dispersal of fish farm effluents

To predict the dispersal of fish farm effluents and sedimentation, we simulated the dispersion MERAMOD model developed for gilthead sea bream *S. aurata* and European sea bass *D. labrax* farming (Cromey et al., 2012). The simulation occurred in 2012, just after the relocation of BI and SW, and before the relocation of EM fish farm. Historical daily current data (2005-2010) of the surface waters and the 10 m depth zone in the study area were extrapolated using 3-D interpolations of the Cyprus Coastal Ocean Forecasting and Observing System (CYCOFOS); a validated hydrodynamic flow model covering the Levantine region (Zodiatis et. al., 2003; 2008). Two scenarios were applied that included the coldest-water period (February) and the warmest-water period (August). The latter accounts for the worst-case scenario since maximum biomass/feed input were used. Data incorporated into the MERAMOD model included: daily average current speed and direction for the months of August and February obtained from the CYCOFOS, bathymetric data at each site and a range of husbandry data collated from the managers of the three fish farms (Table 2).

Table 2. Husbandry data (year 2012) used in MERAMOD for each of the two scenarios.

Data	Scenario 1 (Winter)			Scenario 2 (Summer)		
	SW	BI	EM	SW	BI	EM
Feed input (kg d^{-1})	3665	7500	2200	7500	11000	1200
Feed input (kg $cage^{-1}$ d^{-1})	136	221	183	278	324	200
Max biomass (t)	580	1194	300	600	861	150
Cage diameter (m)	19	22	22	19	22	22
Cage surface area (m^2)	286	390	390	286	390	390
Cage volume (m^3)	4011	5459	5459	4011	5459	5459
No of cages	27	34	12	27	34	6
Feed input per day per unit cage surface area (kg d^{-1} m^{-2})	0.47	0.57	0.47	0.97	0.83	0.51

Waste feed and faeces were assigned a random starting position in the cage volume. An average settling velocity of feed pellets representing 1 to 5 mm pellets and settling velocity of faecal particles for bream and bass (Magill et al., 2006) were assigned to cages according to the percentage of bream and bass being farmed at each site. For particles between sea surface and 5 m depth, surface current speed and direction were used for advection, whereas 10 m current speed and direction were used for particles from 5 m to the sea bed. Predicted flux was scaled to standard units of g m^{-2} yr^{-1} of total dry solids. Numerous default data were used consistently across sites (Table 3), so that differences between predicted impact were primarily driven by differences between the sites in terms of depth, hydrography, feed input and husbandry data in general.

Table 3. MERAMOD default data applied across all scenarios.

Model default parameter	Value
Feed wasted, digestibility, water content	5%, 85%, 9%
Wild fish consumption of waste pellets	50 % of wasted pellets are consumed by wild fish and do not contribute to flux
Horizontal dispersion coefficients: k_x, k_y (m^2 s^{-1})	0.4, 0.1
Vertical dispersion coefficient: k_z (m^2 s^{-1})	0.001
Particle trajectory time step (seconds)	60
Feed settling velocity (cm s^{-1})	Mean = 8.4, standard deviation = 4.3
Faecal settling velocity: Sea Bream (cm s^{-1})	0.4 cm s^{-1} (24%), 1.5 (45%), 2.5 (18%), 3.0 (13%)
Faecal settling velocity: Sea Bass (cm s^{-1})	0.4 cm s^{-1} (6%), 1.4 (9%), 2.5 (20%), 3.6 (38%), 4.6 (27%)

2.3 Monitoring systems and data collection

The four monitoring systems were set up according to the 'Protocol for the setting up of *Posidonia* meadows monitoring systems «MedPosidonia» Programme' (Pergent, 2007). In each monitoring system, 11 numbered cement markers were positioned at 5 m intervals and anchored with 12 mm diameter iron stakes, at the edge of the meadow (total 50 m length). Additionally, 16 mm diameter iron "photostakes", from where photographs were taken, were hammered 50 cm into the sediment and sticking out 1 m, across each marker and the meadow's edge.

At every marker, the following variables were recorded by scuba divers: depth and angle to other markers, % seagrass cover in a 0.36 m^2 quadrat, shoot density and % of plagiotropic (horizontally oriented) rhizomes in three fixed quadrats (0.04 m^2), and shoot exposure or burial of orthotropic (vertically oriented) shoots (three replicates taken at both the edge and another three at the inner side of the meadow). Surface sediment samples were collected from each marker by a diver and granulometry was conducted using an Endecotts Octagon sieve shaker after first drying the samples at 100 ^{o}C until constant weight. The granulometry data were processed with the GRADISTAT particle size analysis software. Fine sediment passing through the 212 μm sieve was homogenised, three replicates of 1.5 g from each marker were combusted at 550 ^{o}C and the organic carbon was determined as % weight loss following ignition. In addition, about 20 randomly selected orthotropic shoots from each monitoring system were removed and leaf morphometric analyses were carried out using the technique of Giraud (1977), including estimating the foliar surface per shoot, which was later used to estimate the Leaf Area Index (LAI). The past annual *P. oceanica* leaf production rate was calculated following a standardised procedure, known as lepidochronology, which uses the thickness of the scales (previous leaf petioles that remain attached on the rhizome) to determine annual cycles (Pergent, 1990; Pergent and Pergent-Martini, 1991). The lepidochronological analysis involves carefully removing the scales from the rhizomes and ordering them from the older (near the rhizome base) to the more recent (near the living leaves). A cross section was made 10-12 mm above the base of each scale, viewed and photographed under an Olympus CX41 microscope attached to a camera. The thickness (μm) of the central/wider portion of the scale was measured with Image Pro Plus software.

About half a decade following deployment, the monitoring systems were revisited. Initially an inspection was carried out to record any missing cement markers, labels, or photostakes. Progression or regression of the edge was measured using a measuring tape from the marker's inner side to the rhizome that was furthest from the marker in progression or closest in regression. Data collection from each monitoring system was repeated (except for lepidochronology) using the same methods. At each of the 44 markers, the measurements of shoot densities and % of plagiotropic rhizomes were repeated from the same fixed quadrats.

2.4 Statistical analysis

To evaluate changes between the two sampling periods for each fish farm, a paired t-test was computed for variables taken from fixed quadrats (i.e. shoot density, plagiotropic rhizomes % and coverage %,), following Elzinga et al. (1998). Variables derived from randomly selected shoots (i.e. number of leaves, foliar surface, shoot exposure) within the meadow were compared with a 2-sample t-test (Elzinga et al., 1998). The assumptions for normality and homogeneity of variances were verified using a Shapiro-Wilk and F test, respectively and if assumptions were violated, $\log_{10}$ or square root transformations were conducted. To calculate the rate of annual leaf production of *P. oceanica* acquired from the lepidochronological analysis and examine the patterns of change over the years (increase, decrease or none), a simple linear regression was performed. When assumptions were not met, the analysis proceeded with the non-parametric Wilcoxon signed rank test for data collected from the fixed quadrats, non-parametric Mann-Whitney U test for data collected from random shoots and non-parametric regression Kendall–Theil Sen Siegel for the lepidochronological data. Shoot progression was compared between the different monitoring sites using the non-parametric Kruskal-Wallis test.

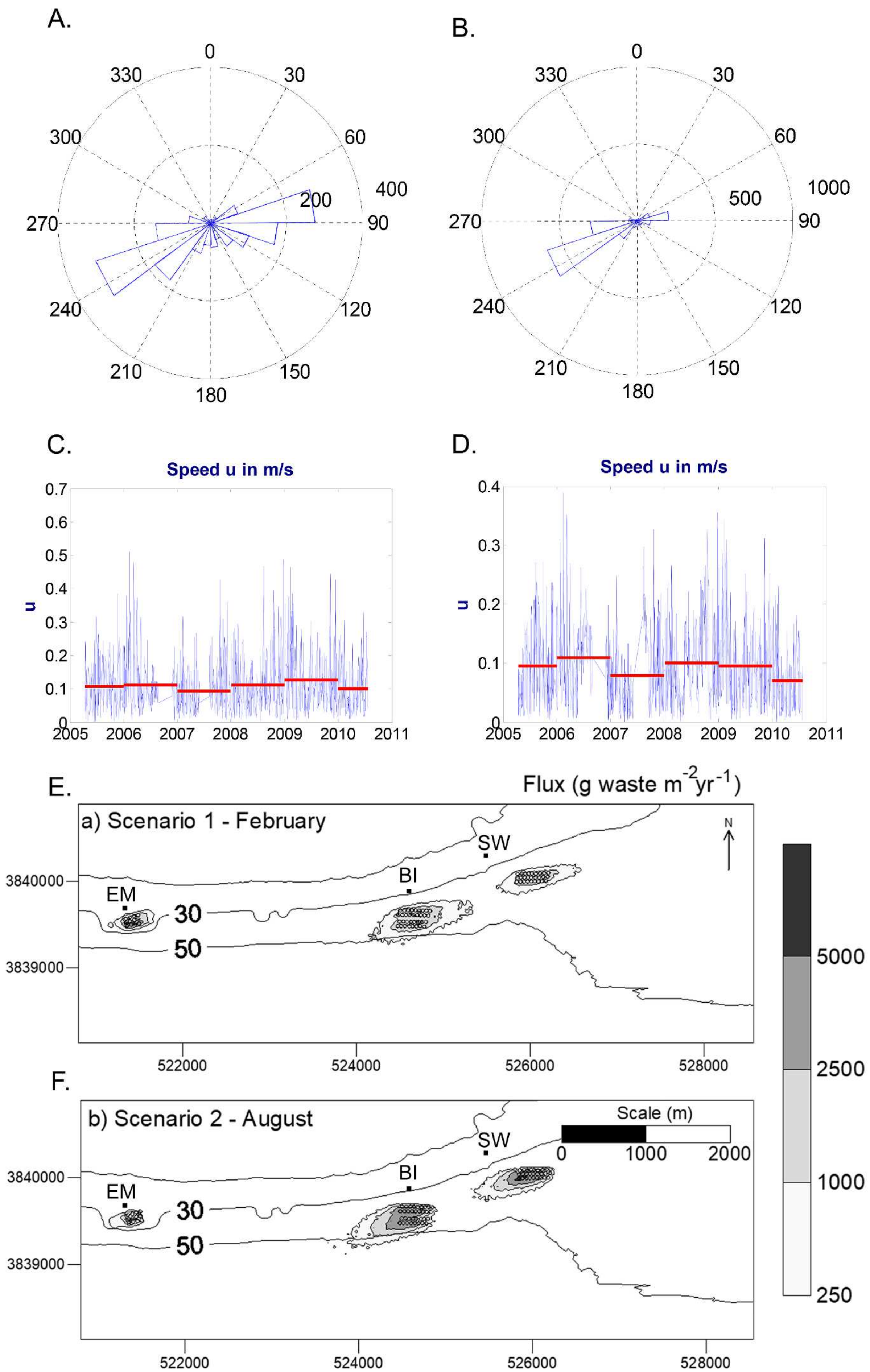
In order to identify the predictors that affect the *P. oceanica* and sediment descriptors, a multiple regression analysis was applied, where all the descriptors were categorised into two environmental predictors (depth and time) and three fish farm associated predictors (distance of a monitoring system to the nearest fish farm, direction of a monitoring system to the nearest fish farm, and the size of production of the nearest fish farm in tonnes). The assumptions for normality and heteroscedasticity were verified via Shapiro-Wilk test and Breusch-Pagan test on either untransformed or cox box transformed data. The multiple regression analysis was further complemented with the relative importance analysis, which aims to identify the factor with the highest controlling effect on the descriptor (Tonidandel and LeBreton, 2011). This was based on the calculation of lmg, the relative contribution of each predictor to the R^2, averaged over the orderings among predictors (Grömping, 2006).

For all the statistical analyses the significance level α was adjusted to 0.05, computation was carried out by R-studio (v 1.0.153) and all the graphic material was generated via the package ggplot2 (Wickham, 2009). The relative importance analysis was conducted via the package relaimpo (Grömping, 2006).

3. RESULTS

3.1 Fish farm effluents

The scalar and vector averages for the entire period considered indicated that there is alternation of surface currents towards the east and west respectively, but the prevailing average direction of the currents at 10 m depth is west – southwest (Figure 2). The predominant direction of the currents during the two scenarios simulated in the MERAMOD, February and August, were towards east and southwest respectively. For all farms, there were virtually no areas predicted to have deposition greater than 5000 g m^{-2} yr^{-1}; areas of 2500 to 5000 g m^{-2} yr^{-1} were evident for farms BI and SW but not for the small EM farm (before relocation) (Figure 2). The extent of the deposition footprints was high as a result of the reasonably high current and depth. According to the model and driven by the currents direction, the main dispersal of the effluents was not in the direction of the *P. oceanica* monitoring systems.



A.
B.
C.
D.
Speed u in m/s
u
E.
Flux (g waste m^{-2}yr^{-1})
a) Scenario 1 - February
F.
b) Scenario 2 - August
SW
BI
EM
30
50
N
Scale (m)
0
1000
2000
5000
2500
1000
250

Figure 2. *Top panel*: Angle histogram in degrees (rose diagram) showing the frequency of current direction for the whole period 2005-2010 at surface (A) and at 10 m depth (B), estimated with CYCOFOS; *Middle panel*: Sea surface current speed data and annual averages at surface (C) and at 10 m depth (D), estimated with CYCOFOS; *Bottom panel:* MERAMOD predicted waste flux (g m^{-2} yr^{-1}) under winter (E) and summer (F) scenarios.

3.2 Field Observations and Sediments

When the monitoring systems were set up in 2012-13, epiphytes and fine particulate matter were covering the leaves of *P. oceanica* (Figure 3). The lower limits investigated were sharp at the edge with high *P. oceanica* cover, surrounded by dead matte covered predominantly by *Caulerpa prolifera* (Forsskål) J.V.Lamouroux. Four to five years following the initial deployment, all 44 markers and 132 iron stakes were still in place. Only one photostake and two labels from the initial 44 were missing. Visually, the ecological condition seemed to be improved in 2017 compared to the first surveys. The fine particulates and the epiphytes covering the seagrass leaves were less pronounced, some calcareous organisms (bryozoans and rhodophytes) were found within the rhizomes under the canopy and *C. prolifera* had almost disappeared from the surface of the dead matte (Figure 4). Improvement of the ecological condition was also reflected in sediment variables. Overall, the organic matter in the sediment was reduced by about 15% and the mean grain size enlarged overall by almost 90%, from very fine sand to sand (Folk and Ward method) (Table 4).

Figure 3. Seagrass meadows near fish farms (SW left, BI right), covered in fine particles and epiphytes during first data collection.

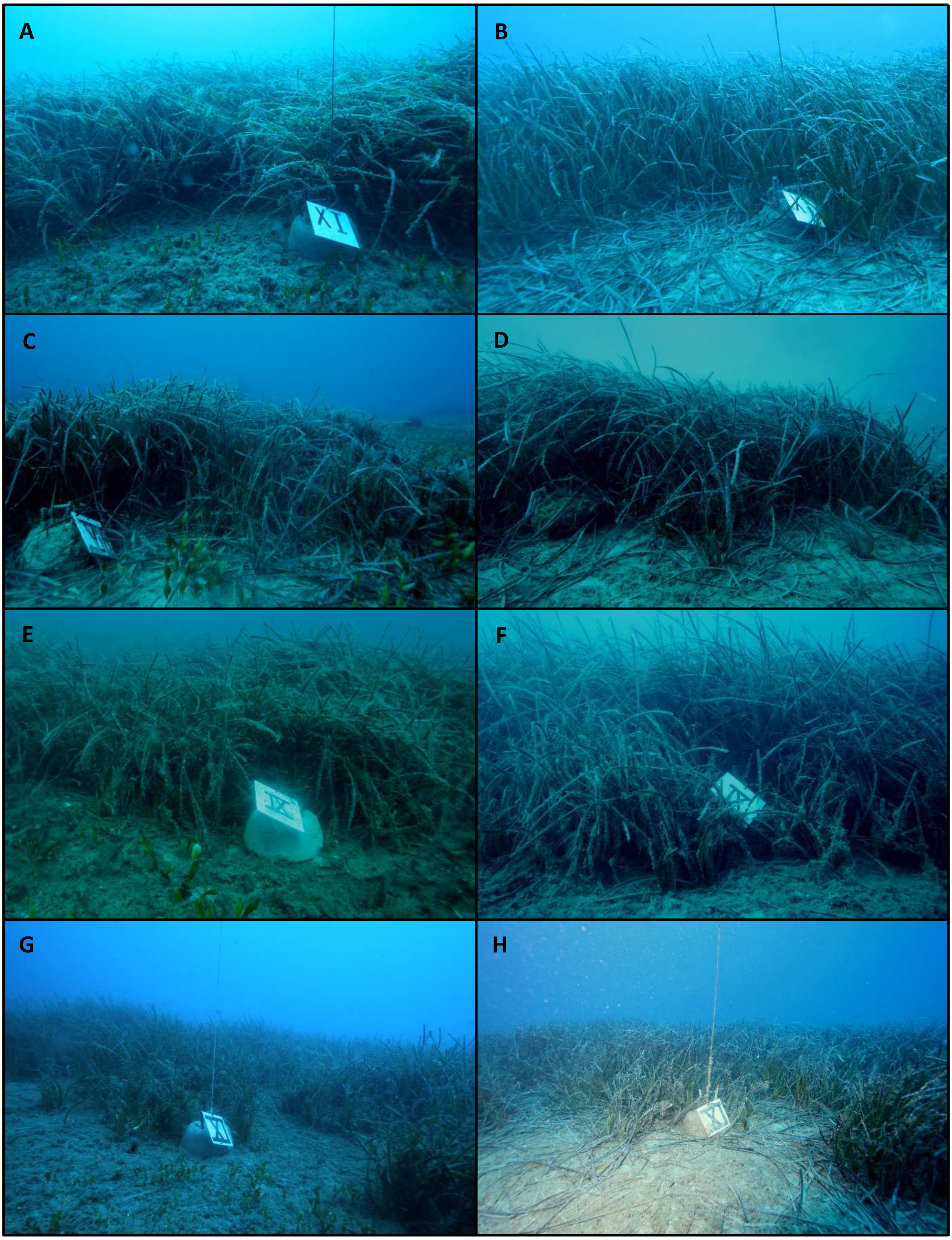


Figure 4. Photographs taken from fixed positions during the first data collection (left) and the follow-up monitoring (right) from: i) SW monitoring system - Marker 11 in 2012 (A) and 2017 (B), ii) BI monitoring system - Marker 8 in 2014 (C) and 2017 (D), iii) EM monitoring system - Marker 9 in 2012 (E) and 2017 (F) and iv) REF monitoring system - Marker 11 in 2013 (G) and 2017 (H).

Table 4. Coordinates, depth, relative abundance of sand, silt and clay, and the % organic carbon for each of the four monitoring systems at both times of sampling.

Monitoring System	Latitude Longitude	Mean depth (m)	Year of sampling	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)
SW	34°42.262'N	25.9	2012	71.54	23.72	4.74	9.41 ± 0.47
	33°16.791'E		2017	76.97	19.19	3.84	8.23 ± 0.17
BI	34°42.086'N	22.7	2014	76.01	19.99	4.00	8.73 ± 0.29
	33°16.105'E		2017	78.85	17.63	3.53	7.87 ± 0.17
EM	34°41.979'N	22.2	2012	68.09	26.59	5.32	8.35 ± 0.19
	33°13.961'E		2017	80.64	16.13	3.23	7.26 ± 0.22
REF	34°41.901'N	28.8	2013	66.49	27.92	5.58	10.43 ± 0.22
	33°11.986'E		2017	80.28	16.43	3.29	7.74 ± 0.19

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3.3 *Posidonia oceanica* metrics

The seagrass limit had not regressed between sample dates; on the contrary, it had progressed at all markers (range 1.2 - 9 cm per year). The slowest progression was recorded at the REF monitoring system, which was the deepest and with no farm in its vicinity (mean progression 14.9 cm in 4 years; Figure 5). Progression was higher at the other monitoring systems, but despite varying distances from the cages and different depths among the stations, the shoot progression was not statistically different (Kruskal-Wallis, $\chi 2 = 4.43$, df = 3, $p > 0.05$).

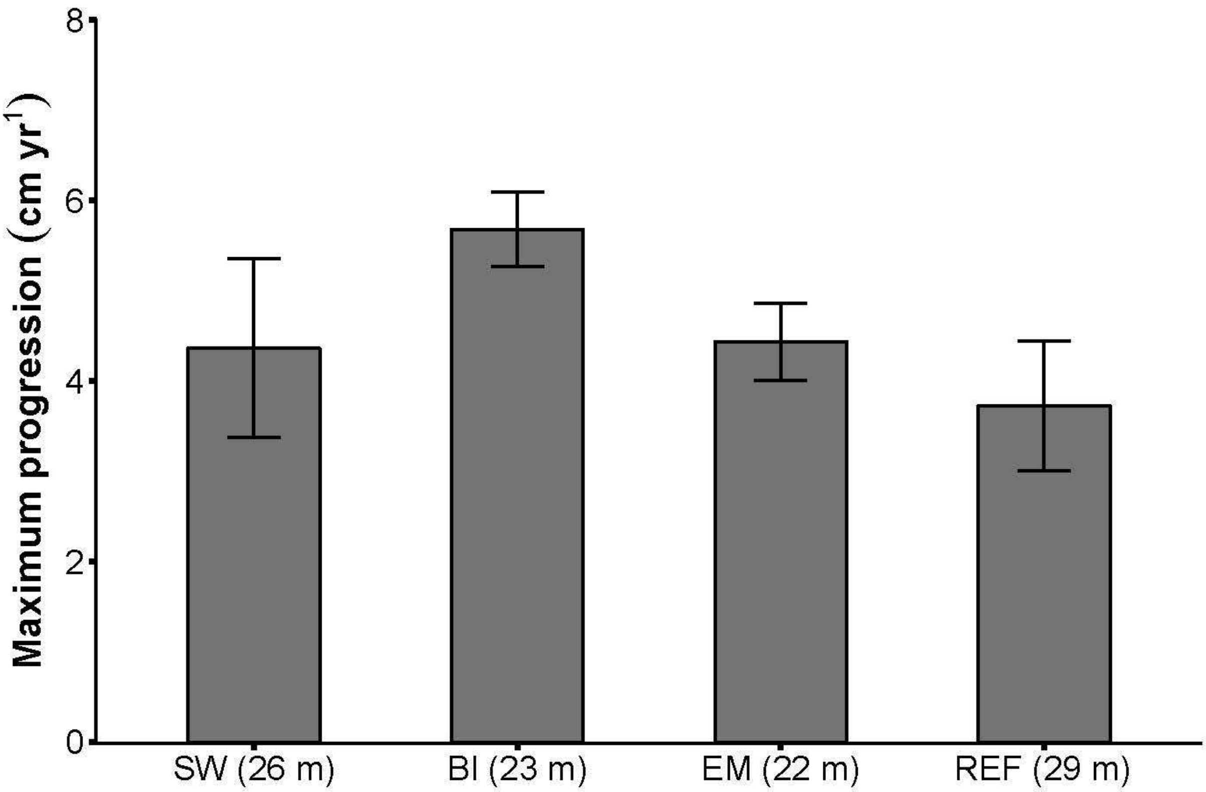


Figure 5. Mean maximum progression (± standard error) of *P. oceanica* measured from all markers at each monitoring system.

The monitoring system REF, which had no fish farm in the near vicinity but was closer to the city of Limassol, appeared the most impacted, exhibiting significant decline in almost all the descriptors measured, including: seagrass % coverage, % of plagiotropic rhizomes, number of leaves per shoot, foliar surface area and shoot exposure. Shoot density from fixed quadrats was not significantly different between the two samplings (Figure 6, Table 5).

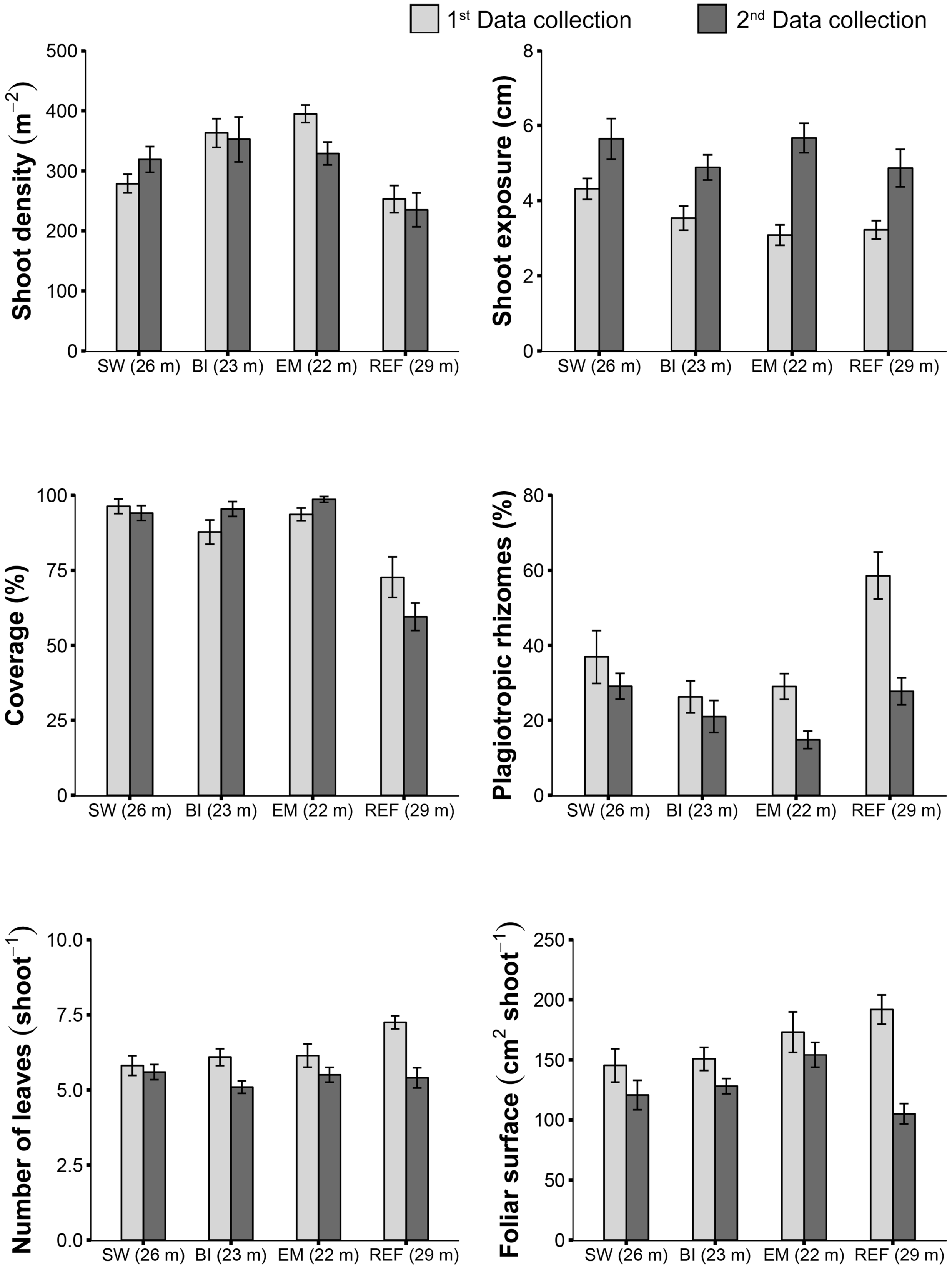
The EM monitoring system, which was approximately 100 m north from a small fish farm that gradually relocated and expanded about 750 m southeast from the monitoring system, had no significant change in seagrass coverage, foliar surface area and number of leaves per shoot. However, a significant increase in shoot exposure and a significant decline in shoot density was apparent, as well as in % of plagiotropic rhizomes, which was around half in 2017 compared to the first values in 2013 (Figure 6, Table 5).

SW monitoring system, that was at a distance between 240-275 m northeast from a major fish farm in both sampling periods, appeared to be unaffected over time, with no significant change in any descriptor being detected. Similar results were found at the BI monitoring system, which is located 250 m north from the largest fish farm (previous distance was 80 m west from the decommissioned mooring). In this case, however, while there was no significant change in seagrass coverage, shoot density, % plagiotropic shoots and foliar surface area, the shoot exposure increased and the number of leaves per shoot decreased (Figure 6, Table 5).

Table 5. Changes of *P. oceanica* and sediment descriptors at each monitoring system between first data collection (2012 for SW and EM, 2013 for REF and 2014 for BI) and follow-up monitoring from the same fixed points (2017). Arrows indicate significant change, – indicates no significant change.

Descriptors	**Monitoring Systems**			
	SW	**BI**	**EM**	**REF**
Coverage (%)	– Wilcoxon signed rank test, V = 14, *p* > 0.05	– Wilcoxon signed rank test, V = 6, *p* > 0.05	– Wilcoxon signed rank test, V = 4, *p* > 0.05	↓ Paired t-test, df = 10, t =-10.09, *p* < 0.05
Shoot Density (m^{-2})	– Paired t-test, df = 10, t = -1.77, *p* > 0.05	– Wilcoxon signed rank test, V = 40, *p* > 0.05	↓ Paired t-test, df = 10, t = 4.25, *p* < 0.05	– Paired t-test, df = 10, t = 0.79, *p* > 0.05
Plagiotropic rhizomes (%)	– Paired t-test, df =10, t = 0.87, *p* > 0.05	– Paired t-test, df = 10, t = 0.95, *p* > 0.05	↓ Paired t-test, df = 10, t = 6.46, *p* < 0.05	↓ Paired t-test, df = 10, t = 6.81, *p* < 0.05
Leaf Number ($shoot^{-1}$)	– 2-sample t-test, df = 46, t = 0.51, *p* > 0.05	↓ Mann-Whitney U test, W = 353, *p* < 0.05	– Mann-Whitney U test, W = 246, *p* > 0.05	↓ Mann-Whitney U test, W = 339, *p* < 0.05
Foliar Surface ($cm^2 shoot^{-1}$)	– 2-sample t-test, df = 46, t = 1.40, *p* > 0.05	– 2-sample t-test, df = 42, t = 1.96, *p* > 0.05	– Mann-Whitney U test, W = 242, *p* > 0.05	↓ 2-sample t-test, df = 38, t = 5.86, *p* < 0.05
Shoot Exposure (cm)	– 2-sample t-test, df = 20, t = -1.97, *p* > 0.05	↑ 2-sample t-test, df = 19, t = -2.83, *p* < 0.05	↑ 2-sample t-test, df = 20, t = -5.44, *p* < 0.05	↑ 2-sample t-test, df = 20, t = -2.92, *p* < 0.05
Organic Matter (%)	– Paired t-test, df =10, t = 2.17, *p* > 0.05	↓ Paired t-test, df =10, t = 4.38, *p* < 0.05	↓ Paired t-test, df =10, t = 3.38, *p* < 0.05	↓ Paired t-test, df =10, t = 11.07, *p* < 0.05
Grain size (µm)	– Wilcoxon rank sum	↑ Paired t-test,	↑ Wilcoxon rank sum	↑ Wilcoxon rank

test, V = 11, $p > 0.05$	df =10, t = -2.74, $p < 0.05$	test, V = 0, $p < 0.05$	sum test, V = 0, $p < 0.05$



309

Figure 6. *P. oceanica* descriptors determined from fixed positions in the summer-autumn period, firstly in 2012 for SW and EM, 2013 for REF and 2014 for BI (in light grey), and follow-up monitoring in 2017 at all the monitoring systems (in dark grey). Mean ± standard error.

The lepidochronological analysis carried out during the first data collection at each of the monitoring systems showed no significant change in leaf production over the years that preceded sampling and when farms operated shallow moorings nearer to *P. oceanica* meadows (Table 6).

The LAI decreased between the two sampling periods across all monitoring systems. The smallest decrease was recorded at SW monitoring system (from 4.06 to 3.85 m^2 of canopy per m^2), while the largest decrease was recorded at the deepest REF monitoring system (from 4.85 to 2.47 m^2 of canopy per m^2). At both times of data collection, the EM monitoring system, which is also the shallowest site, had the highest LAI values (6.84 m^2 of canopy per m^2 in 2012 and 5.07 m^2 of canopy per m^2 in 2017) compared to all other systems.

Table 6. Mean number of leaves per shoot ± SE, during lepidochronological years determined from 15-20 orthotropic shoots collected in 2012-14 from each monitoring system.

Lepidochronological year	**Monitoring System**			
	SW	**BI**	**EM**	**REF**
2012	5.6 ± 0.3	6.9 ± 0.2	7.6 ± 0.4	6.9 ± 0.4
2011	5.7 ± 0.4	6.9 ± 0.4	6.2 ± 0.4	7.2 ± 0.4
2010	5.8 ± 0.4	6.6 ± 0.5	6.6 ± 0.5	7.1 ± 0.4
2009	4.7 ± 0.5	6.4 ± 1.0	6.1 ± 0.3	7.0 ± 0.4
2008	-	-	6.3 ± 0.3	6.5 ± 0.6
2007	-	-	6.1 ± 0.4	6.3 ± 0.5
Rate of change ± SE	0.22 ± 0.17	0.24 ± 0.14	0.06 ± 0.06	0.03 ± 0.08
R^2	0.03	0.04	0.006	0.001
df	50	57	155	84
p	> 0.05	> 0.05	> 0.05	> 0.05
Statistical analysis	Simple linear regression	Simple linear regression	Kendall–Theil Sen Siegel	Kendall–Theil Sen Siegel

3.4 Underlying predictors

Out of the five predictors that were investigated, time and depth had the greatest effect on the descriptors studied, but all the predictors considered seemed to play a key role in explaining most of the descriptors and had similar weight in their contribution (Table 7). The shoot density was the only descriptor that was not affected over time. Depth, distance and direction from the cages were significant predictors of shoot density and had the largest contribution in relative importance (Table 7).

Table 7. The source of variation and the relative importance of two environmental and three fish farm related predictors on seven measured descriptors, acquired from the multiple regression analysis. Note: The source of variation in the model takes in account all the predictors, whereas % lmg takes in account the average relative importance of each predictor without and with all the possible combinations with the rest of the predictors.

Variable	**Source of variation**					**Relative Importance**
	Predictor	Df	Sum of squares	*F*	Prob. > *F*	% lmg
Shoot density	Depth	1	2321	6.83	< 0.05	35.9
	Distance	1	1380	4.06	< 0.05	21.7
	Tonnes	1	192	0.56	ns	16.2
	Direction	2	9184	13.52	< 0.001	22.9
	Time	1	248	0.73	ns	3.2
Plagiotropic rhizomes	Depth	1	2.10	6.80	< 0.05	21.3
	Distance	1	1.56	9.65	< 0.05	16.9
	Tonnes	1	0.46	1.48	ns	11.7
	Direction	2	5.96	5.04	< 0.001	24.8
	Time	1	5.70	18.46	< 0.001	25.3
Number of Leaves	Depth	1	0.00	0.17	ns	3.2
	Distance	1	0.00	0.11	ns	16.2
	Tonnes	1	0.00	3.94	< 0.05	20.1
	Direction	2	0.00	0.91	ns	15.4
	Time	1	0.00	6.55	< 0.05	45.1
Foliar Surface	Depth	1	0.23	7.75	< 0.01	21.5
	Distance	1	0.00	0.29	ns	9.7
	Tonnes	1	0.04	1.52	ns	8.9
	Direction	2	0.00	0.02	ns	11.5
	Time	1	0.50	16.80	< 0.001	48.4
Shoot exposure	Depth	1	15.05	15.04	< 0.01	14.7
	Distance	1	3.11	1.48	ns	16
	Tonnes	1	9.10	9.09	< 0.05	9.5
	Direction	2	14.64	3.48	< 0.05	32.1
	Time	1	19.54	9.29	< 0.01	27.8
Grain size	Depth	1	0.00	0.80	ns	3.7
	Distance	1	0.00	12.66	< 0.001	18.9
	Tonnes	1	0.00	100.73	< 0.001	8.7
	Direction	2	0.00	29.17	< 0.001	19.6
	Time	1	0.00	10.04	< 0.05	49.0
Organic matter	Depth	1	0.00	21.67	< 0.001	14.5
	Distance	1	0.01	52.62	< 0.001	15.7
	Tonnes	1	0.00	5.60	< 0.05	9.2
	Direction	2	0.00	2.06	ns	16.6
	Time	1	0.00	12.06	< 0.001	44.0

4. DISCUSSION

In Cyprus, mariculture activities are concentrated in an area between the cities of Limassol and Larnaca. Small scale production started here in the mid-nineties in shallow water (< 30 m) over *P. oceanica* meadows and this may have contributed to degradation and regression of the lower limits of the meadows. Thereafter, managers followed a precautionary approach (Pergent-Martini et al., 2006) and only allowed new production units to be placed in deeper water while asked for the relocation of the shallow cages when existing farms requested expansion of their production. Our study has shown that this management intervention may have been effective in preventing further declines in the lower limits of the meadows studied. It proves the point that local impacts on *P. oceanica* can be managed at the local level (Guillén et al., 2013).

Our permanent *P. oceanica* monitoring systems are the first in Cyprus and the easternmost seagrass PMN systems in the Mediterranean. Setting up monitoring systems using permanent cement markers is a durable and effective method to monitor the edge of seagrass meadows from fixed positions over medium to long timeframes (Pergent et al., 2015) and is substantially more robust than random plots for monitoring seagrasses (Schultz et al., 2015). About five years following deployment, all cement markers were still in place, despite some major storms. Our results indicate that *P. oceanica* has not regressed during this time and although an overall lower performance was recorded in some *P. oceanica* structural descriptors, this was not detected near fish farms.

Contrary to expectations, *P. oceanica* meadows had progressed at all monitoring stations and although differences among the monitoring systems were not statistically significant, the largest observed progression was recorded near the largest fish farm. There is hope, therefore, that despite major losses of *P. oceanica* from fixed PMN markers at deep meadows in the north-western Mediterranean (Boudouresque et al., 2000, 2012; Pergent et al., 2015), a decline has not been detected in impacted deep meadow limits in the eastern Mediterranean. This is despite the fact that water temperatures are close to the reported upper limit of the species (Celebi et al., 2006). The seagrass horizontal growth rates reported in this study may be overestimated as there was a selection bias for the furthest rhizome from the marker; however the values obtained are consistent with previous estimations but lower than recolonization rates measured along labelled fixed pegs at shallower healthy patches in the western Mediterranean (Gobert et al., 2016). In other studies, *P. oceanica* meadows could survive close to fish farm cages, even though effects on *P. oceanica* descriptors were detected at large distances from the fish cages (Borg et al., 2006; Marbà et al., 2006; Holmer et al., 2008; Rountos et al., 2012). In this study, *P. oceanica* descriptors did not clearly detect impacts of the fish farm operations.

The impacts of mariculture on *P. oceanica* meadows are site-specific and dependant on variables, such as the size of the farm and the intensity of feeding, depth and hydrodynamics. In Cyprus, it seems that the decision to relocate the fish farms deeper (southern), in an area dominated by west and east currents, has been successful in mitigating impacts to the *P. oceanica* meadows that stretch northwards. The model simulations presented in this study, showed that the main dispersal of particulate matter is not in the direction of the seagrass meadows investigated. However, only two months were considered and resuspension, which would tend to increase dispersion of waste particles, was not considered in the model. Furthermore, with the estimated velocity of currents, farm effluents can disperse over a distance covering several kilometres (Sarà et al., 2006) and affect *P. oceanica* meadows even *ca* 3 km away, in ways that are not always reflected by alterations in structural descriptors (Ruiz et al., 2010).

At the sites monitored, improvement was also recorded in sediment variables: the organic matter content decreased, and the mean grain size increased in all monitoring systems, except at SW where the changes were not significant. This, together with the increase in *P. oceanica* shoot exposure measured during the follow-up monitoring, may indicate less sedimentation of suspended fine particulates or resuspension of silty sediments during storms that preceded the second sampling. It is also noteworthy that *C. prolifera*, a highly nitrophilous green seaweed, was very abundant during the first data collection but rare during follow up monitoring, which is another indication of improved water quality condition (Holmer et al., 2009).

Across the four monitoring systems assessed, the lowest rates of progression and the highest reduction in the performance of *P. oceanica* descriptors were recorded at the REF monitoring system, which lies far from any aquaculture operations. At both times of sampling, lower seagrass coverage and shoot densities were measured at this monitoring system compared to its shallower counterparts, although the values of shoot densities measured still indicate high ecological condition of the meadows (Pergent et al., 1995) and progression of the meadow was still recorded despite a strong dynamic regression at other PMN lower limit reference sites in the Mediterranean (Pergent et al., 2015). The deeper water, and consequently the reduced light availability and water circulation, may be the most important limiting factor of the *P. oceanica* descriptors (Martínez-Crego et al., 2008). Furthermore, this site was closer to the anthropogenic footprint of Limassol city, which may be affecting the *P. oceanica* meadow. For example, about 1.4 km to the northeast there is a sewage outlet releasing processed effluents generated from Limassol. This monitoring system can provide valuable baseline data if fish farming begins nearby.

The variation in descriptors considered in this study was explained by the cumulative effects of environmental and farm predictors. The variables having the most cumulative effect on *P. oceanica* descriptors, were: water depth followed by direction, and then distance to the nearest fish farm or production tonnage. The multiple regression analysis to identify main predictors was purely suggestive. The creation of more PMN systems at different directions from the fish farms can enable better discrimination of the factors contributing to the changes in structural and demographic *P. oceanica* descriptors.

The PMN protocol allows microscale detection of regression/progression of seagrass lower limits using structural and morphological *P. oceanica* descriptors that are widely applied in generic ecosystem monitoring. Most of the structural indicators considered exhibit marked seasonality and/or strong bathymetric dependence (Marbà et al., 2013). This bottleneck of inherent patchiness and differences of these indicators across the meadow was removed by sampling around the same time of year (summer - autumn period) and from the same fixed plots (same depth). However, data should be interpreted based on the validity of the structural indicators used to reflect stress. The diversity of *P. oceanica* indicators is striking; structural descriptors of *P. oceanica* used in this study such as coverage and shoot density are widely used in monitoring programmes (e.g. EU Water Framework Directive) as they are linked directly to ecosystem integrity and can detect generalized degradation responses (Martínez-Crego et al., 2008; Marbà et al., 2013). A recent global review of seagrass indicators identified structural indicators such as density, coverage and depth limit among the best suited indicators for generic ecosystem monitoring, stress screening and ecological assessment (Roca et al., 2016).

Lower shoot size, shoot density and coverage are commonly reported responses for *P. oceanica* meadows exposed to fish farm effluents (Pergent-Martini et al., 2006), though this was not detected in our study. The number of leaves per shoot are responding consistently to light stress in seagrasses making them a robust bioindicator of degraded water quality (McMahon et al., 2013). Lepidochronological analysis showed that before our study and relocation of farms, the number of leaves per shoot at each monitoring station was stable and not very different across stations. Between the two sampling periods, the leaf number decreased in two stations but declines of this descriptor near aquaculture are not always consistent (Pergent-Martini et al., 2006). The percentage of plagiotropic rhizomes is correlated with water quality (Gobert et al., 2009). On the other hand, the plagiotropic rhizomes remain plagiotropic when surrounding substrate space is sufficient to allow lateral expansion and revert to orthotropic in dense meadows where space is inadequate for colonization (Molenaar et al., 2000). This makes any comparisons using this descriptor difficult. Plagiotropic rhizomes are the most common rhizome on edges of meadows, while orthotropics are predominant in continuous meadow (Lapeyra, 2016). Hence the lower plagiotropic rhizome measured from the fixed positions of decommissioned sites (REF and EM) in the follow-up monitoring may be partly explained by the fact that the edge has progressed a little and what used to be the edge of the meadow is now a little further inside.

Structural descriptors are responsive to degradation but are not effective in reflecting early improvements and recoveries because they respond slowly and detect impacts much too late for effective management action to be taken (Roca et al., 2015, 2016). Environmental change is first reflected in plant physiology, which modifies seagrass growth and morphology, which induce changes in meadow structure (Collier et al., 2012). Thus, physiological indicators present more stressor-specific responses and can detect degradation responses much faster (Roca et al., 2015, 2016). To the highest level of cellular response, altered gene expression of stress-related genes are the faster predictors of an imminent seagrass collapse (Ceccherelli et al., 2018). One drawback of the PMN protocol is that it only includes structural variables and once a decline in these parameters is sufficiently large to be detected in *P. oceanica* meadows, there is a considerable risk that the seagrass meadow has already degraded irreversibly. The PMN protocol applied in this study can benefit from incorporating early-warning indicators together with the structural *P. oceanica* descriptors considered. The response of the physiological indicators is highly stress-specific so the choice depends on the objectives of the management strategy (Roca et al., 2016).

Despite national and international protection, large declines of *P. oceanica* meadows have been documented since the second half of the 20th century, especially near urban areas of the western Mediterranean (Marbà et al., 2014; Telesca et al., 2015). The loss of *P. oceanica* meadows may result in the erosion and rapid remineralisation of the carbon-rich matte, accelerating climate change (Pergent et al., 2014). Efforts to conserve *P. oceanica* lie mostly in the establishment of marine protected areas, which seem to be insufficient to guarantee the protection of *P. oceanica* meadows (Montefalcone et al., 2009). Across the Mediterranean Sea, seagrass monitoring is extensive, but the adoption of different sampling designs and methods may result in erroneous comparisons (Lopez y Royo et al., 2010). Recently, the PMN has refined a standardised methodology for setting up *P. oceanica* monitoring systems, which has been applied in the Euro-Mediterranean region. Comparable temporal monitoring along the edge of the meadow is possible through photography and measurements of vitality parameters from fixed positions. Slow growing seagrasses such as *P. oceanica* are especially suited to fixed-plot monitoring (Schultz et al., 2015) as small-spatial scale progression or regression of the meadow can be monitored effectively.

The *P. oceanica* monitoring systems set up and monitored in this study are valuable tools for researchers, managers and decision makers and their application should be promoted. Set up at the deepest boundaries of the meadows, near the compensation depth where the plants are most sensitive to changes in water quality, they form an important indicator of ecological integrity and allow for detection of small losses, which is critical for slow growing *P. oceanica* (Holmer et al., 2003; Buia et al., 2004). Future comparisons will guide responsible management and increase our understanding regarding the mariculture impacts on *P. oceanica* in the eastern Mediterranean. They can also be compared with other PMN systems set up in other places across the Mediterranean Sea to assess the *P. oceanica* population dynamics in different regions. The use of fixed plot methods using cement markers, like the PMN method applied in the Mediterranean or using quadrats placed over transects like the SeagrassNet method applied globally (www.SeagrassNet.org), allow reliable and effective microscale monitoring of seagrass descriptors from the same positions using standardised methodologies, have high statistical power, and their use should be encouraged and widely adopted in generic ecosystem monitoring (Schultz et al., 2015). The disadvantage with the used *P. oceanica* descriptors is that they respond late to generalised pressures. If the management strategy aims to achieve effective early detection of stress imposed on *P. oceanica* by specific anthropogenic activities (e.g. burial, metal pollution, eutrophication, organic enrichment, shading), it is recommended to incorporate stress-specific biochemical and genetic indicators in the monitoring program.

5. ACKNOWLEDGEMENTS

The monitoring systems were set up and the first data collection occurred during the research program ‘A holistic approach for the evaluation of ecological status of coastal areas: the case of Vasiliko bay’, conducted in 2011-2014, and co-funded by the Research Promotion Foundation of Cyprus (Republic of Cyprus) and the European Regional Development Fund (grant agreement SMEs/Product/0609/74). The authors are grateful to the Department of Fisheries and Marine Research and the Department of Environment of Cyprus for providing the permits to remove a small number of *P. oceanica* shoots. We are also grateful to Alexis Loucaides who assisted the setting up of the monitoring systems, Fotini Georgiou, Maria Rousou, Polina Polykarpou, Karolina Mukauskaitė, Noemi Mantegazza and all who assisted the lab analyses as well as Dr Chris Cromey for simulating the MERAMOD model, Dr Gérard Pergent for advising and sharing the protocol for setting up the PMN monitoring systems and the Oceanographic Center of Cyprus for providing the current data for the study area.

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Highlights

- Around Cyprus, fish farming initially operated shallow cages over seagrass meadows. The farms expanded rapidly but cages moved away from the seagrass beds to mitigate impacts.
- Four seagrass monitoring systems were set up near fish farms and decommissioned sites.
- Data collection was repeated from the same fixed-plots about five years later.
- Progression of previously impacted seagrass beds was noted and present-day farms, located in deep water, are not preventing nearby seagrass meadow growth.