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The effect of light and heterotrophy in the *ex situ* culture of the soft coral *Sarcophyton* cf. *glaucum*

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The effect of light and heterotrophy in the *ex situ* culture of the soft coral *Sarcophyton* cf. *glaucum*.

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Resumo

O aumento da procura de corais, tanto para bioprospecção de produtos naturais, como para o mercado da aquariofilia marinha, levou à necessidade de cultivo destes organismos. O cultivo de corais *ex situ* permite um maior controlo dos processos de produção, mas implica o desenvolvimento e otimização das infraestruturas e dos protocolos de cultivo. Para proporcionar condições ótimas de cultivo é essencial deter conhecimento da biologia e fisiologia dos organismos a cultivar, permitindo assim o seu desenvolvimento de uma forma rápida e saudável, a redução dos custos associados à produção, bem como a contribuição para a viabilidade económica da exploração. O cultivo de corais é afetado por diversos fatores, bióticos e abióticos. A luz (radiação fotossinteticamente ativa ou o espectro emitido) é um dos fatores abióticos mais importantes no cultivo *ex situ* de corais fotossintéticos, devido à relação ecológica de simbiose que mantêm com dinoflagelados do género *Symbiodinium*, vulgarmente designados por zooxantelas. Adicionalmente, o balanço entre autotrofia e heterotrofia desempenha um papel importante no sucesso do crescimento dos corais. O presente estudo foi desenvolvido com o objetivo de estudar o efeito de três fatores: 1) espectro de luz (luz branca e luz azul), 2) intensidade da radiação fotossintética ativa (50 e 120 µmol quanta.$m^{-2}.s^{-1}$) e 3) alimentação heterotrófica (fornecimento de rotíferos - *Brachionus plicatilis* (Müller, 1786)), na fisiologia, fotobiologia e crescimento do coral mole *Sarcophyton* cf. *glaucum* (Quoy & Gaimard, 1833), cultivado *ex situ* em sistemas recirculados, durante 80 dias. Os resultados obtidos revelaram que o fornecimento de rotíferos como alimento não beneficia diretamente a taxa de crescimento dos corais e promove um aumento de nutrientes inorgânicos na água de cultivo (nitratos e fosfatos). A resposta fisiológica ao espectro e intensidade de luz testados, bem como à interação destes fatores com a alimentação heterotrófica diferiu nos fragmentos de coral provenientes de diferentes colónias. Assim, conclui-se que a variabilidade entre colónias da mesma espécie influencia a resposta dos corais aos diferentes parâmetros estudados, pelo que num cenário de produção este aspeto deve ser acautelado através de uma seleção de colónias que reúnam determinadas características, em função dos objetivos de produção e das condições das instalações de cultivo.

Palavras-chave: aquacultura de corais, sistemas de aquacultura recirculados, fotobiologia, zooxantela, *Symbiodinium*, fluorometria PAM.

Abstract

The increasing demand of corals, either for bioprospecting marine natural products for biomedical purposes, for the marine aquarium trade, or for utilization in coral reefs restoration efforts, has led to the need of cultivating these organisms. The production *ex situ* allows a better control over biomass production through the optimization of culture protocols. Therefore, it is important to understand the biology and physiology of cultivated organisms, in order to improve culture conditions, maximize growth and reduce production costs. These issues are highly relevant for the economic feasibility of coral aquaculture. Among a varied number of factors affecting coral growth, light (either the Photosynthetically Active Radiation – PAR, or the emitted spectrum) is one of the most important issues for symbiotic corals, due to their association with photosynthetic dinoflagellates of genus *Symbiodinium* (commonly termed as zooxanthellae). Additionally, the dynamics between autotrophy and heterotrophy also plays a key role in the success of coral growth. The present study was performed to evaluate the effect of: 1) light spectrum (white and blue light), 2) light PAR intensity (50 and 120 µmol quanta.$m^{-2}.s^{-1}$), 3) heterotrophic feeding (rotifers - *Brachionus plicatilis* (Müller, 1786)), in the physiology, photobiology and growth of fragments obtained from three mother colonies of the mixotrophic coral *Sarcophyton* cf. *glaucum* (Quoy & Gaimard, 1833), cultured *ex situ* in recirculated systems during 80 days. The supply of rotifers did not affect corals growth and promoted the accumulation of inorganic nutrients (nitrates and phosphates) in the culture water. The effect of light PAR intensities and spectrum as well as the interaction of these factors with heterotrophic feeding did not follow a similar pattern in coral fragments originating from different mother colonies. We concluded that the variability between colonies of the same species can play a key role in the response of corals to the studied parameters. Therefore, in a production scenario, mother colonies should be selected according their specific characteristics, to meet production objectives and culture conditions.

Keywords: coral growth, recirculating aquaculture systems, photobiology, zooxanthellae *Symbiodinium*, PAM fluorometry**.**

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1. Introduction

The coral reefs are among the most productive ecosystems in the world, providing food and protection for a wide range of organisms that inhabit these environments. Coral reefs also protect shores from ravages, and are an attraction for tourism and diving (Burke *et al.*, 2011). Near to 10 million of people depend of coral reef resources (Wilkinson, 2008). But even with the ecological and economical importance of coral reefs, they have been suffering an array of threats, principally due to climate changes (Burke *et al.*, 2011; Roberts *et al.*, 2002), or to anthropogenic pressure, such as marine pollution, over fishing or fishing with destructive methods (e.g. dynamite, cyanide) (Calado, 2006; Hodgson, 1999; McClanahan *et al.*, 1996).

Corals are anthozoans, like anemones, sea fans and sea pansies. Most coral species live in compact colonies formed by several polyps, and display a limited organ development (Barnes, 1987). Coral polyps, schematized in figure 1, are composed by two epithelial cell layers, the epidermis (or ectoderm) and the gastrodermis (or endoderm). The epidermis promotes the separation of the coral from the external environment, whereas the gastrodermis limits the gastro-vascular cavity. The boundary between these two layers, the mesoglea, is mostly composed by water but also contain several other substances including fibrous proteins like collagen. The gastro-vascular cavity, where the ingested food is decomposed, opens only at one end, the mouth, that is surrounded by several tentacles with pinnules, and cnidocytes (stinging cells) at the end (Delbeek and Sprung, 1994; Levinton, 1995).

Corals are commonly divided in stony corals (the ones primarily responsible for building reefs) and soft corals (Barnes, 1987). Soft corals unlike hard corals lack a calcium carbonate skeleton, but contain structural sclerites, which contribute to the sustention of polyp structure (Rocha, 2013).

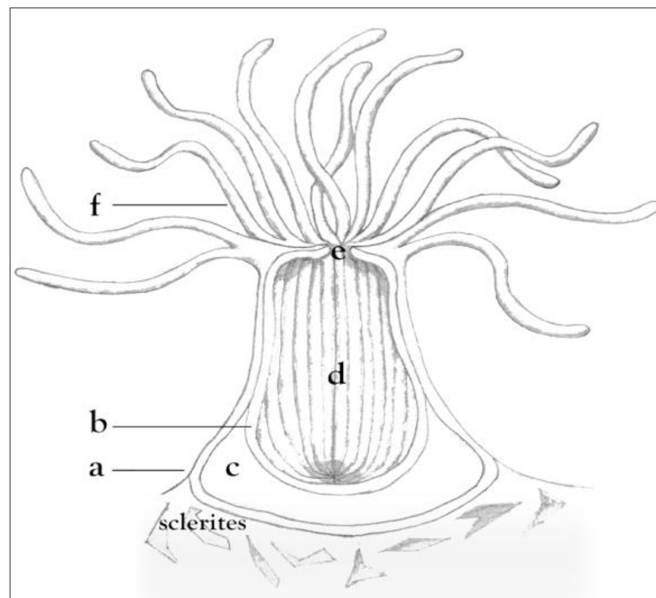

Figure 1 – Soft coral polyp: a) epidermis or ectoderm, b) gastrodermis or endoderm, c) mesoglea, d) gastro-vascular cavity, e) mouth, f) tentacles. Adapted from Rocha (2013).

Many coral species live in association with unicellular photosynthetic organisms (genus *Symbiodinium*), usually termed as zooxanthellae. These dinoflagellates live inside the coral tissue, in perialgal vacuoles (or symbiosomes) in gastrodermis, and are either transmitted to the coral by their parental colony (vertical transmission, less genetic variability) or by uptake from the environment (Oppen, 2004). In this association, the coral provides shelter, carbon dioxide and nutrients, which allow zooxanthellae to perform the photosynthesis; in return, the coral host benefits from organic carbon, amino acids and fatty acids, that are used to respiration, synthesis of mucus, skeletal organic matrix and biomass development (Hoegh-Guldberg *et al.*, 2007; Muscatine *et al.*, 1989; Osinga *et al.*, 2011; Papina *et al.*, 2003). This association between corals and zooxanthellae is fundamental in oligotrophic waters (Al-Moghrabi *et al.*, 1995).

The holobiont (coral and associated microorganisms) has the ability to control the photosynthetic potential in response to the surrounding conditions (Osinga *et al.*, 2011). Under stress conditions, such as disease (Rosenberg and Loya, 1999), high or low levels of light (Banaszak and Trench, 1995), inadequate salinity (Goreau, 1964; Nakano *et al.*, 1997), or temperature values (Gates *et al.*, 1992; Steen and Muscatine., 1987), the coral can reduced the populations of symbionts (Kinzie *et al.*, 2001) by restricting essential nutrients for the zooxanthellae growth (Muscatine and Pool,

1979), as expelling ammonia (Muscatine *et al.*, 1989), or by expelling/digesting the symbiont (Titlyanov *et al.*, 1998); whereas the zooxanthellae can regulate pigment density and composition in response to light spectrum and intensity (Osinga *et al.*, 2011; Rocha *et al.*, 2013a).

The increasing demand of corals for prospection and extraction of bioactive compounds with pharmacological and biomedical potential (Blunt *et al.*, 2008; Khalesi *et al.*, 2008; Leal *et al.*, 2012a, 2012b; Rocha *et al.*, 2011), as well as to the marine aquarium trade (Wabnitz *et al.*, 2003), has led to the increase of coral harvesting in the wild, with consequent pressure over natural populations.

Natural bioactive compounds may be produced by the coral and/or by the microorganisms living in association with the coral (Kobayashi and Ishibashi, 1993; Molinski *et al.*, 2009; Piel, 2009). These production mechanisms are not fully understood, which makes aquaculture *in toto* (coral and microorganisms living associated with it) an important process to deepen the existing knowledge in this area. Marine bioprospection in the past decade has been mainly focused on tropical coral reefs (Leal *et al.*, 2012b). However, this practice entails two potential bottlenecks: sustainability and replicability. The supply of an adequate quantity of pure bioactive compound (Glaser and Mayer, 2009; Mayer *et al.*, 2010), the financial load and complexity of processes for replication of natural molecules in laboratory (Suyama *et al.*, 2011), and development of new chemical synthesis assays for new species (Leal *et al.*, 2014c), are some of the existing pharmaceutical industry constrains.

Despite the highest diversity of secondary metabolites isolated from corals, there are evidences that most of these bioactive compounds are produced by the bacteria living in association with the coral (Leal *et al.*, 2013; Newman and Cragg, 2012; Rocha *et al.*, 2011). However, the isolated culture of these bacterias (ex hospite) is demanding, and only a few successful cases have been achieved (Joint *et al.*, 2010; Leal *et al.*, 2013). Therefore, *in toto* aquaculture (culture of holobiont – coral host and the associated microorganisms) is a viable approach to find solutions and to supply the required biomass to perform the first steps of the drug discovery pipeline (Leal *et al.*, 2014c).

In the last years, the coral production has received more attention by the scientific community, and coral aquaculture is pointed as a sustainable alternative to

the harvest of wild specimens (Ellis and Sharron, 1999; Parks *et al.*, 2003; Sella and Benayahu, 2010). Consequently, the pressure to optimizing culture techniques has grown, aiming to maximize survival and growth rates, and also to reduce the associated production costs (Sella and Benayahu, 2010), in order to assure the economic feasibility of this activity.

Coral aquaculture can be performed *in situ* or *ex situ. In situ* aquaculture have less associated expenses, since produced corals can benefit from natural conditions; however, produced corals are expose to deleterious factors such as sedimentation, pathogens, predators and competitors (Rinkevich, 2005; Rocha *et al.*, 2013c). The production *ex situ* involve higher production costs, but allows a better production control of the biotic and abiotic parameters affecting coral growth, such as: 1) water chemistry (Sella and Benayahu, 2010) 2) illumination (Rocha *et al.*, 2013a, 2013b, 2013c), 3) heterotrophic feeding (Ferrier-Pagès *et al.*, 2003; Houlbrèque and Ferrier-Pagès, 2009), 4) temperature (Gates *et al.*, 1992), 5) nutrients (Muscatine *et al.*, 1989), or 6) water movement (Osinga *et al.*, 2011), among others.

The research on target coral species biology is essential to adjust the biotic and abiotic factors to their needs, in order to: 1) maximize coral growth and survival (Forsman *et al.*, 2006; Leal *et al.*, 2015), 2) guarantee the presence of the symbiont who produces the target compound (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) select different genotypes of species based in the different microbial communities associated and compound production (Leal *et al.*, 2014c). Additionally, *ex situ* aquaculture facilities can be implemented near to the pharmaceutical laboratories, avoiding extra costs associated to packing and shipping (Leal *et al.*, 2014c).

The reproduction of corals can occur by sexual or asexual processes. The sexual reproduction occurs when there is a fusion between the eggs and sperm previously released into the water column, followed by a formation of a planulae (planktonic larval stage) and settlement (Veron, 2000). For some corals fertilization can be internal as well (Veron, 2000).

The asexual reproduction, which occurs naturally, can be a tremendous advantage in the production of these organisms, since it avoid, in opposite to other

cultured aquatic animal species, the production costs with the broodstock and the culture of larval stages. Coral asexual reproduction can be performed by fragmentation, which is a widely used and simple process, with low associated costs, where it is possible to fragment a mother colony into several clones, with high survival rates (Forsman *et al.*, 2006; Rocha *et al.*, 2013c; Sella and Benayahu, 2010)

Light is one of the most important factors for aquaculture *ex situ* of symbiotic corals, due to implementation costs and electrical power consumption (Osinga *et al.*, 2011; Rocha *et al.*, 2013a). Intensity and spectral composition of light are key parameters to the photosynthetic performance of zooxanthellae (Kühl *et al.*, 1995), and this performance can affect directly or indirectly the physiology and growth of the coral host (Osinga *et al.*, 2011; Rocha *et al.*, 2013b, 2013c). As reviewed by Osinga *et al.* (2011), the light limited growth is probably caused by three factors, such as insufficient photosynthesis production, deficit on photosynthetates translocation, and alterations of internal pH due to alterations on photosynthesis.

However, has been shown that photosynthesis exhibit low nitrogen, phosphorus and amino acids concentrations, and is known that a high percentage of the carbon transferred from the zooxanthellae to the coral is lost in respiration or expelled as mucus (Anthony, 1999; Davies, 1984; Falkowski *et al.*, 1984).

Consequently, heterotrophic feeding is necessary to supply the coral with an appropriate biological ratio of both nitrogen, carbon, phosphorus and other several essential components, to increase tissue synthesis rates (Houlbrèque and Ferrier-Pagès, 2009; Osinga *et al.*, 2011; Sella and Benayahu, 2010). Besides, heterotrophy is also crucial to provide energy to coral when bleaching events are occurring, or in aphotic and deep waters (Falkowski *et al.*, 1984).

The processes of autotrophy and heterotrophy are closely linked, reason why corals can be considered mixotrophic organisms. There is species-specific heterotrophic plasticity, where prey catch rates can be dependent on the availability of photosynthetate (Palardy *et al.*, 2005). Nonetheless, Piniak (2002) showed that these capture rates are completely independent from their symbiotic condition. Moreover, it appears that heterotrophic feeding directly depend of several aspects as water flow (Fabricius *et al.*, 1995), zooplankton size, composition (Palardy *et al.*, 2006) and ability to escape, and coral mechanisms of capture (Sebens *et al.*, 1996).

As reviewed by Houlbrèque and Ferrier-Pagès (2009) beside feeding on picoplankton, nanoplankton, or mesomacro-zooplankton, corals also ingest particles of dissolved and particulate organic carbon, phytoplankton and bacteria, by capturing them with their tentacles, mucus adhesion or nematocyst discharges. Apparently food capture and ingestion preferably occurs during the night, but it varies between species and habitats (Anthony and Fabricius, 2000; Johannes *et al.*, 1970; Sebens *et al.*, 1996; Wellington, 1982).

Corals uptake and recycle both inorganic and organic nutrients (Muscatine and Porter, 1977). Besides obtaining essential nutrients from the zooplankton that are not provided by the zooxanthellae (Muscatine and Porter, 1977), the consumption of organic matter as a source of nitrogen is very important for the symbiotic association (Rees and Allard, 1989) as the zooxanthellae seem to be nitrogen limited (Hoegh-Guldberg and Smith, 1989; Muscatine *et al.*, 1989). Nitrogen can also be supplied to corals in the form of particulate matter (Anthony, 1999; Mills *et al.*, 2004), or in dissolved form: ammonium, nitrate and nitrite (D'Elia and Webb, 1977; Snidvongs and Kinzie, 1994).

Muscatine *et al.* (1989) suggested that dissolved nitrogen promotes zooxanthellae growth and particulate nitrogen promotes the coral growth. As nitrogen is available, the C:N ratio of zooxanthellae decreases, leading to an inverse relationship between the consumption of nitrogen and transference of fixed carbon to the coral host (Cook *et al.*, 1988; Muscatine *et al.*, 1989). The nutrient status of the host is dependent on the nutritional condition of the holobiont (D'Elia and Cook, 1988), on the concentration of nutrients present in the water (Grover *et al.*, 2003, 2002; Muscatine and D'Elia, 1978), and on the light availability (Muscatine and D'Elia, 1978).

The assimilation of ammonium (NH_4^+) by the symbiont remains in debate. Some authors defend that NH_4^+ is first assimilated by the zooxanthellae from the host (diffusion-depletion model), creating a diffusion gradient of NH_3 from the seawater to host tissue (Burris, 1983; D'Elia and Cook, 1988). The coral host generate metabolic NH_4^+ which is exploit by the zooxanthellae, by synthesizing amino acids, glutamate and glutamine, and proteins, subsequently transferred to the coral host (Burris, 1983; Swanson and Hoegh-Guldberg, 1998). On the other hand, Miller and Yellowlees (1989) suggested that this depletion could be a result from bacterial assimilation. Some

studies refer the host as the primarily involved in NH_4^+ assimilation, still being the zooxanthellae needed to the process, because provides the coral host with photosynthetically fixed carbon, then acting as ammonium acceptors (Lipschultz and Cook, 2002; Miller and Yellowlees, 1989; Rees, 1987).

Even though ammonium uptake is usually preferred to nitrate uptake (D'Elia and Webb, 1977; Taguchi and Kinzie, 2001), Grover *et al.* (2003) suggested the zooxanthellae drive the nitrate uptake, and moreover the uptake is also ammonium-dependent, being higher when concentrations of NH_4^+ are lower. This may be due to the repression of the nitrate reductase by the NH_4^+ (Taguchi and Kinzie, 2001).

Phosphorus is included in the composition of phospholipids, RNA, DNA and ATP, and is involved in important biological mechanisms such as the control of algae photosynthesis and coral growth (D'Elia, 1977; Ferrier-Pagès *et al.*, 2000; Godinot *et al.*, 2011). D'Elia (1977) suggested that corals uptake phosphorus (P) by active transport. Zooxanthellae deplete the coral tissue of P, creating a gradient between sea water and the coral, leading to a diffusion of active P through coral tissue to zooxanthellae.

Even thought holobiont benefits with 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 calcification (Stambler *et al.*, 1991) and growth (Ferrier-Pagès *et al.*, 2001, 2000; Kinsey and Davies, 1979), negatively affect fertilization and embryo development (Harrison and Ward, 2001), as well recruitment and reestablishment capacities in coral reefs (Bell, 1992).

Several studies have been performed on the light spectrum and intensity effect on corals (Rocha *et al.*, 2013a, 2013b, 2013c), as well as the heterotrophic feeding (Ferrier-Pagès *et al.*, 2003; Houlbrèque and Ferrier-Pagès, 2009; Lewis, 1982; Muscatine *et al.*, 1989). However, the effect of the interaction of these factors on the physiology, photobiology and growth of cultivated soft corals has never been studied.

The Genus *Sarcophyton* (Cnidaria: Anthozoa: Octocorallia: Alcyonacea) is one of the most specious within family Alcyonacea, with 67 validated species ("WoRMS - World Register of Marine Species," 2015). These species have been highly surveyed 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), which make the *Sarcophyton* species good candidates for aquaculture.

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 the soft coral *Sarcophyton* cf. *glaucum* (Quoy & Gaimard, 1833).

2. Materials and Methods

Sarcophyton glaucum (Quoy & Gaimard, 1833) taxonomy is not consensual (Aratake *et al.*, 2012). McFadden *et al.* (2006) suggested that *Sarcophyton glaucum* could 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 confirmation, when the taxonomy of genus *Sarcophyton* reach consensual opinions.

2.1 Coral husbandry and fragmentation

The coral fragmentation was proceeded as describe in (Rocha *et al.*, 2013c). Three colonies of *S.* cf. *glaucum*, collected in Sumbawa, Indonesia approximately between 5 and 15 m depth, were purchased from an ornamental fish wholesaler. *S*. cf. *glaucum* colonies were stocked in the lab for 7 days for acclimatization to water parameters, and observation of any evidence of infection or disease.

The acclimatization modular system was composed of three experimental glass tanks (0.6 m × 0.6 m × 0.25 m; 90 L), connected to a filter tank with a volume capacity of 150 L (fig. 2, C), equipped with a protein skimmer (fig. 2, F; ESC150 ReefSet, São Mamede Negrelos, Portugal), biological filters (fig. 2, G; aprox. 10 L of submerged bioballs and a fluidized sand-bed biological filter with approximately 1 kg of aragonite), a submergible heater (fig. 2, L; Eheim Jäger 150W, Deizisau, Germany).

Water recirculation in the experimental tanks (approximately 1000 $L.h^{-1}$ in each experimental tank) was promoted by a submerged pump (Eheim 1262, Deizisau, Germany) assembled in the filter tank. Additionally, each experimental tank was equipped with a circulation pump (fig. 2, H; Turbelle nanostream-6025 Tunze, Penzberg, Alemanha), which promote an approximated water flow of 2500 $L.h^{-1}$.

Each tank was illuminated from above with a 150 W – 10000 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 salt water, prepared by mixing synthetic salt (Tropic Marin Pro Reef salt – Tropic Marine, Germany) with purified water by a reverse osmosis system (Aqua-win RO-6080, Kaohsiung, Thailand). The salinity was

maintained at 35 using an osmoregulator (fig. 2, K; Deltec Aquastat 1000, Delmenhorst, Germany) which automatically compensates the water loss by evaporation, adding fresh water purified by reverse osmosis.

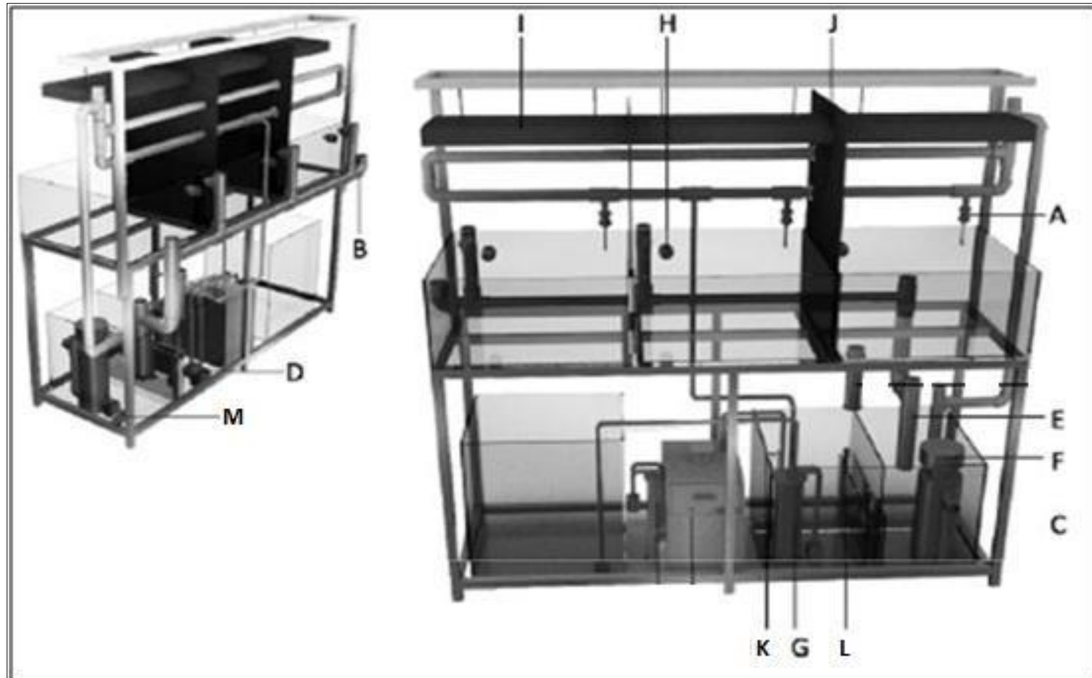

Figure 2 – Illustration of one modular structure of the experimental culture system: A) PVC valve inlet pipe system, B) Outlet pipe system, C) 150 L Filter tank, D) Inlet pipe system submerged pump, E) Mechanical filtration bag (250 µm), F) Protein skimmer, G) Fluidized sand-bed filter, H) Circulation pump, I) Individual lighting system, J) PVC screen, K) Osmoregulator, L) Submergible heater, M) Filter tank connection valves. Adapted from Rocha (2013).

Coral colonies were individually stocked in the glass tanks (1 colony per tank). After the acclimatization period, the capitulum periphery of each mother colony was fragmented with a sterilized scalpel, originating 30 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 plastic stand (Coral Cradle®) and labeled. Coral fragments recovery (fig. 3) occur in the acclimation tank used for the respective *S.* cf. *glaucum* colony from they were fragmented, under the same conditions.

Figure 3 – Coral fragments after recovery from fragmentation.

2.2 Heterotrophic feeding - *Brachionus plicatilis* ingestion

The rotifer *Brachionus plicatilis* (Müller, 1786) was chosen as live prey because is a small species with low mobility, which can be cultured in high densitys, and nutritionally controlled through food administration (Lubzens, 1987; Watanabe *et al.*, 1983).

The suitability of rotifers as live preys for *S*. cf. *glaucum* was assessed in two preliminary trials, performed in a climatized room (25°C) using containers (stocked with one coral fragment) with 1 L of filtered saltwater with the same parameters registered in the coral tank water (please see the values presented in experimental design section below), under a photoperiod of 3 hours dark and 3 hours light.

The first trial was performed during 6 hours in 4 containers with approximately 115 rotifers.mL^{-1}, a density above the values described in the literature for zooplankton supply in corals (Connolly *et al.*, 2012; Hoogenboom *et al.*, 2006; Levas *et al.*, 2013). The excess of preys allows us to assess more accurately whether they are being ingested, since it increases polyp capture probability. A gentle aeration was applied in experimental containers, to promote rotifers distribution through the water column. After 3 hours of experiment each coral fragment was carefully transferred to another recipient (containing water with the same physical and chemical parameters), the water in the experimental containers was thoroughly mixed and 5 samples of 1 mL were taken for rotifer counting in a stereomicroscope (Stemi DV4, Zeiss, Jena, Germany). After this procedure the coral fragment was carefully (to avoid polyp

retraction) transferred back to the experimental recipient and the lights were turned on. After 3 hours, rotifer density was assessed again as described above.

The second trial was performed using the methodologies previously described, but with a density of 4 rotifers.mL^{-1}, the same density to be used in the culture experiment. The density chosen for the main experiment was close to the higher density used in the study by Connolly *et al.* (2012) for scleractinian corals.

2.3 *Brachionus plicatilis* production

Pure cultures of rotifers were maintained in 250 mL conical glass flasks with microalgae (*Nannochloropsis* sp. and *Isochrysis galbana* (Parke, 1949)). Higher culture volumes were performed in 25 L acrylic cylindrical tanks, maintained with constant aeration and fed with PhytoBloom Green Formula (live *Nannochloropsis* sp. concentrate, Necton, Portugal). Before being supplied to corals, rotifers were enriched for 12 hours with *Isochrysis galbana* (at a density of approximately 80000 cells.mL^{-1}). Counts of rotifer cultures were performed daily in a stereomicroscope (Stemi DV4, Zeiss, Jena, Germany). The feeding was performed during 3 consecutive hours of dark and 3 consecutive hours of light.

2.4 Experimental Culture System

The experimental system was composed of two experimental modular systems similar to the system described for the acclimation of *S.* cf. *glaucum* mother colonies.

2.5 Experimental design

The experiment was performed during 80 days in two separated experimental modules, with only one module system being provided with live zooplankton.

Each experimental tank was illuminated from above (with a photoperiod of 10:14, hours light: hours dark) with a 150 W hydrargyrum quartz iodide (HQI) lamp. Two light spectrums (fig. 4) with different wavelength emissions were tested: 1) white light delivered by 150 W HQI, 10000 K lamps (Sylvania, Germany), and 2) blue light delivered by 150 W HQI, 20000 K lamps (Hailea lamp, South Korea). In each spectrum two different PAR light intensities were tested: 1) 50 µmol quanta.m^{-2}.s^{-1}, and 2) 120

µmol quanta.$m^{-2}.s^{-1}$. The distance between the lighting systems and the surface of coral fragments was adjusted to allow the different PAR treatments. During the experiment PAR values were measured at coral fragment level, using an Apogee Quantum Meter (MQ-200, USA), in each experimental tank.

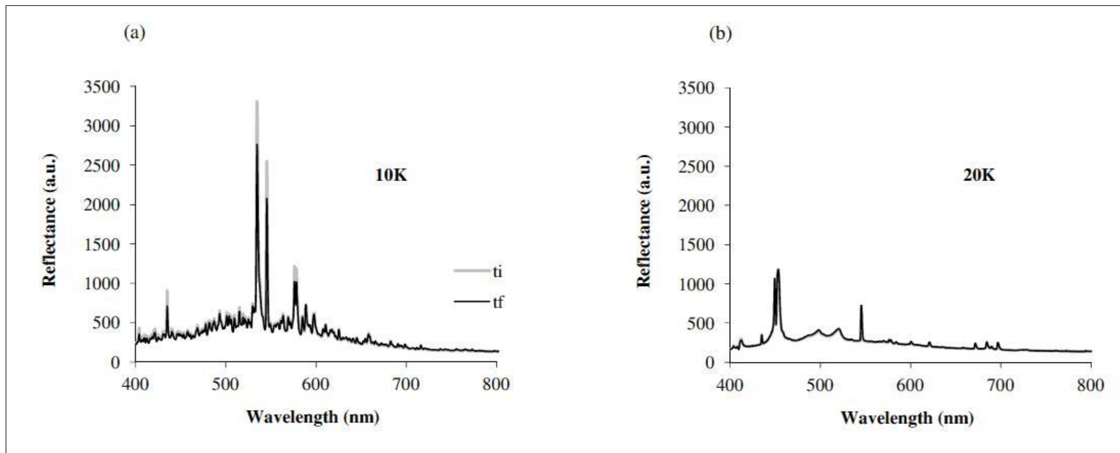

Figure 4 – Two light spectrums with different wavelength emissions: a) white light promoted by 150 W HQI, 10000 K (10K) lamps (Sylvania, Germany); b) blue light promoted by 150W HQI, 20000 K (20K) lamps (Hailea lamp, South Korea).

Each spectrum × PAR intensity combination was performed with and without the supply of heterotrophic feeding. The experimental tanks with feeding treatment were daily supplied with rotifers (approximately 4 ind.mL^{-1}). The feeding was performed every day during 3 consecutive hours of dark and 3 consecutive hours of light. During feeding period the protein skimmers in experimental culture systems were turned-off.

A total of 8 treatments were performed: 1) White light, 120 µmol quanta.$m^{-2}.s^{-1}$, non-feeding (WL 120 NF), 2) White light, 50 µmol quanta.$m^{-2}.s^{-1}$, non-feeding (WL 50 NF), 3) Blue light, 120 µmol quanta.$m^{-2}.s^{-1}$, non-feeding (BL 120 NF), 4) Blue light, 50 µmol quanta.$m^{-2}.s^{-1}$, non-feeding (BL 50 NF), 5) White light, 120 µmol quanta.$m^{-2}.s^{-1}$, feeding (WL 120 F), 6) White light, 50 µmol quanta.$m^{-2}.s^{-1}$, feeding (WL 50 F), 7) Blue light, 120 µmol quanta.$m^{-2}.s^{-1}$, feeding (BL 120 F), 8) Blue light, 50 µmol quanta.$m^{-2}.s^{-1}$, feeding (BL 50 F). Each treatment was composed by 9 coral fragments (n = 9), 3 fragments from each mother colony (fig. 5).

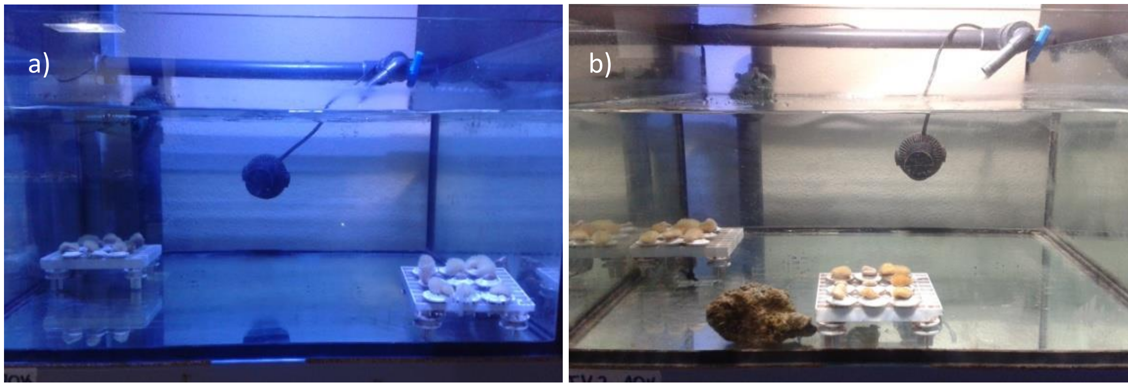

Figure 5 – Two of the four treatment tanks: a) blue light, 50/120 µmol quanta.m^{-2}.s^{-1}, non-feeding tank, and b) white light, 50/120 µmol quanta.m^{-2}.s^{-1}, feeding tank. Each treatment composed by 9 coral fragments.

The water parameters were maintained constant as follows: temperature 26 ± 0.5 °C, pH 8-8.4, salinity 35, NH_3 ≤ 0.1 mg.L^{-1}, NO_2 ≤ 0.1 mg.L^{-1}, NO_3 ≤ 10 mg.L^{-1}, Ca 400-420 mg.L^{-1} and KH 2.50-3.57 meq.L^{-1}. Partial water changes of approximately 10 % of the total system volume were performed on weekly basis.

2.6 Coral fragments specific growth rate

Specific growth rate was estimated based on measurements of coral fragments buoyant weight at the begging (w_i) and at the end (w_f) of the experiment, as described in Davies (1989), using an adapted precision balance (Kern Emb 200-3, Kern & Sohn GmbH, Balingen, Germany) as described by Rocha *et al.* (2013a). Before weighting the coral fragments, coral cradles were rinsed to prevent any artificial increment in total weight promoted by the development of biofouling. To calculate the specific growth rate (SGR) the following formula were used:

$$\mathrm{SGR}\ (\%.\mathrm{day}^{-1}) = \left(\frac{\ln(\mathrm{w_f}) - \ln(\mathrm{w_i})}{\Delta \mathrm{t}}\right) \mathrm{x}\ 100 \tag{1}$$

Where SGR represent the specific growth rate (percentage of growth increase per day), $\ln(w_f)$ and $\ln(w_i)$ represent the natural logarithm of coral fragments buoyant weights (grams) at the beginning and the end of the experiment, and Δt represent time variation (days).

2.7 Spectral reflectance

To analyze spectral reflectance, measurements were performed *in vivo*, at the beginning and at the end of the experiment, under an irradiance of 200 µmol quanta.m^{-2}.s^{-1} emitted by a halogen lamp (Volpi Intralux 5000-1 Volpi, Schlieren, Switzerlan). Each coral fragment was measured in 3 different no overlapping points, using an USB2000 spectrometer (U-VIS-NIR, grating #3, Ocean Optics, Dunedin, Florida, USA) connected to a fiberoptic with 400 µm diameter (QP400-2-VIS/NIR-BX, Ocean Optics, Denedin, Florida, USA) over a bandwidth of 330-1000 nm and a spectral resolution of 0.33 nm. The fiberoptic was maintained always at the same distance from the coral fragment (2 mm).

The light spectrum reflected from the coral fragments was normalized to the spectrum reflected from a reference white panel (WS-1-SL White Reflectance Standart with Spectralon, Ocean Optics, Dunedin, Florida, USA). The reflectance spectrum measured in the dark was subtracted to both spectra to account for the dark current noise of the spectrometer. The Normalized Difference Vegetation Index (NDVI) (Rouse *et al.*, 1973) was then calculated following the next equation:

$$NDVI = \left(\frac{R750 - R675}{R750 + R675}\right) \tag{2}$$

Where R_{750} (reflectance in the infrared spectrum) and R_{675} (reflectance in the red spectrum) represent the average of diffuse reflectance in interval of 749.73-750.39 nm and 674.87-675.55 nm, respectively.

2.8 *In vivo* chlorophyll fluorescence

Chlorophyll fluorescence was determined *in vivo situ* using pulse amplitude modulation (PAM) fluorometry, at the begging and at the end of the experiment, by emitting two different light signals (modulated pulse followed by a saturation pulse) and reading the fluorescence (minimum - F_o, and maximal - $F_{m,}$ dark adapted fluorescence, respectively) emitted back by the photosynthetic organism (Schreiber, 2004). The PAM fluormeter was composed by a computer control unit (Walz) and an emitter-detector unit (Gademann Instruments, GmbH, Würzburg, Germany).

The measurements were performed 2 hours after lights turned on, so that the photosynthetic apparatus could be fully activated. Prior to each measurement, coral fragments were dark adapted for 15 minutes. To determine the photosynthetic efficiency, the maximum quantum yield (F_v/F_m) of photosystem II (PSII) was calculated following the next equation:

$$Fv/Fm = \frac{(Fm - Fo)}{Fm} \tag{3}$$

2.9 Zooxanthellae quantification

Zooxanthellae were quantified at the end of the experiment. One sample of each coral fragment was removed with a scalpel and homogenized in a 15 mL polypropylene conical centrifuge tube with filtered saltwater. Samples were fixed and stained with Lugol's iodine solution. Zooxanthellae were counted in an improved Neubauer hemocytometer chamber (5 counts per sample). Subsequently, the samples were centrifuged (15000 g, 15 min, 4 °C) and, resulting pellets being freeze-dried for 24 hours and weighted. Zooxanthellae density was normalized to coral fragments dry weight.

2.10 Organic and inorganic weight of coral fragments

To determine the organic and inorganic weight, one sample of each coral fragment was removed with a scalpel at the end of the experiment. The dry weight was determined by freeze-drying the samples for 48 h and then weighting them (balance Sartorius BP 2215, Gottingen, Germany). Samples were then burnt at 450 °C in a furnace (Nabertherm, Lilienthal, Germany) and weighted again to determine inorganic weight. The organic weight was obtained by subtracting the inorganic weight from the dry weight.

2.11 Suspended particulate matter

To determine suspended particulate matter (SPM), a sample of 1 L of water was filtered from each experimental system at the end of the experiment, with a pre-

combusted and weighted 0.47 µm glass-fibre filters (Whatman GF/F), and stored in plastic bottles at -32 °C until analysis. The previously weighted filters (balance Sartorius BP 2215, Gottingen, Germany) were dried in an oven (Venticell, MMM Medcenter GmbH, Germany) at 105 °C for approximately 5 hours (until dry weight become constant), and weighted again to obtain total SPM.

2.12 Organic and inorganic matter in culture water

To determine suspended particulate matter (SPM), the water in experimental systems was sampled and filtered at the end of the experiment, as described before. The filters previously weighted (balance Sartorius BP 2215, Gottingen, Germany), were dried in a furnace (Nabertherm, Lilienthal, Germany) at 450 °C for 5 hours, and weighted again to obtain the inorganic matter. The organic matter was obtained by the subtracting the inorganic matter to the total weight.

2.13 Inorganic nutrients

2.13.1 Ammonium

The determination of ammonium (NH_3-N) was made following the method of Limnologisk Metodik (1985). Water samples of each experimental system were filtered (0.47 µm glass-fibre filter, Whatman GF/F). The reagent A, composed by phenol, sodium nitroprussid dehydrate and demineralized water, and the reagent B, composed by sodium hydroxide and sodium hypochlorite, were added to the samples and after 1 hour of reaction the color was measured by spectophotometer at 630 nm. To ensure the quality of the reagents and equipment, a calibration curve was developed at the beginning of the analysis.

2.13.2 Phosphate

The determination of phosphate (PO_4-P) was made following the method of Limnologisk Metodik (1985). Water samples of each experimental system were filtered (0.47 µm glass-fibre filter, Whatman GF/F). Reagent was added and after 15 min of reaction the color was measured by spectrophotometer at 882 nm. To ensure the quality of the reagents and equipment, a calibration curve was developed at the beginning of the analysis.

2.13.3 Sum of nitrate and nitrite

The concentration of nitrate (NO_3-N) and nitrite (NO_2-N) was determined using a flow injection system (FIAstar 5000 Analyzer, Höganäs, Sweden), following Strickland and Parsons (1972) method. A buffer solution was added to the pre-filtered samples (0.47 µm glass-fibre filter, Whatman GF/F) in order to reduce the nitrate to nitrite in a cadmium reducer. By adding an acidic sulphanilamide solution, nitrite formed from reduction will form a diazo compound, measured at 540 nm. To ensure the quality of the reagents and equipment, a calibration curve was developed at the beginning of the analysis and in parallel with the analysis of each sample, using a standard solution.

To determine the quantity of nitrate, the analysis of nitrite was made using a flow injection system (FIAstar 5000 Analyzer, Höganäs, Sweden), as described before and subtracted to the value of the sum of nitrate and nitrite.

2.14 Statistical analysis

Statistical analyses were performed using the Software Statistica version 8.0 (StatSoft Inc.). Factorial ANOVAs were used to assess the existence of significant differences in the growth rate, organic and inorganic weight, Normalized Difference Vegetation Index (NDVI), maximum quantum yield of PSII (F_v/F_m), zooxanthellae density for coral fragments of *S*. cf *glaucum* reared under the different treatments. Light spectrum, PAR intensity, heterotrophic feeding and mother colonies were used as the categorical factors. Assumptions of normality and homogeneity of variance were checked prior to the analysis through the Shapiro–Wilk W and Levene's tests, respectively. Tukey post-hoc comparisons were used to determine the existence of significant differences. The differences in the concentration of suspended particulate matter, organic and inorganic matter and Inorganic nutrients, between experimental systems with and without live food supply, were measured with a t-test.

3. Results

At the end of the experiment (80 days), the registered survival rate was 100 % in all treatments.

3.1 *Bachionus plicatilis* ingestion

The results of rotifers density trials are presented in table 1 and 2, respectively. It is visible, in both tests that the density of rotifers (rot) decreased over time. At the end of the trials, the density was nearly 0 rot.mL^{-1} in almost every recipient, suggesting that *S*. cf. *glaucum* ingested rotifers *B*. *plicatilis*.

Table 1 – *Brachionus plicatilis* (rotifers) density (rot.mL^{-1}, average ± standard deviation) 3 and 6 hours after the beginning of a preliminary trial to access *B. plicatilis* ingestion by *S*. cf. *glaucum* fragments.

	Initial density (rot.mL^{-1})	Density after 3 h (rot.mL^{-1})	Density after 6 h (rot.mL^{-1})
Fragment 1	129 ± 18.69	4 ± 2.16	0.5 ± 0.57
Fragment 2	98 ± 1.36	5 ± 0.81	0.25 ± 0.5
Fragment 3	115 ± 15.25	4.42 ± 0.52	0.25 ± 0.25
Fragment 4	117 ± 13.4	4.25 ± 1.25	0

Table 2 – ***Brachionus plicatilis* (rotifers) density (rot.mL^{-1}, average ± standard deviation) 3 and 6 hours after the beginning of a preliminary trial to access *B. plicatilis* ingestion by *S*. cf. *glaucum* fragments.**

	Initial density (rot.mL^{-1})	Density after 6h (rot.mL^{-1})	Density after 6h (rot.mL^{-1})
Fragment 1	4 ± 0	0	0
Fragment 2	3.66 ± 1.63	0	0
Fragment 3	4.33 ± 1.25	1.5 ± 0.7	0
Fragment 4	4 ± 1.91	0	0

3.2 Coral fragments specific growth rate

The results of coral fragments specific growth rate (SGR %) are presented in figure 6. It is possible to observe that coral fragments from the different colonies displayed contrasting responses within the same treatments, in treatments with supply of food, PAR-120 (white and blue light).

Overall, significant differences were observed in coral SGR between food treatments. In most cases, corals without the supply of live prey had a superior specific growth than those supplied with food (fig. 7).

For the treatments with supply of food, it is possible to observe that coral fragments exposed to a PAR of 120 µmol quanta.$m^{-2}.s^{-1}$ (PAR-120) had a higher percentage of SGR than corals exposed to a PAR of 50 µmol quanta.$m^{-2}.s^{-1}$ (PAR-50). Coral fragments from mother colony (MC) 3, PAR-120, displayed a higher SGR in white light than those from the same mother colony reared under a PAR-50 treatment. Coral fragments from MC1 and MC2, PAR-120, displayed a higher SGR in blue light spectra when respectively compared with the fragments from the same mother colonies reared under the PAR-50. When food was not supplied, there were no differences registered between distinct PAR treatments.

For the differences between the light spectra treatments (white light and blue light), is possible to observe that in treatments without supply of food, the coral fragments from MC1 reared under blue light (PAR-120) displayed a higher percentage of SGR when compared to the corals from the same mother colony reared under white light (PAR-120). In the treatments with food supply, the coral fragments from MC2, PAR-120 blue light, presented higher percentage of SGR, when compared with the corals from the same mother colony grown under the same PAR but in white light. Observing the results for coral fragments reared with food and PAR-50, the percentage of SGR was significantly higher in MC1 and MC2 coral fragments reared with white light when respectively compared to the coral fragments reared under blue light.

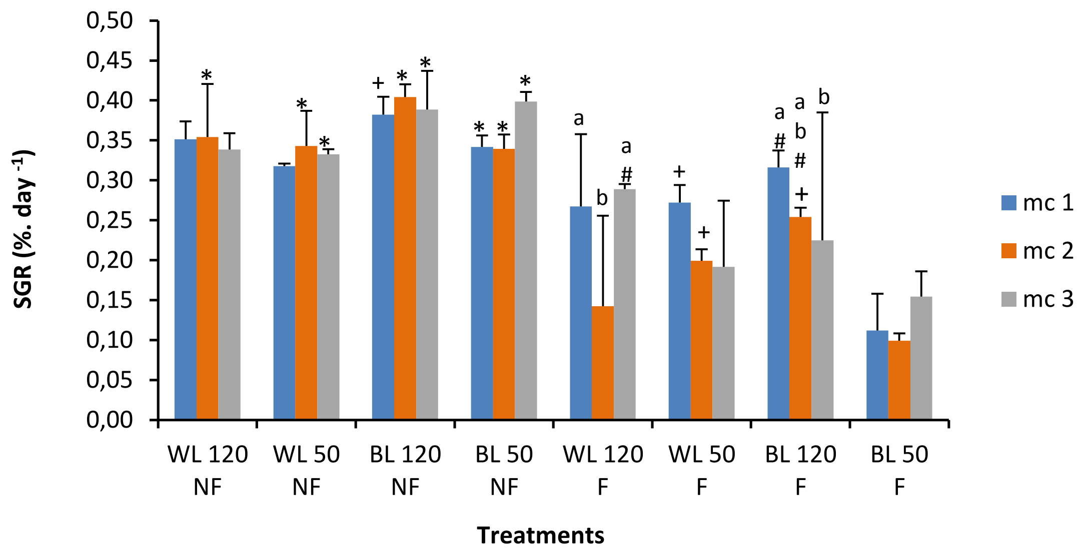

Figure 6 – Average values of specific growth rate (%.day $^{-1}$) for coral fragments from the three mother colonies (MC1, MC2, MC3) reared in the 8 experimental treatments. Statistically significant differences between treatments are distinguishable by symbol ($p < 0.05$ for all Tukey post-hoc comparisons). Significant different values of SGR (%.day $^{-1}$) between mother colonies, for the same treatment, are marked with letters (a,b or c). Significant higher values of SGR (%.day $^{-1}$) between coral fragments, from the same mother colony, with and without food supply with the same Spectra and PAR treatments are marked with (*), between coral fragments reared in different PAR intensities with the same food and spectra treatment (#) and between coral fragments reared in different light spectra with the same food and PAR treatments (+). Vertical lines represent one standard deviation.

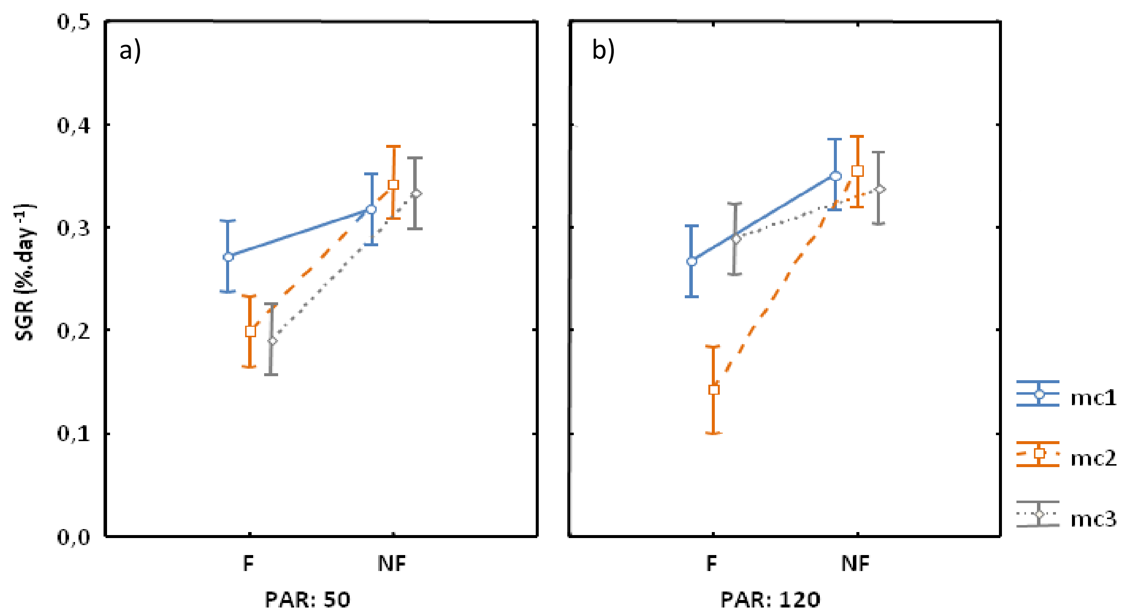

Figure 7 – Average values of specific growth rate (%.day -1) for coral fragments from the three mother colonies (MC1, MC2, MC3) reared with (F) or without food supply (NF), under a) PAR of 50 µmol quanta.m $^{-2}$.s $^{-1}$ and b) PAR of 120 µmol quanta.m $^{-2}$.s $^{-1}$. Current effect: $F (2,189) = 5.2215$, $p=0.00621$. Vertical bars denote 0.95 confidence intervals.

3.3 Spectral reflectance

At the end of the experiment, spectral reflectance was measured in a wavelength range of 400 - 700 nm, the same range of the absorption of light PAR by photosynthetic pigments (fig. 8).

It is possible to observe a triple-peaked pattern approximately at 575, 600, and 650 nm in all coral treatments. An inverted peak is also observed around the 675 nm, and a sharp increase in reflectance values in the 700 nm.

In treatments without food supply, the coral fragments had higher values of spectral reflectance than those with food supply. Also in treatments without food supply, fragments exposed to PAR-120 blue light, presented a higher reflectance compared with fragments from other treatments.

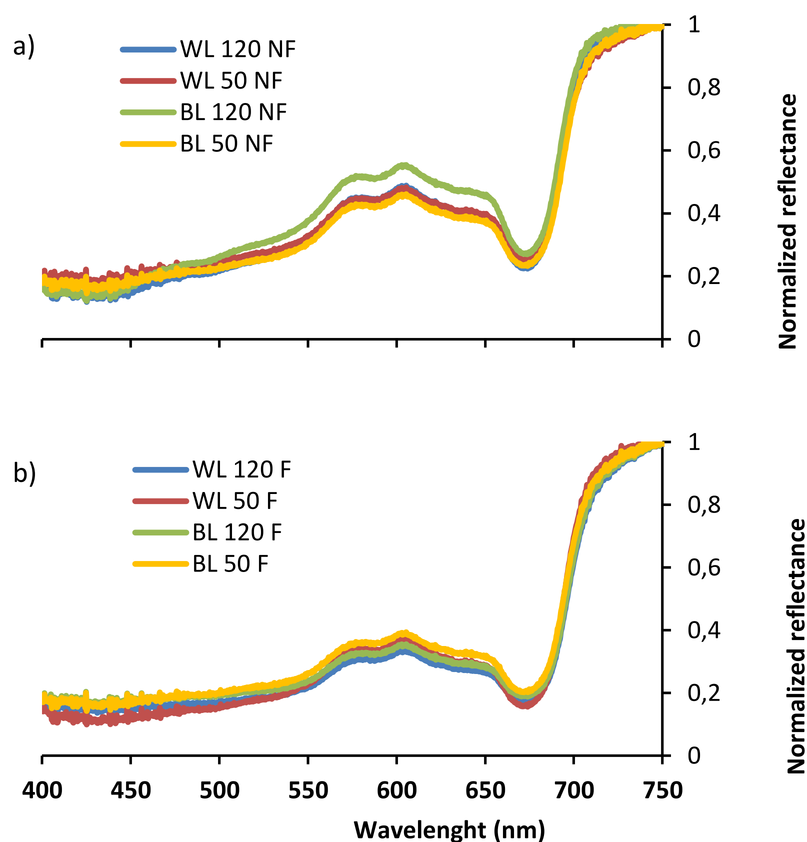

Figure 8 – Average values of reflectance spectra measured in coral fragments at the end of the experiment: a) coral fragments with no supply of food, and b) coral fragments with supply of food.

Differences between the Normalized Difference Vegetation Index (NDVI) obtained from the spectral reflectance measured at the end and at the beginning of the experiment are presented in figure 9.

The difference of NDVI was usually higher for coral fragments when food was provided, excepting coral fragments from MC2 reared under PAR-120 blue light, which had significantly higher difference of NDVI without food supply. In treatments where food is administrated, coral fragments from MC2 reared under PAR-120 white light, coral fragments from MC2 and MC3 reared under PAR-50 white light, and coral fragments from MC3 reared under PAR-120 blue light, had all significantly higher difference of NDVI when respectively compared to coral fragments for the same mother colonies exposed to treatments with food supply.

PAR intensity only affected coral fragments exposed to treatments without food supply. Coral fragments from MC3 exposed to PAR-120 (white light) presented a higher difference of NDVI compared to coral fragments from MC1 exposed to PAR-50 (white light). Coral fragments from MC1 exposed to PAR-50 (blue light) showed higher difference of NDVI than coral fragments from MC1 exposed to PAR-120 (blue light).

Concerning the effect of light spectra, only coral fragments from MC3, reared with food supply under white light (PAR-50) presented higher differences of NDVI, when respectively compared to coral fragments from the same mother colony, exposed to blue light (PAR-50).

Comparing coral fragments from different mother colonies, within the same treatments, we can observe that were only significant differences between colonies when food was not supplied. With exception of treatment white light PAR-120, different mother colonies had distinctively responses to the factors.

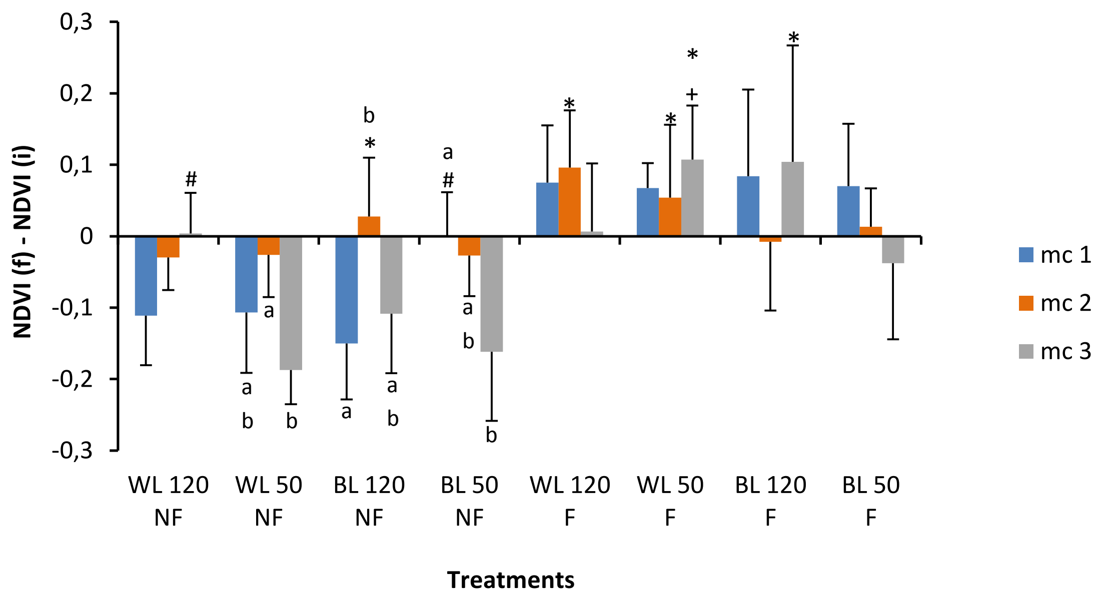

Figure 9 – Average values of the difference of NDVI index measured at the end and NDVI index measured at the beginning of the experiment ($NDVI_{final}$ - $NDVI_{initial}$), for coral fragments from the three mother colonies (MC1, MC2, MC3) reared in the 8 experimental treatments. Statistically significant differences between treatments are distinguishable by symbols ($p < 0.05$ for all Tukey post-hoc comparisons). Significant different values of $NDVI_{final}$ - $NDVI_{initial}$ between mother colonies, for the same treatment, are marked with letters (a,b or c). Significant higher values of the difference of $NDVI_{final}$ - $NDVI_{initial}$ between coral fragments, from the same mother colony, with and without food supply with the same Spectra and PAR treatments are marked with (*),between coral fragments reared in different PAR intensities with the same food and spectra treatment (#) and between coral fragments reared in different light spectra with the same food and PAR treatments (+). Vertical lines represent one standard deviation.

3.4 *In vivo* chlorophyll fluorescence

The differences between maximum quantum yield of PSII (F_v/F_m values) measured at the end and at the beginning of the experiment are presented in figure 10.

In treatments with supply of food, the difference between values of F_v/F_m measured at the beginning and at the end of the experiment was superior for coral fragments of 2 treatments, when compared to fragments without supply of food. For fed coral fragments from MC1 and MC2, reared under PAR-120 white light and blue light had a higher value of F_v/F_m difference when respectively compared to coral fragments from the same mother colonies exposed to treatments without food supply.

When food was not administrated, it is visible that coral fragments from MC3 exposed to PAR-120 white light had higher difference of F_v/F_m when respectively compared to fragments reared under PAR-50. Contrarily, coral fragments from MC2

exposed to PAR-50 white light had higher difference of F_v/F_m when respectively comparing with fragments exposed to PAR-120.

When food was administrated, is possible to observe that coral fragments from MC1 and MC2, exposed to PAR-120 (white light), presented a higher difference of F_v/F_m comparing to coral fragments from the same mother colonies, exposed to PAR-50 (white light). Also, fragments from MC2 exposed to PAR-120 (blue light) showed higher difference of F_v/F_m comparing to coral fragments from MC2, exposed to PAR-50 (blue light).

About light spectrum, only coral fragments from MC2, reared under PAR-120 blue light and without supply of food exhibit significant higher difference of F_v/F_m when compared with coral fragments from MC2 exposed to the same factors, but reared under white light.

Overall, coral fragments originating from the different mother colonies had significant different values of F_v/F_m in response to the factors. Only in treatments with blue light, PAR-120 (when food was not supplied) and PAR-50 (when food was supplied), was no differences recorded between fragments from different mother colonies.

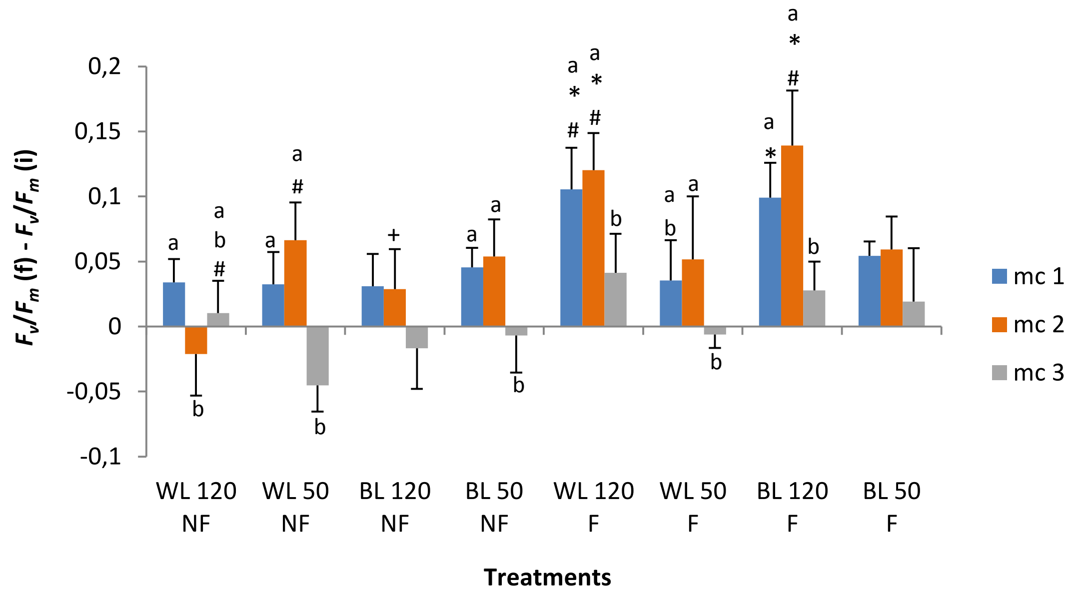

Figure 10 – Average values of the difference between final maximum quantum yield and initial maximum quantum yield (*Fv/Fm* $_{final}$ - *Fv/Fm* $_{initial}$) for coral fragments from the three mother colonies (MC1, MC2, MC3) reared in the 8 experimental treatments. Statistically significant differences between treatments are distinguishable by symbols ($p < 0.05$ for all Tukey post-hoc comparisons). Significant different values of *Fv/Fm* $_{final}$ - *Fv/Fm* $_{initial}$ between mother colonies, for the same treatment, are marked with letters (a,b or c). Significant higher values of $F_v/F_{m\ final}$ - $F_v/F_{m\ initial}$ between coral fragments, from the same mother colony, with and without food supply with the same Spectra and PAR treatments are marked with (*),between coral fragments reared in different PAR intensities with the same food and spectra treatment (#) and between coral fragments reared in different light spectra with the same food and PAR treatments (+). Vertical lines represent one standard deviation.

3.5 Zooxanthellae quantification

Significant differences in the zooxanthellae density (number of cells per gram of coral dry weight - zooxanthellae.g DW^{-1}) were found for the coral fragments from different mother colonies (fig. 11).

Coral fragments of 2 treatments with supply of food had a higher density of zooxanthellae than coral fragments without supply of food. Coral fragments from MC1 and MC2, PAR-120 blue light, presented higher values of zooxanthellae density with food supply when respectively compared with de fragments from the same mother colonies reared without food supply. Coral fragments from MC1, PAR-120 white light, had higher zooxanthellae density in the treatment with food supply when compared with the fragments from the same mother colony reared without food supply.

Concerning the PAR treatments, when food was not administrated, coral fragments from MC1 and MC3 exposed to PAR-50 blue light, had a higher zooxanthellae density when respectively compared to the coral fragments grown under PAR-120. Looking for food supply treatment, coral fragments from MC1, reared

under blue light, PAR-120 had a greater effect on the zooxanthellae density when comparing with coral fragments from the same mother colony exposed to the same factors but with a PAR-50.

Observing the effect of light spectra on zooxanthellae density, it is possible to observe that in treatments without food supply, coral fragments from MC1 and MC3, reared under white light and PAR-120, had higher zooxanthellae density than coral fragments from the same mother colony exposed to the same factors but under blue light. In treatments where food was administrated, coral fragments from MC1, exposed to blue light PAR-120 had a higher zooxanthellae density when respectively compared to the coral fragments reared under white light. However, coral fragments from MC2, exposed to white light PAR-50, presented higher zooxanthellae density when respectively compared to coral fragments reared under blue light.

In all treatments, coral fragments from distinct mother colonies presented significant differences in zooxanthellae density.

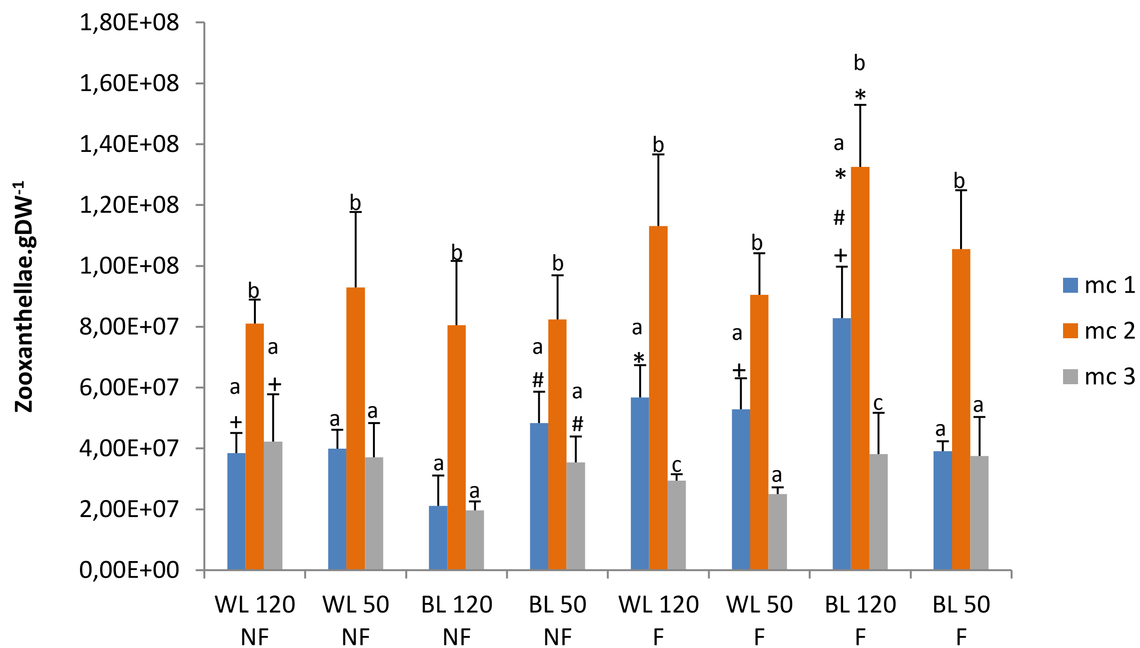

Figure 11 – Average values of number of zooxanthellae per grams of dry weight (zooxanthellae.g DW^{-1}) for coral fragments from the three mother colonies (MC1, MC2, MC3) reared in the 8 experimental treatments. Statistically significant differences between treatments are distinguishable by symbols ($p < 0.05$ for all Tukey post-hoc comparisons). Significant higher values of zooxanthellae.g DW^{-1} between mother colonies, for the same treatment, are marked with letters (a,b or c). Significant higher values of zooxanthellae.g DW^{-1} between coral fragments, from the same mother colony, with and without food supply with the same Spectra and PAR treatments are marked with (*),between coral fragments reared in different PAR intensities with the same food and spectra treatment (#) and between coral fragments reared in different light spectra with the same food and PAR treatments (+). Vertical lines represent one standard deviation.

3.6 Organic and inorganic weight of coral fragments

There were found a few significant differences in percentage of organic weight (% OW) of coral fragments when exposed to the different treatments (fig. 12).

Coral fragments from MC2 without supply of food, reared under PAR-120, with blue and white light spectra presented a higher % OW when compared to the corals from the same mother colony, reared under the same factors but with food supply.

Coral fragments from MC2, exposed to PAR-120 white light without supply of food, showed a higher % OW when compared to coral fragments from the same mother colony, reared under the same spectra but in a PAR-50. However, when food was administrated, coral fragments from MC2 exposed to a PAR-50 and white light demonstrated a higher % OW when compared to coral fragments of MC2 reared under white light but with a PAR-120.

Looking for the light spectra treatments (white light and blue light), it can be observed differences only when food was administrated. Coral fragments from MC1 reared under PAR-50 and white light had a higher % OW than corals from the same mother colony reared under PAR-50 but with blue light.

In all treatments, coral fragments from distinct mother colonies presented significant differences in organic weight.

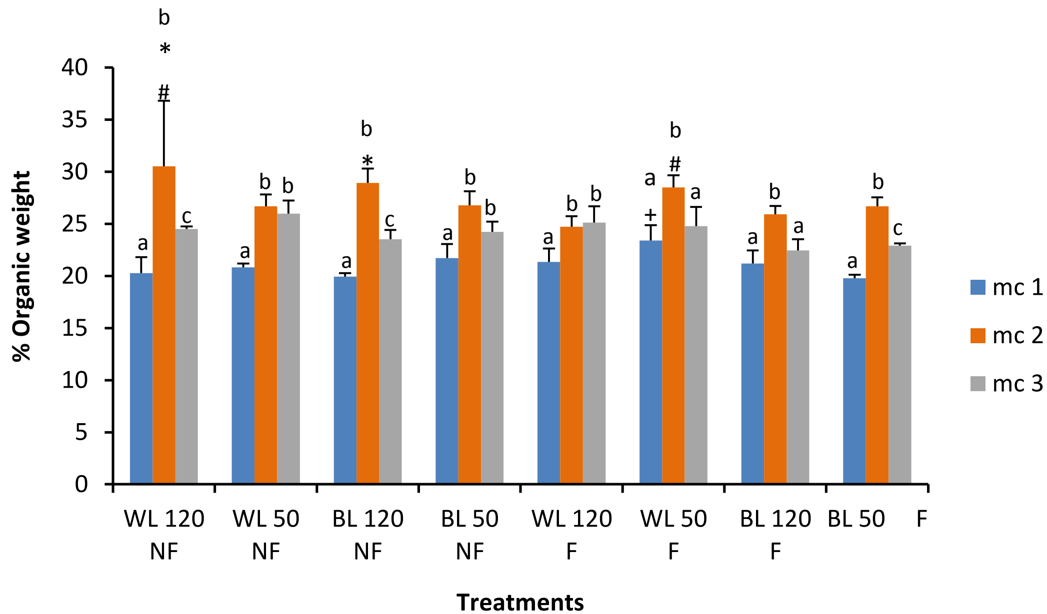

Figure 12 - Percentage of organic weight (% OW) obtained for coral fragments from the three mother colonies (MC1, MC2, MC3) reared in the 8 experimental treatments. Statistically significant differences between treatments are distinguishable by symbols ($p < 0.05$ for all Tukey post-hoc comparisons). Significant different values of % OW between mother colonies, for the same treatment, are marked with letters (a,b or c). Significant higher values of % OW between coral fragments, from the same mother colony, with and without food supply with the same Spectra and PAR treatments are marked with (*),between coral fragments reared in different PAR intensities with the same food and spectra treatment (#) and between coral fragments reared in different light spectra with the same food and PAR treatments (+). Vertical lines represent one standard deviation.

3.7 Suspended particulate matter, organic and inorganic matter in culture water

There were no statistical significant differences ($p > 0.05$ for all Tukey post-hoc comparisons) in the suspended particulate matter, inorganic matter and organic matter, between the tanks were food was not administrated and thanks were zooplankton was provided. Values presented on table 3.

Table 3 – Average (Avg) and standard deviation (StDev) values of suspended particulate matter (SPM), inorganic and organic matter on the experimental water for non food (NF) and food (F) treatments.

	SPM (mg)		Inorganic matter (mg. L^{-1})		Organic matter (mg. L^{-1})	
	Avg	**StDev**	**Avg**	**StDev**	**Avg**	**StDev**
NF	16.9667	0.4082	3.0333	0.4926	13.9333	0.8664
F	17.6333	1.5267	3.6417	1.0782	13.8917	1.1092

3.8 Inorganic nutrients

The values of inorganic nutrients are presented in table 4. There were statistical differences in all the inorganic nutrients. The levels of phosphates (PO_4^-), nitrate (NO_2^-) and nitrates (NO_3^-) were significant higher in the tanks were food was provided in comparison to thanks where no food was administrated.

Table 4 – Average (Avg) and standard deviation (StDev) values of phosphates (PO_4^-), nitrite (NO_2^-) and nitrates (NO_3^-) on the experimental water for non food (NF) and food (F) treatments. Significant statistical differences between the concentration of inorganic nutrients of tanks without food supply and tanks with food supply are marked with an asterisk ($p < 0.05$ for all comparisons).

	PO_4^- (mg. L^{-1})		NO_2^- (mg. L^{-1})		NO_3^- (mg. L^{-1})	
	Avg	**StDev**	**Avg**	**StDev**	**Avg**	**StDev**
NF	0.0106*	0.0031	0.0007*	0.0003	2.9496*	0.2354
F	0.0322	0.0045	0.0040	0.0011	3.3886	0.0233

4. Discussion

The supply of food (rotifers - *Brachionus plicatilis*) did not enhance the specific growth rate of coral fragments nor the organic weight. Furthermore, our results suggest that the food supply with rotifers in the *ex situ* culture of *S.* cf. *glaucum* had a negative effect in coral growth rate.

According to several studies (Anthony and Fabricius, 2000; Houlbrèque and Ferrier-Pagès, 2009; Houlbrèque *et al.*, 2003; Muscatine *et al.*, 1989; Sebens and Johnson, 1991), heterotrophy is essential to biomass buildup and tissue synthesis, since it is the major source of nutrients necessary for coral growth. However, it is important to stress that the supplied zooplankton could not be the only source of food, since corals also fed on bacteria and suspended matter (see review Houlbrèque and Ferrier-Pagès, 2009).

Rotifers, *Brachionus plicatilis*, are widely used as live preys in aquaculture of several marine species (Lubzens, 1987; Lubzens *et al.*, 1989; Rocha *et al.*, 2008), has also been used as live preys in studies with corals (Connolly *et al.*, 2012; Hoogenboom *et al.*, 2006; Levas *et al.*, 2013). Our preliminary tests demonstrate that coral fragments of *S*. cf. *glaucum* ingested the supplied rotifers. However it is known that different species have different feeding capacities (e.g. Lewis, 1982; Sebens *et al.*, 1998). In spite of Sebens *et al.* (1996) and Palardy *et al.* (2006) hypothesize that zooplankton capture rate is related to the mechanisms of capture and the prey escape behavior, rather than selective feeding by the corals, it is possible that some species present selective feeding, ingestion and digestion processes as Leal *et al.* (2014) demonstrated in symbiotic corals feed with phytoplankton. Therefore, due to the remaining gap in knowledge, further investigation is needed to clarify these questions on species specific coral feeding behavior, ingestion and digestion processes.

It is known that the administration of nitrogen influences the zooxanthellae C:N ratio (Grover *et al.*, 2002), which will lead to an increase in the production of amino acids (Wang and Douglas, 1998). The transference of amino acids from the zooxanthellae (Houlbrèque *et al.*, 2004) to the coral host can contribute to increase the synthesis of organic matrix, which consequently will contribute to promote coral growth. However, Sella and Benayahu (2010) suggested that frequent feeding may not be an advantage for coral fragments, probably due to the introduction of microbial

fauna associated to the zooplankton cultivation. Corals can be spending more energy in a cleaning process of developed biofouling in tissues, instead of using the substrates to grow. Therefore, the zooplankton supply on a daily basis can be inappropriate to *S*. cf. *glaucum* cultured in a recirculated system, since the ingestion of rotifers doesn't improve the growth rate during the experimental period.

The concentrations of inorganic nutrients, phosphates and nitrates, can also explain the lower growth rate registered in coral fragments feed with rotifers. Several studies (Marubini and Davies, 1996; Stambler *et al.*, 1991) showed that nitrates can reduce calcification rates in scleractinian corals and a study of Ferrier-Pagès *et al.* (2001) showed a decrease of buoyant weight gain. Phosphates can also decrease the buoyant weight gain, especially when together with nitrates (Ferrier-Pagès *et al.*, 2000). In addition, inorganic nutrients enhance zooxanthellae density, which can lead to a competition between the algae and the coral, and so resulting into a smaller transference of fixed carbon to the host (Cook *et al.*, 1988; Dubinsky *et al.*, 1990; Marubini and Davies, 1996; Muscatine *et al.*, 1989).

In fed coral fragments, the growth rate was greater when they were exposed to PAR-120. Khalesi *et al.* (2008) and Wijgerde *et al.* (2012) demonstrated an optimum growth rate for the soft coral *Sinularia flexibilis* (Quoy & Gaimard, 1833) and the scleractinian coral *Galaxea fascicularis* (Linnaeus, 1758), respectively, reared under a medium light intensities (100-150 µmol quanta.$m^{-2}.s^{-1}$). Blue light was also proved to be the better light for corals growth rate (Kinzie *et al.*, 1984; Levy *et al.*, 2006; Rocha *et al.*, 2013b). However, in this study this was only verified when fragments were exposed to PAR-120. In fragments exposed to PAR-50, it seems that white light had a better effect on coral growth rate.

Overall, non-fed coral fragments presented lower values of spectral reflectance and higher levels of NDVI, leading us to assume that fed corals had higher concentration of pigments than non-fed corals, given that NDVI is widely accepted as a proxy for chlorophyll *a* content (Leal *et al.*, 2014b; Rouse *et al.*, 1974). This result is in agreement with previous studies were chlorophyll *a* and *c* concentration were assessed, and fed corals demonstrated higher concentrations of those pigments then non fed corals (Dubinsky *et al.*, 1990; Houlbrèque *et al.*, 2003; Stambler *et al.*, 1991).

Since the number o zooxanthellae did not increase in lower light intensities, it was at least expected a photosynthetic pigment content increase (Falkowski and Owens, 1980) in order to optimize light absorption. However, this trend was not evident in the present study where most unfed coral fragments don't present a significant increase of NDVI in lower PAR (PAR-50).

Photosynthetic performance reached higher values in fed corals. This not necessarily means that corals receive more photosynthates, as was already demonstrated in sea anemones by Davy and Cook (2001), were algae retain the carbon instead of transfer to the host. In the majority of fragments, PAR-120 had a greater effect on the photosynthetic activity, as happened for zooxanthellae density only in coral fragments from one mother colony.

Previous experimental studies regarding the effect of light spectra in scleractinian corals showed that corals had a higher photosynthetic activity and grew better under blue light rather than under other lights, including the white light PAR range (Kinzie *et al.*, 1984; Levy *et al.*, 2006; Rocha *et al.*, 2013b). Conversely, blue light had a positive effect on the photosynthetic performance of just one group of coral fragments from one mother colony (MC2).

Along with the increase of pigments concentration and photosynthetic performance, zooxanthellae density reached higher values in fed corals, consistent with other reports results. Titlyanov *et al.* (2000) and Houlbrèque *et al.* (2004) concluded that feeding enhanced zooxanthellae density in the host, apparently cell-specific density. Also, in thanks were food was supplied, the concentration of inorganic nutrients was higher, which can lead to an increase in zooxanthellae density (Cook *et al.*, 1988; Dubinsky *et al.*, 1990; Snidvongs and Kinzie, 1994; Stambler *et al.*, 1991).

It was expected a higher number of zooxanthellae in coral fragments exposed to lower PAR values (Rocha *et al.*, 2013c; Titlyanov *et al.*, 2001), however in the present experiment this response was not verified.

5. Conclusions

In the present study, the supply of rotifers did not affect corals growth and promoted accumulation of inorganic nutrients (nitrates and phosphates) in the culture water.

Overall, it is visible an absence of a trend in the responses of coral fragments to the different treatments, which can be explain by the variability between the different mother colonies (which may be related with the genetic characteristics of coral host or with differences in the microbial communities). The results obtained in the present study suggest that variability should be considered as a major issue in a production scenario. Therefore, the colonies used for asexual reproduction should be selected according to their specific characteristics, to cope with the production objectives and culture conditions.

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