

## Impact of environmental conditions on *Posidonia oceanica* meadows in the Eastern Mediterranean Sea

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In the meadow closer to the fish cages, total inorganic nitrogen, ammonium, chlorophyll-a concentrations and organic matter content in sediment were determined. It is relatively higher than those recorded at the control meadow. In order to test significant differences among stations, sampling periods and depths, environmental variables together with morphological characters of the both sites were analyzed by Canoco. Shoot density, adult leaf length, adult leaf surface area, and leaf area index (LAI) showed significant spatial and temporal changes. Additionally, except shoot density, the values obtained other morphological characters showed increasing trend towards deeper stations and further meadow from the cage activities. A coefficient values were relatively higher in the near cages and decreased related with depth.

**[Keywords:** *Posidonia oceanica*, Aquaculture, Pollution, Mediterranean Sea]

### Introduction

*Posidonia oceanica*, an endemic species to the Mediterranean, plays a vital role in the structure and functioning of the Mediterranean ecosystem<sup>1,2</sup>. This unique ecosystem has been protected by both national and European frameworks. The new legislation accepted in 2007 allows cage installation at depths deeper than 30m<sup>3</sup>. The excessive organic matter and nutrient input derived from uneaten fish foods and faecal pellets has led to degraded or in some cases completely destructed seagrass beds, as well as deterioration of water and sediment peculiarities<sup>4,5,6,7,8,9</sup>. The eutrophic environmental condition also encourages dense growth of harmful algae and opportunistic species<sup>10,11</sup>. As a consequence of this eutrophic phenomenon, epiphytic growth increases<sup>12,13</sup> and affects NH<sub>4</sub> and NO<sub>3</sub> uptake rates negatively<sup>14</sup>. The stations closest to fish cages showed reduced shoot density, shoot size, underground biomass, and photosynthetic capacities. Total P content in rhizomes was the physiological descriptors and higher values were determined close to cages<sup>1,15</sup>. In sediment, the accumulation of organic matter leads to the depletion in oxygen with the consequences of sulphide and methane productions<sup>16</sup>. Its impacts on benthic ecosystem are more severe with a consequent reduced biodiversity<sup>17,18</sup>.

The marine fish farm production (particularly sea bass, sea bream) in Turkey is approximately 68.500 tons/year with an economic value of €287.600.000<sup>19</sup>. Increasing request created conflict between the conservation of the meadows and aquaculture development. In the present study, the seagrass meadows' decline caused by near shore fish farming activity was investigated by means of both biotic and abiotic variables at different depth ranges. The aim was to find out factors which are the most responsible for morphological changes of *P. oceanica*.

### Materials and Methods

#### Sampling area

The study area, located at Engeceli Bay (Aegean Sea, 38°27'45.60"N; 26°35'19.42"E), has sandy-muddy sediments (Fig. 1). In this bay, the fish farming activities began in 1990 above *P. oceanica* bed. This facility has 36 cages of 12 m diameter and 20 more cages with dimensions of 5×5×5 m. Floating cages occupy approximately 3500 m<sup>2</sup> area and placed at 8m deep below the water surface. The annual production of sea bass was 300 t in 2005. In the facility, 360 g of faeces were produced per kg of fish production<sup>18</sup>. In the study, two sites were selected as impact and control. In the sampling period, we observed dead *P. oceanica* rhizomes near the cages. This situation could explain the existence of seagrass prior to the farm installation. In the study carried out

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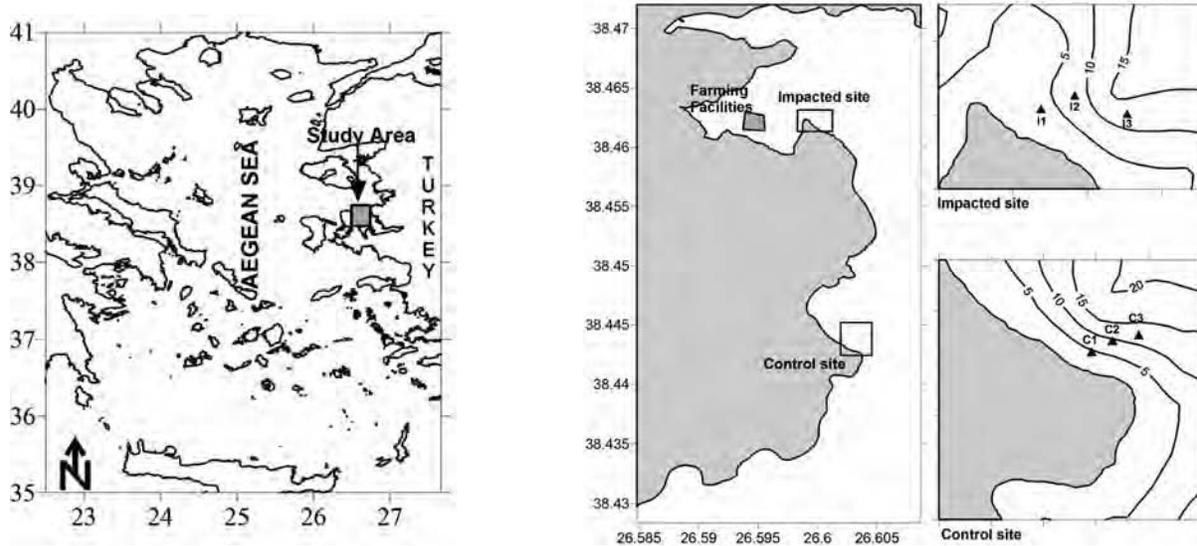


Fig. 1—Location of sampling stations

in 2002, the area was evaluated as poor status according to UNTRIX-based classification frame<sup>20</sup>. In the fish farming area, *P. oceanica* meadow was the 300 m closest to cages and meadow was represented less dense community with respect to control. Three sampling stations (I1, I2 and I3), separated from each other by a distance about 100 m were chosen in different depth ranges (0-5m; 5-10m; 10-15m). The control sites (stations C1, C2 and C3) are near the shore and 1200-1500 m away from fish cages in the same depth range and distance from each other. Three replicates samples for each station were taken randomly by using a quadrat (20×20 cm in dimension). The samples of *P. oceanica* and sediments (0-5 cm) were collected by scuba diving in the autumn 2004, winter and spring 2005. Water samples were taken from just above the seagrass meadow by means of Nansen bottle for nutrients, dissolved oxygen, and chlorophyll-a analysis.

#### Laboratory and data analyses

At each station, shoot density was measured in five replicate quadrates of 400 cm<sup>2</sup>. The density of shoots is expressed as number of shoots per m<sup>2</sup>. Shoot samples collected at each station were fixed with 4% formalin solution and then transferred to the laboratory. For each replicate, randomly selected five shoot samples were washed with tap water in the lab and leaves sorted as defined by Giraud<sup>21</sup>. For each shoot, number of leaves and for each leaf, morphological characters such as length and width were measured using digital compass. Total leaf

number per shoot, leaf surface (cm<sup>2</sup> per shoot), leaf area index (LAI m<sup>2</sup>.m<sup>-2</sup>) and A coefficient (%) which represents the percentage of adult and intermediate leaves that lost their apex, were calculated according to Buia *et al.*<sup>22</sup>.

Water quality parameters such as pH, dissolved oxygen (DO), temperature were measured in situ with the Hanna Instruments HI 8314 membrane model pH meter and Winkler method. The nutrient concentrations were determined using standard methods for all stations. At the time of sampling, 100 ml polyethylene bottles were rinsed twice with the sample and filled, then immediately frozen until analysis. Nutrient analysis was carried out within one week of the completion of the cruise, using a Skalar (two-channel) Autoanalyzer. The colorimetric methods adopted were similar to those described by Strickland and Parsons and Grasshoff *et al.*<sup>23,24</sup>. The total inorganic nitrogen (TIN) is calculated with the sum of nitrate-nitrite and ammonium nitrogen.

Water samples for chlorophyll-a (Chl-a) were collected from surface water and were prefiltered through 210-µm nylon mesh placed in a funnel in order to remove the larger particles (e.g. mesozooplankton). After concentration on GF/F filters (45 µm), Chl-a was extracted with 90% acetone solution. The fluorescence intensity of clear extracts was then measured, using a Sequoia-Turner Fluorometer<sup>23</sup>. A commercially available Chl-a Standard obtained from Sigma was used to quantify the sample intensities.

The amount of organic matter (TOM) was determined spectrophotometrically in sediment samples following the sulfochromic oxidation method. The dried sediment samples were oxidized with  $K_2Cr_2O_7$  and  $H_2SO_4$  and then samples were filtered. The organic matter concentration of sediment samples were determined spectrophotometrically (610 nm) following the method of Hach<sup>25</sup>. The accuracy of this method is  $\pm 0.017$  % organic matter.

The software STATISTICA was used for statistical analysis. Three-way ANOVA was used to determine if there is any significant differences among seasons (Autumn-September; Winter-January; Spring-May), stations and depths by using morphological descriptors of *Posidonia oceanica*: leaf length, leaf number per shoot, leaf surface, leaf area index and A coefficient. Homogeneity of variances was tested by Cochran's C-test and data were log transformed when necessary. Canonical correspondence analysis (CCA) was performed to assess the relationships between changing morphological aspects of *Posidonia oceanica* meadow and environmental variables. The analysis was carried out using the computer programs Canoco and CanaDrow 4.5<sup>26</sup>. This analysis represents direct ordination technique for finding the biggest variability in a morphological descriptors data set explained by environmental variables. The log transformation was applied on the data. The analyses were applied to 6 sampling sites (three different depth ranges: 0-5m; 5-10m; 10-15m for each stations selected as Control and Impact) including 3 replicates, 11 morphological variables of *P. oceanica* species which are the eaten leaves, broken leaves, whole leaves, total leaves, coefficient A, average length, average width, length base, leaf surface, shoot density and LAI, 10 environmental

(C, DO,  $PO_4$ -P,  $NO_3+NO_2$ ,  $NO_2$ -N,  $NH_4$ -N, TIN,  $TPO_4$ -P, Si, Chl-a) and three nominal variables (seasons).

## Results

### Physico-chemical analysis

Dissolved oxygen (DO) values in the water column ranged between  $7.25 \text{ mg l}^{-1}$  and  $7.49 \text{ mg l}^{-1}$  at both the impact and at the control stations (Table 1). Although the lowest dissolved oxygen concentration was observed in summer, there was not found significant spatial variation ( $p=0.904$ ). Temperature among seasons showed important variation ( $p<0.05$ ) with the maximum value ( $22.26 \text{ }^\circ\text{C}$ ) in spring and the minimum ( $15.00 \text{ }^\circ\text{C}$ ) value in winter.

The nutrient concentrations in the water column at the sampling stations are presented in Table 1: The levels of nitrate+nitrite ( $TNO_x$ -N) and nitrite ( $NO_2$ -N) concentrations ranged between 0.40-1.06 and 0.03-0.08  $\mu\text{M}$  among all stations. Both  $TNO_x$ -N and  $NO_2$ -N were recorded with higher values at the control stations. Although, the maximum  $TNO_x$ -N value was measured in autumn at station C2 (1.60  $\mu\text{M}$ ), the highest nitrite value was observed in winter at control station C1 (0.12  $\mu\text{M}$ ). Higher concentrations of ammonium ( $NH_4$ -N) were observed in the impact area (1.43 - 2.11  $\mu\text{M}$ ) than those recorded in the control stations (0.53 - 0.68  $\mu\text{M}$ ). Its concentration increased with depth, and reached a maximum value at station I3 where its value was 3 times higher than that recorded at control station C1. Total inorganic nitrogen (TIN) showed variations between stations. In the impact stations, the TIN level was higher (1.83-2.25  $\mu\text{M}$ ) reaching a maximum mean values in May (2.29  $\mu\text{M}$ ). Dissolved phosphorous concentrations (orthophosphate) showed high values at control stations and the highest mean concentration was recorded at station C2

Table 1—Mean values of some environmental parameters in the control and in the impact stations with  $\pm$ SE

Parameters	I1	I2	I3	C1	C2	C3
DO(mg/l)	7.44 $\pm$ 0.15	7.25 $\pm$ 0.11	7.40 $\pm$ 0.10	7.38 $\pm$ 0.17	7.49 $\pm$ 0.17	7.41 $\pm$ 0.14
Temperature ( $^\circ\text{C}$ )	18.98 $\pm$ 1.18	19.2 $\pm$ 1.06	19.07 $\pm$ 1.06	19.71 $\pm$ 1.05	19.87 $\pm$ 1.09	19.81 $\pm$ 1.05
$o.PO_4$ -P( $\mu\text{M}$ )	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.09 $\pm$ 0.02	0.06 $\pm$ 0.01
( $NO_3+NO_2$ )N( $\mu\text{M}$ )	0.41 $\pm$ 0.06	0.40 $\pm$ 0.04	0.47 $\pm$ 0.03	0.70 $\pm$ 0.05	1.06 $\pm$ 0.14	0.94 $\pm$ 0.10
$NO_2$ -N( $\mu\text{M}$ )	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.03 $\pm$ 0.00	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.01
$NH_4$ -N( $\mu\text{M}$ )	1.43 $\pm$ 0.24	1.43 $\pm$ 0.26	2.11 $\pm$ 0.34	0.53 $\pm$ 0.04	0.55 $\pm$ 0.10	0.68 $\pm$ 0.19
TIN( $\mu\text{M}$ )	1.83 $\pm$ 0.29	1.83 $\pm$ 0.25	2.25 $\pm$ 0.21	1.23 $\pm$ 0.05	1.61 $\pm$ 0.20	1.63 $\pm$ 0.25
T. $PO_4$ -P( $\mu\text{M}$ )	0.56 $\pm$ 0.05	0.51 $\pm$ 0.05	0.36 $\pm$ 0.02	0.41 $\pm$ 0.03	0.53 $\pm$ 0.02	0.33 $\pm$ 0.04
Si ( $\mu\text{M}$ )	1.77 $\pm$ 0.30	1.87 $\pm$ 0.27	1.89 $\pm$ 0.19	1.87 $\pm$ 0.18	1.80 $\pm$ 0.18	1.89 $\pm$ 0.13
Chl.-a( $\mu\text{g/l}$ )	0.15 $\pm$ 0.01	0.16 $\pm$ 0.02	0.12 $\pm$ 0.01	0.11 $\pm$ 0.01	0.10 $\pm$ 0.01	0.12 $\pm$ 0.01
Organic matter (%)	7.03 $\pm$ 0.86	10.67 $\pm$ 1.36	10.67 $\pm$ 2.27	2.47 $\pm$ 0.27	6.10 $\pm$ 0.17	9.73 $\pm$ 1.14

( $0.09 \pm 0.02$ ) The total phosphate phosphorus (T.PO<sub>4</sub>-P) concentration respectively varied from 0.36 to 0.56 and from 0.33 to 0.53  $\mu\text{M}$  in the impact and in the control areas.

Chlorophyll-a concentrations showed significant variation between season and site. However there were no any significant changes among depths. The highest value (0.16  $\mu\text{g/l}$ ) was obtained at the impact station (I2), while the lowest value (0.10  $\mu\text{g/l}$ ) was recorded at the control station (C2).

The impact of fish farming activity on *P. oceanica* meadows was investigated using both morphological adult data and physicochemical data. Fig. 2 shows the position of stations in the ordination space along the first and second axes which respectively explain 80.7% and 13.1% of the variability in stations (meadows) and in physicochemical data. The Monte Carlo test shows that the first ordination axis is significant ( $p=0.0017$ ) and separated stations as control and impact. Four chemical variables [NH<sub>4</sub>-N ( $r=0.5606$ ), Chl-a ( $r=0.5235$ ), TIN ( $r=0.4865$ ) and TPO<sub>4</sub>-P ( $r=0.334$ )] were correlated with the impact meadows. However NO<sub>3</sub>+NO<sub>2</sub>-N and PO<sub>4</sub>-P were positively correlated with the control meadows. The distribution of impact stations along the first axis reflects their relationship with NH<sub>4</sub>-N, TIN and Chl-a. In the control stations, NO<sub>3</sub>+NO<sub>2</sub>-N and PO<sub>4</sub>-P were more effective.

Organic matter (%) content of sediment was related to depth factor in control site. Its mean value was higher at the impact sites where the minimum and maximum levels of organic matter (%) varied from 4.8 to 19.6 and the maximum value was recorded in spring at I3. The sediment samples

showed significant differences in the concentrations of organic matter between stations and across seasons ( $p<0.05$ ).

#### Descriptors of *Posidonia oceanica*

Shoot density is an important structural descriptor. The *P. oceanica* density in terms of shoots per  $\text{m}^2$ , was higher in the control meadows and was inclined to decrease along the depth gradient at both sites. It had the lowest mean density ( $376 \pm 29$ ) in the meadow close to cages at 10 to 15m depth range. According to the classification scale established by Giraud<sup>21</sup> this value indicates a sparse meadow. The values recorded in control meadow were higher than those recorded in the impact. The maximum value was  $540 \pm 25$  shoot per  $\text{m}^2$  at 0-5m depth range (Table 2).

The mean length of adult leaves increased with increasing depth. At each depth in control meadow, the mean length of adult leaves was longer than those measured in the impact site. The lengths of adult leaves showed significant differences among stations, depths and seasons ( $p<0.05$ ). Analysis of variance values for season, depth and station and their interaction regarding *Posidonia oceanica* morphological parameters are given in Table 3. In the intermediate leaf length, no significant difference was found between meadows. However, they showed significant differences between seasons ( $p<0.05$ ). Both adult and intermediate leaf lengths were the highest in the spring period (Table 4).

The leaf width of *P. oceanica* did not show any important temporal and spatial changes for adult leaves. The intermediate leaves showed a significant seasonal variation ( $p<0.05$ ) and the highest leaf width was recorded in winter ( $8.50 \pm 0.02$  mm).

The number of adult leaves was high in spring period and showed significant differences between seasons and depths ( $p<0.05$ ). Although the number of adult leaves was relatively higher in the deeper stations, there was no significant difference among them.

Adult leaf surface area ( $\text{cm}^2/\text{shoot}$ ) was greater at the control meadow than that at the impact meadow. The adult leaf surface area increased at both impact and control stations (I3 and C3) with increasing depth. The highest mean leaf surface value was calculated at station C3 ( $119 \pm 8.7$ ). There were significant differences among stations, depths and seasons in terms of adult leaf surfaces ( $p<0.05$ ) (Table 3), and

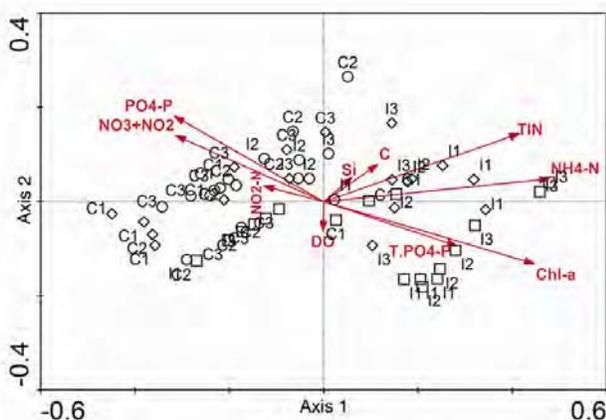


Fig.2—Ordination diagram for biometry of *Posidonia oceanica* and environmental variables (○, Autumn; □, Winter; ◇, Spring)

Table 2—The values of morphological descriptors at the stations (mean±SE)

Variable	I1	I2	I3	C1	C2	C3
Shoot Density (shoots.m <sup>-2</sup> )	510.625±45.48	488.33±18.05	376.66±28.88	540±25.29	508.33±46.96	470±21.79
Mean Adult Leaf Length (cm)	15.71±1.25	26.23±2.05	28.44±1.76	18.91±0.89	29.27±2.41	33.85±2.22
Mean Intermediate Leaf Length (cm)	13.04±1.44	18.31±2.34	18.37±2.09	12.83±0.79	19.80±2.72	18.69±2.37
Mean Adult Leaf Width (mm)	8.10±0.16	8.55±0.19	8.56±0.24	8.02±0.18	8.66±0.20	9.84±0.15
Mean Intermediate Leaf Width (mm)	7.90±0.01	8.35±0.02	8.21±0.02	6.27±0.11	7.57±0.09	9.42±0.01
Number of Adult Leaves per Shoot	3.15±0.14	3.36±0.22	3.41±0.20	3.04±0.25	3.51±0.06	3.62±0.25
Number of Intermediate Leaves per Shoot	2.45±0.31	2.40±0.29	2.42±0.32	1.89±0.15	2.27±0.22	2.18±0.24
Adult Leaf Surface (cm <sup>2</sup> per shoot)	40.07±3.49	75.38±8.02	85.59±8.14	46.20±3.65	95.49±7.18	119±8.7
Intermediate Leaf Surface (cm <sup>2</sup> per shoot)	27.70±4.78	38.43±6.66	39.55±6.85	15.94±3.33	34.91±7.68	39.58±6.92
Adult Leaf Area Index (m <sup>2</sup> .m <sup>-2</sup> )	2.01±0.20	3.78±0.53	3.37±0.55	2.53±0.27	4.91±0.69	5.67±0.58
Intermediate Leaf Area Index (m <sup>2</sup> .m <sup>-2</sup> )	1.41±0.29	1.88±0.34	1.45±0.26	0.81±0.16	1.72±0.45	1.90±0.38
Adult A coefficient (%)	81.10±4.97	61.07±3.80	71.18±4.79	55.20±4.07	40.07±4.30	31.51±5.35
Intermediate A coefficient (%)	20.7±4.26	12.45±3.43	23.62±4.31	5.73±2.87	4.18±3.21	5.10±2.39

Table 3—Anova values for season, depth and station and their interaction regarding morphological parameters (Adult leaves) of *Posidonia oceanica* \* P<0.05

Source of variation	d.f.	Leaf length		Number of leaves per shoot		Leaf surface		Leaf area index		A.coefficient		Shoot density	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Season	2	0.147	8.6*	2.729	13.46*	3520	16.20*	36.97	77.66*	145	0.80	211E3	371.4*
Depth	2	1.714	100.7*	1.002	4.94*	163E2	74.84*	27.54	57.85*	1854	10.20*	439E2	77.08*
Station	1	0.361	21.2*	0.058	0.29	5408	24.88*	23.46	49.28*	114E2	62.55*	300E2	52.80*
Season*Depth	4	0.189	11.1*	0.222	1.10	651	2.99*	3.36	7.05*	189	1.04	17E2	3.08*
Season*Station	2	0.007	0.4	0.461	2.27	156	0.72	0.12	0.26	260	1.43	465	0.82
Depth*Station	2	0.017	1.0	0.078	0.38	734	3.38*	3.35	7.03*	402	2.21	5883	10.33*
Season*Depth*Station	4	0.126	7.4*	0.568	2.80*	641	2.95*	0.82	1.72	212	1.17	9109	15.99*
Cochran's test (C)		0.200		0.416		0.333		0.281		0.133		0.154	
Transformation		Log		None		None		None		None		None	

Table 4—The values of morphological descriptors in seasons (mean ±SE)

Variable	Autumn	Winter	Spring
Shoot Density	499.59±13.74	393.61±13.55	610.44±17.10
Mean Adult Leaf Length (cm)	24.31±2.05	23.95±1.14	28.41±4.45
Mean Intermediate Leaf Length (cm)	10.67±0.43	16.92±0.69	22.80±1.55
Mean Adult Leaf Width (mm)	8.57±0.18	8.92±0.18	8.40±0.38
Mean Intermediate Leaf Width (mm)	8.46±0.02	8.50±0.02	6.94±0.07
Number of Adult Leaves per Shoot	3.11±0.09	3.17±0.18	3.81±0.14
Number of Intermediate Leaves per Shoot	1.48±0.09	2.98±0.11	2.29±0.12
Adult Leaf Surface (cm <sup>2</sup> per shoot)	69.08±6.95	70.48±7.15	92.92±16.83
Intermediate Leaf Surface (cm <sup>2</sup> per shoot)	13.90±1.40	44.20±3.06	39.19±5.03
Adult Leaf Area Index (m <sup>2</sup> .m <sup>-2</sup> )	3.25±0.31	2.62±0.27	5.33±0.92
Intermediate Leaf Area Index (m <sup>2</sup> .m <sup>-2</sup> )	0.66±0.06	1.63±0.10	2.25±0.29
Adult A coefficient (%)	58.13±5.7	54.70±5.23	55.96±8.61
Intermediate A coefficient (%)	6.88±2.47	15.29±3.39	12.95±2.73

the highest leaf surface area ( $92.92 \pm 16.83 \text{ cm}^2/\text{shoot}$ ) was observed in spring. The seasonal mean of leaf surface area varied from  $13.90 \pm 1.40$  (autumn) to  $39.19 \pm 5.3 \text{ cm}^2$  (spring) for the intermediate leaves significant difference was determined among stations.

The leaf area index (LAI  $\text{m}^2.\text{m}^{-2}$ ) is related to the shoot density, the number of leaves, their length and width as recorded at each station. Neither adult nor intermediate leaves at impact stations showed any relationship with depth. However, at the control stations, LAI showed increasing values with increasing depth. Although the meadow density decreased along the depth gradient, the value of LAI for adult leaves ( $5.67 \pm 0.58$ ) was observed to be high in the deepest station at the control site. This index is not only affected by the shoot density but also by the leaf length and width. The leaf area index of adult leaves showed significant differences among stations, depths and seasons ( $p < 0.05$ ) (Table 3). The highest LAI value for adult leaves was calculated for spring and the lowest was recorded in winter. In the leaves of intermediate type, the seasonal mean value of LAI was calculated as  $1.59 \pm 0.17$ .

The A coefficient, which is affected by broken leaf apex, was higher at impact stations close to the cages. In the sea grass bed near the cages, adult leaves showed the higher A coefficient value, which changed among sites in the range of 42% in the control meadow to 70% in the impact meadow. This A coefficient value showed an important variation in adult leaves among all researched stations (stations and depths). However there was no significant change from one season to another.

## Discussion

The fish farm under investigation has been operational in Engeceli Bay for fifteen years. In the present study, decreased shoot density in the vegetated area was found near the cages and the lowest value ( $376 \pm 29$ ) was determined at the deepest impact station (I3). In Italy concerning unwanted sediment conditions close to fish cages, the shoot density of phanerogames declined and the size of unvegetated area increased<sup>15</sup>. The shoot density value in Engeceli Bay determined at the impact area represents a “sparse” meadow according to the classification scale established by Giraud<sup>21</sup>. However, the station C1 ( $540 \pm 25$ ) represents a

“dense” meadow in a good state of health. Light limitation, phytoplankton growth and epiphytic organism development mainly due to increasing nutrient availability could be additional causes contributing to the decline of the meadows<sup>12</sup>.

Significant differences were found for adult leaf length among stations, seasons and depths. Although the highest mean adult leaf lengths was found at the deepest stations in both selected meadows. These values were lower than that recorded ( $43.6 \pm 5.3 \text{ cm}$ ) by Kruzic<sup>27</sup> at the Fulija islet where meadows were influenced by a tuna farm. In the study carried out around the Island of Elba, where *P. oceanica* meadow was not under anthropogenic stress, the mean leaf length changed between  $40.8 \pm 9.9 \text{ cm}$  and  $62.5 \pm 4.2 \text{ cm}$ <sup>28</sup>. In the present study, the maximum length of adult and intermediate leaves was found in spring period. This situation could be explained by species-specific leaf growth dynamics and nutrient parameters<sup>29</sup>. The leaf length of *P. oceanica* also seems to be strongly influenced by age, epiphytic development, available light and competition with algae<sup>8,29</sup>.

The leaf width did not show any important variation among stations for adult leaves and mean values changed from  $8.71 \pm 0.01$  (control site) to  $8.37 \pm 0.01$  (impact site). This range was similar to the data obtained from the sampling sites which were not under the influence of farming activity<sup>27</sup>.

In samples collected from the *P. oceanica* meadow at five meter depth, A coefficient values were higher than the other depth ranges in both meadows. In the El Kantaoui meadow (Tunisia), the greater A coefficient ranged from 60.8% to 51.5% according to depths<sup>30</sup>. Leoni *et al.*<sup>8</sup> showed that the adult leaf A coefficient value was affected by cage ( $30.9 \pm 4.4\%$ ) and fertilized zones ( $41.9 \pm 5.1\%$ ). Variation in A coefficient value could be explained by an increasing herbivore pressure, changes in hydrodynamic and bathymetric conditions<sup>31,32</sup>. Both herbivore pressure and epiphytic development may make apex more fragile<sup>8</sup>. In the vicinity of fish cages, apical parts of *Posidonia* leaves were mostly covered by dense epiphytic organisms<sup>13</sup>. The nitrogen content of the *P. oceanica* leaves was richer near the cages and this would encourage herbivore pressure at the impact stations<sup>33</sup>. In the present study, the significant differences among stations could be explained by changing environmental conditions and depth factor.

Although the mean number of adult leaves per shoot showed significant differences among season and depth, there was not found any important changes between meadows. At the both sides the determined value for number of leaves did not explain that the meadow was under the anthropogenic stress<sup>30</sup>. The leaf length, width and leaf number have strong bearing on the calculation of leaf surface data. At both control and impact stations, leaf surface value increased along the depth gradient and high values were determined in the spring period. The mean leaf surface data were similar to the values obtained from meadows, which were affected by either fish farming activity or other anthropogenic pollution<sup>8,27</sup>. Tsirika *et al.*<sup>34</sup> showed that the parameter increased with depth, peaking at 15 m for the summer period. This increase in leaf surface along the depth gradient can be attributed to hydrodynamic forces as well as to decreasing breakage of plants<sup>31</sup>.

LAI, which was affected by the shoot density of the meadow, showed temporal and spatial variations for adult and intermediate leaves. Wood and Lavery<sup>35</sup> suggested that shoot density and leaf area index, were seasonally changing indicators of seagrass. These parameters were affected by light intensity<sup>36</sup>. When the density decreased, the leaf area index showed a downsite potential<sup>34,27</sup>. In the present study the maximum value was calculated at the deepest control station ( $5.67 \pm 0.58 \text{ m}^2 \cdot \text{m}^{-2}$ ), and this could be attributed to increasing leaf length. At Nasuta Cape (Elba Island), the mean leaf length ( $62.5 \pm 4.2$ ) and shoot density ( $675 \pm 95$ ) were the highest of all other examined stations selected around the island, where LAI was calculated as  $18.6 \pm 9.5$  (SE)<sup>28</sup>.

Additionally the findings in relation to morphometric parameters, organic matter accumulation on the seabeds was also found to be the most efficient predictor of changing environmental conditions with regard to the eutrophication phenomenon<sup>18</sup>. The threshold of inputs was  $1.5 \text{ g of organic matter m}^{-2} \text{ day}^{-1}$  for balanced meadow dynamics. Seagrass decline is accelerated above this threshold<sup>37</sup>. Organic matter accumulation with depth was responsible for reducing shoot density in both meadows. Organic matter level at impact stations were higher than those determined at control points. The max organic matter concentrations were obtained (10.67 %) at the impact stations (I2 and I3). In Fornells Bay (Minorca Island), the value ranged between 10-14.2 % (0-5 cm) at stations located at a

maximum 102 m distance from the cages<sup>1</sup>. In the bay of Figari (Corsica), the high level of organic matter (24%) was recorded beneath the cages. The decline in the seagrass shoot density appears to result from increased organic matter concentration and reduced carbon reserves<sup>1,15</sup>.

Dissolved nutrient concentration showed marked seasonal changes among stations. The  $\text{NH}_4\text{-N}$  and TIN concentrations recorded in control meadow were lower than those measured near the aquaculture cages. The maximum ammonium value ( $2.10 \pm 0.34 \mu\text{M}$ ) was found at station I3 and it was also significantly high ( $1.73 \mu\text{M}$ ) in the spring period. This could be explained by the fact that ammonium is the main excretion product of fish and some fish food contains nitrogenous compounds<sup>38</sup>. In other studies, the  $\text{NH}_4$  value, which is used as an indicator of nutrient enrichment, was recorded at its maximum at sampling stations near the fish farms<sup>8,18</sup>. Total phytoplanktonic biomass determined by Chlorophyll-a, was a bit higher in impact meadows. It showed significant changes between sites and periods. In the spring period the differences between meadows were clear. Chlorophyll-a values measured in either impact or control meadows did not exceed  $0.21 \mu\text{g l}^{-1}$  and were within the range found in oligotrophic coastal areas<sup>18</sup>. In this period total inorganic nitrogen concentration also reached its maximum value ( $2.98 \mu\text{M}$ ). However there was not found any important relationship between TIN and chlorophyll-a concentrations. In this study the high correlation was found between  $\text{NH}_4$  and chlorophyll-a values ( $r=0.81$ ). It revealed that the impact meadow could be under the influence of fish cages. While total inorganic nitrogen (TIN) was always high at the stations close to fish cages, the mean concentrations of  $\text{oPO}_4\text{-P}$  at control stations were higher than those measured at the impact ones. This finding showed that the control meadow near the coastal area could be influenced by local hydrodynamic conditions, nutrient inputs derived from different sources. The nutrient concentrations are not usually found high in the vicinity of cages because of dilution processes and rapid transfer in the food web<sup>39</sup>. Thus total phosphorus and nitrogen concentrations of sea water indicated weakly the nutrient loading by fish farming and cause insignificant deterioration of the water quality<sup>39,40</sup>. The study pointed out that, shoot density, leaf length, leaf surface, leaf area index and A coefficient values were found to be more sensitive indicators of changing environmental conditions.

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