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The effect of mixotrophy in the *ex situ* culture of the soft coral *Sarcophyton* cf. *glaucum*

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ABSTRACT

In toto aquaculture is a promising approach towards the bioprospecting and production of bioactive compounds from the coral holobiont. Coral aquaculture *ex situ* allows a better control of culture conditions to maximize coral growth and reduce production costs. Light (either the Photosynthetically Active Radiation—PAR, or the emitted spectrum) is one of the most important factors affecting the growth of symbiotic corals, due to their association with photosynthetic dinoflagellates. Additionally, the balance between autotrophy and heterotrophy is also known to play a key role in the successful growth of mixotrophic corals. The present study aimed to evaluate the effect of: 1) light spectrum (white and blue light), 2) PAR intensity (50 and 120 μmol quanta m^{-2} s^{-1}), and 3) heterotrophic feeding (rotifers—*Brachionus plicatilis*), in the physiology, photobiology and growth of coral fragments obtained from three mother colonies of the mixotrophic soft coral *Sarcophyton* cf. *glaucum*, cultured *ex situ* in recirculated systems during 80 days. The supply of rotifers did not affect coral growth and promoted the increase of dissolved inorganic nitrogen and phosphorous concentrations (DIN and DIP, respectively) in the water column. Coral fragments originating from the different mother colonies presented distinct responses to the tested light PAR intensities and spectra, as well as to the interaction of these factors with the supply of rotifers. We concluded that the variability displayed between different mother colonies of the same species can affect the growth response of corals under contrasting scenarios of mixotrophy.
Statement of relevance: Improvement of *in toto* aquaculture of *Sarcophyton* cf. *glaucum*.

1. Introduction

Over the past decade, marine bioprospecting for new natural products has been mainly focused on tropical coral reefs (Leal et al., 2012). However, traditional bioprospecting approaches of reef organisms, such as symbiotic corals, entails two potential bottlenecks: sustainability and replicability. Despite the highest diversity of biologically active secondary metabolites isolated from corals, there are growing evidences that several of these bioactive compounds are in fact produced by bacteria living in association with the coral (Leal et al., 2013; Rocha et al., 2011). However, the isolated culture of these bacteria (*ex hospite*) is a challenging task and only a few successful cases have been achieved (Joint et al., 2010; Leal et al., 2013). Therefore, *in toto* aquaculture (culture of the coral holobiont—the cnidarian host, photosynthetic dinoflagellates and associated microorganisms) can be considered a suitable approach to supply the required biomass to perform the first steps of the drug discovery pipeline (Leal et al., 2014c).

In toto aquaculture performed *ex situ*, in recirculated aquaculture systems (RAS), allows to: 1) manipulate culture conditions in order to maximize coral growth and survival (Forsman et al., 2006; Leal et al., 2015), 2) guarantee the presence of target symbionts which can produce target compounds (Isaacs et al., 2009; Kooperman et al., 2007), 3) control the number of symbionts accordingly to their relevance for bioactive compound production (Leal et al., 2014c) and 4) optimize culture protocols for specific genotypes that may yield higher metabolite production or hold contrasting microbial communities with different compound production (Leal et al., 2014c). Additionally, *ex situ* aquaculture facilities, operating in RAS, can be implemented near pharmaceutical laboratories, thus avoiding extra costs associated with packing and shipping (Leal et al., 2014c).

Light is one of the most important factors for *ex situ* aquaculture of symbiotic corals living in association with photosynthetic dinoflagellates of genus *Symbiodinium* (popularly termed as zooxanthellae), due to the implementation and operating costs (Osinga et al., 2011; Rocha et al., 2013a). Intensity and spectral composition of light are key parameter to the photosynthetic performance of the zooxanthellae (Wijgerde et al., 2014) and can directly or indirectly affect the physiology and growth of the coral host (Rocha et al., 2013b, 2013c). However, the

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photosynthetates transferred from the zooxanthellae only provide metabolic energy to the coral (Falkowski et al., 1984), and a high percentage of the carbon transferred is lost in respiration or expelled as mucus (Anthony, 1999; Davies, 1984; Falkowski et al., 1984). Therefore, coral growth is also highly dependent on heterotrophic feeding to obtain an appropriate biological ratio of both carbon, nitrogen, phosphorus and other several essential components that can increase the rate of tissue synthesis (Houlbrèque and Ferrier-Pagès, 2009; Sella and Benayahu, 2010).

The processes of autotrophy and heterotrophy are closely linked in symbiotic corals and species have different feeding capacities and heterotrophic requirements. The need of prey catch can be related with an insufficient availability of photosynthetates (Bessell-Browne et al., 2014; Ferrier-Pagès et al., 2011; Tremblay et al., 2015). Nonetheless, Piniak (2002) showed that capture rates in a facultative symbiotic coral are completely independent from their symbiotic condition. Moreover, it appears that feeding can be directly dependent on water flow (Fabricius et al., 1995), prey size (Palardy et al., 2006), as well as the ability of target prey to escape and the coral's mechanism of capture (Sebens et al., 1996). Corals uptake and recycle both inorganic and organic nutrients (Muscatine and Porter, 1977). Besides obtaining essential nutrients that are not provided by the zooxanthellae (Muscatine and Porter, 1977) through the ingestion of zooplankton, the consumption of other sources of nitrogen is also very important for the symbiotic association, as zooxanthellae seem to be nitrogen limited (Hoegh-Guldberg and Smith, 1989; Muscatine et al., 1989). Nitrogen can be supplied to corals in the form of particulate organic nitrogen (PON) (Anthony, 1999; Mills and Sebens, 2004; Mills et al., 2004) or in dissolved inorganic forms (DIN) as ammonium, nitrate and nitrite (D'Elia and Webb, 1977; Snidvongs and Kinzie, 1994). Dissolved inorganic phosphorus (DIP) is also an important nutrient for zooxanthellae and coral growth (D'Elia, 1977; Ferrier-Pagès et al., 2000). Even though the coral holobiont can benefit from the nitrogen and phosphorus present in the environment (D'Elia, 1977; Grover et al., 2002), elevated concentrations of these nutrients can lead to a decrease in coral growth (Ferrier-Pagès et al., 2001, 2000; Kinsey and Davies, 1979).

The genus *Sarcophyton* (Cnidaria: Anthozoa: Octocorallia: Alcyonacea) is one of the most specious within family Alcyonacea, with 67 valid species to date ("WoRMS—98 World Register of Marine Species", 2015). These species have been highly bioprospected due to their natural compounds with potential for biomedical applications, such as sarcophytolide (Badria et al., 1998), sarcophytol (Wei and Frenkel, 1992), or sarcophine (Sawant et al., 2006a, 2006b). The interest these species arouse among researchers working in the bioprospecting of marine natural products, with potential for the pharmaceutical industry, make them good candidates for aquaculture. Several studies have been performed on the effect of light spectrum and intensity on corals (Rocha et al., 2013a, 2013b, 2013c), as well as on their heterotrophic feeding (Ferrier-Pagès et al., 2003; Houlbrèque and Ferrier-Pagès, 2009; Lewis, 1982; Muscatine et al., 1989). However, the combined effect of these factors on the physiology, photobiology and growth of cultured soft corals, particularly of genus *Sarcophyton*, is yet to be addressed. The present study aims to evaluate the effect of light spectrum, photosynthetic active radiation (PAR) and heterotrophic feeding, in the physiology, photobiology and growth of fragments of the mixotrophic soft coral *Sarcophyton* cf. *glaucum* obtained from 3 mother colonies.

2. Materials and methods

The taxonomy of *Sarcophyton glaucum* is not consensual (Aratake et al., 2012). McFadden et al. (2006) suggested that the species being commonly designated as *Sarcophyton glaucum* could indeed be divided into six different clades, based on sequence analyses of mitochondrial proteins. Therefore, in this study we refer to *Sarcophyton glaucum* as *Sarcophyton* cf. *glaucum* and we preserved samples for future species confirmation (when the taxonomy of *Sarcophyton glaucum* reaches a consensual opinion).

2.1. Coral husbandry and fragmentation

Three colonies of *S.* cf. *glaucum* with a capitulum diameter of approximately 200 mm, collected in Sumbawa, Indonesia, were purchased from a marine ornamentals wholesale company. Colonies were collected in the same area, 3 to 5 m apart, approximately at 8 m depth. *S.* cf. *glaucum* colonies were stocked for 15 days for acclimatization to water parameters and surveyed for any evidence of infection or disease.

The acclimatization modular system was composed by three experimental glass tanks (0.6 m × 0.6 m × 0.25 m; 90 L), connected to a filter tank with a volume of 150 L, equipped with a protein skimmer (ESC—150 ReefSet, Portugal), biological filters (approximately 10 L of submerged bioballs and a fluidized sand-bed biological filter with approximately 1 kg of aragonite), and a submergible heater (Eheim Jäger 300 W, Germany).

Water recirculation in the experimental tanks (approximately 1000 L h^{-1} in each experimental tank) was promoted by a submerged pump (Eheim 1262, Germany) assembled in the filter tank. Additionally, each experimental tank was equipped with a circulation pump (Turbelle nanostream-6025 Tunze, Germany), which promoted an approximated water flow of 2500 L h^{-1}.

Each tank was illuminated with a 150 W–10,000 K Hydrargyrum Quartz Iodide lamp (HQI) (Sylvania, Germany), with a Photosynthetically Active Radiation (PAR) of 120 μmol quanta m^{-2} s^{-1}, under a photoperiod of 10:14 (hours light:hours dark).

The system operates with synthetic saltwater, prepared by mixing synthetic salt (Tropic Marin Pro Reef salt—Tropic Marine, Germany) with water purified by a reverse osmosis system (Aqua-win RO—6080, Thailand). The salinity was maintained at 35 using an osmoregulator (Deltec Aquastat—1000, Germany) which automatically compensates for water loss by evaporation by adding fresh water purified by reverse osmosis. For a more detailed description of the experimental system, please refer to Rocha et al. (2015). Coral colonies were individually stocked in the glass tanks (1 colony per tank). After the acclimatization period, the periphery of the capitulum of each mother colony was fragmented with a sterilized scalpel, originating 35 fragments with approximately 1.5 cm^2, following the procedures described by Rocha et al. (2013b). Each coral fragment was attached with a rubber band to a labelled plastic stand (Coral Cradle®). Coral fragments recovery was performed in the acclimation tank used for their respective mother colony and kept under the same conditions.

2.2. Brachionus plicatilis production

Pure cultures of rotifers were maintained in 250 mL erlenmayers with microalgae (*Nannochloropsis* sp. and *Isochrysis galbana*). Larger volumes were cultured in 25 L acrylic cylindrical tanks, maintained with constant aeration and fed with PhytoBloom Green Formula (live *Nannochloropsis* sp. concentrate, Necton, Portugal). Rotifers were enriched for 12 h with *I. galbana* (at a concentration of approximately 80,000 cells mL^{-1}) before being supplied to corals.

2.3. Heterotrophic feeding—B. plicatilis ingestion

The rotifer *B. plicatilis* was chosen as live prey because of its size and low mobility; additionally this species can be cultured in high concentrations and its nutritional value can be easily manipulated by the administration of different food types (Lubzens, 1987; Watanabe et al., 1983).

The suitability of rotifers as live prey for *S.* cf. *glaucum*, and the better photoperiod for heterotrophic feeding (prey capture), was assessed in a preliminary trial performed in a climatized room (25 °C), using glass containers filled with 1 L of filtered synthetic saltwater with the same