Culture of the seaweed *Ulva ohnoi* integrated in a *Solea senegalensis* recirculating system: Influence of light and biomass stocking density on macroalgae productivity

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Abstract. A growth model was developed to optimize the management of multi-trophic aquaculture systems by analyzing the influence of light and biomass stocking density (SD) in the productivity of *Ulva ohnoi* fed with the effluents from *Solea senegalensis* culture tanks.

Experimental growth rates and productivity were determined in three flat bottom algae tanks with different incident photon irradiances (E_0) (163, 280 and 886 µmol m^{-2} s^{-1}), photoperiod 12:12h and with stocking densities ranging from 82 to 340 gdw m^{-2}. The distribution of photon irradiance in the algae tanks was estimated as a function of the E_0 and SD.

The results obtained showed that, the algae exposed to the highest E_0 (886 µmol m^{-2} s^{-1}) and SD below 170gdw m^{-2} experimented a strong decrease in their growth rate, together with morphological changes.

The model proposed to estimate the specific growth rate (μ_{NET}), on the basis of E_0 and SD, assumed that photosynthetic activity is dependent on the local photon flux density and, therefore, spatially distributed in the tank. The non-linear regression used to estimate the growth kinetic parameters showed a standard deviation of the distance between measured and fitted μ_{NET} data values equal to 0.011 d^{-1}.

In terms of biomass productivity per unit area (BP_A) the model shows, for each E_0 level, a trend to increase with SD, achieving a maximum BP_A, where SD can be considered optimal, and decreasing for higher SD values. The optimal SD and the maximum BP_A achievable can be also determined as a function of E_0.

Keywords: *Ulva ohnoi*, IMTA, growth model, productivity, photon irradiance, biomass stocking density

INTRODUCTION

Green seaweeds belonging to genus *Ulva* Linnaeus (Ulvophyceae, Chlorophyta) have been identified as good candidates for filtering fish effluents due to their capacity to be cultured unattached, their wide environmental tolerances (Cohen and Fong 2004; Bolton et al. 2009), together with their high growth rates and high capacity to absorb nitrogen (Jiménez del Río et

al. 1996; Mata et al. 2010). Furthermore, *Ulva* is gaining interest as ingredient for animal feeds and as a source of ulvans, sulphated polysaccharides with uses in biomedical tissue engineering, regenerative medicine and drug delivery (Lahaye and Robic 2007; Mata et al. 2016). Most of the existing growth models for *Ulva* species have been mainly developed for the management of coasts and estuaries (Bendoricchio et al. 1994; Coffaro and Sfriso 1997; Solidoro et al. 1997; Martins and Marques 2002; Aveytua-Alcázar et al. 2008; Ren et al. 2014).

Ulva ohnoi M. Hiraoka & S. Shimada has been suggested as one of the most suitable species for land-based cultivation, due to its high growth rates, biomass productivity and nitrogen uptake rates (Yokoyama and Ishihi 2010; Lawton et al. 2013; Angell et al. 2014; Mata et al. 2016).

Solea senegalensis Kaup, 1858, has shown to be an adequate fish species for growing at very high stocking densities, compatible with those needed for its intensive commercial farming (Salas-Leiton et al. 2008). The rising intensive production of this species in Spain, Portugal and France (Morais et al. 2016) has increased the interest on developing specific land-based integrated multitrophic aquaculture (IMTA) technologies to minimize the environmental impact of these activities. The similar range of optimal temperature for *U. ohnoi* and *S. senegalensis* cultures, generates great expectations on the feasibility of integrating the production of both species. The main challenges to expand the introduction of this technology are related with the capacity to increase the algae productivity and the nutrient uptake per surface unit, reducing the land area required. Recirculating Aquaculture Systems (RAS) allow the intensification of fish production, as high nutrient concentration in water can be controlled by the flow rate delivered from fish to algae tanks. In these conditions, the light delivered to the algae becomes the main factor determining seaweed productivity.

Effect of light in seaweed growth and productivity

The biomass productivity BP expresses the rate of change in dry biomass B, which is given by the difference between biomass production and biomass loss due to respiration, exudation and mortality, as shown in Eq. 1, were μ_{GROSS} and λ are the specific rates of biomass growth and losses, respectively, expressed in d^{-1} (Duarte and Ferreira 1993; Bendoricchio et al. 1994; Aveytua Alcázar et al. 2008; Duarte et al. 2009).

$$BP = \frac{dB}{dt} = (\mu_{GROSS} - \lambda)\, B \qquad (1)$$

The main limiting factors on μ_{GROSS} are light, temperature (T) and nutrient concentration (N). Their influence can be described with a multiplicative formulation:

$$\mu_{GROSS} = \mu_{max}\, f(E)\ f(T)\ f(N) \qquad (2)$$

Were μ_{max} is the maximum specific growth rate, and light is expressed in terms of photon irradiance (E) as µmol of photosynthetic photons per square meter and second (µmol m^{-2} s^{-1}).

In the case that light is the single limiting variable on growth (e.g. under optimal temperature and not limited nutrient supply), the estimation of the net specific growth rate could be calculated by Eq. 3:

$$\mu_{NET} = \mu_{max}\, f(E) - \lambda \qquad (3)$$

107

108 *Influence of seaweed stocking density on the light delivered to the culture*

109 Most of the tanks used for seaweed production have opaque walls; this implies that only the
110 light incident to the tank water surface can be delivered to seaweeds. In these conditions, the
111 amount of biomass in the tank is frequently expressed in terms of biomass per unit of water
112 surface or stocking density, which will be referred as SD (in gdw m^{-2}) for dry biomass or FSD
113 (in kgfw m^{-2}) for wet biomass. The influence of the SD on the growth of cultured seaweeds is
114 consequential from the light attenuation due to self-shading, that causes light gradients which
115 lead seaweeds to experience different light intensities, depending on their location in the tank.
116 Maximizing the productivity will require a suitable management of seaweed stocking densities,
117 oriented to optimize light distribution along the production process.

118 The incident photon irradiance to the water surface (E_0) attenuates through the water
119 column, by absorption and scattering, and declines exponentially with depth. The photon
120 irradiance at depth z below the surface $E(z)$ is described by the Lambert-Beer law:

$$E(z) = E_0\, exp\,(-K'\, z) \qquad (4)$$

121 Were K' is the apparent light attenuation constant (m^{-1}) that, in a macroalgae tank, will be
122 determined by the water light extinction coefficient K_0 (m^{-1}), by the seaweed biomass per unit
123 volume B_V, expressed in dry weight per unit volume (gdw m^{-3}), and by the seaweed light
124 extinction coefficient K_a (m^2 gdw^{-1}), as shown in Eq. 5 (Bendoricchio et al. 1994; Coffaro and
125 Sfriso 1997; Rorrer and Cheney 2004; Ren et al. 2014).

$$K' = K_0 + K_a B_V \quad \rightarrow \quad E(z) = E_0\, exp\,(-(K_0 + K_a B_V)z) \qquad (5)$$

126 K_0 can be determined by the presence of suspended particles as phytoplankton, particulate
127 organic matter and particulate inorganic matter (Ren et al. 2014). Nevertheless, in macroalgae
128 culture tanks, K_0 has a small relative contribution to the K' value due to the high macroalgae
129 biomass.

130 In a well-mixed flat bottom tank, the stocking density SD, expressed as the seaweed dry
131 biomass per surface unit, will be equal to B_Vx Z, being Z the maximum water depth. The
132 average photon irradiance (E_{av}) can be calculated in function of E_0 and B_V, by integrating Eq.
133 5 from *z=0* to *z=* Z, as shown in Eq. 6.

134

$$E_{av} = \frac{1}{Z}\int_{z=0}^{z=Z} E(z, B_V, E_0)dz = \frac{1}{Z}\int_0^Z E_0 \exp(-(K_0 + K_a B_V)\, z)dz$$
$$= \frac{E_0}{(K_0 Z + K_a SD)}[1 - exp\,(-(K_0\, Z + K_a SD))\,] \qquad (6)$$

135

136 *Modeling the influence of E_0 and SD in seaweed growth*

137 Some models estimate $f(E)$ as a function of the volume average photon irradiance (E_{av}).
138 However, empirical studies have shown that this kind of models should only be used within an
139 established range of: stocking density, incident photon irradiance and tank geometry. It must

be pointed out that algae cultivated in two systems exposed to different E_0 can experience the same E_{av} depending on the SD and the tank geometry, despite differing in the respective fractions of light-limited, light-saturated and light-inhibited cells (Molina Grima et al. 1996; Béchet et al. 2013).

A second kind of model describes the light dependence of algal photosynthesis by assuming that photosynthetic activity is dependent on the local photon flux density and, therefore, spatially distributed within the tank. The measured activity can be considered to be the volume average of the local activity (Evers 1991; Bendoricchio et al. 1994; Coffaro and Sfriso 1997; Yun and Park 2003; Ren et al. 2014). In these models, a Local specific light limitation factor (Lsf) can be calculated in function of depth ($Lsf(z)$) by using a Monod-like function, with a half constant K, coupled with the Lambert-Beer law:

$$Lsf(z) = \frac{E(z)}{K + E(z)} = \frac{E_0}{K\ exp((K_0 + K_a B_V)\ z) + E_0} \qquad (7)$$

In a flat bottom tank, with maximum water depth Z, the model equation for $f(E)$ can be obtained by integrating Eq. 7 thorough the tank depth:

$$f(E) = \frac{1}{Z}\int_{z=0}^{z=Z} Lsf(z)\ dz = \frac{1}{(K_0\ Z + K_a SD)}\ ln\frac{E_0 + K}{K + E_0 exp\ (-(K_0\ Z + K_a SD))} \qquad (8)$$

The aim of this work is to develop a growth model to analyze the effect of stocking density (*SD*) and incident photon irradiance (E_0) on the growth of *Ulva ohnoi* in flat bottom tanks fed with the effluents from *Solea senegalensis* tanks in a water recirculating system. The model must provide some guidelines to maximize *Ulva* biomass production by optimizing the management of the stocking densities in the culture tanks.

MATERIAL AND METHODS

Seaweed and Fish

The thallus of *Ulva ohnoi* were collected at the Ebro Delta, from the bioremediation ponds of an aquaculture facility in “Sant Carles de la Ràpita”, Spain (latitude: 40.62 N, longitude 0.66 E). This species was genetically identified by DNA extraction and PCR amplification of the chloroplast rbcl gene following the protocol described in Hayden et al. (2003) with the primers used by Manhart (1994). It was maintained at the Aquaculture Laboratories of the “Universitat Politècnica de Catalunya” in Castelldefels (Spain) for more than 3 years, in indoor tanks fed by water coming from a *S. senegalensis* recirculation aquaculture system (RAS) equipped with biological and mechanical filter, water temperature control, aeration and oxygen supply.

The fishes were distributed in two vertical-cylindrical tanks with 1 m diameter and 0.3 m water depth. Each tank contained a total biomass around 12.5 kg, and the average individual size was 327±175 g in the first tank and 120±57 g in the second tank. The weekly amount of feed provided to fish was around 600g (120g per day from Monday to Friday). Protein content of feed was 57% and the molar ratio N/P was 12.25.

Ulva culture facilities

Ulva ohnoi was cultivated in three vertical cylindrical indoor tanks (diameter 64 cm and capacity 90 L) illuminated by LED light sources providing different irradiances to each tank and a 12:12h light:dark photoperiod.

The incident photon irradiance E_0 was measured by averaging the irradiance values over the water surface, which decreased from the center to the wall of the tank. The average values were calculated by measuring the photon irradiance along two tank axes, fitting the function $E(r)$, being r the distance from the tank center to a specific point on the water surface, and integrating it from r=0 to r=R; being R the tank radius, as shown in Eq. 9.

$$E_0 = \frac{1}{\pi R^2}\int_0^R 2\pi r\big(E(r)\big)dr = \frac{1}{R^2}\int_0^R 2r\big(E(r)\big)dr \qquad (9)$$

The obtained E_0 for each tank: 163, 280 and 886 µmol m^{-2} s^{-1}, were called low (L), moderate (M) and high (H) E_0 levels, respectively. The maximal values measured at the center of the tanks, achieved, respectively, 217, 516 and 1162 µmol m^{-2} s^{-1}. Considering the 12 h photoperiod, the total photon irradiances delivered daily to the surface of the tanks were, respectively 7, 12, and 38 mol photons m^{-2}.

Water was agitated by continuous aeration through a single air entry (diameter 4mm) placed on the tank bottom center. The air flow rate, measured by a rotameter, was maintained around 6.7 L min^{-1}.

Water and nutrients were provided by the effluents of two *S. senegalensis* tanks, drained to a buffer tank with a nitrifying biofilter. Most of the effluent volume was recirculated to the fish tanks, but a small fraction was delivered to the algae tanks by peristaltic pumps along the lighting period. The water returned from the algae tanks to the fish buffer tank through a perforated half pipe vertically fastened to the internal wall of each algae tank, to avoid losing algae fragments. An external overflow system maintained a constant water level in the tank.

Control of basic water parameters in tanks

To improve the availability of inorganic carbon in algae tanks, sodium carbonate was weekly provided to maintain alkalinity over 100 mg L^{-1} $CaCO_3$, and hydrochloric acid was daily added along the lighting period, to avoid pH levels above 8.5 (Bidwell et al. 1985; Menéndez et al. 2001; Lobban and Harrison 2007; Zou 2014).

Temperature, Dissolved Oxygen (DO) concentration and pH were measured daily, at the end of the dark period, using portable probes (OxyGuard Handy Polaris and OxyGuard Handy pH). The average values obtained ±sd were 20.7±1.3 ºC for temperature, 7.01±0.7 mg L^{-1} for DO and 8.24±0.35 for pH. Total Ammonia Nitrogen (NH_3 +NH_4^+) concentrations were measured in fish tanks with a photometer (Palintest), giving values from 0 to 0.24 mg N L^{-1}.

Water salinity was measured weakly using a salinity refractometer and fresh water was added to compensate for the water lost by evaporation. The average salinity values were 37.5±1.0 g L^{-1}.

Water samples were taken weekly from the buffer tank and filtered by 0.45 µm membrane filter for N-NO_3^- and P-PO_4^{3-} analysis by spectrophotometric methods. The ultraviolet

technique (absorbance at 220 nm and 275 nm) was used for nitrate measure (APHA 1992); and a colorimetric method based on reduction of phosphomolybdic acid for dissolved inorganic phosphate (Grasshoff et al. 1999). The average $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ concentrations ±sd in fish tanks were respectively 21.7±2.5 mg L^{-1} and 0.59±0.088 mg L^{-1}. The concentrations of ammonia N species and nitrite were considered negligible in relation to nitrate, due to the action of the biological filter.

Nutrient supply to algae tanks

The water flow rate delivered to the low and moderate E_0 tanks was 7 L h^{-1}. For the tank with the highest E_0 the flow rate was doubled to avoid the N and P flow becoming a limiting factor. Therefore, the level of N and P load can be estimated about 5.65 g N m^{-2} day^{-1} and 0.153 g P m^{-2} day^{-1} for low and medium irradiance level tanks, and 11.3 g N m^{-2} day^{-1} and 0.306 g P m^{-2} day^{-1} for the highest irradiance tank.

Experimental design

To analyze the influence of light and stocking density on *U. ohnoi* growth, the three tanks with the different levels of incident photon irradiance E_0 abovementioned (L, M and H) were combined with three levels of initial fresh-weight stocking densities (FSD_i) conducted in successive trials, lasting 3 weeks each. Two times per week macroalgae were spun, weighted and restocked to the initial FSD by removing excess biomass. A sample of the removed excess biomass was dried at 60ºC to determine the ratio fw:dw.

The three FSD_i levels were 2.4, 1.6 and 0.8 kgfw m^{-2}. Nevertheless, due to the variability of the water content in fresh samples, the analysis and the model of *Ulva* growth was not based on initial and final FSD but in the respective dry-weight stocking densities (SD). In consequence, in the successive trials, each level of FSD_i will provide a non-negligible range of initial dry biomass stocking densities (SD_i), as shown in Fig. 1. For this reason, the six measurements obtained from each FSD_i level will not be considered as replicates but as independent SD_i values.

Calculating growth rates and biomass productivity per unit area

The net specific growth rate μ_{NET} (d^{-1}) and the biomass productivity per unit area BP_A (gdw m^{-2} d^{-1}) were calculated, for each combination E_0xSD_i, using Eq. 10 and 11 respectively, were $t_f - t_i$ is the time interval and SD is the stocking density (gdw m^{-2}).

$$\mu_{NET} = \frac{\left[ln(SD_f) - ln(SD_i)\right]}{(t_f - t_i)} \qquad (10)$$

$$BP_A = \frac{SD_f - SD_i}{(t_f - t_i)} \qquad (11)$$

To relate μ_{NET} and SD, each μ_{NET} value must be associated to a specific stocking density, which can be calculated as the average value of SD_i andSD_f.

Estimation of growth model parameters

A model was developed to estimate μ_{NET} on the basis of E_0 and SD in a flat bottom *Ulva* tank. It was assumed that photosynthetic activity is dependent on the local photon flux density Lsf (Eq. 7). The model calculates the photon irradiance limiting factor $f(E)$ by volume averaging the local specific light limitation factor (Eq. 8), and the Net specific growth rate μ_{NET} by assuming light to be the single limiting variable on growth, under a fixed temperature and not limited nutrient supply (Eq. 3).

As can be observed in Eq. 3 and 8, the model must include, besides the predictor parameters E_0 and SD, light-transfer-related parameters (K_0, K_a and Z) and growth kinetic parameters (μ_{max}, K and λ). The water light extinction coefficient (K_0) was estimated by measuring the photon irradiance at different water depths in the tank, with the algae biomass laying on the tank bottom. The biomass light extinction coefficient (K_a) was estimated from the values reported in bibliography (Coffaro and Sfriso 1997; Ren et al. 2014). The growth kinetic parameters μ_{max}, K and λ were estimated using the Gauss-Newton nonlinear regression algorithm to minimize the sum of squares of the residual errors (S), and the normal distribution of the residuals was verified.

RESULTS

The net specific growth rates (μ_{NET}) measured along the experiment showed, in a general way, a positive correlation with the incident photon irradiance (E_0), and a negative correlation with the stocking densities (SD) (Fig. 2). The measured μ_{NET} values for moderate and low E_0 ranged, respectively, from 0.045 to 0.126 and from 0.007 to 0.081 d^{-1}. Nevertheless, the higher μ_{NET} measured (0.19 d^{-1}) was not obtained with the combination of the highest E_0 and the lower initial stocking densities (SD_i), as could be expected. It was found that, with the highest E_0, reducing SD_i values below 121 gdw m^{-2} does not contribute to increase μ_{NET} (see crosses in Fig. 2). In the trials with those conditions, the color and morphology of the algae experimented strong changes, becoming yellowish and showing fragmentation and signs of sporulation, with reduction of the thallus area. Subsequent essays in the same conditions corroborated this behavior. In these essays, the analysis of *Ulva* tissue showed a decrease on chlorophyll concentration, together with an increase of nitrogen concentration.

The biomass productivities per unit area (BP_A) are shown in Fig. 4 (dots). The higher values (40 to 51 gdw m^{-2} d^{-1}) were obtained with the combination of the highest E_0 and SD_i above 178 gdw m^{-2}.

DISCUSSION

Growth rates and biomass productivities

The higher biomass productivities per unit area obtained were very close to those obtained in culture tanks by Figueroa et al. (2009) and Neori et al. (1991) for *U. lactuca* (46.5 and 55 gdw m^{-2} d^{-1} respectively); Jiménez del Río et al. (1996) for *U. rigida* (40 gdw m^{-2} $d^{-1)}$; and Mata et al. (2016) for *U. ohnoi* (46.3 gdw m^{-2} d^{-1}).

Change in colour and morphology with the combination of highest E_0 and the lower SD_i can be a photoadaptative response to high irradiance, as observed in wild *Ulva* (Falkowski and LaRoche 1991). Moreover, in algae tanks with continuous water renewal along light period, the reduction of talus area together with the losses in chlorophyll would contribute to diminish light attenuation (see Eq. 5), increasing the total photon irradiance delivered to the algae and contributing to increase the damage. For this reason, the μ_{NET} values corresponding to the damaged trial (highest E_0 and SD_i< 121 gdw m^{-2}) were not included in the nonlinear regression to estimate the kinetic parameters μ_{max}, K and λ. To establish the light threshold which leads to the damage of the algae, is out of the focus of this work and would require considering many aspects related with the flow pattern of the algae into the tank, photoperiod and daily evolution of the incident irradiance.

Predictive model for μ_{NET} on the basis of E_0 and SD

The values of the parameters involved on the model are shown in Table 1. The measured water light extinction coefficient (1.5±0.5 m^{-1}) was, in average, higher than the values reported for *Ulva* models in lagoons, likely due to the turbulence produced by aeration in the algae tanks. Furthermore, the turbidity caused by the management of the fish tanks and the bio-filter, can influence this value.

The non-linear regression used to estimate the growth kinetic parameters, showed a standard deviation between the measured and the fitted values (S) equal to 0.011 d^{-1}. Eq. 12 incorporates the values of the kinetic parameters and allows estimating μ_{NET} on the basis of E_0 and SD. The comparison between experimental and predicted results of the model is shown in Fig. 3.

The value obtained for the most sensitive parameter, μ_{max} , was very close to the reported in literature for *Ulva* growth models (Duke et al. 1989; Bendoricchio et al. 1994; De Guimaraens et al. 2005; Hadley et al. 2015). It must be highlighted that the kinetic parameter μ_{max} (0.416 d^{-1}) is much higher than the highest measured value of μ_{NET} (0.19 d^{-1}) which was also very close to the maximum measured values reported by several authors (Coutinho and Zingmark 1993: 0.21 d^{-1}; Neori et al. 1991: 0.18 d^{-1}).

$$\mu_{NET} = \frac{\mu_{max}}{(K_0\, Z + K_a SD)}\; ln \frac{E_0 + K}{K + E_0 exp\,(-(K_0\, Z + K_a SD))} - \lambda \quad (12)$$

Optimal SD to maximize the biomass productivity per unit area (BP_A)

As can be observed in Fig. 4, with the low and moderate irradiances, the BP_A trends to increase with SD, achieving a maximum BP_A for an optimal SD and decreasing afterwards. With the higher E_0 the higher optimal SD and maximum BP_A is achieved. With the highest photon irradiance it seems to be a minimum SD required to avoid the damage of the seaweed. In our experiment, with E_0 886 µmol m^{-2} s^{-1}, SD values lower than 170 gdw m^{-2} induced damages in the seaweed.

The optimal SD, which provides the maximal biomass productivity for a given E_0, can be estimated from Eq. 12 and the predicted values of the model are shown in Fig. 5. As an example, the graphic shows that, according to our model, with E_0 200 µmol m^{-2} s^{-1} the optimal SD would be 186 gdw m^{-2} and the BP_A achieved would be about 12.6 gdw m^{-2} d^{-1} (see arrows in Fig. 5).

It must be pointed that *Ulva* biomass per unit volume ($B_V = SD / Z$) is limited by the capacity of the tank aeration system to tumble algal biomass. In our experience, this happens when B_V reaches values above 1000 gdw m^{-3}, leading to a worse distribution of the photon irradiance delivered to the algae. Therefore, with high E_0 values, higher water depths (Z) can be needed to achieve the optimal SD without exceeding the mentioned B_V value.

The proposed growth model of *U. ohnoi* will be useful to improve the productivity of algae by managing the culture densities in order to maintain SD as close as possible to the optimal value along the production cycle. Furthermore, it can be a reference to evaluate the productivity achieved.

List of symbols

BP: Biomass productivity (gdw d^{-1})

BP_A: Dry biomass productivity per unit of water surface (gdw m^{-2} d^{-1})

B_V: Dry biomass per unit volume (gdw m^{-3})

E: Photon Irradiance (µmol m^{-2} s^{-1})

E_0: Incident photon irradiance on surface of water (µmol m^{-2} s^{-1})

E_{av}: Volume average photon irradiance (µmol m^{-2} s^{-1})

FSD: Fresh biomass per unit of water surface (kgfw m^{-2})

K: Half constant (µmol m^{-2} s^{-1})

K': Apparent light attenuation constant (m^{-1})

K_0: Water light extinction coefficient (m^{-1})

K_a: Seaweed light extinction coefficient (m^2 gdw^{-1})

Lsf: Local specific light limitation factor (-)

r: Distance to the tank center (m)

R: Tank radius (m)

SD: Stocking density (gdw m^{-2})

z: Distance to water surface (m)

Z: Tank water depth (m)

z: Water depth (m)

λ: Biomass loss specific rate (d^{-1})

μ_{GROSS}: Biomass growth specific rate (d^{-1})

μ_{max}: Maximum specific growth rate

μ_{NET}: Net specific growth rate (d^{-1})

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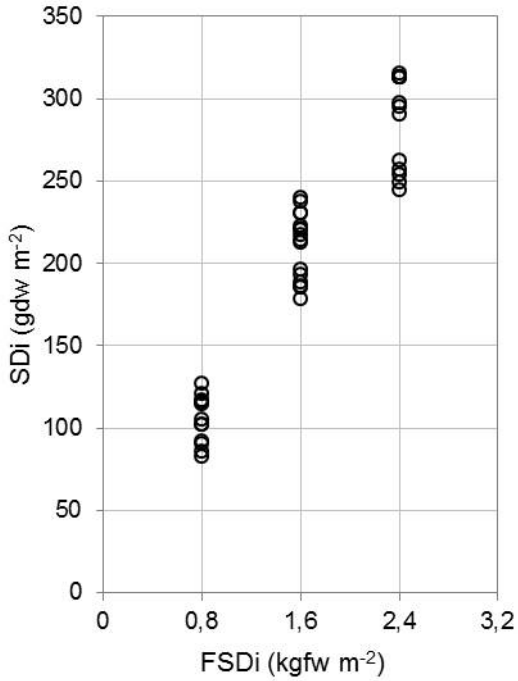

469

470 **Fig. 1** Correspondence between the three levels of initial fresh weight stocking density FSD_i
471 and the values of initial dry weight stocking density (SD_i) calculated after determining the ratio
472 dw:fw

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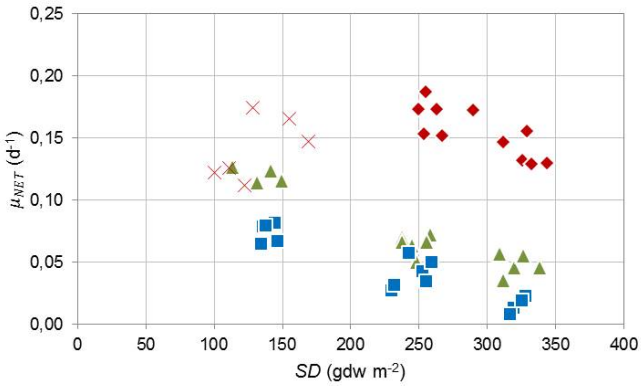

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477 **Fig. 2** Experimental μ_{NET}(d^{-1}) values for the three incident photon irradiances (E_0) 886 (H ◆),
478 280 (M ▲) and 163 µmol m^{-2} s^{-1} (L ■). Red crosses (x) corresponds to the damaged trials with
479 the highest irradiance

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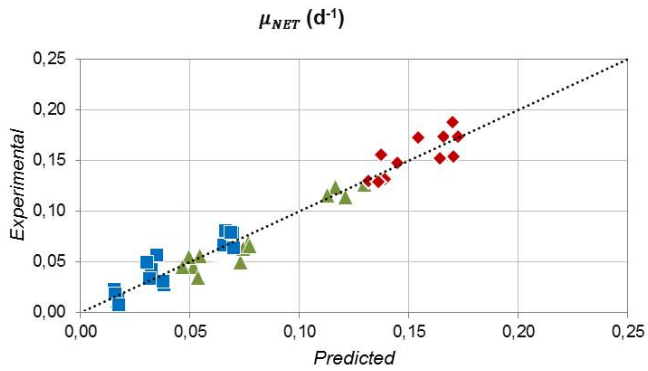

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482 **Fig. 3** Comparison between experimental and predicted μ_{NET}(d^{-1}) values for the three incident
483 photon irradiances (E_0) 886 (H ◆), 280 (M ▲) and 163 µmol m^{-2} s^{-1} (L ■)

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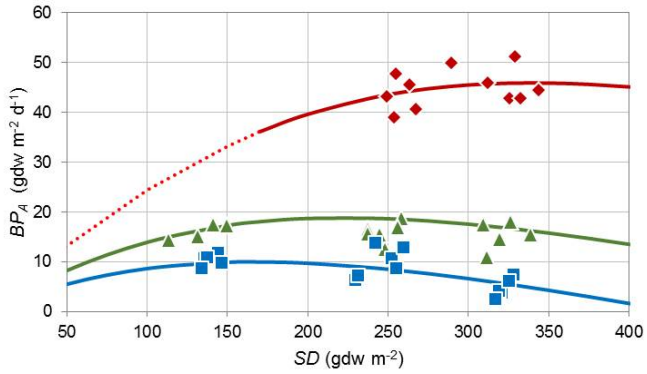

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488 **Fig. 4** Biomass productivity versus SD for the three incident photon irradiances (E_0) 886 (H ◆),
489 280 (M ▲) and 163 µmol m^{-2} s^{-1} (L ■). Continuous line corresponds to predicted values and
490 dots to the experimental values. The dotted line indicates the densities which must be avoided
491 with the highest irradiance to prevent *Ulva* damage

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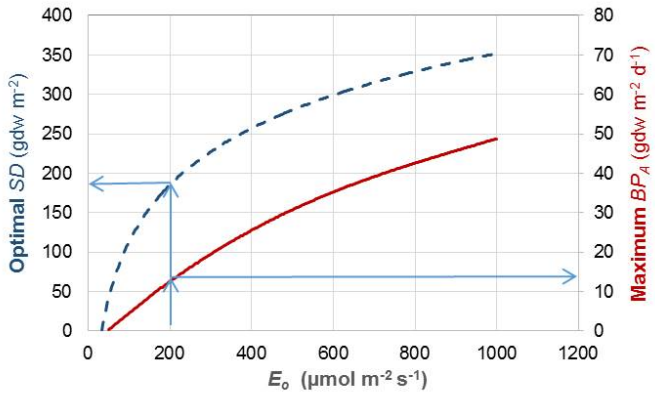

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Fig. 5 Optimal SD and Maximum BP_A depending on the incident photon irradiance (E_0). Dashed blue line shows the optimal SD and continuous red line shows the maximum BP_A achieved. Light blue arrows show, as an example, the optimal SD and Maximum BP_A with E_0 200 µmol m^{-2} s^{-1}

Table 1 Values of the light related and kinematic parameters involved in the estimation of μ_{NET} from the predictors E_0 and SD. S.E.: Standard error

Symbol	**Description of parameter**	**Value**	**Units**	
LIGHT RELATED PARAMETERS				
K_0	Water light extinction coefficient	1.5	m^{-1}	
K_a	*Ulva* biomass light extinction coefficient	0.01	m^2 gdw^{-1}	
Z	Water depth	0.28	m	
ESTIMATED KINEMATIC PARAMETERS				**S.E.**
μ_{max}	Maximum specific growth rate	0.416	d^{-1}	0.0221
K	Half saturation constant	144.8	µmol m^{-2} s^{-1}	33.64
λ	Specific rate of biomass losses	0.066	d^{-1}	0.0129