

## Deliverable 27

### Data of a carbon balance model as integer of plant response to fish farm effluents

Olga Invers, Juan Manuel Ruiz, César Meiners and Marta Pérez  
Departament d'Ecologia, Universitat de Barcelona  
Avda. Diagonal, 645 – 08028 Barcelona

#### Introduction

Fish farm activities cause an increment of organic matter load (i.e. food pellets, faecal excretions) to the water surrounding the cages that have considerable effects on physical and chemical features, such as water transparency and dissolved inorganic nutrient concentrations (Pitta et al., 1999; Ruiz et al., 2001). However, due to generally shallow location of the fish farms (between 20 and 40 m depth) considerable amounts of organic matter reach the sediment and causes profound changes in sediment biogeochemistry (Holmer et al., 2003). Enriched sediments underneath the farm cages present negative redox values, a certain degree of anoxia, increased sulfate reduction rates, high sulfide levels and accumulation of nitrogenous and phosphorous compounds, among others (Holmer, 1991; Hall, 1992; Hargrave et al. 1993). All these changes have important repercussions on the structure and function of benthic communities and result in a loss of biodiversity (Karakassis, 1998, and cites therein).

In the Mediterranean sea, coastal soft bottoms up to 40 m depth are frequently dominated by seagrasses, mainly by the endemic species *Posidonia oceanica* (Procaccini et al., 2003). This seagrass has been shown to be very sensitive to anthropic disturbances, such human and industrial wastes (Pergent-Martini and Pergent, 1995) and organic effluents coming from aquaculture activities (Delgado et al., 1999; Pergent et al., 1999; Ruiz et al., 2001).

Changes on physical and chemical features on water and sediment mentioned above are related with the reduction of seagrass abundance beneath fish cages and surrounding areas, probably through a combined effect of the different factors over plant physiology and growth.

Reduction in water column transparency close to the cages implies a decrease in light availability for the plant, limiting photosynthetic rates. However, *P. oceanica* seems to possess a certain degree of photoadaptation through changes in photosynthetic parameters following light reduction, as has been shown either in natural conditions (changes with depth) or in experimental light reductions (Pirc, 1986; Ruiz and Romero, 2001); this ability of photoadaptation has been also observed in plants submitted to fish farm influence (Ruiz et al., 2001).

Changes on sediment biogeochemistry associated with fish farms also affect negatively *P. oceanica* health and survival. The apparition of sediment hypoxia or the increase in sulphide and ammonium concentrations have deleterious effects over the root metabolism and, hence, over the plant (van Katwijk et al., 1997; Erksine and Koch, 2000; Holmer and Bondgaard, 2001) resulting in high mortality rates and reductions in aboveground and belowground biomass. Changes in the relative proportions of aboveground (photosynthetic tissues) and belowground (respiratory tissues) biomass can alter the proportion of C production vs. losses and affect the net carbon balance of the plant.



Besides changes on water column transparency and sediment features, other factors like changes in the species interactions at the community level can influence *P. oceanica* health and survival. Epiphytic blooms over *P. oceanica* leaves owing to increased nutrient availability produces an important shading to the leaves and can cause a certain degree of light limitation (Tomasko and Lapointe, 1991; Alcoverro et al., submitted). Also, population outbreaks of herbivores, mainly the sea-urchin *Paracentrotus lividus*, has been described to occur near fish farm cages. Such increase in herbivore pressure causes a reduction of leaf biomass that can negatively affect the plant by reducing its ability in light harvesting; however, the herbivorism can reduce epiphyte loads and alleviates shading imposed by increased epiphyte biomass (Alcoverro et al., 1997; Ruiz et al., 2001).

All the mentioned factors have negative effects over *P. oceanica* health and survival and can be summarised into a model of plant carbon balance. Plant photosynthesis and, thus, the organic carbon supply, necessary to maintain plant metabolism and generate carbon reserves, can be severely impaired at the plants submitted to fish farming influence.

The goal of this work is, thus, to use a carbon balance model as an integer of the conditions at which *P. oceanica* can be submitted under the influence of fish farming, using it as a tool to assess the relative importance of the different factors implicated in deterioration of *P. oceanica* meadows under the influence of fish farms.

In this work, C balance between photosynthetic supply and whole plant respiration has been calculated for one month (September) in plants collected at increasing distances from a fish farm, in Sicily. To do the calculations, we measured water column transparency, plant biomass partitioning (leaves, rhizomes, roots), below-ground organs respiration rates and epiphyte biomass. Photosynthetic parameters were obtained from literature (Ruiz et al., 2001).

## Material and Methods

### *Study site*

The fish farm is located at the South of Sicily, close to Porto Palo. The cages are situated at 20 m depth and the bottom presents rocky reefs and dead *P. oceanica* rhizomes. Alive shoots appears at the near surroundings of the fish cages. Three sampling stations were selected in vegetated areas along a gradient of influence of fish farm effluents, following the downstream direction, towards N: *station III* was deployed in the meadow closest to the fish farm, at approximately 10 m from the edge of the cages, and represented the most highly influenced point; *station IV* was situated at 30-40 m from the cages and *station V*, which represents the 'control' meadow out of the fish farm influence, was situated at ca. 1 Km from the cages. All stations were situated at 20 m depth. Sampling was performed during September 2002.

### *Plant and epiphyte biomass*

Plant biomass was obtained by sampling three corers of 17 cm diameter inside *P. oceanica* patches at each station. The samples were sorted into leaf shoots, alive rhizomes and alive roots, dried at 70 °C until constant weight (24-48 hours) and weighed to obtain biomass partitioning into leaves, rhizomes and roots.

Six additional leaf shoots were obtained at each station. Leaves were separated into the four age classes considered: A1, A2, A3 and A4 (0-25, 25-50, 50-100 and >100 days old, respectively; Alcoverro *et al.*, 1998), and the width and length were measured. Epiphytes of the four leaf age classes were gently removed with a razor blade and kept separately. Plant



and epiphyte material was dried as above and weighed to obtain biomass.

### *Photosynthesis and Respiration*

Photosynthetic parameters of *Posidonia oceanica* A2 leaf age class were obtained from Ruiz et al. (2001); parameters for A1, A3 and A4 leaf age class were estimated following the relationship between photosynthetic parameters among leaf age class found by Alcoverro et al. (1998). We assumed that photosynthetic parameters of plants from st. III and st. IV were similar.

Respiration rates were measured *in situ*. Five shoots were sampled at each station and rhizomes and roots were separated for respiration measurements. Below-ground respiration rates were measured as changes in oxygen concentration incubating rhizomes and roots (0.4 and 0.3 gdw, respectively), per triplicate, in 100 ml bottles in the field. Incubation lasted for two hours at *in situ* seawater temperature. Bottles were tied to a structure, which was hanged from a buoy which allows natural agitation.

At the end of the incubation period, seawater oxygen concentration was measured using an oximeter (WTW Oximeter, OXI196) and was compared to that of control bottles (without plant). More details on the method can be found in Alcoverro et al. (1998). Plants were dried at 70°C for 24 hours and subsequently weighed, and photosynthetic rates were expressed in mg O<sub>2</sub> g<sup>-1</sup> dw h<sup>-1</sup>.

### *Light data*

Underwater irradiance was estimated using values of sea surface irradiance (SSI), from a meteorological station. Due to difficulties in find light data from a place close to the study site, and considering the main objective of this work is to find differences between stations, we have used light data from a meteorological station in Spain.

Light data from the meteorological station was transformed into underwater PAR quantum irradiance using empirical factors and formulae in Kirk (1983) to account for reflection at the sea-air interface; irradiance reaching the plant canopy was calculated applying the Beer-Lambert law of light attenuation:

$$I_z = I_0 e^{-kz} \quad \text{Equation 1}$$

where  $I_z$  was the irradiance at the considered depth,  $I_0$  the irradiance at the surface,  $k$  the coefficient of light extinction and  $z$  the depth considered.

Light extinction coefficient ( $k$ ) was estimated from light data obtained in vertical profiles measured with a Li-Cor sensor attached to a CTD (Karakassis and Tsapakis, unpublished data) at the stations III and V; we considered the same coefficient  $k$  for st. III and for st. IV.

Light reduction due to epiphyte biomass was estimated following Alcoverro et al. (submitted), with the equation:

$$A = (1 - \exp(-Bep/2.11)) \quad \text{Equation 2}$$

where  $A$  is the percentage of light absorption caused by epiphytes, and  $Bep$  is the epiphyte biomass in mg dw / leaf cm<sup>2</sup>. This reduction in light was applied to the estimated light that reach the plant canopy to obtain the resultant light available to the plant.

### Carbon budget calculations

The carbon budget was based mostly on Alcoverro et al. (2001). An estimate of the daily carbon budget of the plant was obtained as follows: daily integrated respiration of non-photosynthetic parts was subtracted from daily-integrated net carbon gains by leaves, which were computed on photosynthesis-irradiance curves for the four different leaf-age classes considered, A1-A4. Photosynthesis-irradiance curves followed the equation of Edwards and Walker (1983) which was modified by Pérez and Romero (1992):

$$P = P_{\max} \times (I - I_c) / (I_k + (I - I_c)) \quad \text{Equation 3}$$

where P was the net oxygen release ( $\text{mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) at irradiance I ( $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ),  $P_{\max}$  was the light-saturated photosynthesis (net oxygen release,  $\text{mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ),  $I_c$  was the compensation irradiance ( $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ), and  $I_k$  was the threshold for light-saturated photosynthesis ( $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ), as classically defined by most authors (Pirc, 1986). Integration was done on an hourly basis. Results were expressed as  $\text{mg C shoot}^{-1} \text{ month}^{-1}$  using the quotient  $\text{O}_2/\text{C}$  of 1/1.2 (Alcoverro et al., submitted).

We tested the importance over *P. oceanica* carbon balance in plants submitted to changes in: i) plant features (biomass and respiration of below-ground organs) ii) water-column transparency, iii) adaptation of photosynthesis to reduced light availability (photoadaptation), iv) epiphyte biomass, and iv) an integration of all these factors to simulate the real situation that plants experienced at each station.

### Statistics

The statistical significance of shoot biomass partitioning, epiphyte biomass of the whole leaf shoot, and respiration variability among stations (factor 'zone') was tested using a one-way ANOVA. Variability among stations (factor 'zone') of epiphyte biomass at each leaf age class (factor 'leaf') was tested using a two-way ANOVA. Whenever necessary, significance of differences between zones was assessed using the Tukey post-hoc test.

## Results

### Plant measurements

#### Plant biomass

There was a significant decrease in biomass of leaves, rhizomes and roots from st. V to st. III (Fig. 1; Table 1;  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , respectively). The effect was more evident in leaves, with a decrease of ca. 80 % in leaf biomass, while in rhizomes and roots was ca. 60%. The reduction of biomass per unit area was due to a reduction in shoot density (see Ruiz et al., 2003). In addition, there was a decrease in shoot size, being ca. 78% and 54% smaller at st. III and IV, respectively, than shoots from st. V. This reduction could be attributed to the important herbivore pressure by *Paracentrotus lividus* that has been described in these stations closest to fish cages, that was higher closest to cages (Ruiz et al., 2003).

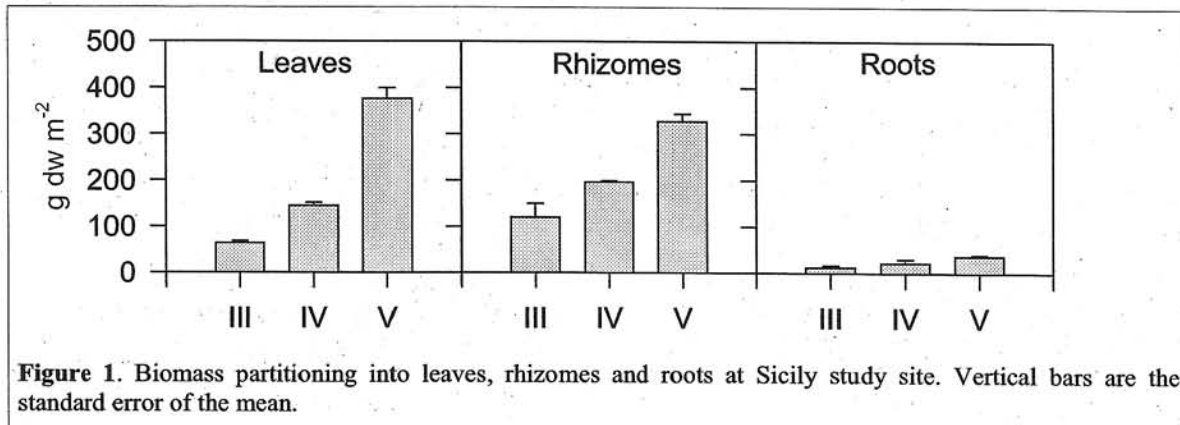
Despite the difference in shoot size, the proportion of the four age classes of leaves considered into a shoot was similar at the three stations, corresponding 6.4%, 16.9%, 53.6% and 23,1% of the leaf shoot biomass to A1, A2, A3 and A4, respectively.

#### Respiration of below-ground organs

Rhizome respiration rates were similar among shoots of the three stations. Roots, however, showed significant differences, with respiration rates at st. III ca. 50% lower than those



measured in roots from st. IV and V (one-way ANOVA,  $p < 0.05$ , Table 1; see Fig.2 and Table 2).



**Table 1.** Summary of ANOVA results.  $H_0$ : there are not significant effects between “zones” (i.e. between st III, st IV and st V stations). NS = not significant, \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

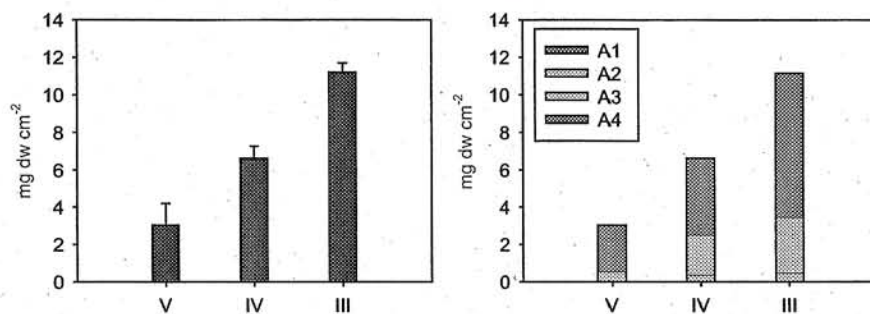
Variable	Sicily Campaign			
	d.f.	MS	% Variance	p
<i>Biomass partitioning</i>				
Leaves	2	393.93	99.2	***
Rhizomes	2	164.31	96.4	**
Roots	2	2.39	83.9	*
<i>Epiphyte biomass</i>	2	0.359	99.9	***
<i>Respiration rates</i>				
Rhizomes	2	0.0005	45.3	NS
Roots	2	0.0103	95.26	*

#### Epiphyte biomass

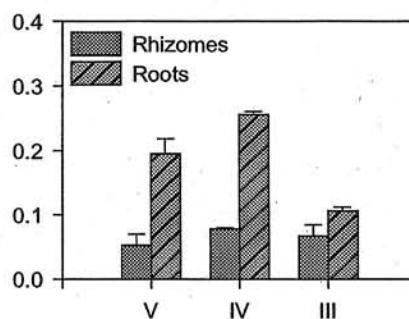
Epiphyte biomass per leaf shoot unit area was significantly higher at st. III and IV than at st. V ( $p < 0.001$ , one way ANOVA, Table 1; Fig. 3A). This reflects an intense stimulation of the colonization of *P. oceanica* leaves due to the effect of fish farm effluents. The increase in epiphyte biomass was more important with increasing leaf age at all three stations (two way ANOVA, significant interaction ‘zone’ x ‘leaf’,  $p < 0.01$ ; see Fig. 3B), from almost none epiphyte in the youngest leaf A1, to a ca. 65-80% of the whole shoot epiphyte biomass in the oldest leaf A4.

Despite this general trend, a slight shift of epiphyte distribution among leaf ages was detected, from leaf A4 towards leaf A3. In effect, there was a significant lower percentage of the shoot epiphyte biomass in leaf A4 at st. III and IV (ca. 65%) than at st. V (80%) (post-hoc Tuckey test,  $p < 0.01$ ), and the percentage in leaf A3 was significantly higher at st. IV (33%) than at st. V (16%).

The epiphytes cause a significant shading in control plants (st. V) as shown by a increase in the percent of light absorption (Table 3). At plants of st. III and IV, this light absorption increases highly, up to more than 80% of the incident light in the oldest leaves (Table 3).



**Figure 2.** A. Epiphyte biomass (mg dw cm<sup>-2</sup>) in *Posidonia oceanica* leaves. B. Epiphyte biomass at each leaf age tissue of *Posidonia oceanica* shoots. Vertical bars are the standard error of the mean.



**Figure 3.** Respiration of below-ground organs from shoots collected at the stations III, IV and V at Sicily study site. Vertical bars are the standard error of the mean.

**Table 2.** Photosynthetic parameters and respiration rates used to estimate the carbon balance in *Posidonia oceanica* shoots at the three stations.  $P_{max}$  (mg O<sub>2</sub> gdw<sup>-1</sup> h<sup>-1</sup>),  $I_k$  ( $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) and  $I_c$  ( $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) for A2 were obtained from Ruiz et al. (2001), and values corresponding to A1, A3 and A4 were estimated following the relationship between leaf age classes found by Alcoverro et al. (1998). Respiration rates (mg O<sub>2</sub> gdw<sup>-1</sup> h<sup>-1</sup>) of rhizomes and roots were measured *in situ* at the Sicily fish farm.

Station	Plant tissue	Photosynthetic parameters			Respiration
		$P_{max}$	$I_k$	$I_c$	
Stations III and IV	A1	11.7	52.9	2.0	
	A2	10.8	74.3	2.6	
	A3	6.4	74.3	4.8	
	A4	2.1	33.4	5.4	
Station V	A1	6.5	47.3	3.0	
	A2	6.0	66.4	3.9	
	A3	3.6	66.4	7.2	
	A4	1.2	29.9	8.1	
All stations	Rhizomes				-0.066
Stations IV and V	Roots				-0.225
Station III	Roots				-0.106



**Table 3.** % Light absorption due to epiphyte biomass calculated with equation 2, following Alcoverro et al. (submitted).

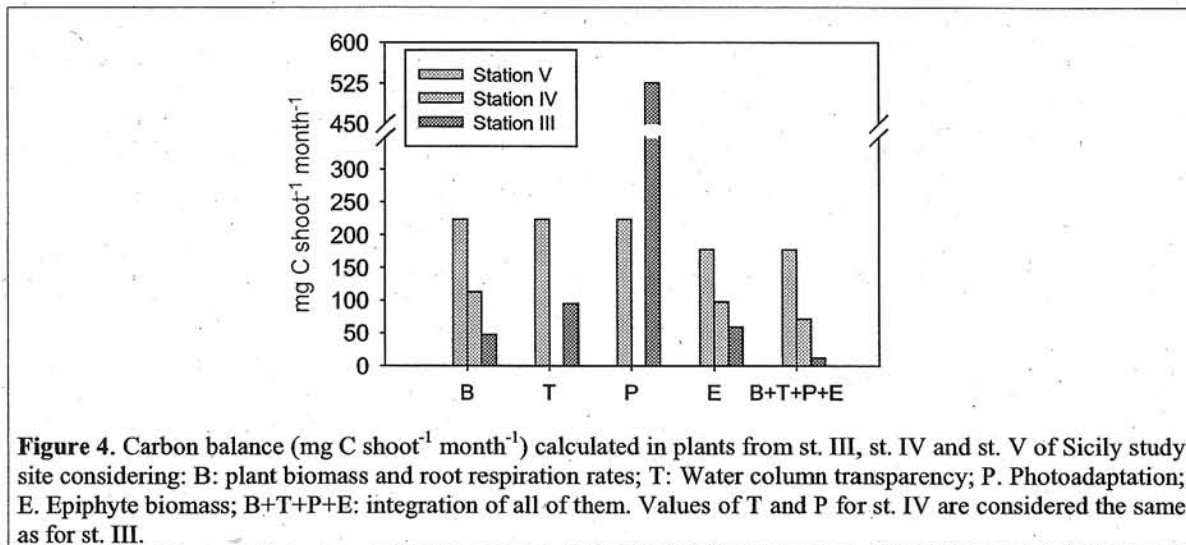
Station	% Light absorption			
	A1	A2	A3	A4
III	0.00	17.97	65.30	84.03
IV	0.00	14.73	55.12	73.82
V	0.00	2.35	19.46	59.21

### Carbon balance

#### *Plant features*

The reduction of plant biomass in st. III and IV affected negatively the carbon balance. Despite C losses as respiration were reduced due to low biomass, the decrease in C gain due to the decrease in photosynthetic biomass is much more important (see Table 4 and Figure 4). Thus, C balance decreases a 80% in st. III plants, and a 49.6% in st. IV plants relative to st. V plants.

The significant reduction in root respiration found in st. III can only alleviate the reduction in C balance in a 1.3%.



**Figure 4.** Carbon balance ( $\text{mg C shoot}^{-1} \text{ month}^{-1}$ ) calculated in plants from st. III, st. IV and st. V of Sicily study site considering: B: plant biomass and root respiration rates; T: Water column transparency; P: Photoadaptation; E: Epiphyte biomass; B+T+P+E: integration of all of them. Values of T and P for st. IV are considered the same as for st. III.

#### *Water column transparency*

There is a higher light attenuation close to the fish cages as shown by the values of  $k$  obtained at the three stations ( $k = 0.122 \text{ m}^{-1}$  for both st. III and IV, and  $k = 0.093 \text{ m}^{-1}$  for st. V). The increased light attenuation caused a reduction in the light availability at the plant canopy close to cages of 47.2 %, which caused a reduction of 57.7% in the carbon balance of plants compared to st. V (see Figure 4 and Table 4).

#### *Photoadaptation*

The result of a change in photosynthetic parameters (same for st. III and IV) like the considered made increase substantially the C gain of the plants close to cages, ca. 134% of the value calculated for plants from st. V (Table 4, Fig. 4). It would mean an important response of the plants to unfavourable conditions of light availability.

### *Epiphyte biomass*

The shading effect of epiphytes was important (see Fig. 4 and Table 4) even in plants from st. V, which showed a reduction in carbon balance of 20% due to the epiphytes. This reduction was more important in plants from st. III and IV with a decrease of 67% and 45%, respectively, compared with values of st. V plants.

### *Integrating factors: simulation of the real situation*

From the carbon balance data obtained above, the effect of biomass decrease and shading by epiphytes seemed to be the most important among those tested. The effect of the photoadaptation, potentially important, would alleviate partially the negative influence of the light attenuation by a decreased water column transparency and by epiphyte shading. However, the integration of the changes in all the above parameters results in a substantial reduction of the carbon balance of plants from st. III and IV that accounts for 93% and 60% from values calculated in plants from st. V (Fig. 4).

**Table 4.** Carbon balance calculations at the plants of the three stations considering changes in different parameters: plant features (plant biomass and below-ground respiration); water column transparency; photoadaptation; epiphyte biomass, and the resultant of all of them combined.

Carbon balance mg C shoot <sup>-1</sup> month <sup>-1</sup>	Station V	Station IV	Station III	% variation	
				St. IV	St. III
Plant features (B)	223.6	112.7	46.9	49.6	79.0
Water column transparency (T)	223.6		94.7		57.7
Photoadaptation (P)	223.6		525.3		134.9
Epiphyte biomass (E)	177.6	97.7	58.8	45.0	66.9
B + T + P + E	177.6	71.1	11.7	60.0	93.4

### **Discussion**

Fish farming effluents have an important influence over the surrounding *Posidonia oceanica* ecosystem. Similarly as occurred in other study cases (Delgado et al., 1997; Ruiz et al., 2001), the meadow and the community structure were highly affected, causing the seagrass decline at the surroundings of the cages (for a better description, see Ruiz et al., 2003). The decrease in *P. oceanica* density and cover drove to a decrease in plant biomass per area, although not similarly in all plant parts, with more important reduction of leaf biomass than of rhizomes and roots. This effect over the leaves can be attributed to changes in other components of the community triggered by fish farm effluents. The increase of epiphyte proliferation in *P. oceanica* leaves and the increase of *Paracentrotus lividus* abundance, an herbivore of this seagrass, are two of the more evident responses at the community level. The important proliferation of epiphytes over leaf shoots implied an increase in the food supply to sea-urchins that facilitated the increase of *P. lividus* abundance, which caused an important reduction of leaf shoot size and, hence the reduction of the potential C gain through reduction in photosynthetic biomass.



In addition to changes in shoot size, the rising epiphyte biomass close to cages imply an important shading to *P. oceanica*, highly decreasing its light availability. This effect was even more important due to the fact that the epiphyte proliferation affected not only the oldest leaves (A4), which have the lowest photosynthetic rates (Alcoverro et al., 1998), but also intermediate ages, which showed the highest proportion of biomass in the leaf shoot (more than 50%) and the highest photosynthetic rates.

The effect of fish farming on below-ground organs was only evidenced by the reduced respiration rates in roots from plant closest to fish cages. Due to reduction in root respiration is one of the earliest responses of plants under anoxia (Liao and Lin, 2001), and there were evidences of increased sulphide concentrations close to fish cages (Frederiksen and Holmer, 2003), the changes in respiration rates can not be related to a physiological strategy of *P. oceanica* to reduce C losses but to a physiological response to increased anoxia.

At the station closest to cages, the reduction of plant biomass was the factor that affected more negatively to *P. oceanica* C balance. There, the lower below-ground biomass implied a lower C loss by respiration, thus, a reduced C gain was attributed to the smaller shoot size due to herbivory. Shading by epiphyte biomass was the second factor in importance, followed by the effect of the reduced water-column transparency. All these factors drove to a reduction in the *P. oceanica* capability of light utilization. In this situation, *P. oceanica* could respond changing its photosynthetic parameters, as it has been described in other works (Ruiz et al., 2001; Ruiz and Romero, 2001). However, photoadaptation could only partially alleviate the decrease in C budget. In plants from the st. IV, the three factors (plant and epiphyte biomass and water column transparency) account for a similar negative effect over the C balance and were neither compensated by the photoadaptation considered.

The decrease in C balance values fitted with a decrease in the content of C reserves in the rhizomes of those plants. Carbohydrate reserves in temperate seagrasses are crucial for survival during episodes of low light availability, especially during winter, in which the C balance can be negative (Pirc, 1985; Burke et al., 1996; Alcoverro et al., 2001). Specifically, the use of internal C reserves allows *P. oceanica* to maintain a high leaf growth rate while nutrients are abundant, and develop a foliar canopy well before maximum summer irradiance (Alcoverro et al., 1995). A reduction in these reserves could lead to a decrease in the winter production of leaf tissue, fact that would make worse the situation of the plants close to cages.

At the light of the results obtained, the situation of the plants at the st. III and IV was critical due to its low carbon balance, and the decline of the meadow probably will continue if environmental conditions do not change.

#### **Acknowledgements**

We thank Dr. Fiona Tomàs for field and laboratory work, to IMBC team for vertical profiles of light data, and to Pere Renom for assessment on carbon balance calculations.

#### **References**

Alcoverro, T., Duarte, C., and Romero, J., 1997. The influence of herbivores on *Posidonia oceanica* epiphytes. *Aquatic Botany* 56 (2): 93-104.



- Alcoverro, T., Duarte, C. M., and Romero, J., 1995. Annual growth dynamics of *Posidonia oceanica*: contribution of large-scale versus local factors to seasonality. *Marine Ecology Progress Series* 120: 203-210.
- Alcoverro, T., Manzanera, M., Romero, J., 1998. Seasonal and age-dependent variability of *Posidonia oceanica* (L.) Delile photosynthetic parameters. *Journal of Experimental Marine Biology and Ecology* 230: 1-13.
- Alcoverro, T., Manzanera, M., and Romero, J., 2001. Annual metabolic carbon balance of the seagrass *Posidonia oceanica* (L.) Delile: the importance of carbohydrate reserves. *Marine Ecology Progress Series* 211, 105-116.
- Alcoverro, T., Pérez, M., and Romero, J., submitted. Importance of within-shoot epiphyte distribution for the carbon budget of seagrasses: the example of *Posidonia oceanica*. *Marine Ecology Progress Series*.
- Burke, M.K., Dennison, W.C., and Moore, K.A., 1996. Non-structural carbohydrate reserves of eelgrass *Zostera marina*. *Marine Ecology Progress Series* 137: 195-201.
- Delgado, O., Grau, A., Pou, S., Riera, F., Massuti, C., Zabala, M., and Ballesteros, E., 1997. Seagrass regression caused by fish cultures in Fornells Bay (Menorca, Western Mediterranean). *Oceanol. Acta* 20: 557-563.
- Delgado, O., Ruiz, J.M., Pérez, M., Romero, J., and Ballesteros, E., 1999. Effects of fish farming on seagrass (*Posidonia oceanica*) in a Mediterranean bay: seagrass decline after organic loading cessation. *Oceanol. Acta* 22: 109-117.
- Edwards, G., and Walker, D., 1983. C<sub>3</sub>, C<sub>4</sub> mechanisms and cellular and environmental regulation of photosynthesis. Blackwell Scientific, Oxford.
- Erskine, J.M., and Koch, M.S., 2000. Sulfide effects on *Thalassia testudinum* carbon balance and adenylate energy charge. *Aquatic Botany* 67: 275-285.
- Frederiksen, M.F., Holmer, M., 2003. Fish farm influence on sulfur dynamics in *Posidonia oceanica* meadows. Annual report 2003, Annex 4.5, MedVeg Project.
- Hall, P.O.J., Holby, O., Kollberg, S., and Samuelsson, M.O., 1992. Chemical fluxes and mass balances in a marine fish cage farm. IV. Nitrogen. *Marine Ecology Progress Series* 89: 81-91.
- Hargrave, B.T., Duplisea, D.E., Pfeiffer, E., and Wildish, D.J., 1993. Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. *Marine Ecology Progress Series* 96: 249-257.
- Holmer, M., 1991. Impacts of aquaculture on surrounding sediments: generation of organic-rich sediments. In: De Pauw N, Joyce J (eds) *Aquaculture and the environment*. Eur. Aquacult. Soc. Spec. Publ. 16: 155-175.
- Holmer, M., and Bondgaard, E. J., 2001. Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. *Aquatic Botany* 70: 29-38.



- Holmer, M., Pérez, M., and Duarte, C., 2003. Benthic primary producers-a neglected environmental problem in Mediterranean maricultures? *Marine Pollution Bulletin* 46: 1372-1376.
- Invers, O., Tomàs, F., Pérez, M., and Romero, J., 2002. Potential effect of increased CO<sub>2</sub> availability on the depth distribution of the seagrass *Posidonia oceanica* (L.) Delile. *Bulletin of Marine Science* 71(3): 1191-1198.
- Jassby, A. D., and Platt, T., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography* 21: 540-547.
- Karakassis, I., 1998. Aquaculture and coastal marine biodiversity. *Océanis* 24 (4): 271-286.
- Karakassis, I., Tsapakis, M., Hatziyanni, E., Papadopoulou, K.N., and Plaiti, W., 2000. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES Journal of Marine Science* 57: 1462-1471.
- Kirk, J.T.O. 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, London: 24-41.
- Pérez, M., and Romero, J., 1992. Photosynthetic response to light and temperature of seagrass *Cymodocea nodosa* and the prediction of its seasonality. *Aquatic Botany* 43: 51-62.
- Pergent-Martini, C., and Pergent, G., 1995. Impact of a sewage treatment plant on the *Posidonia oceanica* meadow: assessment criteria. In *Proceedings of the Second International Conference on the Mediterranean Coastal Environment, MEDCOAST'95*, ed. E. Özhan, pp. 1389-1399. Tarragona, Spain.
- Pirc, H., 1985. Growth Dynamics in *Posidonia oceanica* (L.) Delile. I. Seasonal changes of soluble carbohydrates, starch, free amino acids, nitrogen and organic anions in different parts of the plant. *P.S.Z.N.I: Marine Ecology*: 6, 141-165.
- Pirc, H., 1986. Seasonal aspects of photosynthesis in *Posidonia oceanica*: influence of depth, temperature and light intensity. *Aquatic Botany* 26: 203-212.
- Pitta, P., Karakassis, I., Tsapakis, M., Zivanovic, S., 1999. Natural vs. mariculture induced variability in nutrients and plankton in the eastern Mediterranean. *Hydrobiologia* 391: 181-194.
- Procaccini, G., Buia, M.C., Gambi, M.C., Pérez, M., Pergent, G., Pergent-Martini, C., and Romero, J., 2003. The seagrasses of the Western Mediterranean. In: Green, E.P., and Short, F.T (eds). *World atlas of seagrasses*. Prepared by the UNEP World Conservation Monitoring Centre. University of California Press, Berkeley, USA.
- Renom, P., Pérez, M., Alcoverro, T., Manzanera, M., Romero, J. 2000. Assessing temporal variability in the carbon balance of the seagrass *Posidonia oceanica* (L.) Delile. *Biologia Marina Mediterranea* 7 (2): 119-122.
- Ruiz, J.M., and Romero, J., 2001. Effects of *in situ* experimental shading on the Mediterranean seagrass *Posidonia oceanica*. *Marine Ecology Progress Series* 215: 107-120.

Ruiz, J.M., Pérez, M., and Romero, J., 2001. Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. *Marine Pollution Bulletin* 42: 749-760.

Ruiz, J.M., Invers, O., and Pérez, M., 2003. Changes of community structure under the influence of cages. Reports of MedVeg project.

van Katwijk, M. M., Vergeer, L. H. T., Schmitz, G. H. W., and Roelofs, J. G. M., 1997. Ammonium toxicity in eelgrass *Zostera marina*. *Marine Ecology Progress Series* 157: 159-173.