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Effect of light intensity on post-fragmentation photobiological performance of the soft coral *Sinularia flexibilis*

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ABSTRACT

The soft coral *Sinularia flexibilis* is currently considered as a suitable candidate for aquaculture. This soft coral is commonly traded for marine aquariums, is used in reef restoration efforts, as well as in the bioprospecting of marine natural products. The production of this coral under controlled laboratory conditions may be the best option for a sustainable and continuous supply of its biomass. It is known that the fragmentation of corals harboring photosymbiotic unicellular dinoflagellates of genus *Symbiodinium*, commonly termed zooxanthellae, can be influenced by light, as the photosynthetic performance of zooxanthellae can affect coral physiology and growth. This study aimed to investigate the effect of different light intensities on the photobiology of *S. flexibilis* following *ex situ* fragmentation. *S. flexibilis* mother colonies were fragmented after being acclimated for 5 months to a photoperiod of 12 h light with an irradiance of 120 μmol quanta m^{-2} s^{-1}. Fragments were then distributed by three light treatments (50, 80 and 120 μmol quanta m^{-2} s^{-1}) for monitoring of their photosynthetic performance, photosynthetic and accessory pigment concentration, zooxanthellae density, and growth. No significant differences were recorded one month post-fragmentation on the maximum quantum yield of PSII (F_v/F_m), neither on zooxanthellae density, between fragmented corals placed under tested light intensities. However, zooxanthellae density significantly increased after 5 months in fragments exposed to 50 and 80 μmol quanta m^{-2} s^{-1}, while F_v/F_m and pigment concentration decreased under the highest light intensity (120 μmol quanta m^{-2} s^{-1}). This study showed that the use of low light levels after fragmentation, in the absence of heterotrophic feeding, do not significantly affect coral growth. Moreover, light levels used after fragmentation should be adjusted according to the intended stocking time of produced coral fragments.

1. Introduction

The increasing demand for soft corals (Octocorallia), either for biotechnological research on marine natural products (Blunt et al., 2008, 2009) or to supply the marine aquarium trade (Wabnitz et al., 2003), has prompted an increase on their harvest (Castanaro and Lasker, 2003). However, the dependence on organisms collected from the wild compromises the sustainability of this approach. In this way, it has been recommended that future research on marine natural products should consider the use of specimens produced in captivity (Mendola, 2003; Proksch et al., 2003) and that the marine aquarium industry should promote the trade of cultured soft corals, rather than wild specimens (Calfo, 2007; Olivotto et al., 2011).

In this context, coral aquaculture can be a potential solution for a continuous and sustainable supply of soft coral biomass (Sella and Benayahu, 2010). Coral propagation by asexual reproduction is a relatively simple and inexpensive process, which has been commonly used for the production of new colonies, with a high survival rate of fragments and a reduced impact on mother colonies (Fox et al., 2005; Soong and Chen, 2003). Coral fragments can be produced either *in situ* or *ex situ*. *In situ* fragmentation and grow-out may benefit from natural environmental conditions and requires no adaption to artificial propagation systems. However, fragments are exposed to potential deleterious factors, such as sedimentation, pathogens, predators, competitors and other natural hazards, which can reduce survival (Rinkevich, 2005). In contrast, *ex situ* fragmentation has the advantage of maximizing survival and growth rates through the manipulation of culture conditions, such as light, flow and food availability (Forsman et al., 2006; Khalesi, 2008a).

Light is a key factor for symbiotic corals due to their association with photosymbiotic unicellular dinoflagellates from genus *Symbiodinium* (commonly termed as zooxanthellae) (Osinga et al., 2011; Schutter et al., 2012; Wijgerde et al., 2012). The photosynthates produced by the zooxanthellae are transferred to the coral host and fulfill a significant part of its energetic requirements (Falkowski et al., 1984; Hoogenboom et al., 2006). Light variation is known to affect zooxanthellae density, photosynthetic pigment concentration and photosynthetic efficiency (Frade

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et al., 2008b; Kühl et al., 1995; Lesser et al., 2010). Ultimately, changes in the density of zooxanthellae can affect coral physiology and its response to stress (Venn et al., 2008). As the fragmentation process *per se* induces stress to both coral mother colony and produced fragments, it is expected that light can play an important role on the post-fragmentation photophysiological processes and, therefore, on coral recovery.

The captive culture of the soft coral *Sinularia flexibilis*, one of the dominant benthic invertebrate inhabitants of Indo-Pacific reefs (Bastidas et al., 2004; Van Ofwegen, 2002) has been recently addressed by several studies (Khalesi, 2008a, 2008b; Khalesi et al., 2009). However, a gap of knowledge persists in some practical issues related to coral recovery post-fragmentation. The present study aimed to investigate the effect of different light intensities in the physiology and photobiology of the soft coral *S. flexibilis* following *ex situ* fragmentation, namely photosynthetic performance, zooxanthellae density, photosynthetic and accessory pigments concentration and coral fragments growth.

2. Materials and methods

2.1. S. flexibilis husbandry and fragmentation

Five colonies of *S. flexibilis* were kept for 5 months in a recirculating system with synthetic saltwater (prepared by mixing Tropic Marin Pro Reef salt — Tropic Marine, Germany — and freshwater purified by a reverse osmosis unit). The glass tank holding the mother colonies (90 L water volume) was connected to a 100 L filter tank. The mother colonies' tank was equipped with a circulation pump (Turbelle nannostream, 6025 Tunze, Germany), which provided an approximate water flow of 2500 L h^{-1}. The filter tank was equipped with a protein skimmer (APF-600 Deltec, Germany), a biological filter (composed of 20 kg of live rock and submerged bio-balls), two submersible heaters (Eheim Jäger 300 W, Germany) and a submerged pump (EHEIM 1260, Germany) that supplied a flow of approximately 1500 L h^{-1} to the coral stocking glass tank. The tank holding the mother colonies was illuminated with a 150 W (10,000 K) metal halide lamp (BLV, Germany) delivering a photosynthetic active radiation (PAR) of 120 μmol quanta m^{-2} s^{-1} at the level of the colonies with a 12 h light:12 h dark photoperiod. Salinity was maintained at 35 using an osmoregulator (Deltec Aquastat 1000) that provided automatic compensation of evaporated water with freshwater purified by a reverse osmosis unit. Other water parameters were maintained as follows: temperature 26 ± 0.5 °C, total ammonia nitrogen 0.05 ± 0.01 mg L^{-1}, NO_2^-–N 0.03 ± 0.01 mg L^{-1}, NO_3^-–N 1.0 ± 0.1 mg L^{-1}, PO_4^{3-}–P 0.01 ± 0.01 mg L^{-1}, pH 8.2 ± 0.2, alkalinity 3.90 ± 0.20 mEq L^{-1}, Ca^{2+} 420 ± 20 mg L^{-1}, and Mg^{2+} 1300 ± 20 mg L^{-1}.

S. flexibilis colonies were fragmented using a scalpel producing 6 similar sized fragments (about 10 cm) per colony, with each one being individually attached with a rubber band to a plastic coral stand (Coral Cradle®), labeled and randomly distributed among the different experimental treatments (see below).

2.2. Experimental design

Twenty-seven fragments of the pool of 30 fragments produced (6 fragments × 5 mother colonies) were randomly selected and distributed by the stocking tanks of the 3 coral propagation modules, each with 3 stocking tanks. Each tank was stocked with 3 coral fragments. Each coral propagation module was composed of three 90 L glass tanks (0.6 m × 0.6 m × 0.25 m) connected to a 150 L filter tank equipped with a protein skimmer (ESC150 ReefSet, Portugal), a biological filter (composed of 30 kg of live rock and submerged bio-balls), two submergible heaters (Eheim Jäger 300 W, Germany), a calcium hydroxide reactor (KM500 Deltec, Germany) connected to an osmoregulator (Deltec Aquastat 1000) and a submerged pump (EHEIM 1262, Germany; providing an approximate flow of 1000 L h^{-1} to each tank). Additionally, each tank was equipped with a single circulation pump (Turbelle nannostream, 6025 Tunze, Germany; approximate flow of 2500 L h^{-1}). Each tank was illuminated from above with a 150 W (10,000 K) metal halide lamp (BLV, Germany) with 12 h light:12 h dark photoperiod. The distance between the lamps and water surface were adjusted to provide one of the following PAR intensities (± 5%) at the level of coral fragments: 50, 80 and 120 μmol quanta m^{-2} s^{-1}. During the experiment, PAR values were measured once a week at the level of coral fragments using a Quantum Flux meter (Apogee, MQ-200) with a submergible sensor. The position of each coral fragment in the tank was adjusted so that all fragments in each light treatment had the same PAR value. The use of three independent modules allowed the use of three replicates per light intensity treatment, each replicate being composed of three coral fragments. Water parameters were kept as described above for mother colonies. Partial water changes using synthetic saltwater (10% of total experimental system volume) were performed every week. The experiment was performed for 5 months.

2.3. In vivo chlorophyll fluorescence

Pulse amplitude modulation (PAM) fluorometry was used to monitor photosynthetic activity by measuring non-intrusively variable chlorophyll fluorescence (Schreiber et al., 1986). The PAM fluorometer comprised a computer-operated PAM-control unit (Walz) and a WATER-EDF-Universal emitter-detector unit (Gademann Instruments, GmbH, Würzburg, Germany) (Cruz and Serôdio, 2008). Actinic and saturating lights were provided by a blue LED-lamp (peaking at 450 nm, half-bandwidth of 20 nm) that was delivered to the sample by a 1.5 mm-diameter plastic fiber optic bundle. The fiber optic bundle was positioned perpendicularly to the surface of the coral cutting. Measurements were carried out 2 h after the start of the daylight period, to ensure the full activation of the photosynthetic apparatus, in 9 different points in the initial coral colonies immediately before fragmentation (T0), and in the coral fragments 1 month (T1) and 5 months (T5) after fragmentation and transfer to the experimental system. Coral fragments were kept in a recirculating water bath (Frigiterm-10, Selecta, Spain) at 25 °C during PAM measurements. At each measuring occasion, the corals were dark-adapted for 15 min, after which one saturation pulse (0.8 s) was applied to determine the minimum- or dark-level fluorescence, F_o, a parameter expected to correlate with the Chl *a* content (Serôdio et al., 2001), and the maximum fluorescence, F_m. F_o and F_m were used to determine the maximum quantum yield of PSII (Schreiber et al., 1986):

$$F_v/F_m = \frac{(F_m - F_o)}{F_m}. \quad (1)$$

2.4. Zooxanthellae

Following *in vivo* PAM measurements, a sample of coral tissue was removed with a scalpel to analyze zooxanthellae density and determine photosynthetic and accessory pigments (see below). Zooxanthellae density was assessed in 9 tissue fragments of the initial coral colonies immediately before fragmentation (T0), at T1 and at T5. The time between sampling events allowed the complete cicatrisation and total recovery of the surveyed coral fragments. Sampled coral tissue was homogenized in tubes containing 15 mL of filtered (0.2 μm) seawater. The homogenate was diluted and homogenized before zooxanthellae counting in a hemacytometer with improved Neubauer ruling (5 cell counts for each coral fragment). After counting the total volume of each sample was centrifuged (10 min, 5000 rpm), the supernatant water was discarded and the pellet freeze-dried for 24 h to determine total dry weight. Zooxanthellae concentration was normalized to *S. flexibilis* dry weight.

The genotype of the *Symbiodinium* sp. (commonly termed as zooxanthellae) harbored by *S. flexibilis* mother colonies was identified by

sequencing of the entire ITS1-5.8S-ITS2 region of the ribosomal gene according to Santos et al. (2001).

2.5. Photosynthetic and accessory pigments

The concentration of the following photosynthetic and accessory pigments was determined in coral colonies before fragmentation (T0) and in coral fragments at T1 and at T5: chlorophyll *a* (Chl *a*), chlorophyll c_2 (Chl c_2), diadinoxanthin (DD), diatoxanthin (DT), peridinin (Per) and β-carotene (β-Car). Freeze-dried samples of 0.04 to 0.12 g were extracted with 3–5 mL of 95% cold buffered methanol (2% ammonium acetate) for 30 min at −20 °C in total darkness. The samples were sonicated (Bransonic, model 1210) for 30 s at the beginning of the extraction period. The extracts were filtered (Fluoropore PTFE filter membranes, 0.2 μm pore size) and immediately injected in a Shimadzu HPLC system with photodiode array (SPD-M10AVP) and fluorescence (RF-10AXL) detectors. Chromatographic separation was carried out using a C18 column for reverse phase chromatography (Supelcosil; 25 cm long; 4.6 mm in diameter; 5 μm particles) and a 35 min elution program. The solvent gradient followed (Kraay et al., 1992) with a flow rate of 0.6 mL min^{-1} and an injection volume of 100 μL. Pigments were identified from absorbance spectra and retention times and concentrations were calculated from signals in the photodiode array detector. Calibration of the HPLC peaks was performed using commercial standards from DHI (Institute for Water and Environment, Hørsholm, Denmark).

2.6. Coral fragments growth

Buoyant weight measurements (Spencer Davies, 1989) were made using a Kern Emb 200-3 balance (Kern & Sohn GmbH) to determine the growth of coral fragments between T0 and T1, and between T1 and T5. The buoyant weights of each coral cradle and rubber bands used to attach each coral fragment was also determined prior to fragmentation. The buoyant weight of all coral fragments was determined and corrected with the weight of the respective cradle and rubber band to obtain net and total weights. Coral cradles were cleaned thoroughly with seawater and a tooth-brush the day before each measurement, in order to minimize any potential bias promoted by the development of biofouling. To ensure reproducibility, each coral fragment was weighted 3 times at T0-5. Water temperature and salinity were kept stable during all buoyant weight measurements. To calculate the percentage of daily coral growth (% CG day^{-1}) for each coral fragment, the following formula was used:

$$\%\mathrm{CG\,day^{-1}} = \left(\left(\frac{w_f - w_i}{w_i}\right)/\Delta t\right) \times 100 \quad (2)$$

where w_f and w_i are the final and initial coral net weights expressed in grams (g), and Δt is the time interval in days. CG is expressed in percentage of coral weight increase per day.

2.7. Statistical analysis

Statistical analyses were carried out using the software Statistica version 8.0 (StatSoft Inc.). Repeated measurements ANOVAs was used to evaluate the existence of significant differences in the maximum quantum yield of PSII (F_v/F_m), zooxanthellae density, photosynthetic pigment concentrations, relation between zooxanthellae and photosynthetic pigments, and coral growth recorded for fragments of *S. flexibilis* kept with different light regimes. Mauchly's test of sphericity was used to determine if the variances of the differences between all combinations of related groups were equal. Post hoc Tukey HSD test was used to determine differences between light treatments and between sampling points of each treatment. When the assumptions of homogeneity of variances and homoscedasticity were not met, data transformation methods were used. Particularly, zooxanthellae counting and photosynthetic pigment data were square root and log transformed, respectively.

3. Results

3.1. In vivo chlorophyll fluorescence

Maximum quantum yields of PSII (F_v/F_m) measured on *S. flexibilis* exposed to different light intensities are presented in Fig. 1. At the end of the experiment (T5) *S. flexibilis* fragments reared under the lowest PAR treatment (50 μmol quanta m^{-2} s^{-1}) showed significantly higher F_v/F_m values ($P<0.001$) when compared to the fragments reared under the highest PAR (120 μmol quanta m^{-2} s^{-1}). During the experimental period the mean values of F_v/F_m decreased significantly ($P<0.001$) in the corals under the highest PAR light treatment (120 μmol quanta m^{-2} s^{-1}).

3.2. Zooxanthellae

The reduction of photosynthetic active radiation (PAR) increased zooxanthellae density (Fig. 2). Zooxanthellae concentration in *S. flexibilis* fragments reared with a PAR of 50 μmol quanta m^{-2} s^{-1} was significantly higher ($P<0.01$) at the end of the experiment (T5) ($3.09\times10^8 \pm 0.80\times10^8$ cells g^{-1} DW; average ± standard deviation) when compared to cell concentration at T0 ($2.46\times10^8 \pm 0.16\times10^8$ cells g^{-1} DW). At the end of the experiment the fragments reared under a PAR of 80 μmol quanta m^{-2} s^{-1} also showed significantly higher ($P<0.001$) zooxanthellae density ($3.20\times10^8 \pm 0.65\times10^8$ cells g^{-1} DW) when compared to the results at T0 ($2.46\times10^8 \pm 0.16\times10^8$ cells g^{-1} DW). No significant differences were registered throughout the experiment for fragments reared under the highest PAR (120 μmol quanta m^{-2} s^{-1}). At T5 corals reared under 50 and 80 μmol quanta m^{-2} s^{-1} PAR had significantly higher zooxanthellae density ($P<0.01$) when compared to corals reared under the highest PAR (120 μmol quanta m^{-2} s^{-1}), as shown in Fig. 2.

Symbiodinium sp. present in the *S. flexibilis* was identified as type B_1 after their successful genotyping.

3.3. Photosynthetic and accessory pigments

The results of photosynthetic pigment analysis are displayed in Fig. 3. Overall, each pigment concentration (μg g^{-1} DW) increased in all light treatments one month after fragmentation and decreased

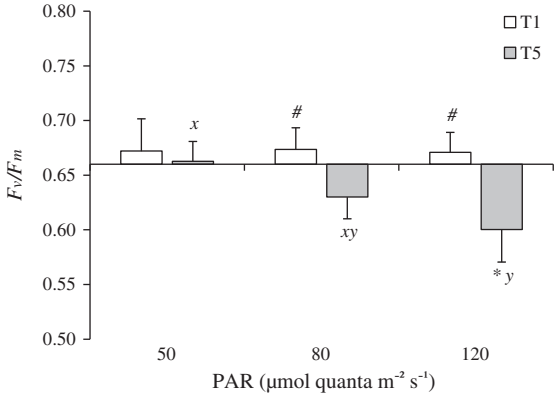

Fig. 1. Average values of maximum quantum yield of PSII (F_v/F_m) measured on 9 *S. flexibilis* fragments (one month — T1 and 5 months — T5 after the beginning of the experiment) exposed to three different light treatments (50, 80 and 120 μmol quanta m^{-2} s^{-1}). Horizontal axis crosses vertical axis in the mean value obtained before fragmentation — T0. $n=9$ coral fragments per light treatment. Vertical lines represent standard deviation. Significantly different from T0 are marked (*); # represents significant differences between T1 and T5 in the same light PAR treatment; different superscript letters represent significant differences within the same time ($P<0.001$ for all comparisons; Tukey HSD post-hoc comparisons).

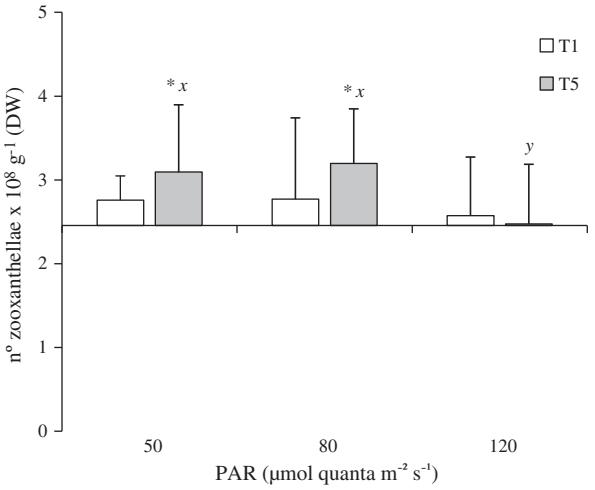

Fig. 2. Zooxanthellae density (per gram of coral dry weight). Average measurements made on 9 *S. flexibilis* fragments per light treatment (50, 80 and 120 μmol quanta m^{-2} s^{-1}). Vertical lines represent one standard deviation. Horizontal axis crosses vertical axis in the mean value obtained before fragmentation — T0. Significantly different from T0 are marked (*); # represents significant differences between T1 and T5 in the same light PAR treatment; different superscript letters represent significant differences within the same time ($P<0.01$; Tukey HSD post-hoc comparisons) in and between light treatments during the experimental period (T0 — beginning, T1 — one month and T5 — 5 months after the beginning of the experiment).

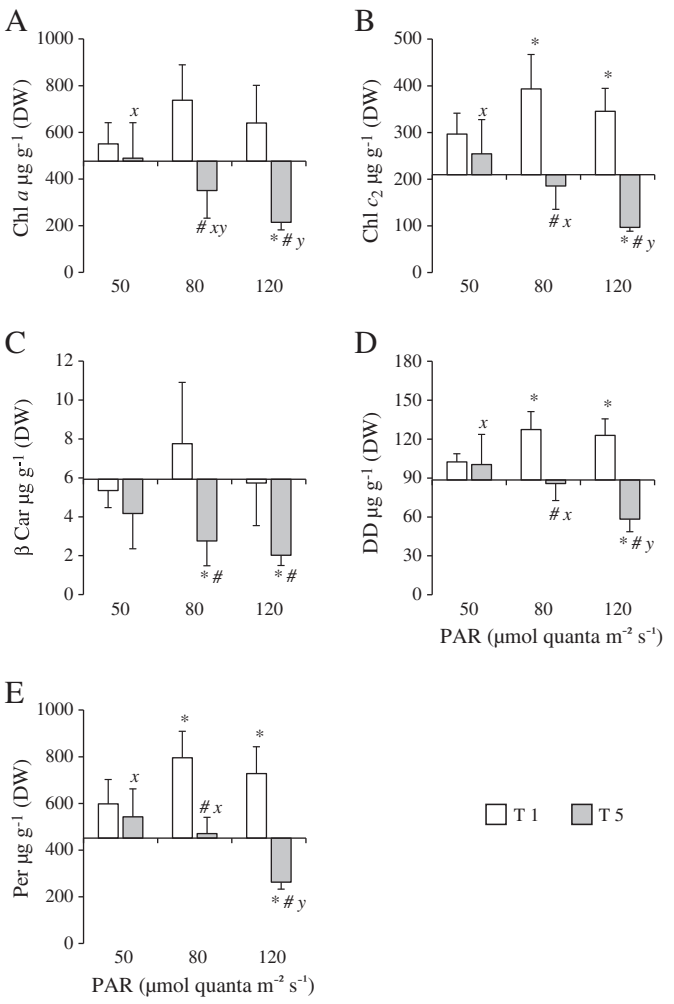

Fig. 3. Average concentration of photosynthetic pigments (μg.g^{-1} soft coral dry weight) measured at T0 (beginning of the experiment, before fragmentation), T1 (one month) and T5 (5 months) after fragmentation in 9 *S. flexibilis* fragments from each light treatment. (A) Chlorophyll *a* (Chl *a*), (B) chlorophyll c_2 (Chl c_2), (C) β-carotene (β-Car), (D) diadinoxanthin (DD) and (E) peridinin (Per). Horizontal axis crosses vertical axis in the mean value obtained before fragmentation — T0. Vertical lines represent one standard deviation. Significantly different from T0 are marked (*); # represents significant differences between T1 and T5 in the same light PAR treatment; different superscript letters represent significant differences within the same time ($P<0.05$; Tukey HSD post-hoc comparisons).

after 5 months. The concentration of Chl *a* recorded at T5 under the lowest light intensity treatment (50 μmol quanta m^{-2} s^{-1}) was significantly higher ($P<0.001$) than the mean value obtained for *S. flexibilis* fragments reared with the highest PAR value (120 μmol quanta m^{-2} s^{-1}). Under 80 and 120 μmol quanta m^{-2} s^{-1}, Chl *a* concentration was significantly lower at T5 than at T1 ($P<0.001$). The mean value of Chl c_2 concentration (Fig. 3B) in the light treatment with the lowest PAR value (50 μmol quanta m^{-2} s^{-1}) did not change significantly during the experimental period. The mean value of Chl c_2 (Fig. 3B) concentration in the light treatment with the highest PAR (120 μmol quanta m^{-2} s^{-1}) was significantly higher at T1 than in the mother colonies, T0 ($P<0.05$). At the end of the experiment, Chl c_2 concentration was significantly lower ($P<0.001$) than the results observed at T0 and T1. β-Car concentration (Fig. 3C) in coral fragments reared under a PAR of 80 and 120 μmol quanta m^{-2} s^{-1} were significantly lower at T5 than at T1 ($P<0.01$) and T0 ($P<0.05$). The mean values of DD (Fig. 3D) and peridinin concentration (Fig. 3E) did not change significantly for *S. flexibilis* fragments reared under the lowest light PAR treatment (50 μmol quanta m^{-2} s^{-1}). However, in fragments reared under a PAR of 80 μmol quanta m^{-2} s^{-1}, the mean values of both these two photosynthetic pigments were significantly higher at T1 than at T5 ($P<0.001$ and $P<0.01$ for peridinin and DD, respectively), as well as those determined for mother colonies (T0) ($P<0.001$ and $P<0.05$ for peridinin and DD, respectively). *S. flexibilis* fragments reared under 80 and 120 μmol quanta m^{-2} s^{-1} showed higher DD and peridinin concentration at T1 than at T0 and T5 ($P<0.05$ and $P<0.01$) for DD and peridinin, respectively.

3.4. Photosynthetic pigments/zooxanthellae

The results of the ratio of photosynthetic pigment (μg) per zooxanthellae (both normalized to *S. flexibilis* DW) were similar to those described above for photosynthetic pigment alone and are represented in Fig. 4. No significant differences were recorded throughout the experiment for any of the tested ratios in coral fragments cultured under a PAR of 50 μmol quanta m^{-2} s^{-1}. Significantly lower values ($P<0.05$) of μg Chl *a* per zooxanthellae (Fig. 4A), μg Chl c_2 per zooxanthellae (Fig. 4B), μg DD per zooxanthellae (Fig. 4D) and μg peridinin per zooxanthellae (Fig. 4E) were found in coral fragments reared under the PAR values of 80 and 120 μmol quanta m^{-2} s^{-1} at the end of the experiment, when compared to the values recorded at T1 results.

3.5. Coral fragments growth

The mean values of coral growth registered in the coral fragments (% CG mean ± standard deviation, $n=9$ coral fragments per treatment) in the first time interval (between T0 and T1) were 0.042 ± 0.012%day^{-1}, 0.041 ± 0.009%day^{-1} and 0.043 ± 0.014%day^{-1} for coral fragments from the light PAR treatments of 50, 80 and 120 μmol quanta m^{-2} s^{-1}, respectively. In the second time interval (between T1 and T5) the mean values of coral growth were 0.040 ± 0.010%day^{-1}, 0.039 ± 0.012%day^{-1} and 0.039 ± 0.009%day^{-1} for coral cuttings from the light PAR treatments of 50, 80 and 120 μmol quanta m^{-2} s^{-1}, respectively. No significant differences were found in the growth of coral fragments between light PAR treatments in both time intervals (T0 to T1 and T1 to T5).

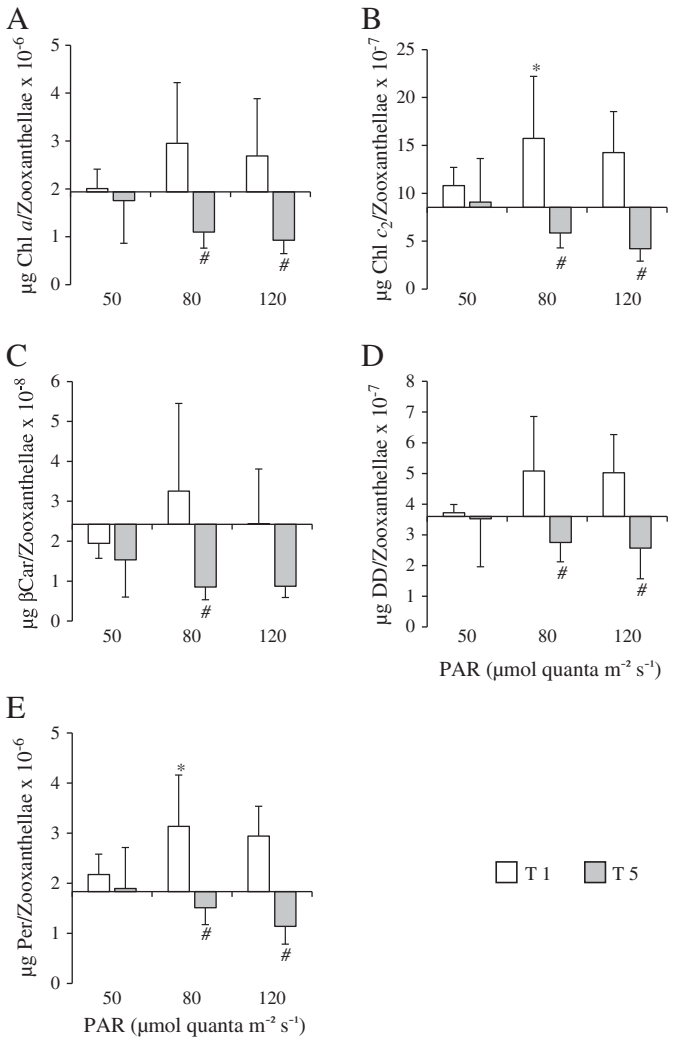

Fig. 4. Average ratios of photosynthetic pigment (μg) per zooxanthellae (both normalized to *S. flexibilis* dry weight in three sampling points (T0, T1 and T5) in the 9 fragments in each light treatment (50, 80 and 120 μmol quanta m^{-2} s^{-1})). (A) Chlorophyll *a* (Chl *a*), (B) chlorophyll c_2 (Chl c_2), (C) β-carotene (β-Car), (D) diadinoxanthin (DD) and (E) peridinin (Per). Horizontal axis crosses the vertical axis in the mean value obtained before fragmentation — T0. Vertical lines represent one standard deviation. Significantly different from T0 are marked (*); # represents significant differences between T1 and T5 in the same light PAR treatment; different superscript letters represent significant differences within the same time ($P<0.05$; Tukey HSD post-hoc comparisons).

4. Discussion

An excessive increase in light levels is known to commonly damage the photosynthetic apparatus of zooxanthellae, while an increase in zooxanthellae density is usually recorded when corals are exposed to suboptimal light intensities (Frade et al., 2008a; Hoegh-Guldberg and Jones, 1999). High and low light levels will ultimately lead to an adaptive response of the coral holobiont, either through the action of photoprotective mechanisms (such as the increase of photoprotective pigments) or adapting the photosynthetic apparatus to maximize light capture (Titlyanov and Titlyanova, 2002a). According to the previous authors, acclimation to low light involves the maximization of the light harvesting capacity through: 1) the increase of photosynthetic pigment concentration in zooxanthellae; and 2) the multiplication of zooxanthellae (increased density). However, while changes in pigment concentrations present in zooxanthellae usually occur within 2–4 days, changes in the number of zooxanthellae (thus zooxanthellae densities) only commonly occur within 40 days (Titlyanov et al., 2001).

In the present study, the soft coral *S. flexibilis* was initially adapted to a light intensity of 120 μmol quanta m^{-2} s^{-1}. Overall, results of photosynthetic efficiency of zooxanthellae and zooxanthellae density one month after fragmentation were similar to those recorded prior to fragmentation. This suggests a recovery from fragmentation stress to initial photophysiological conditions within a one month period. However, five months after fragmentation, significantly lower photosynthetic efficiencies were observed in corals reared on 120 μmol quanta m^{-2} s^{-1}. While no significant changes on zooxanthellae concentration were observed, an increasing concentration of photosynthetic and accessory pigments was observed at T1, even for corals from 120 μmol quanta m^{-2} s^{-1} PAR treatment. Despite the same PAR level being provided before and after fragmentation, the overshadow effect after fragmentation is expected to be lower in coral fragments than in large mother colonies, thus maximizing the light intensity reaching the coral tissue of fragments. The increasing trend observed for pigment concentration per zooxanthellae might have been promoted by the fragmentation process, as a result of the reduction of the overshadow effect (thus shifting the light environment affecting the coral tissue of produced fragments). The use of a lower PAR after fragmentation induced a contrasting effect on the density of zooxanthellae as it significantly increased after 5 months in corals reared at 50 and 80 μmol quanta m^{-2} s^{-1}. These results are in accordance with previous studies that report an increase in zooxanthellae density to maximize light absorption under low PAR values (Titlyanov et al., 2001). In contrast, results for corals reared under the highest PAR level show decreasing pigment concentrations per zooxanthellae after 5 months, suggesting an adaptive response to high light levels (Titlyanov and Titlyanova, 2002b). Although the mother colonies of *S. flexibilis* were acclimated to a light intensity of 120 μmol quanta m^{-2} s^{-1}, this light level may be inadequately high to coral fragments and can potentially induce light stress. As part of light-protecting reactions it would be expected to observe an increase on β-Car concentration (Bandaranayake, 2006; Mobley and Gleason, 2003). Curiously, the concentration of this pigment decreased, which may be associated with a potential high light stress that may have prompted the zooxanthellae to reach a point of no return. As no fragment mortality was observed and, in general, photobiological performance decreased over time in higher light PAR treatments, we can hypothesize that fragmentation recovery processes took over light-stress photoprotective mechanisms. This scenario resulted in a decrease of photosynthetic efficiency and photosynthetic pigment concentration, which may ultimately culminate in a decrease of photosynthate translocation from endosymbiotic zooxanthellae to their cnidarian host (Levy et al., 2003; Titlyanov and Titlyanova, 2002a). The same authors reported that fragmented corals which allowed to regenerate under lower light levels were able to acclimate and maximize light absorption. This acclimation probably promoted an increase on the production and translocation of photosynthetates and a larger contribution of autotrophy to the coral's mixotrophic nutrition (Levy et al., 2003).

No significant effects were recorded on coral growth between the different light treatments. Therefore, the present results suggest that the use of lower light levels can be a suitable option following fragmentation. Additionally, this option can contribute to decrease the costs associated with coral culture, as Osinga et al. (2011) have already reported that the use of artificial light is one of the factors influencing the economic viability of *ex situ* coral aquaculture. It must also be stressed that the trend recorded in the present work may be species specific, may shift for the same coral species harboring different zooxanthellae clades or for corals recovering *in situ*. Kuguru et al. (2008) performed an experimental study using different light levels in the field and in the laboratory and found different photoacclimation results, particularly in terms of pigment concentration per zooxanthellae. Furthermore, as the morphology and physiology of symbiotic invertebrates can vary widely among species (Gates and Edmunds, 1999), a given *Symbiodinium* type may experience very dissimilar environments, depending on the symbiotic invertebrate species that it inhabits (Goulet et al., 2005).

5. Conclusion

Our work showed that keeping *S. flexibilis* fragments under the same light conditions as their mother colonies seems to be photobiologically acceptable for a short-term husbandry (*e.g.* when producing a large number of small sized fragments for research studies), lower light intensities than those used for mother colonies may favor the photobiological performance of coral fragments intended to be stocked for longer periods and contribute to a reduction of production costs (*e.g.* when producing large sized colonies that can yield a larger biomass production for biotechnological applications and need to be stocked in captivity for several months).

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