



## The effect of irradiance on long-term skeletal growth and net photosynthesis in *Galaxea fascicularis* under four light conditions

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### ABSTRACT

The relation between irradiance, skeletal growth and net photosynthesis was studied for the scleractinian coral *Galaxea fascicularis* to provide experimental evidence for mediation of light-enhanced calcification through photosynthesis. The hypothesis was tested that skeletal growth and photosynthesis are linearly correlated.

A long-term experiment was performed in a closed-circuit aquarium system, in which four series of nine nubbins (single polyp clones of a coral colony) of *Galaxea fascicularis* were exposed to four light treatments (10L:14D): 144 W T8 fluorescent lighting providing an irradiance of 68  $\mu\text{E}/\text{m}^2/\text{s}$  and 70, 250 and 400 W Metal Halide lighting providing an irradiance of 38  $\mu\text{E}/\text{m}^2/\text{s}$ , 166  $\mu\text{E}/\text{m}^2/\text{s}$  and 410  $\mu\text{E}/\text{m}^2/\text{s}$ , respectively. Growth of these nubbins was measured as buoyant weight at different time intervals in a 294 day experiment. A light-saturation curve for photosynthesis was measured in a respirometric flow cell using a 54 week *Galaxea fascicularis* colony grown at 60  $\mu\text{E}/\text{m}^2/\text{s}$ .

No saturation of net photosynthesis of *Galaxea fascicularis* was found at the irradiances tested. The specific growth rate ( $\mu$ , in  $\text{day}^{-1}$ ) of the coral nubbins increased with irradiance. Whereas irradiance varied 11-fold (38 to 410  $\mu\text{E}/\text{m}^2/\text{s}$ ), buoyant weight (increase after 294 days) increased 5.7 times (2243 to 12374 mg), specific growth rate (1–294 days) increased 1.6 times (0.0103 to 0.0161  $\text{day}^{-1}$ ), while net photosynthetic rate increased 8.9 times (0.009  $\mu\text{mol O}_2/\text{min}/\text{cm}^2$  to 0.077  $\mu\text{mol O}_2/\text{min}/\text{cm}^2$ ). The increase of specific growth rate with irradiance was less than expected based on the increase in net photosynthetic rate with irradiance. This discrepancy between potential energy produced in photosynthesis and energy used for skeletal growth indicates that skeletal growth is not limited by photosynthetic potential at high irradiance levels.

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### 1. Introduction

Light is one of the most important abiotic factors influencing the growth of scleractinian corals. Scleractinian corals live in symbiosis with unicellular algae, known as zooxanthellae, that reside in their endodermal tissue layers. In the light, zooxanthellae perform photosynthesis, during which process they produce oxygen and organic compounds. When their own respiratory needs are satisfied, zooxanthellae translocate the excess photosynthetic products to the coral host (Muscatine and Cernichiaro 1969; Muscatine et al., 1981). Zooxanthellae can thus provide a considerable part of the energy needed for coral growth.

Growth of scleractinian corals can be divided in two components: first, skeletal growth due to the deposition of an external skeleton of calcium

carbonate aided by the synthesis of an organic matrix in a process called calcification, and second, tissue growth. According to the light-enhanced calcification theory (see Gattuso et al., 1999 and Allemand et al., 1998 for review), the symbiosis with zooxanthellae is aiding to the process of skeletal growth. According to this theory, calcification of the coral host is enhanced by photosynthesis of zooxanthellae (Goreau and Goreau 1959; Pearse and Muscatine 1971; Allemand et al., 2004). Indeed, on average, calcification in light is found to be around three times higher than calcification in darkness (review by Gattuso et al., 1999). Although photosynthesis and calcification are spatially separated processes (photosynthesis occurs in the oral tissue layer and calcification in the aboral tissue layer), they do share a common pool of inorganic carbon inside the coelenteron of the coral host, accounting for the interaction between these two processes. The exact mechanisms of the enhancement of calcification by photosynthesis are still a matter of debate (Gattuso et al., 1999; Furla et al., 2000). Some of the proposed mechanisms include that: 1) photosynthesis provides energy for the energy-demanding processes associated with calcification, such as calcium transport and organic matrix synthesis

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(Wainwright 1963; Chalker and Taylor 1975), and 2) photosynthesis raises intracellular pH and intracellular saturation state of calcium carbonate, thereby favoring the precipitation of calcium carbonate (Goreau and Goreau 1959; Allemand et al., 1998).

The relation between light and photosynthesis can be quantitatively described using light-dependent models (Chalker, 1981), resulting in photosynthesis-irradiance curves. At low irradiance, the rate of photosynthesis is nearly directly proportional to irradiance. At higher irradiance, the rate of photosynthesis rapidly approaches a horizontal asymptote, which is the point where saturation of photosynthesis is reached (the maximum gross photosynthetic rate,  $P_{\text{max}}^g$ ). Calcification can be described using the same light-dependent models (Chalker, 1981).

Since scientists started to study coral calcification some 50 years ago, several authors have found a positive correlation between light and calcification, either in the field (Bosscher and Meesters, 1993) or through experimental work (e.g. Goreau, 1959; Marubini et al., 2001; Reynaud-Vaganay et al., 2001; Reynaud et al., 2004; and Schlacher et al., 2007).

However, none of these authors coupled their growth data to a photosynthesis-irradiance curve. Although it has been demonstrated that higher rates of skeletal growth in *Galaxea fascicularis* are supported by higher rates of photosynthesis and respiration in the adjacent polyp parts (Al-Horani et al., 2005), it cannot be derived from either of these studies to what extent an increase in photosynthesis leads to a proportional increase in skeletal growth.

To the best of our knowledge, a study describing the relation between light, photosynthesis and skeletal growth – i.e. the result of calcification – of individual corals followed in time under controlled conditions is still lacking. We examined this relation by measuring the growth of the scleractinian coral *Galaxea fascicularis* under four different irradiance levels in a closed-circuit aquarium system and comparing the results to a photosynthesis-irradiance curve of this species. In this study, the hypothesis was tested that skeletal growth and photosynthesis are linearly correlated.

## 2. Materials and Methods

Thirty-six (36) coral nubbins (single polyp clones) of *Galaxea fascicularis* were created of colonies that were grown at a light intensity of 60  $\mu\text{E}/\text{m}^2/\text{s}$  (144 W HQI) in a closed-circuit coral aquaculture system “Quarantine system QU4” of Burgers Ocean, Arnhem, The Netherlands. Each coral nubbin was fixed to a 5 × 5 cm perforated PVC plate using Reef Construct (Aquamedic). Nine plates of coral nubbins were fixed to one single square plate and assigned to each of the following four experimental treatments: 70 W Metal Halide (MH) lighting (BLV Hit-Lite, HIT-DE, 10.000 K), 144 W fluorescent T8 lighting (2x Philips TLD 36 W/950 (5300 K), 2x Osram L 36 W/67 (blue)), 250 W MH lighting and 400 W MH lighting. A light dark cycle of 10L:14D was applied. As a result of working inside a public aquarium such as Burgers Ocean, we were constrained to incorporate our experiments into existing systems, which limited our ability to standardize the experimental setup. To standardize the light regimes, the average irradiance level was determined within each experimental treatment by measuring irradiance (or photosynthetic photon flux density) at different locations under the light source. Irradiance was measured using a Li-Cor 192SA quantum underwater sensor, which measures light in the photosynthetic active region (PAR, 400–700 nm). The metal halide light sources had a quite variable light distribution compared to the T8 light source. Using these light distribution patterns, the average irradiance experienced by the coral nubbins was calculated for each treatment at the start of the experiment: 38 (range: 35–45)  $\mu\text{E}/\text{m}^2/\text{s}$  for 70 W MH treatment, 68 (range: 65–70)  $\mu\text{E}/\text{m}^2/\text{s}$  for 144 W T8 treatment, 166 (range: 125–200) for 250 W MH treatment and 410 (range: 300–500) for 400 W MH treatment. Irradiance levels were measured at different times during the experiment and were found to decrease in time (at most, a 17% decrease in 274 days).

Each PVC plate containing 9 coral nubbins was placed randomly in culture system QU4, directly under the middle of each of the

light sources, receiving bidirectional flow (2.5 minute interval) at flow speeds of respectively  $5 \pm 0$  cm/s,  $5 \pm 2$  cm/s,  $15 \pm 3$  cm/s and  $6 \pm 0$  cm/s as measured using a SENA-RC2 electromagnetic current meter (Aquadata).

Culture system QU4 is a 4000 l system consisting of two 1000 l aquaria and two 1000 l sumps. The circulation system cycles 18  $\text{m}^3/\text{h}$  and the system is connected to a trickle tower, a Schuran Jet Stream 2  $\text{Ca}^{2+}$  reactor, and a Schuran Aquafloater AQ250 protein skimmer.

Seawater was made up from Tropical Marine salt (Zoomix). Temperature was maintained at 26 °C and salinity at 34 ppt. The system was fed 7 days a week using *Artemia nauplii* (Salt Lake) that were hatched on site and subsequently enriched using Rich Advanced feed for 24 hours. Since hatching efficiency is not constant, the amount of artemia fed each day is estimated to vary between 4–8 artemia/ml. Water quality parameters were measured at regular intervals.

### 2.1. Growth parameters

To measure growth, the buoyant weight of the coral nubbins was measured four times during a 294 day period, at  $t=0$ ,  $t=111$ ,  $t=179$  and  $t=294$  days. These intervals were the result of practical circumstances prevailing at the facility where the experiments were carried out (e.g., a public aquarium). In spite of their irregularity, both the frequency of measuring points and the covered time range give sufficient security that the data enable to test the hypothesis. Time is expressed as days after preparation of the nubbins.

Buoyant weight is a good approximation of skeletal weight, since coral tissue has a density which is similar to that of seawater and therefore does not contribute significantly to the buoyant weight. Tissue only comprises 1% of the total buoyant weight when tissue does not penetrate deep into the skeleton (Davies, 1989).

Buoyant weight was measured in the laboratory by suspending each coral (plus PVC plate) on a hook in a defined volume of seawater at a constant depth. Seawater was maintained at 26 °C and 34 ppt salinity. The hook was attached to an underweighing analytical balance (Kern&Sohn D-72458 Albstadt, type 870-13) using a thin nylon string (Osinga et al., 1999). Buoyant weight of each coral was measured and the average of three measurements was taken. The initial weight of the nubbins before their attachment to their PVC-plate at  $t=0$  was estimated by weighing 5 similar-sized nubbins of a *Galaxea fascicularis* colony on a weighing glass and taking the average. Using this parameter, it was possible to estimate the weight of the PVC plate and the amount of Reef Construct that was used to attach each coral to its plate. All our buoyant weights were corrected for this weight in order to obtain the buoyant weight of the coral colony itself. This weight was used as parameter for data analysis.

The growth data of buoyant weight were also used to calculate specific growth rate ( $\mu$ ) using the formula:

$$\mu = (\ln BW_n - \ln BW_{n-1}) / \Delta t \quad [\text{day}^{-1}]$$

where  $\mu$  is the specific growth rate ( $\text{day}^{-1}$ ),  $BW_n$  is buoyant weight at the end of a growth interval,  $BW_{n-1}$  is buoyant weight at the start of a growth interval and  $\Delta t$  is time between measurements of buoyant weight in this growth interval.

### 2.2. Photosynthesis-irradiance curve

A photosynthesis-irradiance curve was measured for a *Galaxea fascicularis* colony that was grown under a 250 W Metal Halide lamp at an irradiance of 60  $\mu\text{E}/\text{m}^2/\text{s}$ . Since Goiran et al. (1996) already measured a photosynthesis-irradiance curve for *Galaxea fascicularis*, it was repeated only once to verify its applicability for this study. Net photosynthetic production of oxygen was measured by means of

intermittent flow respirometry in a 3500 cm<sup>3</sup> respirometric flow cell at irradiances ranging from 40 to 500  $\mu\text{E}/\text{m}^2/\text{s}$  using irradiance intervals of ca 60  $\mu\text{E}/\text{m}^2/\text{s}$ . It was not possible to reach irradiances higher than 500  $\mu\text{E}/\text{m}^2/\text{s}$  using our setup. However, the range of irradiances used corresponds to the irradiances applied in the growth experiment. Respiratory consumption of oxygen was measured in the dark. Lighting was provided by a T5 lighting system (ATI) containing eight 24 W Aquablue Spezial bulbs. Irradiance was measured using a Li-Cor 192SA quantum underwater sensor. A flow speed of  $\pm 10$  cm/s was applied to ensure adequate mixing and to simulate the situation in the aquarium environment (5–15 cm/s). At each irradiance, the increase in oxygen concentration was measured every 10 seconds using a luminescent oxygen probe (Hach) until a difference in concentration was detected of  $\pm 1$  mg O<sub>2</sub>/l. After each measurement the flowcell was flushed with “fresh” seawater from the Quarantine tank to return the oxygen concentration to the initial value before the start of the experiment and to remove possible accumulated waste products. Temperature inside the respirometric flowcell was maintained at  $26 \pm 0.5$  °C and salinity at  $34 \pm 0.1$  ppt. Surface area and polyp number of the coral were determined in order to normalize the respirometric data. The volume of the coral was determined using the water displacement technique in order to correct flowcell volume for the space taken in by the coral.

Photosynthetic rates at each irradiance were estimated by regressing oxygen concentration against time. Net photosynthetic rates were calculated according to the following equation:

$$P_{\text{net}} = ((V_{\text{cell}} - V_{\text{coral}}) \times \text{slope}) / S \quad [\mu\text{molO}_2/\text{min}/\text{cm}^2]$$

Where  $P_{\text{net}}$  is the rate of net photosynthesis ( $\mu\text{mol O}_2/\text{min}/\text{cm}^2$ );  $V_{\text{cell}}$  is volume of respirometric flowcell (l);  $V_{\text{coral}}$  is volume of coral; slope is regression coefficient of dissolved oxygen against time ( $\mu\text{mol O}_2/\text{l}/\text{min}$ ), and  $S$  is surface area of coral ( $\text{cm}^2$ ).

The P/I curve was fitted according to the model of Barnes and Chalker (1990) using Sigmaplot 8.0.

$$P_{\text{net}} = R_{\text{dark}} + P_{\text{max}}^g \times \tanh(I/I_k) \quad [\mu\text{molO}_2/\text{min}/\text{cm}^2]$$

where  $P_{\text{net}}$  is net photosynthesis as measured during respirometry in light and  $R_{\text{dark}}$  is rate of respiration as measured during respirometry in darkness.  $P_{\text{max}}^g$  is the maximum gross photosynthetic rate (defined as maximum net photosynthetic rate ( $P_{\text{max}}^n$ ) minus dark respiration ( $R_{\text{dark}}$ )),  $\tanh$  is the hyperbolic tangent,  $I_k$  is sub-saturation irradiance (i.e. irradiance at which the initial linear slope of the curve intercepts the horizontal asymptote).

### 2.3. Data analysis

Normality ( $p > 0.05$ ) and homogeneity of variance ( $p > 0.05$ ) of the data were tested using Shapiro-Wilk and Levene's test in SAS 9.1. Since our data did not satisfy the assumptions for ANOVA testing, we used Kruskal Wallis as a non-parametric test to detect statistical differences between treatments.

## 3. Results

### 3.1. Culture system parameters

During the experiment (22/03/2005 till 03/07/2005) the alkalinity in the system was  $3,23 \pm 0.54$  S.D. mEq/l, calcium concentration  $393.75 \pm 14.36$  S.D. mg/l, magnesium concentration  $1290 \pm 51.29$  S.D. mg/l, nitrate concentration  $0.19 \pm 0.08$  S.D. mg NO<sub>3</sub>-N/l, nitrite concentration  $0.014 \pm 0.002$  S.D. mg NO<sub>2</sub>-N/l and phosphate concentration  $0.015 \pm 0.022$  S.D. mg PO<sub>4</sub>/l.

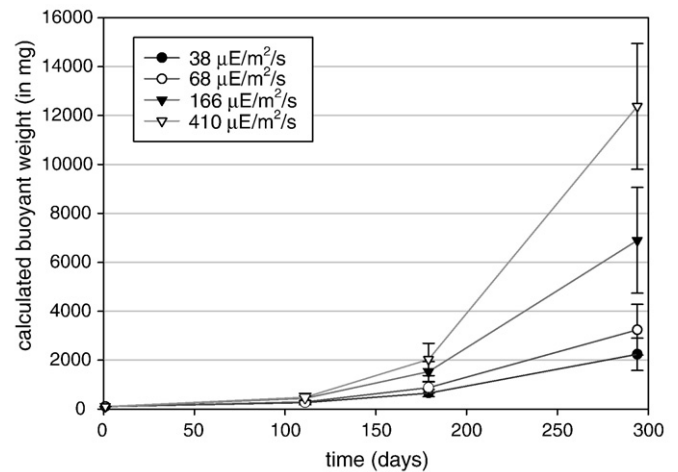


Fig. 1. Effect of irradiance on the calculated buoyant weight of *Galaxea fascicularis* colonies. Values are mean  $\pm$  S.D.,  $N=9$ . Error bars indicate standard deviations.

### 3.2. Growth parameters

#### 3.2.1. Buoyant weight

All corals grew during the experiment. The buoyant weight of the corals (Fig. 1) increased significantly in time in each treatment ( $p < 0.001$ ).

An increase in growth as buoyant weight with increasing irradiance was observed: at day 111 and day 179, the corals in the two highest light treatments (166  $\mu\text{E}/\text{m}^2/\text{s}$  and 410  $\mu\text{E}/\text{m}^2/\text{s}$ ) had a significant higher calculated buoyant weight ( $p < 0.01$ ) compared to corals in the two lowest light treatments (38  $\mu\text{E}/\text{m}^2/\text{s}$  and 68  $\mu\text{E}/\text{m}^2/\text{s}$ ). At Day 294, the corals in the highest light treatment (410  $\mu\text{E}/\text{m}^2/\text{s}$ ) had a significant higher calculated buoyant weight compared to the 166  $\mu\text{E}/\text{m}^2/\text{s}$  treatment ( $p < 0.01$ ). On its turn, the corals in the 166  $\mu\text{E}/\text{m}^2/\text{s}$  treatment had a significant higher calculated buoyant weight ( $p < 0.001$ ) compared to the corals in the two lowest light treatments (38  $\mu\text{E}/\text{m}^2/\text{s}$  and 68  $\mu\text{E}/\text{m}^2/\text{s}$ ).

Differences between treatments became more pronounced during the course of the experiment. Whereas irradiance level varied 11-fold (38 to 410  $\mu\text{E}/\text{m}^2/\text{s}$ ), the average buoyant weight at Day 111 of the corals grown in the highest light treatment (410  $\mu\text{E}/\text{m}^2/\text{s}$ ) compared to the corals grown in the lowest light treatment (38  $\mu\text{E}/\text{m}^2/\text{s}$ ) was only 1.8 times increased (265 to 484 mg). At Day 179, this difference had increased to 3.1 times (652 to 2030 mg), and to 5.5 times at Day 294 (2243 to 12374 mg).

#### 3.2.2. Specific growth rate

The specific growth rate ( $\mu$ ) of coral colonies grown under different light conditions (38  $\mu\text{E}/\text{m}^2/\text{s}$ , 68  $\mu\text{E}/\text{m}^2/\text{s}$ , 166  $\mu\text{E}/\text{m}^2/\text{s}$  and 410  $\mu\text{E}/\text{m}^2/\text{s}$ ) was calculated using the calculated buoyant weight of the corals at different time intervals (1–111 days, 111–179 days, 179–294 days and 1–294 days), see Table 1.

It is found that the specific growth rate was not constant during the experiment. In the second growth interval (111–179 days), the specific growth rates were significantly higher compared to those in the first time interval ( $p < 0.01$ ). In the third growth interval (179–294), the specific growth rates had decreased ( $p < 0.001$ ) compared to the second growth interval, except for the 38  $\mu\text{E}/\text{m}^2/\text{s}$  treatment ( $p = 0.3084$ ). Apparently, our corals did not follow first order kinetics.

In most cases, higher irradiance supported a higher specific growth rate. When calculated over the entire 294 days time interval (Fig. 2), again, the specific growth rate significantly increased with irradiance in each light treatment ( $p < 0.01$ ) except for the difference between the 38  $\mu\text{E}/\text{m}^2/\text{s}$  and 68  $\mu\text{E}/\text{m}^2/\text{s}$  light treatment, which was not significant ( $p = 0.08$ ).

**Table 1**

Specific growth rates ( $\mu$  in  $\text{day}^{-1}$ ) calculated using the calculated buoyant weight of the corals are given for growth interval 1 (1–111 days), interval 2 (111–179 days), interval 3 (179–294 days) and the entire 294 days for each light condition

	interval 1		interval 2		interval 3		1-294 days	
	average	S.D.	average	S.D.	average	S.D.	average	S.D.
38 $\mu\text{E}/\text{m}^2/\text{s}$	0.0078	0.0033	0.0137	0.0055	0.0106	0.0014	0.0103	0.0011
68 $\mu\text{E}/\text{m}^2/\text{s}$	0.0090	0.0016	0.0161	0.0014	0.0112	0.0009	0.0115	0.0010
166 $\mu\text{E}/\text{m}^2/\text{s}$	0.0127	0.0022	0.0181	0.0013	0.0130	0.0011	0.0141	0.0011
410 $\mu\text{E}/\text{m}^2/\text{s}$	0.0134	0.0028	0.0211	0.0007	0.0159	0.0011	0.0161	0.0007

Average and standard deviation are given.

### 3.3. Photosynthesis-irradiance: comparison with specific growth rate

The relationship between photosynthetic rate and irradiance was determined for a 54 week old *Galaxea fascicularis* colony that was grown at 60  $\mu\text{E}/\text{m}^2/\text{s}$  receiving 144 W MH lighting (Fig. 3, black dots) and compared with the effect of irradiance on the specific growth rate (from 1 to 294 days) (Fig. 3, bar graph).

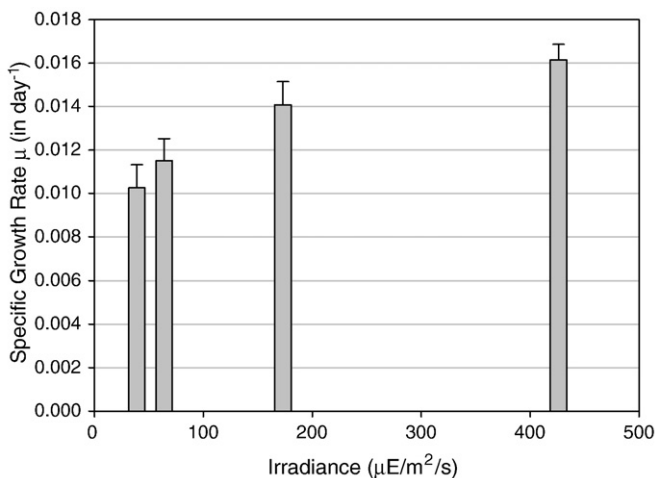
The photosynthesis-irradiance curve was fitted according to the model of Barnes and Chalker (1990) (Fig. 3, line graph) and was found to be similar to the one measured by Goiran et al. (1996), verifying our result and its applicability for this study. Although our data did not allow a legitimate estimation of  $P_{\text{max}}^{\text{g}}$ , we can assert and confirm from the photosynthesis-irradiance curve measured by Goiran et al. (1996) that the irradiance experienced by the coral nubbins in the highest light treatment in the long-term growth experiment was close to saturation.

Both specific growth rate and net photosynthesis increase with irradiance. As light varied 11-fold (38 to 410  $\mu\text{E}/\text{m}^2/\text{s}$ ), specific growth rate increased 1.6 times (0.0103 to 0.0161  $\text{day}^{-1}$ ) while net photosynthetic rate increased 8.9 times (0.009  $\mu\text{mol O}_2/\text{min}/\text{cm}^2$  to 0.077  $\mu\text{mol O}_2/\text{min}/\text{cm}^2$ ). Specific growth rate does not increase proportionally with net photosynthetic rate.

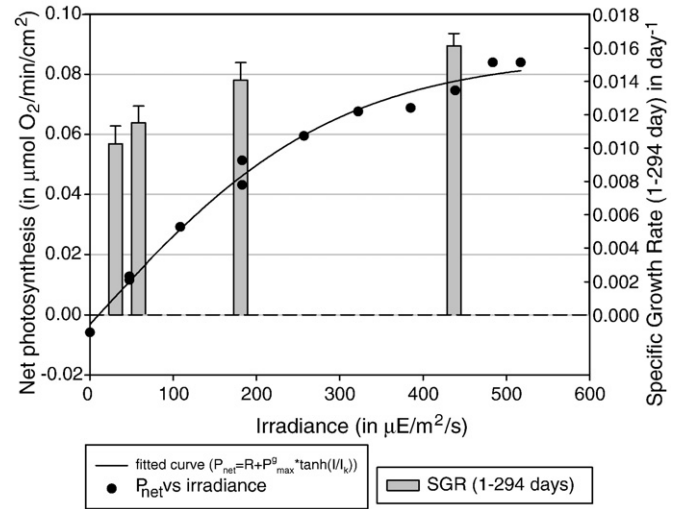
## 4. Discussion

### 4.1. Growth and irradiance

Skeletal growth of *G. fascicularis* increased with increasing irradiance, which is in agreement to the positive correlations of calcification with light found by Marubini et al. (2001) for the stony



**Fig. 2.** Specific growth rate of *Galaxea fascicularis* colonies grown under different light conditions calculated over the total growth period (1–294 days) and plotted against irradiance. Values are mean  $\pm$  S.D.,  $N=9$ .

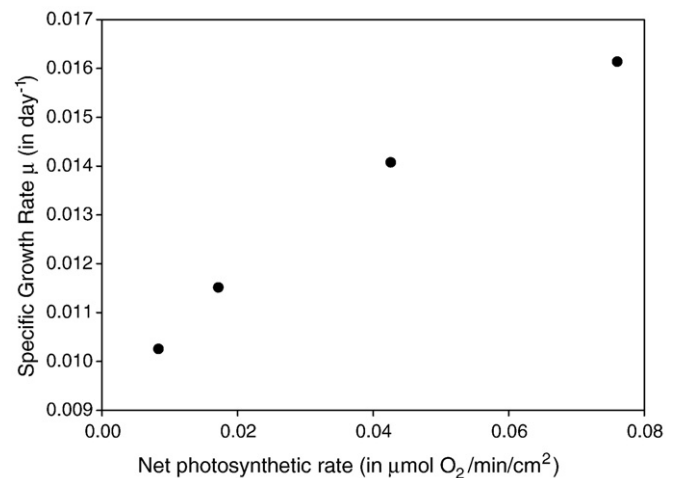


**Fig. 3.** Effect of irradiance on net photosynthesis in  $\mu\text{mol O}_2/\text{min}/\text{cm}^2$  (plotted on left axis) and specific growth rate calculated from day 1 to 294 in  $\text{day}^{-1}$  (plotted on right axis). The light-saturation curve was fitted according to the model of Barnes and Chalker (1990) using Sigmaplot 8.0. Values of specific growth rate are mean  $\pm$  S.D.,  $N=9$ .

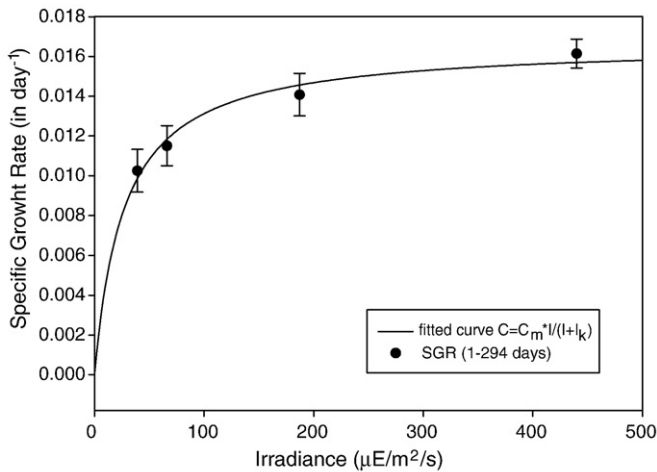
coral *Porites compressa*, by Reynaud-Vaganay et al. (2001) for *Stylophora pistillata* and *Acropora* sp., by Reynaud et al. (2004) for the stony coral *Acropora verweyi*, and by Schlacher et al. (2007) for *Acropora solitaryensis*.

The specific growth rate of *G. fascicularis* also increased with increasing irradiance. Inherent to the assumption of exponential growth, the relative increase in specific growth rate is less than the increase in buoyant weight over a 294 day period. However, for coral breeding in captivity, small differences in specific growth rate can result in large differences in buoyant weight increase over long time intervals.

Growth of *G. fascicularis* in this experiment did not follow first order kinetics, since specific growth rates differed between growth intervals. The first growth interval is biased because of a lag phase in growth due to regeneration after nubbing (Meesters et al., 1994). However, when comparing the specific growth rates in the second and third growth interval, it is notable that specific growth rates decrease in time. The same trend was found for *Galaxea fascicularis* in another long-term study (M. Schutter, unpublished results). Although the



**Fig. 4.** Specific growth rate plotted against net photosynthetic rate, which was calculated at the same irradiances using the equation of the photosynthesis-irradiance curve.



**Fig. 5.** Light saturation curve (right rectangular hyperbola, Chalker (1981)) fitted using SigmaPlot 8.0 on specific growth rate (day<sup>-1</sup>) from day 1 to day 294 against irradiance. Values of specific growth rate are mean ± S.D., N=9.

exponential growth model is thus not applicable to the growth of *G. fascicularis*, it remains a proper tool to evaluate differences in growth.

#### 4.2. Growth and photosynthesis

The relationship between net photosynthetic rate and irradiance followed a hyperbolic tangent function. The irradiance experienced by the coral nubbins in the highest light treatment in the long-term growth experiment was close to saturation.

When comparing specific growth rates (from 1 to 294 days) in the different light treatments with the photosynthesis-irradiance curve, it was observed that net photosynthesis increases relatively faster with irradiance than specific growth rate. In other words, specific growth rates did not increase as much as expected from the increase in net photosynthetic rate. Plotting the specific growth rate against net photosynthetic rate (Fig. 4), shows that their relationship is not linear but levels off with increasing net photosynthetic rate.

It is possible, that the coral's heterotrophic metabolism obscures the relation between its phototrophic metabolism and its specific growth rate in this long-term experiment. This line of thinking would suggest that the major source of energy and/or building blocks for skeletal growth is heterotrophic feeding, while phototrophic feeding is only a minor source. However, the potential energy produced in photosynthesis should not be underestimated. Part of the photosynthetically produced oxygen and photosynthates are instantly used by the coral and its zooxanthellae in the process of light respiration, generating ATP while releasing carbon dioxide and water. Light respiration was found to be ca. 12 times higher than respiration in the dark, as measured using oxygen micro-sensors inside the tissue of *G. fascicularis* (Al-Horani et al., 2003).

Although it is not tested whether this ratio changes with increasing irradiance, it is very plausible that it does. This notion would signify that the poor increase in specific growth rate compared to the increase in net photosynthetic rate is probably not due to a lack of energy, but more likely due to a lack of (nitrogen-rich) building blocks. It is therefore unlikely in this experiment that heterotrophic feeding obscured the relation between phototrophic metabolism and specific growth rate.

It is generally assumed that photosynthesis and calcification are tightly coupled and that an increase in photosynthesis will lead to an increase in calcification. Both processes follow a hyperbolic tangent function when plotted against irradiance (Barnes and Chalker, 1990). However, it is not established whether both processes are saturated at the same irradiances. Comparing the studies from Houlbreque et al. (2004) and Moya et al. (2006) on *Stylophora pistillata*, provides

indications that these processes are not linearly correlated. Houlbreque et al. (2004) fitted a hyperbolic tangent function to the photosynthetic rate of *S. pistillata* (nmol O<sub>2</sub>/h/cm<sup>2</sup>) grown at 175 μE/m<sup>2</sup>/s and found an I<sub>k</sub> (i.e. sub-saturation irradiance) of ~203 μE/m<sup>2</sup>/s for starved corals and ~404 μE/m<sup>2</sup>/s for fed corals. Moya et al. (2006) used the same coral species grown at the same irradiance and measured the calcification rate (nmol Ca<sup>2+</sup>/mg protein/h) at different irradiances. They found an optimal calcification rate at 100 μE/m<sup>2</sup>/s. Although these studies do not use the same parameter to express their results (protein vs. surface area), these results do imply that calcification rate reaches a maximum far before photosynthetic rate does.

Our results do fit in this view, considering the fact that no direct 1:1 relation between calcification and photosynthesis was observed. Specific growth rate and net photosynthetic rate continued to deviate with increasing irradiance. If photosynthesis were to support calcification until saturation of photosynthesis, then it would be expected that a higher photosynthetic rate would lead to a higher calcification rate. To test whether maximum calcification was already reached at an intermediate irradiance level, we applied a right rectangular hyperbola function, according to the procedure of Chalker (1981) (Fig. 5). The fitted curve shows that skeletal growth (and hence: calcification) was close to saturation at the highest irradiance level we applied. Thus, it is not likely that calcification rate already reaches a maximum far before photosynthetic rate does, in contrast to what our comparison of the studies of Houlbreque et al. (2004) and Moya et al. (2006) suggested. Further research describing the relation between photosynthesis, calcification and irradiance in short-term experiments is in progress.

Our results confirm earlier suggestions in literature on the use of photosynthetically derived resources by corals: Moya et al. (2006) suggested that at an irradiance of 100 μE/m<sup>2</sup>/s most requirements for optimizing skeletal growth (both short- and long-term) are already met and a further increase of photosynthetic rate does not add much to calcification rate. Davies (1984), Falkowski et al. (1984) and Muller-Parker (1985) suggested that at higher irradiances, corals are not able to deal economically with their resources, and potential energy for organic matrix synthesis and calcium carbonate deposition is lost. These views suggest that at higher irradiance levels, calcification is not limited by light (and hence: photosynthesis). It either becomes inhibited by light (e.g. Ralph et al., 1999; Winters et al., 2003) or is limited by another factor, such as the availability of bicarbonate (Marubini and Thake, 1999) or the availability of planktonic food, which may be needed for the synthesis of the organic matrix (Allemand et al., 1998; Houlbreque et al., 2004). The results of the current study did not provide evidence to support the hypothesis that skeletal growth and photosynthesis are linearly correlated and therefore this hypothesis has to be rejected. Most probably linearity cannot be reached because at high irradiance, growth will be limited by other factors than irradiance.

#### 5. Conclusion

This study demonstrates that the relationship between net photosynthesis and calcification is not directly proportional. Thus it seems that enhancement of calcification is not entirely photosynthesis-driven: light enhanced calcification seems only to be mediated by photosynthesis at lower irradiances, while at higher irradiances the relation between calcification and photosynthesis is distorted. This finding has implications for the aquaculture of corals for aquarium/restoration purposes, since it is generally believed that more light leads to more (skeletal) growth.

The discrepancy between potential energy produced in photosynthesis and energy used for skeletal growth can be caused by several possible factors which have been discussed in this paper. Future studies should focus on the question to what extent these factors influence the relationship between photosynthesis and calcification.

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