Universidade de Aveiro Departamento de Biologia
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Filipa Fonseca Ribeiro

Otimização da alimentação heterotrófica do coral mole *Sarcophyton* cf. *glaucum*

Optimization of heterotrophic feeding of the soft coral *Sarcophyton* cf. *glaucum*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor Rui Rocha, Investigador de pós-doutoramento do Departamento de Biologia da Universidade de Aveiro e sob a coorientação do Doutor Ricardo Calado, Investigador Principal no Departamento de Biologia da Universidade de Aveiro.

Dedico aos meus pais.

o júri

presidente

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agradecimentos

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palavras-chave

Alimentação heterotrófica, crescimento do coral, alimentação com microalgas, zooxantela, fluorometria PAM.

resumo

A alimentação heterotrófica desempenha um papel importante nos processos de crescimento e reprodução dos corais mixotróficos. O coral mole *Sarcophyton* cf. *glaucum* é uma espécie com potencial para a aquacultura, devido ao seu interesse económico no comércio de organismos ornamentais e na bioprospecção de produtos naturais marinhos. A insuficiente informação sobre a alimentação heterotrófica desta espécie conduziu ao desenvolvimento deste estudo, que teve como objetivo avaliar o efeito dos processos de conservação de microalgas na sua adequabilidade como alimento heterotrófico para o coral mixotrófico *S*. cf. *glaucum*. Adicionalmente, pretendeu-se identificar qual a espécie de microalga mais adequada, assim como a quantidade mais apropriada a aplicar no cultivo desta espécie. Realizaram-se duas experiências: na primeira experiência, a microalga *Nannochloropsis oculata* foi fornecida como alimento a fragmentos de coral em três formas de preservação distintas (pasta de alga viva, alga congelada e alga liofilizada) na dosagem de 10^6 cell mL^{-1}; na segunda experiência, foram testadas três espécies de microalga liofilizada (*Nannochloropsis oculata*, *Isochrysis galbana* e *Phaeodactylum tricornutum*) em duas dosagens diferentes: 7.33 mg L^{-1} (correspondente à concentração de 10^6 cell mL^{-1} de *N. oculata*) e 3.66 mg L^{-1}. Em ambas as experiências foram avaliados os seguintes parâmetros: taxa de crescimento dos fragmentos de coral, sobrevivência, peso orgânico, fotobiologia e qualidade da água. Os resultados não mostraram diferenças na taxa de crescimento e peso orgânico, entre os fragmentos de coral alimentados com as três formas de preservação de microalga, no entanto, foram observadas diferenças na qualidade da água. A microalga liofilizada evidencia ser uma boa alternativa como alimento heterotrófico para a aquacultura de corais, uma vez que apresenta os melhores resultados nos parâmetros analisados, tem um tempo de prateleira maior e poucos custos associados ao seu armazenamento. Entre as espécies avaliadas na segunda experiência, a microalga *I. galbana* foi a que promoveu uma taxa de crescimento mais elevada e uma maior percentagem de peso orgânico nos fragmentos de coral. Adicionalmente, os tanques em que se forneceu *I. galbana* como alimento heterotrófico apresentaram um maior equilíbrio na qualidade de água de cultivo.

keywords Heterotrophic feeding, coral growth, microalgae feeding, zooxanthellae, PAM fluorometry

abstract

Heterotrophic feeding has an important role in the processes of growth and reproduction of mixotrophic corals. The soft coral *Sarcophyton* cf. g*laucum* is a good candidate for aquaculture due to its economic interest for the marine aquarium trade and for the bioprospection of marine natural products. The lack of information on heterotrophic feeding of this species with preserved microalgae conducted to development of this work. The present study aimed to evaluate the effect of the conservation processes of microalgae in its suitability as heterotrophic feeding for the mixotrophic coral *S*. cf. *glaucum*. Additionally, we aimed to identify the most suitable freeze-dried microalgae species and cell density to be employed in the culture of this mixotrophic coral species. Two experiments were performed: in the first experiment the microalgae *Nannochloropsis oculata* was supplied to coral fragments in three different preservation forms (live paste, frozen and freeze-dried) at the concentration of 10^6 cell mL^{-1}; in the second experiment three different microalgae species (*Nannochloropsis oculata*, *Isochrysis galbana* and *Phaeodactylum tricornutum*) were tested in two different amounts: 7.33 mg L^{-1} (corresponding to the concentration of 10^6 cell mL^{-1} of *Nannochloropsis oculata*) and 3.66 mg L^{-1}. Growth rate, survival, organic weight and photobiology of coral fragments, as well as water quality in culture tanks, were evaluated in both experiments. Preserved forms of microalgae did not demonstrated differences in growth rate, organic weight and survival rate of coral fragments, but affected water quality. Freeze-dried microalgae seems to be a good feed supply for coral aquaculture, as it has the best results and it has the higher shell-life time and the lower associated costs. Between the species evaluated in second experiment, *Isochrysis galbana* promoted higher specific growth rate and higher percentage of organic weight in the coral fragments; additionally the culture tanks supplied with this microalgae species also presented a better water quality in the end of the experiment.

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1. Introduction

1.1. Coral biology and ecology

Coral reefs are one of the most productive ecosystems on the earth, being a habitat for hundreds of thousands of species (Hoegh-Guldberg, 1999; Moberg and Folke, 1999; Nyström *et al.*, 2000).

Coral reefs have an undoubted value for tropic regions, providing ecological goods and services (Moberg and Folke, 1999; Halpern *et al*., 2012), functioning as coastline protectors, and builders of islands and white sand beaches, a natural attraction for tourism (Pendleton, 1995). Ecologically speaking, coral reefs represent an important local of spawning and nursery for many species; additionally, allow the formation of lagoons and sedimentary environments, creating favourable conditions for seagrass and mangrove growth (Moberg and Folke, 1999; Nagelkerken *et al*., 2000). In spite of biodiversity found in these ecosystems, their essential foundation is based on a group of animals: corals (Rocha, 2013).

These important eecosystems are still threatened by both natural and anthropogenic factors (Hughes *et al*., 2003; Bellwood *et al*., 2004; Baums, 2008). It is estimated that 30 % of coral reefs are already severely damaged and close to 60 % may be lost by 2030 (Hughes *et al*., 2003). The over-exploration, deleterious methods of fishing (e.g. dynamite blasting or cyanide), pollution, massive tourism, diseases, and climate change, have threatened corals survival (Moberg and Folke, 1999; Shafir *et al.*, 2006; Baums, 2008).

The designation of coral is commonly applied to identify cnidarians from class Anthozoa. Most corals are colonial organisms, typically living in a compact colony of polyps (Veron, 1995, 2000; Reed, 2002; Hughes *et al.*, 2003; Wafar *et al.*, 2011). Corals are informally divided in two groups: (*i*) hard corals, and (*ii*) soft corals, based in the presence or absence of a calcium carbonate skeleton to support the colony. In spite of hard (or stony) corals being the major contributors for the structure of coral reefs, some soft corals are able to cement sclerites and build solid structures through the consolidation of dense sand agglomerates in their basis, contributing also to reef building (Jeng *et al.*, 2011).

Anatomically, coral polyps are composed by two epithelial cell layers, the epidermis and the gastrodermis. The epidermis promotes the separation between the coral and the external environment, whereas the gastrodermis lines the gastro-vascular cavity, which is separated from the external environment by a mouth, surrounded by tentacles. The two epithelial layers (epidermis and gastrodermis) are connected by the mesoglea, a translucent substance mostly composed by water, and other substances such as fibrous proteins like collagen, nerve fibres, muscle bundles and amoebocytes, which are involved in phagocytosis processes (Sarras *et al*., 1991; Fosså and Nielse, 1998; Adey and Loveland, 2007).

Most coral species live in symbiosis with dinoflagellate algae of genus *Symbiodinium*. These algae, commonly termed zooxanthellae, provide the coral host with organic carbon, amino acids and fatty acids. In return, they benefit of protection, carbon dioxide, and nutrients such as ammonia and phosphates (Muscatine and Porter, 1977; Ferrier-Pagé *et al.*, 1998; Hoegh-Guldberg *et al.*, 2007). Zooxanthellae release up to 95 % of its photosynthetic activity, while living in vacuoles formed inside the cells of the gastrodermis layer of corals (Hoegh-Guldberg, 1999; Delbeek and Sprung, 1994).

Heterotrophic feeding is also an important aspect for coral nutrition, since corals are able to ingest bacteria, sediments, suspended particular matter (Ferrier-Pagé *et al.*, 1998; Anthony and Fabricius, 2000), plankton, invertebrates and even small fishes (Adey and Loveland, 2007), depending on the species. Prey catching is promoted by nematocysts discharges, tentacle grabbing or mucus adhesion (Houlbrèque and Ferrier-Pagès, 2009).

Therefore, coral species living in symbiosis with zooxanthellae can be considered mixotrophic organisms, since they combine the photosynthates provided by their endosymbionts (autotrophy) with heterotrophic feeding (Muscatine and Porter, 1977; Ferrier-Pagé *et al.*, 1998; Hoegh-Guldberg *et al.*, 2007, Leal *et al.*, 2014a).

Corals are able to reproduce sexually and asexually, depending on the genus, species and geographic location (Richmond and Hunter, 1990; Hellström *et al*., 2010). Corals have three different modes of sexual reproduction: 1) internal brooding, through internal fertilization, with development of planula larvae inside the gastrovascular cavity and release of mature planula; 2) external surface brooding,

where released eggs adhere to the mucus on the polyp surface until maturation, and then are released; and 3) broadcast spawning, with release of gametes in the water, where fertilization occurs (Richmond and Hunter, 1990).

Most corals are hermaphrodites, having both sexes in a single polyp, whereas a few species are gonochoric (Richmond and Hunter, 1990; Schleyer *et al.*, 2004). Depending on species and location, reproductive cycles can be annually, seasonally, monthly or continuous. Corals use clues like temperature, photoperiod and lunar cycle to knowing exactly when they should release their gametes (Richmond and Hunter, 1990; Delbeek and Sprung, 1994; Hellström *et al.*, 2010; Leal *et al.*, 2014a).

Asexual reproduction can occur by fragmentation, budding, fission, pedal laceration and asexual production of planulae (Delbeek and Sprung, 1994; Delbeek and Sprung, 2005; Hellström *et al*., 2010). Asexual reproduction is more dominant in corals living at their ecological limits, including stressful environments such as locals with high wave energy and instable substrate (Willis and Ayre, 1985; Richmond and Hunter, 1990). This reproduction strategy is important to increase coral population (Willis and Ayre, 1985), and apparently the propagules have the advantage of being large size and locally adapted (Richmond and Hunter, 1990).

1.2. Aquaculture of corals

The increasing demand of corals, either for biotechnological research on marine natural products (Brown and Bythell, 2005; Blunt *et al*., 2008, 2009; Leal *et al*., 2014b), or to supply the marine aquarium trade (Wabnitz *et al.*, 2003; Olivotto *et al*., 2011; Osinga *et al*., 2011), has motivated an increase on their harvest (Castanaro and Lasker, 2003). However, the dependence on wild organisms is not sustainable (Rocha, 2013). Therefore, it has been recommended that researchers and marine aquarium traders should consider the use of specimens produced in aquaculture, rather than wild organisms (Mendola, 2003; Proksch *et al*., 2003; Calfo, 2007; Olivotto *et al*., 2011).

Coral aquaculture can be performed *in situ* or *ex situ*. Aquaculture *in situ* is a low-cost solution since it takes advantage of natural conditions to growth corals (Rocha, 2013). However, the control of culture conditions is not effective and corals are susceptible to stressors as predation, pollution and adverse meteorological conditions (Leal *et al*., 2013a). The production *ex situ*, in recirculating aquaculture

systems, is more expensive than *in situ* aquaculture, but has the advantage to allow the control of abiotic and biotic factors (Osinga *et al*., 2011; Forsman *et al*., 2012; Leal *et al*., 2013a) that can influence coral physiology and growth performance under culture. The manipulation of culture conditions is the key of success of coral culture (Rocha, 2013). Several parameters can influence coral physiology and growth performance, such as water flow, light, nutrients, heterotrophic feeding and temperature (Forsman *et al*., 2012; Rocha *et al.*, 2013a; Leal *et al.*, 2014a). Besides, aquaculture *ex situ* can be performed everywhere, which permits production structures to be located closed to output market, solving the problem of shipping and handling (Leal *et al.*, 2014b). *In toto* aquaculture is the culture of the invertebrate host and the associated community of microorganisms, allowing bacteria and other important microorganisms, contributors for production of marine natural products, to be present in coral production (Molinski*et al.*, 2009; Leal *et al.*, 2013a; Leal *et al*., 2014a). This strategy can be essential to pharmacology industry, since most of bioactive compounds are produced by the bacteria living in association with the coral (Piel, 2006; Leal *et al.*, 2013b).

Heterotrophic feeding has an important role in the processes of growth and reproduction of mixotrophic corals, since most of photosynthetic products from zooxanthellae lack essential compounds, as nitrogen and phosphorus (Ferrier-Pagé *et al.*, 2003). Besides, heterotrophy is a significant source of carbon when photosynthesis cannot be performed, which happens in bleaching events and in turbid or deep waters (Houlbrèque and Ferrier-Pagès, 2009). A study by Ferrier-Pagès *et al.* (2003) demonstrate that feeding have a strong effect on coral growth and even in optimal light conditions, photosynthetic activity of corals cannot fulfil the requirements of maintenance and growth of the colony. This study also showed that feeding increase the symbiotic zooxanthellae concentration.

As mentioned before, corals can feed on a large range of food sources, including microalgae (Widdig and Schlichter, 2001; Leal *et al*., 2014c). Microalgae have been applied in aquaculture as nutritive supply for some marine organisms' larval stages and as complement feeding supply for some marine animals, due its nutritive composition (Raja*et al.*, 2014). Nutritional value of microalgae is mainly determined by the content of protein, vitamin and polyunsaturated fatty acids (PUFAs). A study by

Leal *et al.* (2014c), showed that microalgae were captured by symbiotic and asymbiotic corals. However, this study also demonstrated that corals are selective on the intake of microalgae species, and the capacity of feeding on microalgae is not only a matter of prey size, involving other factors which need to be better understood.

As reviewed by Hemaiswarya *et al.* (2011), some strategies can be used to improve the utilization of microalgae as heterotrophic feeding, such as the combination of different algae species to provide a better balanced nutrition, and the improvement of PUFA content by the manipulation of culture conditions (e.g. light intensity, photoperiod, carbon dioxide supply or temperature).

Microalgae employed in coral aquaculture can be cultured or acquired in preserved forms (live, frozen and freeze-dried), in order to reduce the production costs involved in microalgae production (infrastructures and manpower) (Lubzens *et al.*, 1995; Spolaore *et al.*, 2006). The different forms of microalgae preservation presents different shelf life times and associated storage costs, which should be taken into consideration in the elaboration of the business plan of an aquaculture (Hemaiswarya *et al.*, 2011). Freeze-dried microalgae have the most extended shelf-life and don't need special equipment's for storage, whereas the utilization of frozen microalgae brings more cots, due the need of a freezer to its conservation. Notwithstanding, the costs involved in the utilization of preserved microalgae still lower when compared with the costs associated with microalgae production.

According with Hemaiswarya *et al.* (2011) live microalgae have a better nutritive value when compared with other preserved forms. Nonetheless, the nutritional profile of live cultured microalgae can differ a lot, depending on the production methodologies and culture harvest stage (Brown, 2002). The utilization of freeze-dried microalgae has been pointed out as an alternative to live microalgae, since they preserve the original cell shape and texture and preserve the biochemical profile (Hemaiswarya *et al.*, 2011).

1.3. The culture of the species *Sarcophyton glaucum*

The genus *Sarcophyton* (Octocorallia: Alcyoniidae) is composed of 36 species inhabiting coral reefs from the eastern Africa to the western Pacific Ocean and also on the Red Sea (Verseveldt, 1982; Benayahu and Loya, 2013).

Sarcophyton glaucum is a common species of the genus, characterized by a mushroom-shaped with a capitulum and solitary stalk (Aratake *et al.,* 2012). This mixotrophic species is a good candidate for aquaculture, due to its value as ornamental species, for the marine aquarium trade (Rocha *et al*., 2013a), but especially due to the biotechnological potential that results from the production of bioactive natural products, such as sarcophytolide (Badria *et al*., 1998), sarcophytol (Wei and Frenkel, 1992), or sarcophine (Sawant *et al.*, 2006a, 2006b).

Bioactive natural products are secondary metabolites produced by the holobiont (composed of the coral animal host and its associated microorganisms) for different reasons, such as competition for space, inhibition of growth and survival of neighbours, or protection against predation (Aratake *et al.,* 2012).

Several studies have been performed on the effect of light (intensity and spectra) and heterotrophic feeding in the culture of the mixotrophic coral *S. glaucum* (Sella and Benayahu, 2010; Rocha *et al*., 2013a, 2013b, 2013c). However, the suitability of microalgae as heterotrophic feeding for this species is yet to be addressed.

1.4. Objectives

The present study aims to evaluate the effect of the conservation processes of microalgae in its suitability as heterotrophic feeding for the mixotrophic coral *S.* cf. *glaucum*. Additionally, we aimed to identify the most suitable microalgae species and cell density to be employed in the culture of this mixotrophic coral species.

Tow experiments were performed. In the first experiment, microalgae *Nannochloropsis oculata* was supplied to coral fragments in three different preservation forms (live paste, frozen and freeze-dried). In the second experiment, after confirmation that results of survival and growth of coral fragments fed with freeze-dried microalgae do not differ from the results obtained for coral fragments fed with live paste concentrates or frozen microalgae, three different microalgae species were tested in two concentrations. In both experiments the corals fragments growth, survival, photobiology and organic weight were evaluated.

2. Materials and methods

2.1. Coral husbandry and fragmentation

Six colonies of *Sarcophyton* cf. *glaucum* (figure 1), collected in Sumbawa, Indonesia were purchase from a wholesaler aquarium company, and kept in a recirculating system for acclimatization to laboratory conditions.

Figure 1– Mother colony of *Sarcophyton* cf. *glaucum.*

The acclimatization system was composed of three glass tanks of 90 L (0.6 m × 0.6 m × 0.25 m) with circulation pumps (Turbelle nanostream-6025 Tunze, Penzberg, Germany; approximate flow of 2500 L h^{-1}), connected to a 150 L filter tank, equipped with a biological filter (submerged bio-balls), a fluidized bed filter (FLF100 ReefSet, Portugal), a submersible heater (Eheim Jäger 300 W, Deizisau, Germany), a protein skimmer (ESC150 ReefSet, São Mamede Negrelos, Portugal), and a submerged pump (Eheim 1262, Deizisau, Germany; with an approximate flow of 1000 L h^{-1} in each tank) (figure 2). For each tank a 150 W hydrargyrum quartz iodide (HQI) blue light lamp (BLV, Steinhöring, Germany) was used, with a photoperiod of 12 h light: 12 h dark and a PAR (Photosynthetically Active Radiation) intensity of 120 µmol quanta m^{-2} s^{-1}. These procedures were based on methods described by Rocha *et al.* (2013b).

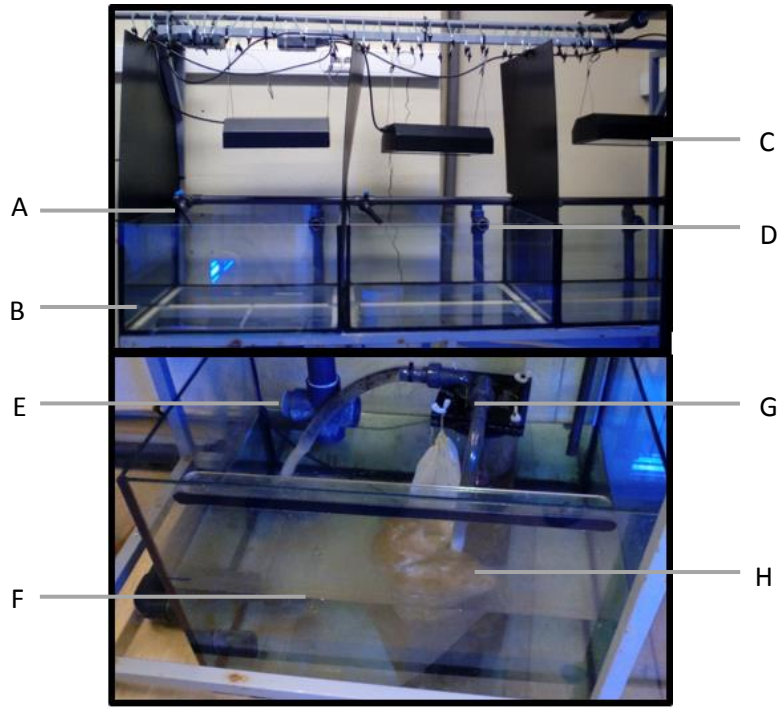

Figure 2 - Modular culture system basic set up: A) PVC valve inlet pipe system, B) 90 L glass tank, with circulation pumps C) blue light, D) outlet pipe system, E) tank outlet pipe to filter tank, F) 150 L filter tank with protein skimmer, G) fluidized sand-bed biological filter, H) submergible heater.

The water parameters were monitored and adjusted to optimal levels: temperature 26 ± 0.5 °C, ammonia < 0.01 mg L^{-1}, nitrites < 0.1 mg L^{-1}, nitrates < 20 mg L^{-1}, phosphates 0.01 mg L^{-1}, pH 8 – 8.2, alkalinity 3 ± 0.5 mEq L^{-1}, calcium 440 ± 20 mg L^{-1} and salinity at 35. Freshwater (purified by a reverse osmosis unit) was regularly added to the system to compensate the losses by evaporation and maintain the salinity. The system operated with synthetic saltwater, prepared by mixing synthetic sea salt (Tropic Marin Pro Reef, Wartenberg, Germany) with freshwater purified by reverse osmosis. A solution of calcium hydroxide was daily added to the system in order to maintain alkalinity; partial water changes of nearly 10 % were performed weekly.

After acclimatization, *Sarcophyton* cf. *glaucum* colonies were fragmented using a scalpel, and each fragment was attached with a rubber band to a plastic stand (TMC coral cradle) and labelled. Coral fragments recovery occurs in the acclimation tanks for two weeks, along with the respective mother colonies (figure 3).

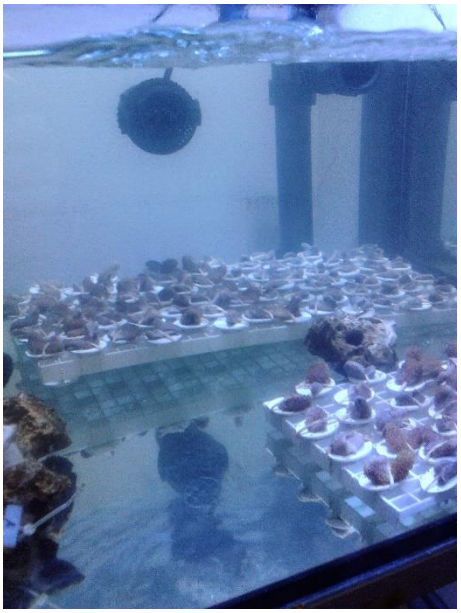

Figure 3 - Coral fragments recovery in a plastic stand (TMC coral cradle) after fragmentation, before the beginning of the experiments.

2.2. Experimental design

Experiments were carried out in four modular experimental systems, similar to the one described in the previous section. In the first experiment (see below in 2.2.1 section) one system was used for each microalgae conservation process. In the second experiment (see below in 2.2.2 section) one system was used for each microalgae species and one system used for control treatment (non-feeding). Water parameters were kept similar to values referred before in fragmentation procedures.

2.2.1. Influence of conservation processes of microalgae

In the first experiment, the microalgae *Nannochloropsis oculata* was supplied to coral fragments in three different forms: 1) live, 2) frozen and 3) freeze-dried (Phytobloom-Necton, Portugal) to study the influence of conservation process in the suitability of microalgae as coral feeding. Two different PAR intensities were tested: 50 and 120 µmol quanta m^{-2} s^{-1}, delivered by a 150 W HQI blue light lamp (BLV, Steinhöring, Germany) with a photoperiod of 12 h light: 12 h dark.

A total of 6 treatments were performed: 1) 120 µmol quanta m^{-2} s^{-1}, live microalgae, 2) 50 µmol quanta m^{-2} s^{-1}, live microalgae, 3) 120 µmol quanta m^{-2} s^{-1}, frozen microalgae, 4) 50 µmol quanta m^{-2} s^{-1}, frozen microalgae, 5) 120 µmol quanta

m^{-2} s^{-1}, freeze-dried microalgae, 6) 50 µmol quanta m^{-2} s^{-1}, freeze-dried microalgae. Each treatment was composed by 7 coral fragments, randomly selected from the pool of coral fragments.

The coral fragments were fed 5 days a week during 3 months, with 10^6 cell mL^{-1}. According to our better knowledge this was the first time that microalgae were tested as heterotrophic feeding in *S.* cf. *glaucum* aquaculture. Due to the lack of information applied on this species, we established this concentration following the concentrations described in experiments performed with other coral species: 10^4cell mL^{-1} of *Thalassiosira pseudonana*, *Isochrysis galbana* and *Phaeocystis globosa* (see Miguel Leal *et al.*, 2014c), 6x10^4cell mL^{-1} of *N. oculata* (Widdig and Schlichter, 2001) and 8 – 9x10^6cell mL^{-1} of *N. Oculata* (Varghese*et al.*, 2012). The cell concentration was determined using a Neubauer haemocytometer. Five samples of live paste, frozen and freeze dried microalgae, from the batch used for the experiment, were resuspended in filtered salt water to allow cell counting. After cell counts, an average value of 2.3 mL of microalgae live paste, 3.3 g of frozen microalgae, and 0.66 g of freeze-dried microalgae was determined to be used in each 90 L experimental culture tank, resulting in feeding concentrations of 25.56 µL L^{-1} of microalgae live paste, 36.67 mg L^{-1} of frozen microalgae, and 7.33 mg L^{-1} of freeze-dried microalgae. Coral fragments were fed during 6 h, 3 h of dark and 3 h of light. During this time, the protein skimmer was switched off and the water flow through the experimental culture tank cut to a minimum flow. The circulation pumps inside the experimental culture tanks were maintained in operation to guarantee an efficient distribution of microalgae in the water column.

2.2.2. Suitability of different microalgae species as exogenous feed

After the first experiment, three different species of freeze-dried microalgae with different fatty acids profile, available in the market, were tested: *Nannochloropsis oculata* (rich in ARA and EPA), *Isochrysis galbana* (rich in DHA) and *Phaeodactylum tricornutum* (rich in EPA) (Phytobloom-Necton, 2013). Coral fragments were fed with two amounts of each microalgae species: 7.33 mg L^{-1} (the amount used in the previous experiment) and 3.66 mg L^{-1} (used as a lower feeding amount). A PAR intensity of 120

µmol quanta m^{-2} s^{-1} was delivered by a blue light lamp (150 W HQI; BLV, Steinhöring, Germany), with a photoperiod of 12 h light: 12 h dark.

A total of 7 treatments were performed: 1) 120 µmol quanta m^{-2} s^{-1}, non-feeding, 2) 120 µmol quanta m^{-2} s^{-1}, 3.66 mg L^{-1} *N. oculata,* 3) 120 µmol quanta m^{-2} s^{-1}, 7.33 mg L^{-1} *N. oculata*, 4) 120 µmol quanta m^{-2} s^{-1}, 3.66 mg L^{-1} *I. galbana*, 5) 120 µmol quanta m^{-2} s^{-1}, 7.33 mg L^{-1} *I. galbana*, 6) 120 µmol quanta m^{-2} s^{-1}, 3.66 mg L^{-1} *P. tricornutum*, 7) 120 µmol quanta m^{-2} s^{-1}, 7.33 mg L^{-1} *P. tricornutum*. Each treatment was composed by 15 coral fragments.

Feeding was performed with the same methods describe for experiment one.

2.3. Coral fragmentsspecific growth rate (SGR)

Buoyant weight were performed at the beginning and at the end of the experiments, as described by Rocha *et al*. (2013a), to determine the specific growth rate, using a Kern Emb 200-3 balance (Kern & Sohn GmbH, Balingen, Germany). The buoyant weight of coral cradle and rubber bands used to attach the coral fragments was determinate and deducted to the total weight of coral fragments. During weighting procedures the light and water circulation in the experimental culture tanks were switched off. To minimize the error by accumulation of biofouling, corals cradles were cleaned with seawater and a toothbrush before the measurements. Specific growth rate (SGR) of coral fragments (% day^{-1}), was calculated using the formula:

$$\text{SGR (\% day}^{-1}) = \left(\frac{\ln(wf) - \ln(wi)}{\Delta t}\right) \times 100$$

Where ln(wf) and ln(wi) are the natural logarithm of final and initial coral net weights expressed in grams (g), and Δt is the growth interval in days. SGR is expressed as a percentage of coral weight increase per day.

2.4. Organic and inorganic weight

At the end of the experiment, one sample of each coral fragment was extracted with a scalpel to determine the organic and inorganic weight. Samples were freeze-drying for 48 h and weighted to obtain the dry weight. Next, to determinate the inorganic weight, samples were burnt at 450°C (muffle furnace, Nabertherm, Lilienthal,

Germany) and weighted again. Organic weight was obtained by subtracting the inorganic weight from the dry weight.

2.5. *In vivo* chlorophyll fluorescence F_v/F_m

Chlorophyll fluorescence was measured *in vivo* using a PAM (pulse amplitude modulation) fluorometer (Junior PAM, Walz, Effeltrich, Germany). This non-intrusively method allows to monitor photosynthetic activity photosystem II (PSII) of zooxanthellae (Schreiber *et al.,* 1986). Measurements were initiated 2h after the start of the day light period for the full activation of the photosynthetic apparatus. Previous to each measurement, fragments were dark-adapted for 15 min, the minimum- or dark- level fluorescence (Fo) was determined, followed by one saturation pulse (0.8 s) to determine the maximum fluorescence (Fm). The maximum quantum yield of photosystem II (F_v/F_m) was calculated through the next equation (Schreiber *et al.*, 1986):

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

2.6. Water analyses

2.6.1. Organic matter

At end of experiments, water samples (1.5 L) were collected from each experimental system for analyses. To determine organic matter, the 0.47 µm glass-fiber filters (Whatman GF/F) were dried in a muffle furnace at 450°C (Nabertherm, Lilienthal, Germany) for approximately 4 h and weighted. Then, samples of water were filtered, the filters were dried in an oven at 50°C (Venticell, MMM Medcenter GmbH, Germany) for approximately 12 h and weighted. After weighting, filters were dried at 450°C for approximately 4 h and weighted again. The organic matter was obtain by the subtracting the inorganic matter to the total weight.

2.6.2. Suspended particulate matter

The same samples of water were used to determine suspended particulate matter (SPM). Filters GF/F were dried in an oven (Venticell, MMM Medcenter GmbH,

Germany) at 105°C for 5 h and weighted. After, water samples were filtered and the water was stored in plastic bottles at -20°C for future analysis of nutrients. The filters were dried again at 105°C for 5 h and weighted to obtain total SPM.

2.6.3. Nutrients

Water samples previous filtered and stored at -20 °C were used for nutrient analysis. Analysis was perform following the standard methods described in Limnologisk Metodik (1992) for ammonia (NH_3-N) and phosphate (PO_4-P) and following Strickland and Parsons (1972) for nitrate (NO_3-N) and nitrite (NO_2-N).

2.7. Statistical analysis

Statistical analyses were performed using the software Statistica version 8.0 (StatSoft Inc.). Chi-square test was used to compare survival percentage of treatments in the first and second experiment. Analysis of variance (ANOVAs) were used to evaluate the existence of significant differences in the maximum quantum yield of PSII (F_v/F_m), coral growth, organic and inorganic weight on fragments of *S.* cf. *glaucum* in the two experiments performed. In the first experiment two-away ANOVA were performed (with feed and PAR as categorical factors); in the second experiment one-way ANOVA was applied. Normality and homogeneity of variance were tested before the analysis through Shapiro–Wilk and Levene tests, respectively. Unequal-N HSD post-hoc comparisons were performed to determine the differences between the different conservation processes of microalgae used as feed and between different PAR treatments in the first experiment, and between different species of microalgae used as feed in the second experiment.

3. Results

3.1. Influence of conservation processes of microalgae

3.1.1. Survival of coral fragments

At the end of experiment, coral survival did not show significant differences among the treatments, nonetheless, it can be observed (table 1) that coral survival had lowest percentage for treatments of live microalgae with PAR of 50 µmol quanta m^{-2} s^{-1} and freeze-dried microalgae with PAR of 120 µmol quanta m^{-2} s^{-1}.

Table 1 – Percentage of survival of *S.* cf. *glaucum* fragments reared in the different treatments after three months. No significant statistical differences were found at P<0.05.n= 3 for treatments L 50 and FD 120; n = 4 for treatments L 120, F 50, F 120 and FD 50.

Microalgae	**PAR (µmol quanta m^{-2} s^{-1})**	**Survival (%)**
Live	**50**	37,5
Live	**120**	50,0
Frozen	**50**	57,1
Frozen	**120**	57,1
Freeze-dried	**50**	62,5
Freeze-dried	**120**	37,5

3.1.2. Coral fragments specific growth rate (SGR)

No significant differences were found in the SGR of coral fragments reared with the different food treatments (frozen, freeze-dried and live paste microalgae) and PAR treatments (50 and 120 µmol quanta m^{-2} s^{-1}) (ANOVA, F (2, 17) = 1.729, P=0.207). However, it was perceptible that the mean values (± standard deviation) of specific growth rate (% day^{-1}) were higher for treatment of 120 µmol quanta m^{-2} s^{-1}, freeze-dried microalgae (0.552 ± 0.282 % day^{-1}) (figure 4).

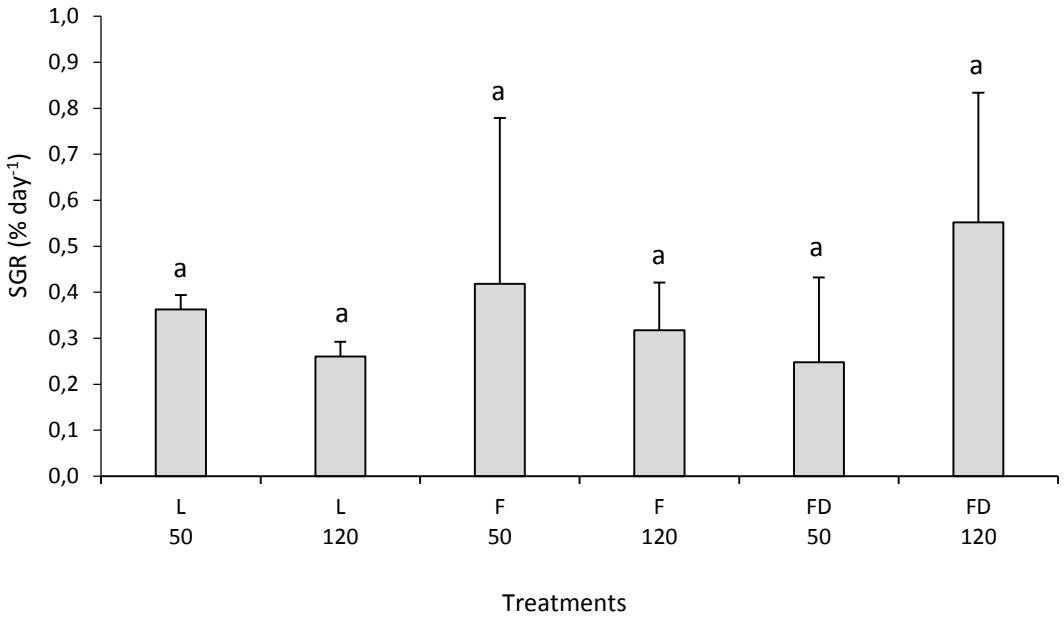

Figure 4 - Mean values (± standard deviation) of SGR (% day^{-1}) measured in the *S*. cf. *glaucum* fragments reared in the different treatments: L – live *Nannochloropsis oculata*, F – frozen *N. oculata*, and Fd – freeze-dried *N. oculata*; 50 - PAR 50 µmol quanta m^{-2} s^{-1}, and 120 - PAR 120 µmol quanta m^{-2} s^{-1}, after three months of experiment. No significant statistical differences were found ($P > 0.05$). n= 3 for treatments L 50 and FD 120; n = 4 for treatments L 120, F 50, F 120 and FD 50.

3.1.3. Organic and inorganic weight

Average values of organic and inorganic weights percentage, obtained at the end of experiment, are presented in figure 5. The highest percentage of organic weight were obtained in coral fragments reared with frozen microalgae with PAR of 120 µmol quanta m^{-2} s^{-1} with (22.4 %) and freeze-dried microalgae with PAR of 120 µmol quanta m^{-2} s^{-1} with (22.5 %). Yet, no significant differences were observed between PAR and feed treatments (ANOVA, $F(2, 17) = 3.327$; $P = 0.060$).

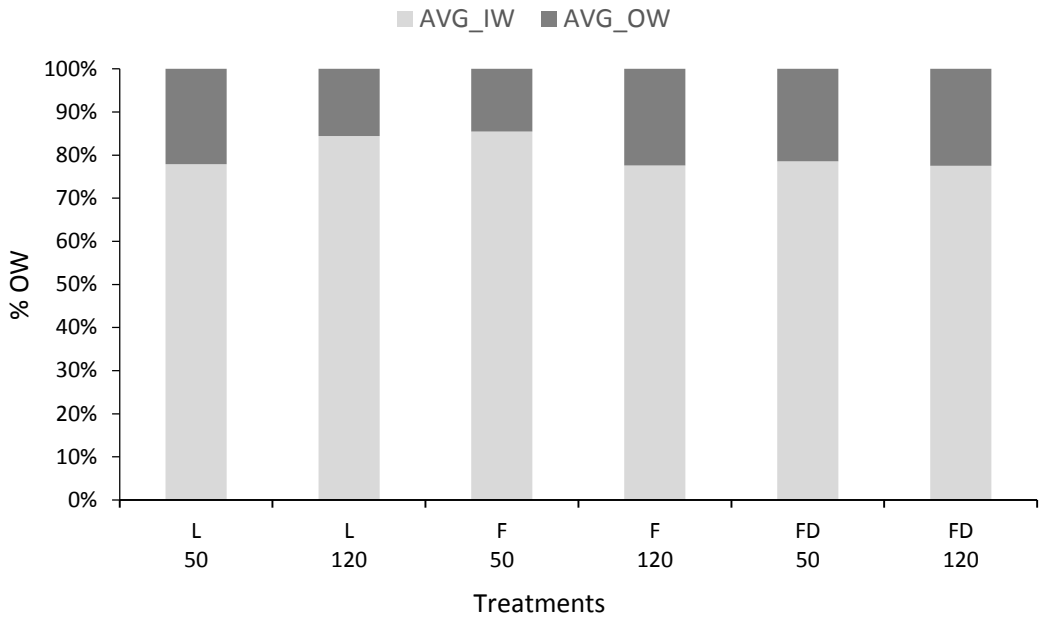

Figure 5 - Percentage of organic weight (% OW) and inorganic weight (% IW) measured in the *S.* cf. *glaucum* fragments reared in the different treatments: L – live *Nannochloropsis oculata*, F – frozen *N. oculata*, and Fd – freeze-dried *N. oculata*; 50 - PAR 50 µmol quanta m^{-2} s^{-1}, and 120 - PAR 120 µmol quanta m^{-2} s^{-1}, after three months of experiment. No significant statistical differences were found (P > 0.05). n = 3 for treatments L 50 and FD 120; n = 4 for treatments L 120, F 50 and F 120; n = 5 for treatment FD 50.

3.1.4. *In vivo* chlorophyll fluorescence F_v/F_m

Means of maximum quantum yield of PSII F_v/F_m for all the treatments are presented in figure 6. Tanks with lower PAR (50 µmol quanta m^{-2} s^{-1}) treatments had higher values of chlorophyll fluorescence compared with the treatments with highest PAR (120 µmol quanta m^{-2} s^{-1}). There was not significant interaction between the factors PAR and food (ANOVA, F (2, 17) = 1.44, P = 0.265). However, corals fragments fed with freeze-dried microalgae and PAR of 50 µmol quanta m^{-2} s^{-1} had significant higher values of F_v/F_m comparing to live microalgae and PAR of 120 µmol quanta m^{-2} s^{-1} ($P<0.05$).

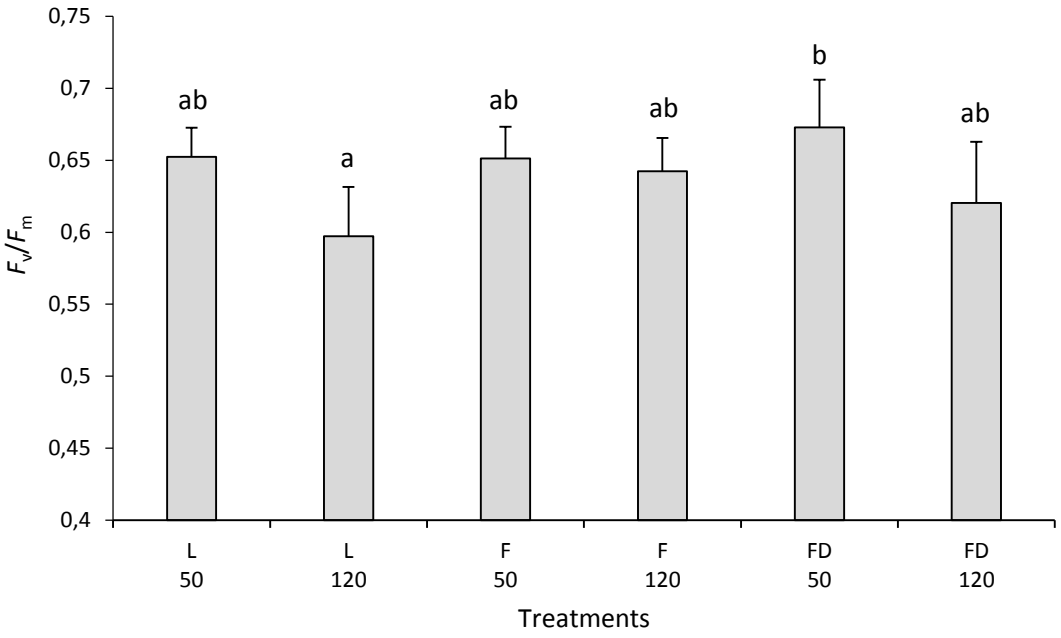

Figure 6 - Mean values (± standard deviation) of maximum quantum yield of PSII (F_v/F_m) measured in the *S.* cf. *glaucum* fragments reared in the different treatments: L – live *Nannochloropsis oculata*, F – frozen *N. oculata*, and FD – freeze-dried *N. oculata*; 50 - PAR 50 µmol quanta m^{-2} s^{-1}, and 120 - PAR 120 µmol quanta m^{-2} s^{-1}, after three months. Different letters represent significant differences at $P < 0.05$. n = 3 for treatments L 50 and FD 120; n = 4 for treatments L 120, F 50 and F 120; n = 5 for treatment FD 50.

3.1.5. Water analyses

3.1.5.1. Organic matter and suspended particulate matter

No significant differences were found for suspended particulate matter (SPM) (ANOVA, F (2, 6) = 0.014, P=0.986) and for organic matter among the three treatments (table 2; ANOVA, F (2, 6) = 1.453, P=0.306).

Table 2 – Average values (± standard deviation) of suspended particulate matter (SPM) and organic matter on the experimental water of culture tanks of *S.* cf. *glaucum* fragments reared in the different treatments: live *N. oculata*, frozen *N. oculata* andfreeze-dried *N. oculata*. No significant differences were recorded (P>0.05). n = 3 for all the treatments.

Treatment	**SPM (mg L^{-1})**	**Organic matter (mg L^{-1})**
Live	102.3333 ± 10.7742	27.6667 ± 2.3629
Frozen	102.6667 ± 15.8850	28.8333 ± 1.4434
Freeze-dried	100.5000 ± 22.4889	25.1667 ± 3.7528

3.1.5.2. Inorganic nutrients

Mean values of inorganic nutrients, measured in water of experimental culture systems employed for the different treatments, are described in table 3. Significant differences were observed in all the inorganic nutrients except for ammonium, which was not detected in all the treatments. The concentrations of NO_3-N and PO_4 (mg L^{-1}) where significantly different in all the tested treatments (ANOVA, $F(2, 6) = 219.498$, $P < 0.001$, for NO_3-N; and ANOVA, $F(2, 6) = 1002.198$, $P < 0.001$, for PO_4). The detected values were higher in the culture systems where live paste microalgae was used as feed, followed by the systems used to test freeze-dried microalgae, being the lowest values obtained in the culture system used to test frozen microalgae as feed. NO_2-N concentration (mg L^{-1}), measured in the culture system where microalgae live paste was tested as feed, was higher than in culture systems used to test frozen and freeze-dried microalgae (ANOVA, $F(2, 6) = 38.113$, $P < 0.001$)

Table 3 - Average (±standard deviation) values of ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4-P) on the experimental water of culture tanks of *S.* cf. *glaucum* fragments reared in the different treatments: live *N. oculata*, frozen *N. oculata* and freeze-dried *N. oculata*. Significant statistical differences between treatments were distinguish with letters at $P<0.05$. n = 3 for all the treatments.

Treatment	**NH_3-N (mg L^{-1})**	**NO_2-N (mg L^{-1})**	**NO_3-N (mg L^{-1})**	**PO_4-P (mg L^{-1})**
Live	0.0000 ± 0.0000^a	0.0173 ± 0.0016^a	2.8257 ± 0.1916^a	0.3534 ± 0.0055^a
Frozen	0.0000 ± 0.0000^a	0.0053 ± 0.0019^b	0.0191 ± 0.0176^b	0.0235 ± 0.0009^b
F-dried	0.0000 ± 0.0000^a	0.0071 ± 0.0019^b	1.1265 ± 0.2119^c	0.2880 ± 0.0156^c

3.2. Suitability of different microalgae species as exogenous feed

3.2.1. Survival of coral fragments

Coral fragments survival did not presented statistical differences (table 4). Yet, the treatments with high feeding amount of microalgae (7.33 mg L^{-1}) had higher mortality, being the lowest percentage of survival observed in the treatment 7.33 mg L^{-1} of *P. tricornutum.*

Table 4 - Percentage of survival of *S*. cf. *glaucum* fragments reared in the different treatments: NF – non-feeding, Iso - *Isochrysis galbana*, Nanno – *Nannochloropsis oculata* and Phaeod – *Phaeodactylum tricornutum*, in amounts of 3.66 mg L^{-1} (0.33 - total of 0.33 g day^{-1} per tank) and 7.33 mg L^{-1} (0.66 - total of 0.66 g day^{-1} per tank), after three months of experiment. No significant statistical differences were found ($P > 0.05$). n = 3 for treatment Phaeod 0.66; n = 4 for treatments Iso 0.66; n = 5 for treatment Phaeod 0.33 and Nanno 0.66; n= 6 for treatments NF and Iso 0.33; n = 7 for treatment Nanno 0.33.

Treatment		Survival (%)
NF		46,2
Nanno	**0.33**	53,8
Nanno	**0.66**	46,2
Iso	**0.33**	46,2
Iso	**0.66**	28,6
Phaeod	**0.33**	38,5
Phaeod	**0.66**	23,1

3.2.2. Coral fragments specific growth rate (SGR)

Results of SGR are presented in figure 7. No statistical differences were registered between the treatments with microalgae and the control (non feeding). However, it is possible to observe that coral fragments supplied with lower amounts of microalgae (3.66 mg L^{-1}) presented highest values of SGR than coral fragments feed with higher amounts of microalgae (7.33 mg L^{-1}). Coral fragments feed with 3.66 mg L^{-1} of *I. galbana* presented the highest SGR values (1.685 ± 0.977 % day^{-1}), significantly higher (ANOVA, $F(6, 30) = 3.457$, $P = 0.01$) when compared with the SGR, obtained for coral fragments fed with 3.66 and 7.33 mg L^{-1} of *N. oculata* ($P = 0.028$ and $P = 0.006$, respectively).

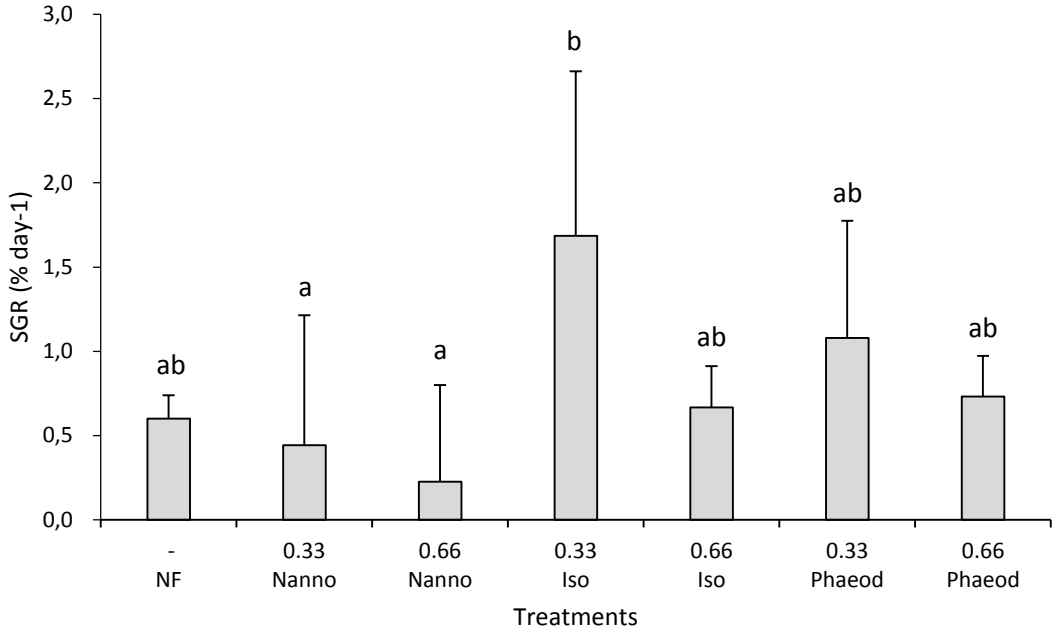

Figure 7 - Mean values (± standard deviation) of SGR (% day^{-1}) measured in the *S.* cf. *glaucum* fragments reared in the different treatments: NF – non-feeding, Iso - Isochrysis galbana, Nanno – *Nannochloropsis oculata* and Phaeod – *Phaeodactylum tricornutum*; in amounts of 3.66 mg L-1 (0.33 - total of 0.33 g day $^{-1}$ per tank) and 7.33 mg L^{-1} (0.66 - total of 0.66 g day $^{-1}$ per tank), after three months of experiment. Different letters represent significant differences at $P < 0.05$. n = 3 for treatment Phaeod 0.66; n = 4 for treatments Iso 0.66; n = 5 for treatment Phaeod 0.33 and Nanno 0.66; n= 6 for treatments NF and Iso 0.33; n = 7 for treatment Nanno 0.33.

3.2.3. Organic and inorganic weight

No statistical differences were found in the percentages of organic and inorganic weight (figure 8) obtained in the different treatments (ANOVA, $F(6, 30) = 1.270$; $P= 0.300$).However, the higher percentage of organic weight was obtained in treatments supplied with 3.66 mg L^{-1} of *I. galbana* (32.6 %).

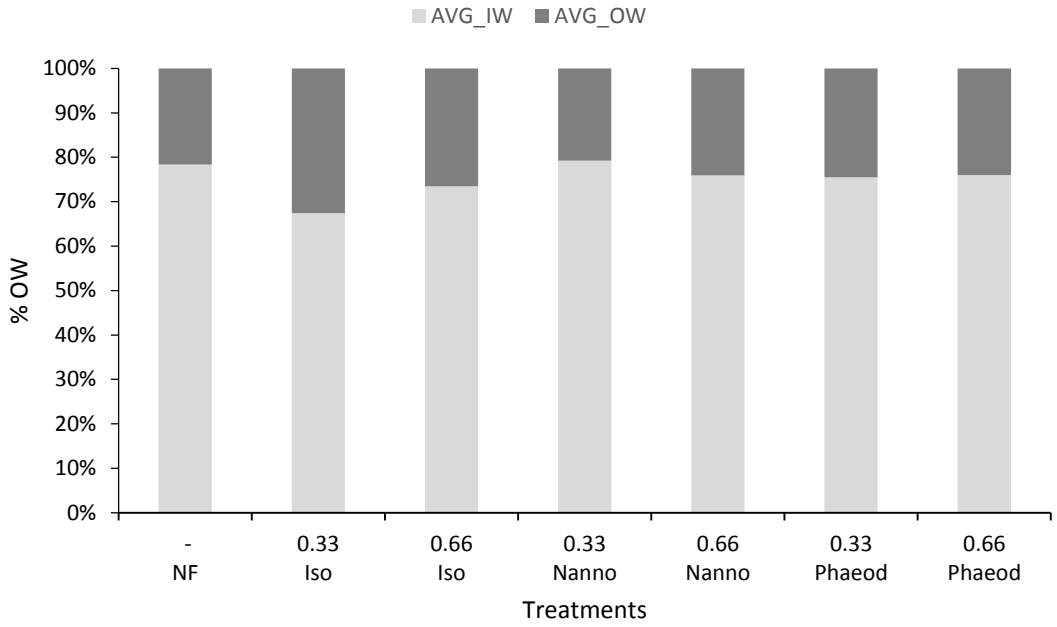

Figure 8 - Percentage of organic weight (% OW) and inorganic weight (% IW) for coral fragments of *S.* cf. *glaucum* fragments reared in the different treatments: NF – non-feeding, Iso - *Isochrysis galbana*, Nanno – *Nannochloropsis oculata* and Phaeod – *Phaeodactylum tricornutum*; in amounts of 3.66 mg L^{-1} (0.33 - total of 0.33 g day $^{-1}$ per tank) and 7.33 mg L^{-1} (0.66 - total of 0.66 g day $^{-1}$ per tank), after three months of experiment. No significant differences between treatments were distinguished ($P > 0.05$). n = 3 for treatment Phaeod 0.66; n = 4 for treatments Iso 0.66; n = 5 for treatment Phaeod 0.33; n= 6 for treatments NF, Iso 0.33 and Nanno 0.66; n = 7 for treatment Nanno 0.33.

3.2.4. *In vivo* chlorophyll fluorescence F_v/F_m

Values of F_v/F_m obtained in coral fragments feed with the three species of microalgae (*I. galbana*, *N. oculata* and *P. tricornutum*) are featured in figure 9. All treatments presented highest values of F_v/F_m in the tanks with lowest amount of feed (3.66 mg L^{-1}). Significant differences of F_v/F_m were recorded between coral fragments feed with 3.66 mg L^{-1} of *I. galbana* and 7.33 mg L^{-1} of *P. tricornutum* (ANOVA, $F(6, 30) = 3.08$; $P = 0.018$).

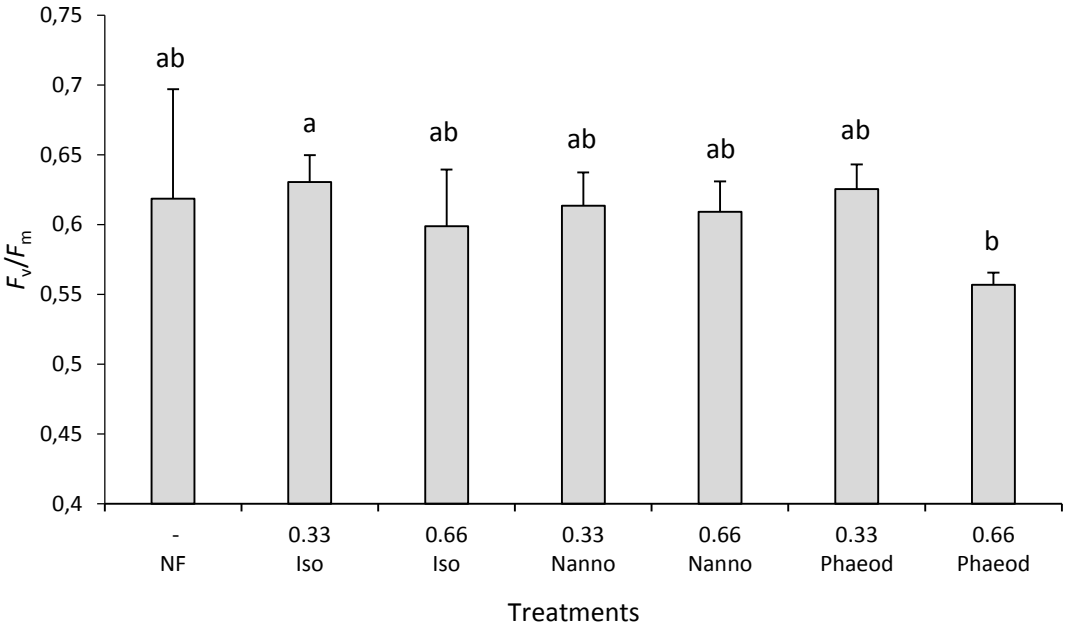

Figure 9 - Mean values (± standard deviation) of maximum quantum yield of PSII (F_v/F_m) measured in the *S.* cf. *glaucum* fragments reared in the different treatments: NF – non-feeding, Iso - *Isochrysis galbana,* Nanno – *Nannochloropsis oculata* and Phaeod – *Phaeodactylum tricornutum*; in amounts of 3.66 mg L^{-1} (0.33 - total of 0.33 g day $^{-1}$ per tank) and 7.33 mg $^{L-1}$ (0.66 - total of 0.66 g day $^{-1}$ per tank), after three months. Different letters represent significant differences at $P < 0.05$. n = 3 for treatment Phaeod 0.66; n = 4 for treatments Iso 0.66; n = 5 for treatment Phaeod 0.33; n= 6 for treatments NF, Iso 0.33 and Nanno 0.66; n = 7 for treatment Nanno 0.33.

3.2.5. Water analyses

3.2.5.1. Organic matter and suspended particulate matter

All the treatments presented similar values of SPM and organic matter (table 5). No significant differences were found for suspended particulate matter and to organic matter (ANOVA, F (3, 8) = 0.244, P = 0.864; ANOVA, F (3, 8) = 1.501, P = 0.287).

Table 5 - Average values (± standard deviation) of suspended particulate matter (SPM) and organic matter) on the experimental water of culture tanks of *S.* cf. *glaucum* fragments reared in the different treatments: NF - non-feeding, Iso - *Isochrysis galbana*; Phaeod – *Phaeodactylum tricornutum* and Nanno – *Nannochloropsis oculata*. No significant statistical differences were found (P > 0.05). n = 3 for all the treatments.

Treatment	SPM (mgL^{-1})	Organic matter ($mg\ L^{-1}$)
NF	72.8000 ± 7.7149	20.9333 ± 2.6026
Iso	82.4000 ± 7.9699	19.8667 ± 1.6166
Phaeod	74.2667 ± 2.0526	21.6000 ± 0.6928
Nanno	80.9333 ± 7.4009	20.1333 ± 4.5490

3.2.5.2. Inorganic nutrients

Mean values of inorganic nutrients are reported in table 6. Significant statistical differences were observed in all the inorganic nutrients except to ammonium, which was not detected. NO_3-N concentration ($mg\ L^{-1}$) measured in culture system feed with *N. oculata* were higher than in other treatments (ANOVA, F (3, 8) = 26.846, P=0.000). NO_2-N concentration ($mg\ L^{-1}$) measured in culture system feed with *I. galbana* was significantly higher than the value obtained in non-feeding treatment (ANOVA, F (3, 8) = 16.364, P=0.000). PO_4 concentration ($mg\ L^{-1}$) measured in the systems feed with *N. oculata* and *I. galbana* were significantly higher than the concentration obtained for non-feeding and *P. tricornutum treatments* (ANOVA, F (3, 8) = 155.927, P = 0.000). Tanks where *N. oculata* was provided had significantly higher concentration of NO_3-N and PO_4-P compared with the other treatments.

Table 6 - Average (± standard deviation) values of ammonia (NH3-N), nitrite (NO2-N), nitrate (NO3-N) and phosphate (PO4-P)) on the experimental water of culture tanks of *S.* cf. *glaucum* fragments reared in the different treatments: NF - non-feeding, Iso - Isochrysis galbana; Phaeod - *Phaeodactylum tricornutum* and Nanno - *Nannochloropsis oculata*. Significant statistical differences between the treatments (P < 0.05) are distinguish with different letters. n = 3 for all the treatments.

Treatment	NH_3-N ($mg\ L^{-1}$)	NO_2-N ($mg\ L^{-1}$)	NO_3-N ($mg\ L^{-1}$)	PO_4-P ($mg\ L^{-1}$)
NF	0.0000 ± 0.0000	0.0040 ± 0.0008 [a]	0.0127 ± 0.011 [a]	0.0092 ± 0.0036 [a]
Iso	0.0000 ± 0.0000	0.0104 ± 0.0009 [b]	0.0906 ± 0.0451 [b]	0.1515 ± 0.0028 [b]
Phaeod	0.0000 ± 0.0000	0.0075 ± 0.0018 [b]	0.1078 ± 0.0137 [b]	0.0089 ± 0.0023 [a]
Nanno	0.0000 ± 0.0000	0.0094 ± 0.0011 [b]	0.2143 ± 0.027 [c]	0.5851 ± 0.0755 [c]

4. Discussion

4.1. Influence of conservation processes of microalgae

*Nannochloropsis oculata*is commonly used in aquaculture industry as feed for live preys (e.g. rotifers), fish larvae, and corals (Lubzens *et al*., 1995; Gwo *et al*., 2005; Raja *et al*., 2014). However, there are some concerns on the utilization of preserved forms, since freezing process can affect the cell integrity of algae (Hemaiswarya, 2011).

In the experiment performed to test the effect of preservation processes of microalgae and their suitability as heterotrophic feeding for corals, coral survival did not show significant differences among treatments, although survival rate presented different values. These results evidence that there isn´t a relationship between different preserved microalgae and survival. Nonetheless, there is the possibility of differences be camouflage by the low number of replicates. The mortality of fragments during the experimental procedure can be explained by values of organic and inorganic matter, as it is explained further in this discussion.

The data from specific growth rate and organic weight suggested that coral fragments fed with the three preserved microalgae presented an acceptable growth rate and were healthy, since values of SGR were approximated to values described in previous studies for healthy corals (Ferrier-Pagès *et al*., 2003; Rocha *et al.* 2013a, 2013c). The same conclusion can be established for organic weight, since our results are in concordance with results from literature, which should be between 10 to 25 % for octocorals (Sorokin, 1995).

Coral fragments of *S.* cf. *glaucum* fed with three preserved microalgae for three months did not show significant differences in survival, growth rate or organic weight gain. Results suggest that preserved microalgae tested, have equal nutritional value for coral fragments and identical feed uptake, leading to identical growth rate and organic tissue synthesis. Nonetheless, previous studies have referred differences in quality and conservation of these three forms.

Although algae pastes or concentrates have some potential as alternative to live algae (Brown, 2002), some studies demonstrated that algae paste only should be used to replace 50 % of live algae, because of its rapid quality loss (Hemaiswarya,

2011). In contrast, frozen microalgae have good results for *N. oculata* compared with other diets (Yamasaki*et al*., 1989), being already present in the aquaculture market (Spolaore *et al*., 2006). *N. oculata* persists to long freezing periods, having little alterations in its chemical composition (Lubzens*et al*., 1995). Additionally, freeze-dried microalgae preserve the original cell shape and texture after being frozen (Hemaiswarya, 2011), and some studies have shown high values of growth and survival of organisms fed with freeze-dried microalgae (Albentosa *et al*., 1997; Navarro, 1999; Pedro and Fernández-Díaz, 2001).

Concerning the photo-physiology of coral fragments, values of maximum quantum yield of photosystem II (F_v/F_m) were close to maximum recorded in other reported researches (Rodrigues *et al*., 2008; Winters *et al*., 2009; Rocha *et al*., 2013a, 2013c), indicating that coral fragments remained photo-physiologically healthy when fed with the tested forms of preserved microalgae. Our results do not show significant differences between treatments of feeding, neither between both PAR tested (50 and 120 µmol quanta m^{-2} s^{-1}), except in corals fragments fed with freeze-dried microalgae under a PAR of 50 µmol quanta m^{-2} s^{-1} and live microalgae under a PAR of 120 µmol quanta m^{-2} s^{-1}. However, this data does not allows a conclusion, due the fact of the comparison being between two different algae and two different PAR.

The water quality of culture tanks did not exhibit significant differences between treatments for organic matter. Nonetheless, water of tanks where frozen algae were provided to coral fragments showed the highest concentration of organic matter. Although organic matter can enhance coral growth, being an extra food source (Osinga *et al*., 2011), it can, on the other hand, lead to an increase of water turbidity, reducing light penetration and probably affecting zooxanthellae and inhibiting coral growth (Fabricius, 2005). The concentration of inorganic nutrients was higher in tanks where coral fragments were fed with live paste than in the other treatments. Phosphates and nitrates are essential nutrients for corals. However, nitrates can lead to lower growth rates, as verified by Ferrier-Pagès *et al.* (2001), where results showed a decrease of buoyant weight gain. Phosphates can also decrease the buoyant weight gain, and when together with nitrates, the reduction of growth rate is even higher (Ferrier-Pagès *et al.*, 2000). Besides, inorganic nutrients, as nitrogen and phosphates,

enhance zooxanthellae density, which can lead to a smaller transference of fixed carbon to the host (Marubini and Davies, 1996; Muscatine *et al*., 1989).

The present study demonstrated that the differences between preservation processes of microalgae and their suitability as heterotrophic feeds to corals are found essentially in water quality of culture systems. In spite of coral survival, growth and organic weight percentage are not change by the preservation of microalgae supplied to corals, freeze-dried algae was the one with less impact in the quality of experimental water. Additionally, freeze dried microalgae has the longer shelf-life and the lower associated storage costs (not requiring freezing).

The freeze-dried microalgae seems to be an acceptable food source to be employed in *in toto* aquaculture of *S.* cf. *glaucum*, since had the similar results as the other preserved microalgae and has higher conservation time, additionally, permit the maintenance of water quality.

4.2. Suitability of different microalgae species as exogenous feed

To study the suitability of different microalgae species as exogenous feed, *I. galbana*, *P. tricornutum* and *N. oculata* were used in the experiment, since these species belong to the group of algae most used in aquaculture (Spolaore *et al*., 2006). Results of coral fragments survival did not show differences as in previous experiment, nonetheless, rates of survival are even more different among themselves, as explained, it can be relate with the number of replicates, and consequently, did not allow observed differences between treatments.

Corals fragments fed with *I. galbana* had higher specific growth rate when compared with corals fed with *N. oculata.* Although not significant, there are some visible differences in SGR of coral fragments fed with higher amounts (7.33 mg L^{-1}) comparing to fragments fed with lower amounts (3.66 mg L^{-1}), being register higher growth for the amount of 3.66 mg L^{-1}, suggesting that higher amounts of feeding lead to lower growth rates. Some studies demonstrated a coral weight gain with phytoplankton supply (Roushdy and Hansen, 1961; Sorokin, 1991; Fabricius *et al.,* 1995; Leal *et al*., 2014c). Nonetheless, if excess feeding is introduced in the system, this could lead to a rapid degradation of the experimental water, affecting normal

development and growth of coral fragments (Muscatine *et al*., 1989; Marubini and Davies, 1996; Fabricius, 2005; Sella and Benayahu, 2010).

Data from organic weight did not show significant differences between treatments. However, corals fed with *I. galbana* showed the highest percentage of organic weight. Adding the results of growth rate, we can conclude that *I. galbana* promoted a higher increase of coral fragments comparing to the other two species. Contrarily, results from growth rate and organic tissue synthesis in relation to coral fragments reared with feed and coral fragments reared without feed did not show significant differences. These observations suggest that there wasn't uptake of microalgae or, in alternative, microalgae do not add nutrition value to enhance coral growth. Nonetheless, we don´t have results of ingestion to draw a conclusion. But, we know by other studies that corals uptake microalgae as feed (Migné and Davoult, 2002; Leal *et al.,* 2014c) and they also present a selective feeding (Leal *et al.,* 2014c). It is also known that ingestion of microalgae is related with the shape, size, digestibility and biochemical composition of algae (Brown, 2002).The size of food can be a limitation factor, when addressed to the upper size capture by polyps and type of tentacles and nematocysts (Houlbrèque *et al*., 2009). Additionally, nutrients in water can also influence coral growth (Ferrier-Pagès *et al.*, 2000; Ferrier-Pagès *et al.,* 2001).

The three tested algae species are composed by different PUFAs. *N. oculata* is rich in ARA and EPA, while *P. tricornutum* is more rich in EPA and *I. galbana* is rich in DHA (Phytobloom-Necton, 2013).These fatty acids are essential for marine animals as precursors of cellular membrane (Guedes *et al*., 2011; Guedes and Malcata, 2012). *I. galbana* composition of protein and carbohydrate is richer than *P. tricornutum* (Albentosa *et al*., 1997).The three supplied algae have the same spherical shape, but different sizes: *N. oculata* has 2 - 4 µm, *I. galbana* has 3 - 6 µm, and *P. tricornutum* has 5 - 12µm (Phytobloom-Necton, 2013). Consequently, it is possible that our results are related with preferences of size of prey by *Sarcophyton* cf. *glaucum.*

The values of maximum quantum yield of photosystem II (F_v/F_m) were close to maximum recorded in other reported researches (Rodrigues *et al*., 2008; Winters *et al*., 2009; Rocha *et al*., 2013a, 2013c), and as explained in first experiment, they indicate that coral fragments remained photo-physiologically healthy. Photosynthetic efficiency was higher in coral fragments fed with lower amount of feed and corals fed

with 3.66 mg L^{-1} of *I. galbana* were significant higher that corals fed with 7.33 mg L^{-1} of *P. tricornutum*, which enhance the good physiologic condition of fragments fed with lower amounts.

Experimental water of the treatments where *I. galbana* and *P. tricornutum* were supplied had less nitrates and phosphates than treatments where *N. oculata* was supplied. These nutrients are important components of nutrition of soft corals, but also can decrease of buoyant weight gain (Ferrier-Pagès *et al.*, 2000; Ferrier-Pagès *et al.,* 2001). As mentional before, frequent feeding or supply large amounts of feed could lead to higher values of mortality (Sella and Benayahu, 2010).

I.galbana seems to be an adequate option as feeding supply for *Sarcophyton* cf. *glaucum*, since it was the microalgae inducing higher specific growth rate and higher percentage of organic weight in the coral fragments; and did not induce an accumulation of nutrients in the experimental water.

5. Conclusion

The suitability of the three preserved microalgae as heterotrophic feeding for corals was similar and differences are essential in water quality of tanks. The preservation processes of microalgae do not affect its suitability as heterotrophic feeding for the soft coral *Sarcophyton* cf. *glaucum*. Freeze-dried microalgae can be employed as feed instead of live microalgae, since it has the higher shell-life and the lower associated storage costs (not requiring freezing). Additionally, freeze-dried microalgae does not affect the water quality.

Isochrysis galbana promoted higher growth rates and seems to be an adequate feed for *Sarcophyton* cf. *glaucum*, since it was the microalgae promoting the higher specific growth rate and percentage of organic weight in the coral fragments, also does not affect the water quality. Feeding amount and frequency should be considered as an important issue in mixotrophic corals aquaculture *ex situ*.

In future studies, it should be investigated if different PUFAs have a relation with coral uptake of nutrients and coral growth rate. Additionally, should be tested blends of different species of microalgae in the *ex situ* culture of mixotrophic corals.

Experiments should also focus on feeding frequency and feeding amounts, digestibility and assimilation of microalgae species.

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