

# Photosynthetic activity of the solar-powered lagoon mollusc *Elysia timida* (Risso, 1818) (Opisthobranchia: Sacoglossa)

F. Giménez Casalduero<sup>1\*</sup> and C. Muniain<sup>2</sup>

<sup>1</sup>Departamento de Ciencias del Mar y Biología Aplicada, Universidad de Alicante, Ap C 99, 03080, Spain, Email. Francisca.Gimenez@ua.es;

<sup>2</sup>CONICET – Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", C1405DJR, Buenos Aires, Argentina, Email. cmuniain@macn.gov.ar

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## Abstract

The sacoglossan *Elysia timida* (Risso, 1818) is typical of shallow sea areas and coastal lagoons. It retains symbiotic functional chloroplasts derived from its algal food *Acetabularia acetabulum* (Linnaeus) for 45 days. However, some aspects of the photosynthetic behaviour of the mollusc-chloroplast association are unknown. This work studies the photosynthetic characteristics of *E. timida* from a lagoon environment (Mar Menor), defined between 25 and 600  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  by the photosynthetic-irradiance curve. Experimental data were fitted to different exponential models and models based on Michaelis-Menten kinetics. Among the latter, the model that bests fits to the experimental data was identified. Furthermore, it incorporates respiration values and allows an estimation of compensation irradiance ( $I_c$ : 11.89  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ). This model showed a rapid saturation of the photosynthetic apparatus at relatively low irradiance values ( $I_k$ : 31.33  $\mu\text{mol quanta m}^{-2}$ ). Excess light seems to be modulated by the mollusc's opening and closing of their parapodia. Mean P/R values (Production versus Respiration relationship) are above 0.91; considered high in coral-zooxanthellae symbioses. The photosynthetic efficiency of the symbiotic chloroplasts is fairly high, taking into account that sacoglossans are more active than corals and have a higher respiration rate.

**Keywords:** *Elysia timida*, sacoglossa, kleptoplasts, photosynthesis

## 1. Introduction

Sacoglossan gastropods are a vast group of herbivorous opisthobranch molluscs that feed suctorially, mainly on green syphonaceous algae through a uniseriate radula (Jensen, 1996, 1997; Marín and Ros, 2004). Retention of plastids is common among these molluscs, which engulf chloroplasts phagocytotically into the digestive cells during feeding. Some sacoglossans studied maintain the chloroplasts photosynthetically active for weeks or months (Kawaguti and Yamasu, 1965; Trench, 1975; Clark and Bussacca, 1978; Hinde, 1980; Stirts and Clarks, 1980; Clark, 1992; Marín and Ros, 1993; Rumpho et al., 2000; Muniain et al., 2001). Functional chloroplasts photosynthesize inside the mollusc cell, evolving oxygen and producing photosynthates like glucose and other carbohydrates, lipids, and even proteins (Greene, 1970; Greene and Muscatine, 1972; Trench et al., 1973; Hinde and Smith, 1975; Hinde, 1978; Scheuer, 1979).

Furthermore, these molluscs have shown both morphological and behavioural adaptations associated with a better use of photosynthesis and control of energy production. One of these adaptations is an enlargement of the digestive gland surface area, either generating branching in the form of cerata where chloroplasts are stored, or flattening and increasing the area in the form of a leaf, thus increasing the photosynthetic area. In species with photosynthetic ability, the photosynthetic symbiont is often orientated towards the light; these species are capable of regulating excessive irradiance by closing the parapodia (Rahat and Monseline, 1979; Monseline and Rahat, 1980; Weaver and Clark, 1981).

Photosynthesis-irradiance fit curves have been traditionally used in physiological studies of algae since they provide important information on the photosynthetic apparatus. Values of the photosynthesis versus irradiance relationship are often fitted to mathematical functions to obtain a series of parameters to describe predictive models of primary production (Gattuso and Jauvert, 1985). These curves can be an interesting tool to describe the energy

\*The author to whom correspondence should be sent.

production parameters of symbiotic sacoglossan-chloroplasts (Wägele and Johnsen, 2001).

*Elysia timida* is a Mediterranean sacoglossan that lives in well-lit and shallow littoral habitats (Bouchet, 1984; Marín and Ros, 1988; Thompson and Jacklin, 1988) and is very common in the Mediterranean coastal lagoons. Marín and Ros (1992, 1989, 2004) have found that *Acetabularia acetabulum* (Linnaeus) is the exclusive food source in Mediterranean waters, although recent observations would demonstrate that the mollusc exhibits a greater feeding versatility, incorporating brown algae in the months when *A. acetabulum* is absent (Giménez Casaldueiro, 2004).

Several studies found that *E. timida* obtains kleptoplasts from algal food sources (Rahat, 1976; Rahat and Monseline, 1979; Marín and Ros, 1989; 1992; Ros and Marín, 1991), which remain active during 45 days in the absence of food (Marín and Ros, 1993). Previous studies on *E. timida* have focused on the description of its symbiotic chloroplasts (Bouchet, 1984; Ros and Rodríguez, 1985), and its photobiology (Monseline, 1979; Rahat and Monseline, 1979). Also, recent studies have demonstrated some genetic differences between the population of *E. timida* from a coastal lagoon (Mar Menor) and the Mediterranean populations (González-Wangüemert et al., 2006). There is abundant information available on this species; however, little is known about the sacoglossan-chloroplast energy production relationship. This work studies the photosynthetic parameters of *E. timida* individuals occurring in a Mar Menor lagoon environment.

## 2. Material and Methods

### *Specimens*

Sacoglossans were collected from the coastal lagoon of Mar Menor (SE Spain), at a depth of 0.5–2 m, and transported to the laboratory in an ice box within 3 h following collection. The specimens were deposited in 36 L aquaria in aerated and filtered sea water at a temperature of 23–25°C, similar to the mean temperature values estimated in the field collection sites. A total of 184 individuals were used. Experiments were conducted during the two days following collection.

### *Estimation of dry weight/length relationship*

Dry weight/length relationship was estimated for a total of 61 molluscs to obtain biomass of individuals prior to treatment, with the aim of avoiding possible alterations in size during the experiment. Sacoglossans were recorded with a video camera in a Petri dish at a known distance. By watching the recording, the images of individuals reaching maximum stretching were selected. The exact length (mm) was estimated by analyzing the images. The size value used resulted from the arithmetic mean of all length values

obtained for a given individual (Giménez-Casaldueiro, 1999). Then, individuals were placed in a heater at 100°C for 24 h to estimate dry weight. The relationship between length (mm) of the individual and its biomass (g) was determined by linear regression analysis.

### *Photosynthesis versus irradiance curve*

Photosynthesis versus irradiance (P-I) curve for *E. timida* was estimated between 25 and 600  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ , using a total of 123 individuals. Biomass of sacoglossans was estimated as described above. Net oxygen production rate was measured using closed-chamber respirometry techniques, similar to those described by Chapelle and Peck (1995), and oxygen concentration was obtained by comparisons with control chambers (experimental chambers containing no molluscs and run in parallel).

Three of the individuals measured were placed in a 15 ml incubation chamber containing seawater filtered through 0.47  $\mu\text{m}$ -pore Millipore filters. There was enough room in the chambers for each slug to unfold its parapodia. The initial oxygen was calculated from oxygen values obtained from two aliquots of 2.5 ml; then the chamber was hermetically closed and placed in the incubation system at constant light and temperature (23°C) for the entire experimental period (2 h). The treatment was repeated for the different irradiance values (25, 50, 100, 200, 300, 400, and 600  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ), which were obtained by combining 40 W halogen lamps and natural filters. Respiration rate was based on results obtained with incubation chambers kept in the dark; the chambers were prepared following the method described above, but were covered with aluminium film to avoid photosynthesis. A total of 20 dark chambers were measured. Three incubation chambers plus one in dark conditions and the control one were placed for each irradiance value simultaneously. After the incubation period, dissolved oxygen concentration was estimated by a modification of the Micro-Winkler methodology (Fox and Wingfield, 1938) based on the adaptation by Peck and Uglow (1990).

Oxygen concentration in the incubation chambers was measured using coulometric techniques (Peck and Uglow, 1990). Measurements were performed by adding sodium thiosulfate (0.001 N), previously standardized with potassium iodide (Parsons et al., 1984). A 0.1 ml syringe containing thiosulfate, attached to a micrometer and connected to the water sample from the incubation chamber was used. Thiosulfate was gradually added until endpoint was reached, which was identified by the change of colour in the sample and was detected with the luxometer. During measurement, the sample was homogenized using a magnetic stirrer. The volume added to the sample until endpoint was reached with a micrometer. This chemical method of oxygen measurement is especially sensitive, with 2% accuracy (Fox and Wingfield, 1938; Ramus, 1981).

Table 1. Fitness models used of photosynthesis versus irradiance relationship. P: Photosynthesis rate;  $P_{\max}$ : maximum photosynthesis rate at saturation irradiances; I: irradiance; R: respiration rate in darkness; a: photosynthetic efficiency at irradiances below saturation.

Model		Reference
	Exponential	
(1)	$F = a \cdot I \cdot e^{(-a \cdot I / (P_{\max} \cdot e))}$	Jassby and Platt (1976)
(2)	$F = P_{\max} \cdot (1 - e^{(-a \cdot I / P_{\max}})$	Gattuso and Jaubert (1985)
(3)	$F = P_{\max} \cdot (1 - e^{(-a \cdot I / P_{\max}}) + R$	Terrados (1991)
	(Michaelis and Menten, 1913)	
(4)	$F = (P_{\max} \cdot a \cdot I) / (P_{\max} + a \cdot I)$	Gattuso and Jaubert (1985)
(5)	$F = (P_{\max} \cdot a \cdot I) / (P_{\max} + a \cdot I) + R$	Pérez (1989)
(6)	$F = P_{\max} \cdot (I - R/a) / ((P_{\max}/a) + (I - R/a))$	Pérez (1989)

Photosynthetic parameters were estimated using the (P-I curve). Numerous models were fitted to describe the P-I curve, depending on the material analyzed (Platt et al., 1980; Lapointe et al., 1984; Carpenter, 1985). The models most widely mentioned in the literature are exponential (Jassby and Platt, 1976; Gattuso and Jaubert, 1985; Pérez, 1989; Terrados, 1991) and those based on Michaelis-Menten kinetics (1913) (Gattuso and Jaubert, 1985; O'Neal and Prince, 1988; Pérez, 1989). Six models were selected among the different ones analyzed, three exponential models and three based on Michaelis-Menten kinetics (Table 1). Curves were fitted using nonlinear techniques. The ability of each model to explain the variation ( $R^2$ ) in the data was compared. The goodness of fit of each equation was estimated on the basis of the fidelity index (FI) used by Nelson and Siegrist (1987):

$$FI = (\sum^n (O_{oi} - O_{ei})^2 / n$$

where  $O_o$ : Oxygen production values observed;  $O_e$ : Oxygen production values expected. The equation yielding the lowest FI values is the best fit. Maximum photosynthesis rate ( $P_{\max}$ ) was estimated as the mean of the highest irradiance values obtained experimentally. Photosynthetic efficiency or initial slope ( $\alpha$ ) was estimated by fitting the nonlinear regression models between 20–600  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ . The saturation irradiance ( $I_k$ ) value was estimated from the intercept of initial slope tangent and  $P_{\max}$  (Pollard, 1999).

$$I_k = P_{\max} / \alpha$$

The photosynthesis-respiration (P/R) ratio in organisms with symbiotic associations is frequently used to interpret the Production (P) versus Respiration (R) relationship (Beyers, 1966; Kanwisher and Wainwright, 1967; Pillai and Nair, 1972). In general, (P) and (R) values used to calculate P/R coefficients are obtained in 24 hours. P is expressed as the amount of oxygen produced in 24 hours, and R as the oxygen consumed by an organism in 24 h dark period. The

formula most commonly used is based on work by Muscatine and Porter (1977):

$$P/R = \text{Gross } P_s (24 \text{ h}) / R_c (24 \text{ h})$$

where P/R is the percentage of the contribution by the symbiotic photosynthesizing organism; gross  $P_s$  is the photosynthetic production of the symbiotic organism, and  $R_c$  is the oxygen consumed by the host-symbiont association.

An approximation to the method proposed by Muscatine and Porter (1977) was employed for the sacoglossan-chloroplast association. The photosynthetic production of the symbiotic organism (gross  $P_s$ ) was estimated from theoretical values obtained in the P-I curve, taking into account daily irradiance variation in the lagoon, following Terrados (1991) and Ruíz (2000), as a measure of oxygen production at different irradiance conditions. Furthermore, 24 h respiration values were calculated based on the mean of all hourly respiration values obtained experimentally in the incubation chamber in the dark and multiplied by 24. Data were fitted to the different P-I curve models using SIGMA PLOT nonlinear curve fitter (Jandel Scientific, 1992).

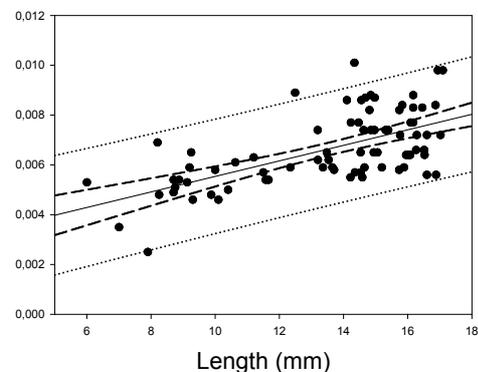


Figure 1. Linear regression of the length (mm) of *E. timida* with respect to its dry weight (g). Medium dash line corresponds to 95% confidence intervals; dotted line is the prediction intervals.

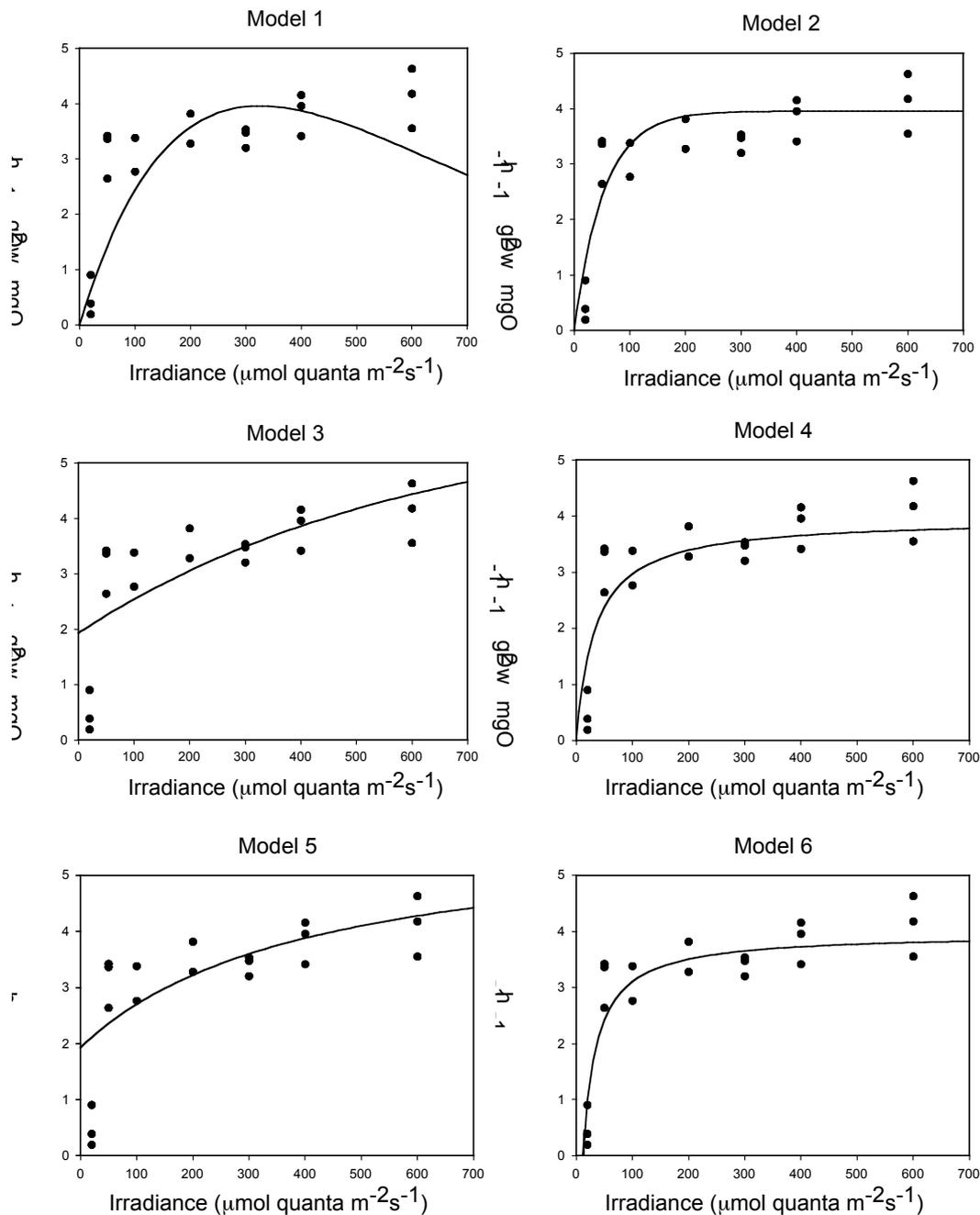


Figure 2. Irradiance-production curves; effect of light intensity on net photosynthesis rate for *E. timida*, at 23°C. Regression estimated for the interval of 20–600  $\mu\text{mol quantum m}^{-2} \text{s}^{-1}$  for the exponential models tested (models 1, 2 and 3) and models based on Michaelis-Menten Kinetics (models 4, 5 and 6).

### 3. Results

Results of fitting dry weight and length data revealed a linear regression of positive slope (Fig. 1) that corresponds to the equation:

$$\text{DW} = 0.00242 + 0.00031L \text{ (a)}$$

where DW is dry weight as a measure of biomass (g) and L is length of individual at maximum stretching (mm). The fitness model for the length versus biomass relationship was highly significant, although the variance explained is relatively low ( $r^2 = 0.39$ ,  $p < 0.001$ ).

### Estimation of photosynthesis parameters

Photosynthesis saturation or maximum photosynthesis ( $P_{\max}$ ) value obtained from experimental data was  $3.96 \pm 0.14 \text{ mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ , and the mean value for respiration rate in the dark ( $R$ ) was  $-1.93 \pm 0.07 \text{ mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ . Using these basic parameters, the values of photosynthetic efficiency at low irradiances ( $\alpha$ ) were estimated for each model described along with the values that show the goodness of fit, both for the coefficient of variable ( $R^2$ ) and (FI) (Table 2). The parameters  $P_{\max}$ ,  $R$ , and  $\alpha$ , allow us to represent P-I curves according to the different models tested (Fig. 2). After calculating the basic parameters, the data were fitted to the different models. Only model 1 had a photoinhibitory response, which is not observed in experimental data. In all cases, the analysis of the response of the P-I curve showed that at low irradiance values, photosynthesis is directly proportional to an increase of irradiance, with values of photosynthetic efficiency ( $\alpha$ ) varying from 0.0033 in model 1 to 0.1624 in model 6. The models that best fit to the data are models 2, 4 and 6. Model 2 is exponential, but the other two are based on Michaelis-Menten kinetics (Fig. 2), with FI values of 0.34, 0.38, and 0.25, respectively (Table 2).

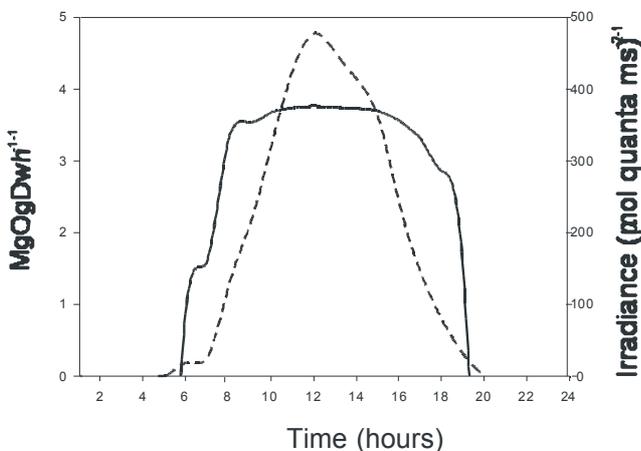


Figure 3. Daily oxygen production ( $\text{mg O}_2 \text{ dw}^{-1} \text{ h}^{-1}$ ) of *E. timida*, estimated on the basis of model 6 (Pérez, 1989) (continue line); daily cycle of incident irradiance ( $\mu\text{mol quantum m}^{-2} \text{ s}^{-1}$ ) in Mar Menor Lagoon, according to Terrados (1991) and Ruiz (2000) (discontinued line).

Saturation irradiance ( $I_k$ ), the interaction between  $\alpha$  and  $P_{\max}$ , is the irradiance value at which  $P_{\max}$  occurs. Fairly low  $I_k$  values were obtained for lagoon *E. timida* using the best fit curves. The value of daily photosynthetic production of chloroplasts is obtained based on the production curve using model 6, with Mar Menor lagoon

Table 2. Results of values of photosynthesis efficiency ( $\alpha$ ) and fidelity index FI estimated for each model employed for fitness of production data as a function of irradiance. Basic parameters of PI curve for the best fit models (2, 4 and 6).  $I_c$ : the compensation irradiance;  $I_k$ : saturation irradiance;  $R$ : respiration rate.

Model	Initial slope ( $\alpha$ )	$R^2$	IF	$I_c$	$I_k$
(1)	$0.033 \pm 0.005$	0.44	0.82		
(2)	$0.074 \pm 0.013$	0.76	0.34	–	53.59
(3)	$0.007 \pm 0.001$	0.53	0.69		
(4)	$0.118 \pm 0.027$	0.74	0.38	–	33.53
(5)	$0.009 \pm 0.002$	0.55	0.65		
(6)	$0.124 \pm 0.020$	0.82	0.25	11.89	31.33

Table 3. Summary of optimal photosynthesis and optimal irradiance for Sacoglossans. Taken from Weaver and Clark (1981).

Species	$I$ ( $\mu\text{e}/\text{m}^2/\text{s}$ )	Reference
<i>Costasiella liliana</i>	200	Weaver and Clark (1981)
<i>Elysia tuca</i>	<100	Weaver and Clark (1981)
<i>Elysia viridis</i>	120–220	Gallop et al. (1980)
<i>Tridachia crispata</i>	200–300	Weaver and Clark (1981)
<i>Oxynoe antillarum</i>	<100	Weaver and Clark (1981)
<i>Berthelinia caribbea</i>	<100	Weaver and Clark (1981)

daily irradiance data (Fig. 3). A total of  $44.11 \text{ mg O}_2 \text{ gps}^{-1} \text{ h}^{-1}$  of daily production was obtained. The daily respiration value was obtained from the respiration mean experimentally estimated and multiplied by 24 h, yielding  $46.32 \text{ mg O}_2 \text{ gps}^{-1} \text{ h}^{-1}$ . A value of 0.91 was calculated for the production versus respiration ratio.

## 4. Discussion

The analysis of the different models of P-I curves shows that the model that best interprets behaviour of the photosynthetic characteristics observed in *Elysia timida* is the one described by Pérez (1989) (model 6), which is based on the kinetics of Michaelis-Menten (1913) (Table 1). This model also incorporates respiration values, which allow us to estimate the compensation irradiance value ( $I_c$ :  $11.89 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ), the minimum irradiance value to compensate for respiration. Model 6 shows a rapid saturation of the photosynthetic apparatus at relatively low irradiance values ( $I_k$ :  $31.33 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ) (Fig. 2).

The low saturation value seems to contradict the individual's habitat (shallow, well-lit areas); however, this rapid saturation might be influenced by a control of parapodia, which would avoid pigment photodestruction under light oversaturation conditions, as mentioned later. During the experimental period a closing and opening of parapodia was observed, which agrees with descriptions by Rahat and Monseline (1979); parapodia were completely open when exposed to low irradiance and gradually closed with increasing irradiance values.

Another outstanding characteristic of the P-I curve is the lack of photoinhibition at high irradiance. The curves introducing photoinhibition strategies described in the literature yield bad data fitness. The one that shows best fit is that based on Michaelis-Menten kinetics, probably, once again, because of the ability of sacoglossans to regulate photosynthetic production. Thus, the photosynthetic apparatus would not function at its maximum capacity in an ordinary situation, but would be controlled by the parapodia behaviour of the sacoglossan. *E. timida* is found in the shallowest areas of the lagoon, where the influence of irradiance varies seasonally. From March to June, irradiance at midday is close to  $500 \mu\text{E}^{-2} \text{s}^{-1}$  (Fig. 3), whereas from January to August, it is below  $200 \mu\text{E}^{-2} \text{s}^{-1}$  (Terrados, 1991). These irradiance values are higher than those indicated in the literature as the optimum for other photosynthesizing *Elysia* species (Table 3). Furthermore, these high irradiances contrast with the low saturation irradiance value found ( $31.33 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ); however, this could be an indicator of the great dependence on photosynthetic energy that the mollusc has for survival. With such low saturation values, the mollusc ensures a maximum yield at low light intensity, whereas at high intensity, the sacoglossan keeps the parapodia closed, thus avoiding the problems produced by light excess described earlier. Furthermore, in the field, the sacoglossan is commonly found with completely closed parapodia on a well-lit day (per. obs.).

The Production versus Respiration relationship (P/R) was initially used to estimate compensation irradiance for symbiotic associations between corals and zooxanthellae. A ratio equal to 1 indicated that most of the carbon fixed in photosynthesis was consumed in respiration by the host and the "guest". A  $P/R > 1$  indicated that the amount of carbon fixed by photosynthesis exceeded the basal metabolic requirement of the host; whereas,  $P/R < 1$  indicated that the host needed to feed on other sources to complete the amount of carbon required for its basal metabolic needs (McCloskey et al., 1978). It has been demonstrated that the real compensation depth for each plant is usually shallower than that estimated with this model. However, the high values of this index described in some works on symbiotic organisms of coral reefs (Verwey, 1930; Kawaguti, 1937) seem to demonstrate that photosynthesizing symbiotic organisms substitute at least part of the basal energy requirements of the host species. At least they have certain

ability to replace these requirements, since there is extra energy availability in the host organisms. Theoretically estimated mean P/R values of lagoon *E. timida* are above 0.91, which are considered high in the coral-zooxanthellae symbioses. This value should be regarded as an approximation, since respiration has been considered constant based on the mean obtained for individuals during periods of activity. If a study of 24 h respiration was conducted, the total value would probably decrease. If we consider that sacoglossans are more active than corals and possess higher respiration values, production values of chloroplasts should also be higher to obtain similar indices. Therefore, photosynthetic efficiency of symbiotic chloroplasts is fairly high.

The parameters of the P-I curve obtained in this work provide useful baseline information to perform further studies on photosynthetic energy generated from the relationship between the mollusc and the acquired chloroplasts, as well as on the importance of such energy for primary metabolism of lagoon sacoglossans.

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