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

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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 (Fv/Fm), 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 Fv/Fm 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
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 10 L filter tank. The mother colonies’ tank was equipped with a circulation pump (Turbelle nanostream, 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 submerged heaters (Eheim Jäger 300 W, Germany) and a submerged pump (HEIM 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 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, Fm, a parameter expected to correlate with the Chl a content (Serôdio et al., 2001), and the maximum fluorescence, Fm. Fe and Fm were used to determine the maximum quantum yield of PSII (Schreiber et al., 1986):

\[ F_v/F_m = \frac{(F_m - F_o)}{F_m} \]  

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 c2 (Chl c2), 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 (Branson, 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. To calculate the percentage of daily coral growth (% CG day−1) for each coral fragment, the following formula was used:

\[
\text{%CG day}^{-1} = \left( \frac{W_f - W_i}{W_i} \right) \times 100
\]

where \(W_f\) and \(W_i\) are the final and initial coral net weights expressed in grams (g), and \(\Delta t\) 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 \(F_{v}/F_{m}\), zoxanthellae density, photosynthetic pigment concentrations, relation between zoxanthellae 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, zoxanthellae 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. Zoxanthellae

The reduction of photosynthetic active radiation (PAR) increased zoxanthellae density (Fig. 2). Zoxanthellae concentration in S. flexibilis fragments reared with a PAR of 50 μmol quanta m−2 s−1 was significantly higher \((P<0.001)\) at the end of the experiment (T5) \((3.09 \times 10^8 \pm 0.80 \times 10^8\) cells g−1 DW; average ± standard deviation) when compared to cell concentration at T0 \((2.46 \times 10^8 \pm 0.16 \times 10^8\) 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 \((P<0.001)\) zoxanthellae density \((3.20 \times 10^8 \pm 0.65 \times 10^8\) cells g−1 DW) when compared to the results at T0 \((2.46 \times 10^8 \pm 0.16 \times 10^8\) cells g−1 DW). No significant differences were registered throughout the experiment for fragments reared under the highest PAR \((120 \mu\text{mol quanta m}^{-2}\text{ s}^{-1})\). At T5 corals reared under 50 and 80 μmol quanta m−2 s−1 PAR had significantly higher zoxanthellae density \((P<0.01)\) when compared to corals reared under the highest PAR \((120 \mu\text{mol quanta m}^{-2}\text{ s}^{-1})\), as shown in Fig. 2.

Symbiodinium sp. present in the S. flexibilis was identified as type B1 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 \((\mu g \text{ g}^{-1} \text{ DW})\) increased in all light treatments one month after fragmentation and decreased...
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).
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 photosynthetic 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 differences 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 photacclimation 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).

4. Discussion

An excessive increase in light levels is known to commonly damage the photosynthetic apparatus of zooxanthellae, while an increase in zooplankton 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).
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 bio-technological applications and need to be stocked in captivity for several months).

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