

# Modification of light utilization for skeletal growth by water flow in the scleractinian coral *Galaxea fascicularis*

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Received: 29 October 2009 / Accepted: 4 December 2010  
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**Abstract** In this study, we tested the hypothesis that the importance of water flow for skeletal growth (rate) becomes higher with increasing irradiance levels (i.e. a synergistic effect) and that such effect is mediated by a water flow modulated effect on net photosynthesis. Four series of nine nubbins of *G. fascicularis* were grown at either high ( $600 \mu\text{E m}^{-2} \text{s}^{-1}$ ) or intermediate ( $300 \mu\text{E m}^{-2} \text{s}^{-1}$ ) irradiance in combination with either high ( $15\text{--}25 \text{ cm s}^{-1}$ ) or low ( $5\text{--}10 \text{ cm s}^{-1}$ ) flow. Growth was measured as buoyant weight and surface area. Photosynthetic rates were measured at each coral's specific experimental irradiance and flow speed. Additionally, the instantaneous effect of water flow on net photosynthetic rate was determined in short-term incubations in a respirometric flowcell. A significant interaction was found between irradiance and water flow for the increase in buoyant weight, the increase in surface area, and specific skeletal growth rate, indicating that flow velocity becomes more important for coral growth with increasing irradiance levels. Enhancement of coral growth with increasing water flow can be explained by increased net photosynthetic rates. Additionally, the need for costly photo-protective

mechanisms at low flow regimes could explain the differences in growth with flow.

## Introduction

Light and water flow are two of the most important factors influencing the growth of scleractinian corals. Light stimulates coral growth by the process of light-enhanced calcification (Gattuso et al. 1999; Allemand et al. 2004), which is mediated by zooxanthellar photosynthesis. Consistent with this hypothesis, a higher photon flux density increases net photosynthetic rate and long-term skeletal growth (Marubini et al. 2001; Reynaud-Vaganay et al. 2001; Reynaud et al. 2004; Schutter et al. 2008). These increases might be different at different flow velocities. Water flow reduces the diffusive boundary layer around the coral. This layer acts not only as barrier to the supply and uptake of dissolved gasses, nutrients, and heterotrophic food but also as a barrier to the removal of sediment and metabolic waste products, such as, oxygen, oxygen radicals, and possibly mucus. Possibly because one or more of the above mechanisms, increasing water flow rates correlate with increased skeletal growth (Jokiel 1978; Montebon and Yap 1997; Sebens et al. 2003; Nakamura et al. 2005; Schutter et al. 2010).

Both abiotic factors have only been studied separately, while they might interact strongly (Lesser et al. 1994). At higher irradiance and therefore with higher photosynthetic rate, the removal of photosynthetically produced oxygen (Finelli et al. 2006, 2007) and the supply of inorganic carbon (Lesser et al. 1994) become more important to support photosynthesis and prevent (photo)inhibition. Thus, the thickness of the boundary layer needs to be smaller at higher irradiance than at lower irradiance and

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Communicated by R. H. Richmond.

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consequently water flow needs to be higher at the higher irradiance level.

To investigate this interaction, the influence of water flow on light utilization for skeletal growth of the scleractinian coral *G. fascicularis* was studied using a factorial design. To test whether the observed effects on coral growth are related to the influence of water flow on net photosynthesis, photosynthetic rates were determined of three coral colonies from each experimental treatment by measuring them at their specific irradiance and flow speed inside a respirometric flowcell.

## Materials and methods

### Study species

Thirty-six (36) coral nubbins (single polyp clones) of *G. fascicularis* were created of colonies of the same genotype that were grown at an irradiance of  $60 \mu\text{E m}^{-2} \text{s}^{-1}$  (70 W HQI) in a close-circuit coral aquaculture system in Burgers Ocean, Arnhem, The Netherlands. Each coral nubbin was fixed to a  $7 \times 7 \times 0.4$  cm PVC plate using Reef Construct (Aquamedic). Nine PVC plates with coral nubbins were randomly fixed to each of four square pegboards. After recovering for 1 week, they were transported to a 600 l closed-circuit coral culture system at Wageningen University.

### Coral culture system

The coral culture system in Wageningen consisted of a 400-l aquarium and a 200-l sump containing life rock. The system was connected to an ATI protein skimmer and a self-assembled calcium reactor (6.2–6.4 pH, coral sand). Sea-water was made up from Reef Crystals<sup>®</sup>. Temperature was maintained at  $26.1 \pm 0.4^\circ\text{C}$  and salinity at  $34.4 \pm 1.5$  ppt. Water quality during experimental time was measured at regular intervals and maintained. Alkalinity in the system over the course of the experiment was  $3.3 \pm 1.1$  SD mEq  $\text{l}^{-1}$ , calcium concentration  $388.1 \pm 38.4$  SD mg  $\text{Ca}^{2+} \text{l}^{-1}$ , magnesium concentration  $1348.8 \pm 77.6$  SD mg  $\text{Mg}^{2+} \text{l}^{-1}$ , nitrate concentration between 0 and 2 mg  $\text{NO}_3^- \text{l}^{-1}$ , and phosphate concentration between 0 and 0.5 mg  $\text{PO}_4^{3-} \text{l}^{-1}$ .

### Experimental setup

Each pegboard containing 9 nubbins was assigned to each of the following experimental treatments: high irradiance  $\times$  high flow (HI HF), high irradiance  $\times$  low flow (HI LF), intermediate irradiance  $\times$  high flow (II HF) and intermediate irradiance, low flow (II LF). During the

experiment, the intermediate light regime was kept at an irradiance of approximately  $300 \mu\text{E m}^{-2} \text{s}^{-1}$  and the high light regime was kept at an irradiance of approximately  $600 \mu\text{E m}^{-2} \text{s}^{-1}$ . Water flow was kept between 15 and 25  $\text{cm s}^{-1}$  for high flow, and between 5 and 10  $\text{cm s}^{-1}$  for low flow.

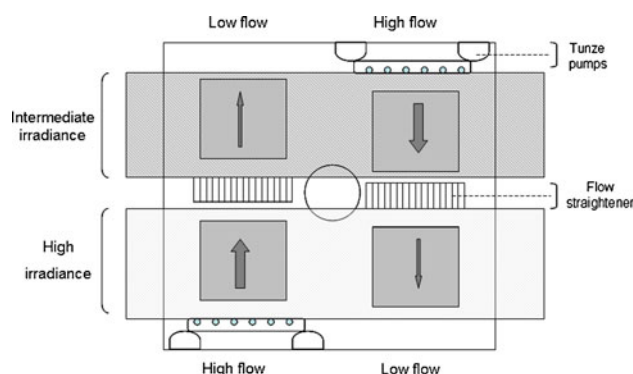
Lighting was supplied using two ATI lighting armatures that each contained four 39 W ATI Aquablue Spezial bulbs. A light:dark cycle of 12L:12D was applied. Irradiance was measured using a Li-Cor 192SA quantum underwater sensor. A broad stream of unidirectional water flow was created by connecting two Tunze TURBELLE<sup>®</sup> nanostream 6 055 pumps to a perforated PVC pipe. The pumps were controlled using a Tunze 7095 Multicontroller. A flow straightener was placed after the first set of corals in the “high flow” treatment, so to reduce the flow that reached the corals in the “low flow” treatment (Fig. 1). Water flow was measured using a SENSE RC-2 electromagnetic velocity meter (Aquadata). The corals were fed 7 days a week with approximately 150,000 freshly hatched *Artemia nauplii* each day, yielding concentrations of 250 *Artemia* per liter in the rearing system.

### Growth parameters

Buoyant weight and surface area were determined according to Schutter et al. (2008). Pictures were taken of the coral nubbins before fixation to their PVC plates. Our data were used to calculate increase in growth and specific growth rates. Specific growth rates were calculated using the following formula:

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

where *SGR* is the specific growth rate ( $\text{day}^{-1}$ ),  $BW_n$  is buoyant weight (or surface area) at the end of the



**Fig. 1** Schematic drawing of the top view of the experimental setup of the light  $\times$  flow experiment. Water flow was created at the high flow side (thick arrows) and reduced by a flow straightener to reach a lower flow rate at the low flow side (thin arrows). The lighting armatures were placed perpendicular to the water streams

experiment,  $BW_{n-1}$  is buoyant weight (or surface area) at the start of the experiment, and  $\Delta t$  is time between the measurements of buoyant weight.

Since our observations suggested that the corals suffered from growth retardation by an unknown cause during the first part of the experiment (1–125 days, in total 125 days), it was decided to use only the growth data from the second part of the growth experiment (125–400 days, in total 275 days) for analysis. Increase in buoyant weight and surface area was calculated from this moment, and specific growth rates were calculated between this moment and the end of the experiment. The size of the coral colony at the start of the second part of the experiment was taken as covariate (see “Data Analysis”).

### Respirometric measurements

Net photosynthetic rate was measured of three different nubbins from each experimental treatment inside a respirometric flowcell (3,500 ml, Fig. 2), which allowed measurement of net photosynthesis at exactly the same irradiance and flow speed as the corals experienced in the experiment. The irradiance applied was either 280 or 560  $\mu\text{E m}^{-2} \text{s}^{-1}$  (corresponding to ambient irradiance at that time) and water flow speed either 5 or 20  $\text{cm s}^{-1}$  (low flow vs. high flow).

Additionally, the instantaneous effect of water flow on net photosynthetic rate at intermediate irradiance (280  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) was determined using paired measurements: first, net photosynthetic rate was measured at a flow speed of 5  $\text{cm s}^{-1}$ , and then, the flow speed was increased to 20  $\text{cm s}^{-1}$  while the respirometric measurement was continued. Five coral colonies were used that were grown in the intermediate irradiance treatment.

Water flow speeds were created using a modified paddlewheel that was powered by a Maxon DC motor with a 3-channel incremental encoder and line driver that allows precise control of rotational speed. Water flow speeds across the coral section were calibrated using video recordings (30 fps, 480 × 860 pixels) of *Artemia* cysts ( $\varnothing$  max. 500  $\mu\text{m}$ ) moving in a 5-mm light plane created by

a slide projector with a slitted cover across the lens. Video recordings were analyzed using a tailor-made program for particle tracking in Matlab, which allowed less time-consuming and more efficient tracking of particles across successive frames. Only particles that remained in the beam plane for 4 or more successive frames were used for calculations. Lighting was provided by a T5 lighting system (ATI) containing eight 24 W Aquablue Spezial bulbs. Irradiance was measured submerged in seawater and underneath the plastic lid, at the exact same distance from the light source for each coral.

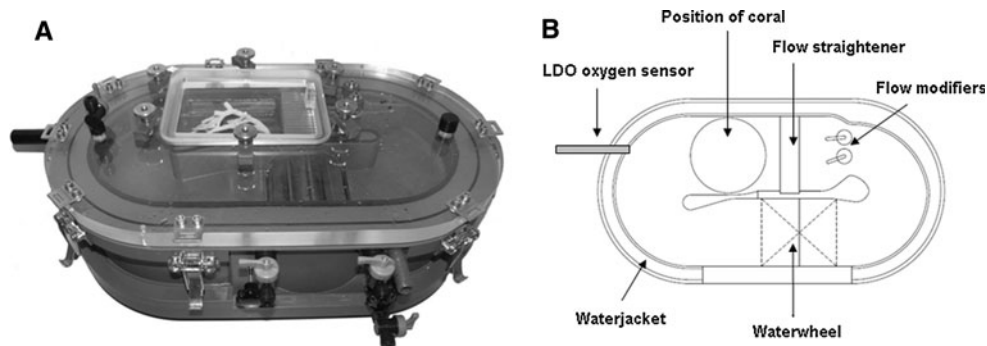
Each incubation with a coral was preceded by a control incubation (seawater only). The increase in oxygen concentration was measured every 10 s using a luminescent oxygen probe (Hach) until a difference in concentration was detected of  $\pm 1 \text{ mg O}_2 \text{ l}^{-1}$ . Each incubation or measurement took approximately 15–20 min. Seawater was replaced after each measurement with a coral, to prevent potential effects of hyperoxia or hypoxia on the performance of the experimental corals and to remove possible accumulated waste products.

Temperature inside the respirometric cell was maintained at 26°C and salinity at 35 ppt. Surface area and volume were determined of each coral in order to normalize the respirometric data. Photosynthetic and respiratory rates were calculated according to Schutter et al. (2008).

### 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. A two-way analysis of covariance (ANCOVA) was used to test the main and interaction effects of light and water flow on the different growth parameters, taking initial colony size at the start of the second growth period (i.e. day 125) as a covariate. Homogeneity of regression was tested ( $p > 0.05$ ) to make sure this assumption for ANCOVA was not violated. Post hoc comparisons were made using the Bonferroni correction. Whenever a covariate was found not to be significant,

**Fig. 2** Respirometric flowcell (version III). **a** Picture of the top/side view of the respirometric flowcell. **b** schematic drawing of top view of respirometric flowcell, indicating the location of the different parts



statistical differences were tested using a two-way ANOVA, followed by multiple comparison using the Bonferroni correction. The same approach was followed for testing statistical differences in net photosynthetic rate. A two-sided paired *t*-test was used to test whether the instantaneous effect of water flow on net photosynthetic rate was significant.

## Results

### Growth

#### Increase in biomass

All corals grew during the experiment. Increase in buoyant weight and surface area during the second growth period was highest in the high light  $\times$  high flow treatment. Although buoyant weight and surface area of the coral colonies at the start of this growth period accounted for a significant proportion of the variation in growth ( $p < 0.005$ ), a significant interaction between irradiance and water flow was found for both buoyant weight ( $p = 0.0031$ ) and surface area ( $p < 0.0003$ ) (Table 1; Fig. 3).

At high irradiance, increasing water flow resulted in a significant increase in both buoyant weight ( $p < 0.0001$ ) and surface area ( $p < 0.0001$ ). At intermediate irradiance, the effect of increasing water flow also resulted in a significant increase in both buoyant weight ( $p = 0.017$ ) and surface area ( $p = 0.0007$ ). At low flow, increasing irradiance resulted in a significant increase in buoyant weight ( $p = 0.041$ ), but no significant increase in surface area ( $p = 0.146$ ). Whereas at high flow, increasing irradiance resulted both in a significant increase in buoyant weight ( $p < 0.0001$ ) and surface area ( $p < 0.0001$ ) (Table 2, Fig. 3). No significant difference in increase in biomass

was found between high light  $\times$  low flow and intermediate light  $\times$  high flow for either buoyant weight ( $p = 1$ ) or surface area ( $p = 0.2912$ ) (not shown in Table).

#### Specific growth rates

Specific growth rates were calculated over the second part of the growth experiment. The buoyant weight of the coral colonies at the start of this growth period accounted for a significant portion of variation in specific growth rate as buoyant weight ( $p = 0.0120$ ), while surface area of the coral colonies at the start of this growth period did not significantly influence the specific growth rate as surface area ( $p = 0.7841$ ). A significant interaction was detected between irradiance and water flow for specific growth rate of buoyant weight ( $p = 0.0163$ ). Interaction between irradiance and water flow for specific growth rate of the surface area was detected at a probability level of 9%. ( $p = 0.0884$ ). The main effects of both irradiance and water flow were significant for specific growth rate of the surface area ( $p < 0.0001$ ). (Table 3, Fig. 4).

Specific growth rate of buoyant weight was highest in the high irradiance  $\times$  high flow treatment, compared to all other treatments ( $p < 0.0001$ ). Higher water flow resulted in a significantly higher specific growth rate of buoyant weight at high irradiance ( $p < 0.0001$ , 43.7% increase), but this increase was not significant at intermediate irradiance ( $p = 0.2987$ , 19.6% increase). Higher irradiance also resulted in a significantly higher specific growth rate of buoyant weight at high flow ( $p < 0.0001$ , 48% increase), but this increase was not significant at low flow ( $p = 0.2548$ , 23.2% increase).

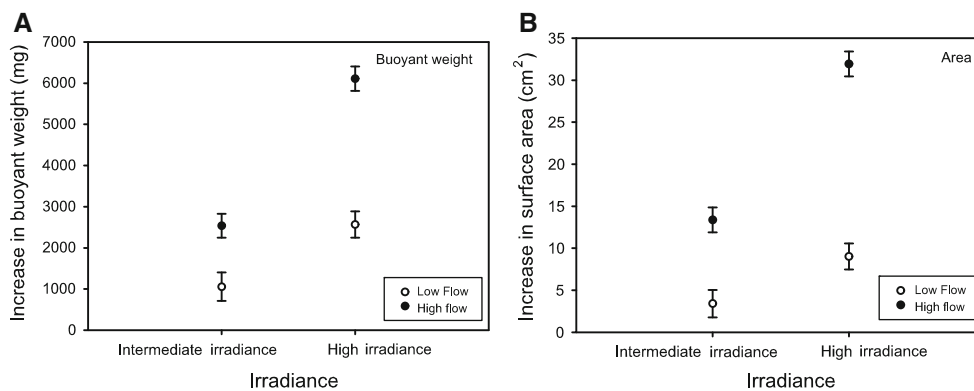
Specific growth rate of the surface area showed a similar picture, although in contrast to specific growth rate of buoyant weight, all differences were significant. Specific growth rate as surface area was highest in the high

**Table 1** ANCOVA interaction table giving the main effect of irradiance and flow and the interaction effect between irradiance and flow (I  $\times$  F) for increase in buoyant weight and surface area between day 125 and day 400

	Light regime Flow rate	II		HI				Covariate effect <i>p</i> -value	Main effects		Interaction effect I $\times$ F <i>p</i> -value		
		LF		HF		LF			HF				
		Adj. means	S.E.	Adj. means	S.E.	Adj. means	S.E.		Adj. means	S.E.		<i>p</i> -value	<i>p</i> -value
Increase in buoyant weight	(in mg)	1058.6	348.1	2537.1	292.2	2567.5	318.9	6108.4	297.6	0.0018	<0.0001	<0.0001	0.0031
Increase in surface area	(in cm <sup>2</sup> )	3.42	1.64	13.38	1.49	9.03	1.54	31.92	1.49	0.0008	<0.0001	<0.0001	0.0003

*p*-values indicate the level of significance of the effects of independent variables, covariables and interactions between independent variables on the dependent variables

Adjusted means  $\pm$  S.E. are given for each experimental treatment. *N* = 9. *II* intermediate irradiance, *LF* low flow, *HF* high flow, *HI* high irradiance



**Fig. 3** Average increase in buoyant weight (a) and surface area (b) per treatment during the second growth period. Values are adjusted means  $\pm$  S.E.  $N = 9$

**Table 2** Results of the post hoc comparison test for the effect of light and flow on increase in buoyant weight and increase in surface area

	Increase in buoyant weight		Increase in surface area	
	<i>p</i> -values	% increase	<i>p</i> -values	% increase
Effect flow				
At HI	<0.0001	<b>137.9</b>	<0.0001	<b>253.5</b>
At II	0.0172	<b>139.6</b>	0.0007	<b>291.4</b>
Effect irradiance				
At HF	<0.0001	<b>140.7</b>	<0.0001	<b>138.6</b>
At LF	0.0411	<b>142.5</b>	0.146	<b>164.2</b>

The percentage increase between treatments is also given. *p*-values indicate the level of significance of the effects of irradiance and flow on specific growth rates

irradiance  $\times$  high flow treatment, compared to all other treatments ( $p < 0.0001$ ). Higher water flow resulted in a significantly higher specific growth rate of surface at both high irradiance ( $p < 0.0001$ , 55.6% increase) and intermediate irradiance ( $p < 0.0001$ , 48.9% increase). Higher

irradiance also resulted in a significantly higher specific growth rate of the surface area at both high flow ( $p < 0.0001$ , 34.7%) and low flow ( $p < 0.0084$ , 28.9%) (Table 4).

No significant difference in specific growth rate is found between high irradiance  $\times$  low flow and intermediate irradiance  $\times$  high flow for either buoyant weight ( $p = 1$ ) or surface area ( $p = 0.1784$ ) (not shown in Table).

Respirometric measurements

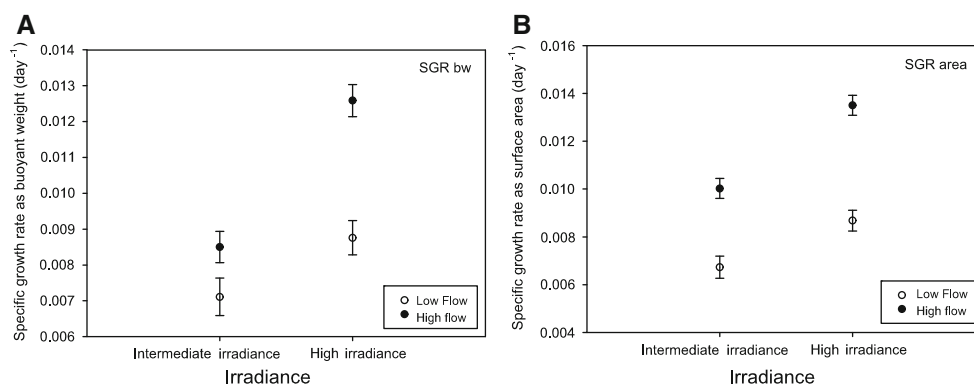
Since surface area was not a significant covariate for net photosynthesis ( $p = 0.6212$ ), a two-way ANOVA was used to test our data for significant differences. No significant interaction is found between the effect of irradiance and water flow on net photosynthesis ( $p = 0.0573$ ) and neither the main effect of irradiance ( $p = 0.8319$ ) or flow ( $p = 0.2374$ ) were significant. None of the specific effects were significant either ( $p > 0.20$ ). Average photosynthetic rate at  $560 \mu\text{E m}^{-2} \text{s}^{-1}$  was twofold higher at high flow ( $0.057 \pm 0.011 \mu\text{mol O}_2 \text{cm}^{-2} \text{s}^{-1}$ ) compared

**Table 3** ANCOVA interaction table giving the main effect of irradiance and water flow and the interaction effect between irradiance and water flow (I  $\times$  F) for specific growth rate of buoyant weight (SGR<sub>bw</sub>) and specific growth rate of the surface area (SGR<sub>area</sub>)

	Light regime	ANOVA	II		HI		Covariate effect	Main effects		Interaction effect			
			Flow rate		Flow rate			Irradiance	Flow				
			LF	HF	LF	HF							
Adj. means	S.E.	Adj. means	S.E.	Adj. means	S.E.	Adj. means	S.E.	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value			
SGR (in bw day <sup>-1</sup> )	ANOVA	0.0071	0.0005	0.0085	0.0004	0.0088	0.0005	0.0126	0.0004	0.0120	<0.0001	<0.0001	0.0163
SGR (in area day <sup>-1</sup> )	ANOVA	0.0067	0.0005	0.0100	0.0004	0.0087	0.0004	0.0135	0.0004	0.7841	<0.0001	<0.0001	0.0960
	ANOVA	0.0067	0.0004	0.0100	0.0004	0.0087	0.0004	0.0135	0.0004	-	<0.0001	<0.0001	0.0884

*p*-values indicate the level of significance of the effects of independent variables, covariables and interactions between independent variables on the dependent variables

Adjusted means  $\pm$  S.E. are given for each experimental treatment.  $N = 9$ . II intermediate irradiance, LF low flow HF high flow, HI high irradiance

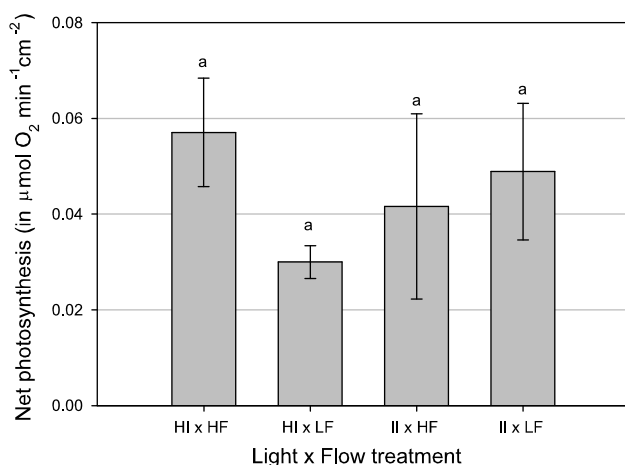


**Fig. 4** Interaction plot for the effect of irradiance and water flow on **a** specific growth rate of buoyant weight, and **b** specific growth rate of the surface area. Values are adjusted means  $\pm$  S.E.  $N = 9$

**Table 4** Results of the post hoc comparison test for the effect of irradiance and flow on specific growth rate of buoyant weight (SGR bw) and specific growth rate of the surface area (SGR area)

	SGRbw		SGR area	
	<i>p</i> -values	% increase	<i>p</i> -values	% increase
Effect flow				
At HI	<0.0001	<b>43.7</b>	<0.0001	<b>55.6</b>
At II	0.2987	<b>19.6</b>	<0.0001	<b>48.9</b>
Effect irradiance				
At HF	<0.0001	<b>48.0</b>	<0.0001	<b>34.7</b>
At LF	0.2548	<b>23.2</b>	0.0084	<b>28.9</b>

The percentage increase in growth rates is also given. *p*-values indicate the level of significance of the effects of irradiance and flow on specific growth rates



**Fig. 5** The interacting effect of irradiance and water flow on net photosynthetic rate at growth irradiance (280 vs 560  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and flow (5  $\text{cm s}^{-1}$  vs 20  $\text{cm s}^{-1}$ ). See text for details.  $N = 3$  corals. Values are mean  $\pm$  SD Means lacking a common superscript differ significantly ( $p < 0.05$ )

to low flow ( $0.030 \pm 0.003 \mu\text{mol O}_2 \text{cm}^{-2} \text{s}^{-1}$ ). Average photosynthetic rate at 280  $\mu\text{E m}^{-2} \text{s}^{-1}$  was  $0.042 \pm 0.019 \mu\text{mol O}_2 \text{cm}^{-2} \text{s}^{-1}$  at high flow and  $0.049 \pm 0.014 \mu\text{mol O}_2 \text{cm}^{-2} \text{s}^{-1}$  at low flow (Fig. 5). A significant increase in net photosynthetic rate ( $p = 0.02$ ) was found in response to an instantaneous increase in water flow from 5  $\text{cm s}^{-1}$  to 20  $\text{cm s}^{-1}$ .

## Discussion

### Growth

A significant interaction was found between irradiance and water flow for increase in buoyant weight and increase in surface area. Similarly, a significant interaction was found between irradiance and water flow for specific growth rate of buoyant weight. This significant interaction term in combination with interpretation of the interaction plots implies that light and water flow act synergistically to increase coral growth for these parameters (Dunne 2010). The importance of water flow for (skeletal) growth rate was higher at high irradiance than at intermediate irradiance, in agreement with our hypothesis. The interaction between irradiance and water flow for specific growth rate of the surface area was not significant at the 5% probability level, but reached significance at a probability level of 10%. Growth as surface area was consistently much higher than growth as buoyant weight. Since our aquarium system was dominated by crustose coralline algae, this higher rate of surface area growth could be explained by the lack of competition with nuisance/turf algae, which allowed the corals to grow more in the periphery.

Interaction between irradiance and water flow was hypothesized to be the result of a limiting effect of low water flow at higher irradiance levels. Indeed, the limiting effect of low water flow becomes obvious when comparing

the specific growth rates of corals cultured at high irradiance ( $600 \mu\text{E m}^{-2} \text{s}^{-1}$ ) and low flow conditions with intermediate irradiance ( $300 \mu\text{E m}^{-2} \text{s}^{-1}$ ) and high flow conditions. Despite having a twofold difference in irradiance, the specific growth rates at high light  $\times$  low flow are comparable to the specific growth rates at intermediate light  $\times$  high flow. Low water flow can limit growth by several mechanisms: (1) limitation of nutrient and/or gas transport (Lesser et al. 1994), and/or (2) inhibition of metabolism as a result of accumulation of oxygen and/or oxygen radicals, accompanied with light stress (Nakamura et al. 2005; Finelli et al. 2006; Finelli et al. 2007). Light stress reduces coral growth due to energy allocation to (costly) stress responses (Anthony et al. 2002) such as the synthesis of heat shock protein and protecting pigments. (3) Reduced photosynthetic rates as a result of oxidative stress (Lesser 1996; Nakamura and van Woesik 2001; Finelli et al. 2006), potentially impairing light-enhanced calcification. (4) Decreased encounter rate of potential food particles (Sebens et al. 1997), and (5) increased chance for sedimentation (Rogers 1990) and attachment of (and competition with) algae (Smith and Birkeland 2007).

Specific growth rates were lower than expected based on previous studies (49% lower for intermediate irradiance, 45% lower for high irradiance; comparing low flow values) (Schutter et al. 2008). Two reasons can be given to explain this: (1) Specific growth rates were calculated over a different time span (i.e. longer time after nubbing). Since specific growth rates of *G. fascicularis* decrease in time (Schutter et al. 2008), calculating them over a longer time span will result in lower specific growth rates. In the current study, specific growth rates were calculated between day 125 and day 400 after nubbing, while Schutter et al. 2008 calculated them between day 1 and day 294 after nubbing. Thus, absolute values should not be compared between these studies. (2) The amount of *Artemia* fed in this study was 16–32 times lower than in Schutter et al. 2008. Heterotrophic feeding increases skeletal growth in an additive way (Ferrier-Pagès et al. 2003). More feeding would therefore probably have resulted in higher growth rates and even more significant results.

## Net photosynthesis

### *Net photosynthetic rate and increasing water flow*

No significant difference was detected between the net photosynthetic rate of corals grown in the high water flow regime and of corals grown in the low water flow regime, neither at intermediate nor at high irradiance. Possibly, the natural variability between corals and the low number of replicates prevented the detection of a significant difference. Nevertheless, additional measurements on the

instantaneous effect of water flow on net photosynthetic rate did confirm that water flow has a significant stimulating effect on photosynthesis at  $280 \mu\text{E/m}^2/\text{s}$ . This is consistent with the findings of Mass et al. (2010), who demonstrated that water flow enhances photosynthesis by increasing the efflux of oxygen. Likely, this flow-driven enhancement of photosynthesis becomes more important at higher photosynthetic rates (hence: higher irradiance levels). The twofold difference in photosynthetic rate between high irradiance  $\times$  low flow and high irradiance  $\times$  high flow ( $0.030 \pm 0.003$  versus  $0.057 \pm 0.011 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ min}^{-1}$ ), although not significant, also suggests that the decreased efflux of oxygen and consequently increased the accumulation of oxygen within coral tissues resulted in photo-inhibition of photosynthesis (Lesser 1996; Nakamura and van Woesik 2001; Finelli et al. 2006). Two mechanisms can play a role to inhibit photosynthesis: (1) the inhibition of photosynthesis due to photorespiration: a decreased ratio of  $\text{CO}_2:\text{O}_2$  inside coral tissues enhances the competitive advantage of  $\text{O}_2$  as a substrate for RuBisCo compared to  $\text{CO}_2$ , and (2) the production and accumulation of reactive oxygen species (ROS) in the Mehler reaction (Badger et al. 2000).

Differences in skeletal growth rate with increasing flow can therefore be explained by differences in net photosynthetic rate. Additionally, the differences in skeletal growth could be explained by the occurrence of oxidative stress (e.g. photorespiration) and the subsequent need for photo-protection at low flow regimes. Photo-protective mechanisms (e.g. Asada 1999; Leggat et al. 1999; Shick and Dunlap 2002) are likely to be energetically costly (Finelli et al. 2006; Hoogenboom et al. 2009). Possibly, more energy is allocated to photo-protective mechanisms at low flow regimes, resulting in lower growth rates compared to the corals in the high flow regimes. It is therefore likely that increased flow rates lead to increased coral growth at high irradiance levels through the relief of oxidative stress. Possibly, increased heterotrophic feeding (e.g. *Artemia* and/or DOM) and/or nutrient uptake with increasing flow (Sebens et al. 1997; Atkinson and Bilger 1992) also played a role. Kaandorp et al. (2005) demonstrated the role of (hydro-dynamically created) gradients of inorganic carbon and nutrients in controlling coral growth and morphology using diffusion—dominated computer models. The importance and contribution of these factors should to be quantified in future experimental research.

### *Net photosynthetic rate and increasing irradiance*

No significant difference was detected between the net photosynthetic rate of corals grown in the high irradiance regime and of corals grown in the intermediate irradiance regime, neither at low, nor at high flow. Our data for net

photosynthetic rates could therefore not explain differences in growth with increasing irradiance. This is in contradiction with the light-enhanced calcification hypothesis that assumes that the enhancement of calcification is mediated by photosynthesis (Allemand et al. 2004; Schutter et al. 2008). Schutter et al. (2008) demonstrated a positive relationship between specific growth rate and net photosynthesis for *G. fascicularis*. However, these findings were based on a (short-term) photosynthesis-irradiance curve, which is possibly different from photosynthetic measurements of corals that are long-term adapted to a certain irradiance. Corals are known to optimize their net photosynthetic rate in order to minimize losses in their metabolic activity (Titlyanov and Titlyanova 2002). It is therefore possible that the net photosynthetic rates of both the corals at  $280 \mu\text{E m}^{-2} \text{s}^{-1}$  and at  $560 \mu\text{E m}^{-2} \text{s}^{-1}$  were optimized to similar rates. Moreover, differences in net photosynthetic rate are likely so subtle that significance of measurement is not easily reached.

Besides photosynthesis, the most important factor likely to be co-responsible for increased growth with increasing irradiance is light respiration. Light respiration occurs in the light and is generally much higher than dark respiration due to the presence of photosynthetically produced oxygen and possibly also due to the direct use of translocated photosynthetically fixed carbon. Gross photosynthesis is postulated to be much higher at higher irradiance, but after subtracting the postulated increased light respiration at higher irradiance, the resulting net photosynthesis might be the same (postulated according to Al-Horani et al. 2003). Light respiration results in a higher availability of metabolic  $\text{CO}_2$ , which is the major form of dissolved inorganic carbon that is used for calcification (70%, Furla et al. 2000).

The phototrophic enhancement of coral growth with increasing irradiance (i.e. light-enhanced calcification) might thus not only be related to increased (net) photosynthesis, but also to light respiration. A limited number of studies on light respiration in corals is known, since it requires sophisticated techniques such as oxygen micro-sensors (Al-Horani et al. 2003) or other methods that can separate respiratory fluxes from concurrent photosynthetic fluxes (e.g. oxygen isotopes ( $^{18}\text{O}$ )) (Grande et al. 1991). To the best of our knowledge, the enhancement of light respiration with increasing irradiance has not been studied in scleractinian corals. However, a positive relationship between irradiance and light respiration has been found for several phytoplankton species (Lewitus and Kana 1995).

Nevertheless, differences in light respiration are not likely to explain the interaction between irradiance and water flow. Since photosynthesis is postulated to supply the coral with more oxygen and carbon compounds for respiration than water flow, the enhancing effect of water flow

on light respiration is postulated to be small compared to the enhancing effect of irradiance.

## Conclusion

Water flow and irradiance act synergistically to increase coral growth. The importance of flow is demonstrated at high irradiance levels, since coral growth at high irradiance  $\times$  low flow is comparable or even less than coral growth at lower irradiance levels. Thus, the importance of water flow for coral growth becomes higher with increasing irradiance levels.

The enhancement of coral growth with increasing flow can be explained by an increase in net photosynthetic rate. It is postulated that water flow enhances the efflux of oxygen and thereby prevents a decrease in photosynthetic rate due to oxygen accumulation. On the other hand, the enhancement of coral growth with increasing irradiance could not be explained by a corresponding change in photosynthetic rate. It is postulated that photosynthetic rates were optimized in each treatment, but that a significant amount of (photosynthetic) energy was allocated to photo-protective mechanisms and the response to oxidative stress in the low flow treatments. Enhancement of coral growth with increasing water flow would therefore be related to the relieve of oxidative stress. The potential effect of light respiration needs further study.

**Acknowledgments** This research is part of the European CORALZOO project that aims at improving coral husbandry techniques for sustainable coral breeding in zoo's and public aquaria. In this project, scientists and aquarists collaborate to provide a scientific basis for coral husbandry techniques. The experiment described in this paper was aimed to determine the effect of interaction between light and water flow on growth of scleractinian corals. This work was funded by the European Commission (Project CORALZOO-012547). We thank Eric Karruppanan, Evert Janssen and VINK kunststoffen B.V. for helping us with the design and construction of the respirometric flowcell. Additionally, we like to thank Imke Crucq for performing the additional measurements to determine the instantaneous effect of water flow on net photosynthetic rate. All procedures described herein comply with current laws of the Netherlands. The authors declare that they have no conflict of interest.

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