Universidade de Aveiro
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Pedro Miguel da Silva Soares Pinto de Seabra

Vantagens da utilização de dietas microencapsuladas no cultivo *ex situ* da espécie *Montipora digitata*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha Aplicada, realizada sob a orientação científica do Doutor Rui Jorge Miranda Rocha (Investigador Auxiliar do Departamento de Biologia e CESAM, Universidade de Aveiro) e da Doutora Andreia do Carmo Martins Rodrigues (Investigadora em Pós-Doutoramento do Departamento de Biologia e CESAM, Universidade de Aveiro).

Dedico este trabalho às minhas duas avós por tudo o que sempre fizeram por mim.

o júri

Presidente	Prof. Doutora Maria Marina Pais Ribeiro da Cunha Professora associada, Departamento de Biologia, Universidade de Aveiro
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Agradecimentos

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palavras-chave

Aquacultura; Dietas microencapsuladas; Crescimento; Reservas Energéticas; *Montipora digitata*

resumo

Os recifes de coral encontram-se sob forte ameaça devido às alterações climáticas globais, podendo perder irremediavelmente a sua biodiversidade num futuro próximo. Para além dos fatores ambientais, a crescente procura destes organismos para fins ornamentais e de investigação científica coloca ainda mais pressão sobre estes ecossistemas já fragilizados. A aquacultura de corais surge como uma potencial solução para a elevada procura de corais duros e moles, mas necessita de estudos para que seja otimizada e rentável. Neste estudo foram avaliadas as vantagens da utilização de dietas microencapsuladas no cultivo *ex-situ* da espécie de coral duro *Montipora digitata* (Subclasse Hexacorallia; Ordem Scleractinia). Os parâmetros avaliados foram a sobrevivência, o crescimento, o consumo de energia, as reservas energéticas e a fotobiologia dos corais. A partir de uma colónia mãe foram obtidos 80 fragmentos, posteriormente colocados em aclimatização sem adição de dietas microencapsuladas durante 5 meses. Após o período de aclimatização foram divididos por quatro aquários com diferentes dietas: controlo (dieta autotrófica), dieta microencapsulada à base de fitoplâncton, dieta microencapsulada à base de peixe e lula e dieta microencapsulada à base de *Artemia*. Os corais foram alimentados duas vezes por semana (0,585 mg/L) durante 80 dias. Durante toda a experiência não ocorreu mortalidade. Os corais alimentados com a dieta microencapsulada à base de peixe e lula demonstraram os melhores resultados em termos de crescimento. Relativamente aos parâmetros energéticos, os corais cultivados com dieta autotrófica mostraram os melhores resultados. De uma forma geral, as dietas microencapsuladas mostraram ser adequadas e vantajosas para o cultivo desta espécie, à exceção da dieta microencapsulada à base de *Artemia*.

keywords

Aquaculture; Microencapsulated diets; Growth; Energy Reserves; *Montipora digitata*

abstract

Coral reefs are under serious threat due to global climate change. In addition to the negative impact of several environmental factors, the marine aquarium trade for ornamental and research purposes causes even more pressure on these ecosystems. *Ex-situ* aquaculture rises as a potential solution for the high coral demand but still needs more studies to improve its production and quality. In this study we evaluated the advantages of microencapsulated diets in the *ex-situ* aquaculture of *Montipora digitata* (Subclass Hexacorallia; Order Scleractinia). We evaluated the microencapsulated diets' effect on survival, growth, energy consumption, energy reserves and photobiology. After fragmentation of a single colony, 80 fragments were obtained and acclimated for 5 months in an autotrophic diet regime. After that we distributed the coral fragments in four treatments: control (autotrophic diet), phytoplankton microencapsulated diet, fish and squid meal microencapsulated diet and *Artemia* microencapsulated diet. Corals were fed twice per week (0.585 mg/L) for 80 days. After the experiment no coral mortality was registered. The corals fed with fish and squid meal microencapsulated diet showed the best results in growth. Regarding energy reserves and energy consumption, the non-fed corals showed the best results. All microencapsulated diets showed positive results on coral growth, except the *Artemia* microencapsulated diet.

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1. Introduction

Corals are marine invertebrates from the phylum Cnidaria, class Anthozoa. These animals are colonial, living in a symbiotic relationship with other organisms and forming compact colonies of numerous individual polyps. They are commonly divided in two major informal groups: the hard corals (Subclass Hexacorallia, Order Scleractinia) and the soft corals (Subclass Octocorallia, Order Alcyonacea). Scleractinia corals form their skeleton by calcium carbonate precipitation, being responsible for the formation of the coral reefs. However, the octocorals have no skeleton, forming only tiny calcium carbonate spicules which give shape to the colony (Goldberg and Benayahu, 1987). Several coral species also live in a symbiotic relationship with unicellular algae called zooxanthellae, which are dinoflagellates of genus *Symbiodinium.* The coral provides protection and contributes with nutrients and carbon dioxide that the zooxanthellae need for photosynthesis. The zooxanthellae retribute with several carbon compounds derived from photosynthesis as well as amino acids and fatty acids, providing up to 90% of the host's nutritional requirements (Muscatine and Porter, 1977; Berkelmans and Oppen, 2006).

In terms of their feeding habits, corals that live in symbiotic relationship with zooxanthellae are considered mixotrophic. Due to the presence of these unicellular organisms corals get a very considerable amount of their food by photosynthesis of the zooxanthellae and can complement this by filter feeding mainly on zooplankton or particle organic matter (POM) (Fox *et al*., 2019).

Corals are also capable of reproducing sexually and asexually. Sexually, corals can be hermaphrodites or have separated sexes. In many tropical reefs, spawning occurs as a mass synchronized event, when corals release both eggs and sperm to the water column. Asexually, corals can reproduce in three different ways: fragmentation, fission and polyp bail-out to generate new offspring of identical genetics (clones) (Fossa and Nilsen, 1998).

Tropical coral reefs are among the most stunning and spectacular ecosystems on Earth. Unparalleled in their biological and habitat diversity, coral reefs are key ecosystems not only because of their role on global marine ecology, but also due to the major importance of their economic, social and cultural services, especially for

maritime tropical and subtropical nations (Hughes et al., 2003). These coastal areas depend heavily on coral reefs' goods and services to survive and continue to develop their economy. In addition to the huge amount of income they provide to tropical and subtropical nations' economy obtained by fishing, mining and tourism, coral reefs also protect these countries' beaches and shorelines from the erosive and destructive power of oceanic waves.

Despite the importance and crucial provision of food and resources for over 500 million people, coral reefs are in decline and under serious threat (Hoegh-Guldberg, 2011). This threat is a result of a combination of local and global factors that together contribute to acute and long-term stresses to the coral communities. Some of these stresses and their intensity were never experienced by these organisms in hundreds of thousands of years of existence on our planet (Hoegh-Guldberg *et al.*, 2007).

Among the most threatening global stresses, are the rapid climate change and ocean acidification, responsible for the growing intensity and frequency of local stresses and mass coral bleaching events (Hughes *et al.*, 2003; Hoegh-Guldberg *et al.*, 2007). Ultimately, these two severe global stresses have been caused by the increasing concentration of carbon dioxide in Earth's atmosphere. During the 20th century, the rapidly increasing atmospheric CO_2 concentrations led to an increase in the global oceans' average temperature by 0.74°C and sea level by 17 cm as well as a decrease of seawater carbonate concentrations by approximately 30 μmol kg^{-1} seawater and acidity by 0.1 pH units (IPCC, 2007).

In addition to the global warming threat, the international marine aquarium trade has significantly increased in this century and is now a serious threat to the biodiversity of ornamental species in which corals are inserted, since the majority of the specimens involved in the trade are caught in the wild (Rhyne *et al.*, 2014). The demand for coral specimens is rising significantly due to their ornamental value and more recently for the bioprospecting of marine bioactive compounds with therapeutical properties (Rocha *et al.*, 2011).

To solve this high demand for coral supply ex-situ aquaculture has risen as a potential solution. In comparison with in-situ marine culture of corals, the ex-situ aquaculture method has several advantages. Despite being more expensive, this method ensures the manipulation of biotic and abiotic factors that can maximize coral growth

and promote a better environment for recovery after fragmentation (Leal *et al.*, 2014). In order to develop better methods and improve ex-situ aquaculture of tropical coral species several studies have been released in the past decade (Rocha *et al.*, 2013a, 2013b; Costa *et al*., 2016). However, more studies are needed to fully understand the optimal settings for coral growth in ex situ aquaculture systems, considering the high variability in corals biodiversity, which implicates different conditions for different species.

One of the most important factors to ensure coral growth is the diet. Corals are known to be capable of heterotrophic feeding to find a supplement of nutrients to the photosynthates given by the zooxanthellae, so it can be hard to find diets that suit these organisms needs in an ex-situ type of system.

Microencapsulated diets have been used to feed aquatic filter feeders since the 1970s (Knauer and Southgate, 1999). These artificial diets offer the possibility to put together the nutritional requirements of a target species on a digestible micro capsule, helping to solve long lasting problems in *ex-situ* aquaculture such as food supply, diseases and product quality (Willer and Aldridge, 2019). There are several processes to combine core and shell materials into a micro capsule, namely atomization, spray coating and coextrusion (Oxley, 2014). Each process offers methods to produce unique encapsulated diets with different advantages to the target species. Since the majority of tropical corals are known to be filter feeders and mixotrophic, microencapsulated diets could be used to fulfil coral requirements and allow the development of coral *ex-situ* aquaculture.

In this thesis, we evaluated the usage of microencapsulated diets on the ex-situ aquaculture of *Montipora digitata*, a Scleractinia coral with high economic value and very popular in the aquarium trade. We aimed to provide better understanding of the advantages of microencapsulated diets in the growth of hard corals in a recirculated aquaculture system by evaluating the diet effects on survival, growth, metabolism and photobiology of *M. digitata* after fragmentation of husbandry colonies.

2. Material and Methods

2.1. Experimental system configuration

For the acclimatization of the *M. digitata* fragments and feeding trials performed in this thesis four experimental recirculated aquaculture systems (RAS) were used. Each system was composed by one main tank with capacity of 240 litres (150 cm × 40 cm × 40 cm), a filtration sump with capacity of 180 litres (80 cm × 45 cm × 50 cm) and an osmosis tank with capacity of 54 litres (30 cm × 30 cm × 60 cm). The water circulation inside the main tank was provided by two circulation pumps (Turbelle nanostream 6055 Tunze, Germany), installed on each side of the tank and a T5 fluorescent light (Sea REEF-SPEC, 80W) was collocated above the aquarium with one red light and one actinic light.

The filtration system was installed in the sump and was composed of five components: 1) a skimmer (Deltec SC 500) to remove the dissolved inorganic compounds from the water, 2) active carbon which does chemical filtration by adsorbing organic compounds and other compounds that can negatively affect the water quality, 3) bio-balls which make biological filtration through nitrifying bacteria, oxidizing ammonia and nitrites dissolved in the water and 4) a Kalkwasser reactor (Kalkwassermischer km 500) used to maintain the value of water pH and supplement calcium, which are connected to 5) the osmosis water replacement system (Deltec aquastat 1001).

The tank water enters in the sump and passes by the skimmer and two thermostats (Eheim Jagger 300 W) which control the water temperature. After that, it passes by the active carbon and bio-balls. Next, the water is pumped using a pump (EHEIM universal 1200 l/min) into the Ultraviolet (UV) filtration (Vecton 600, TMC), which leads to a sterilization process of the water. In the end, the water moves from the UV filter to the chiller (Hailea Model: HC-500 A), previously set for 25 °C, and then goes back to the aquarium by another pump (EHEIM universal 2400 l/min). In addition to this circuit, an osmosis water replacement system was set to regulate the water level and salinity. When water evaporation occurs in the main tank the osmosis sensor activates the osmosis water pump (Deltec aquastat 1001) which replaces osmosis water into the system maintaining water salinity.

All systems operated with synthetic seawater. The synthetic seawater was obtained by mixing purified osmosis water with synthetic salt (Coral PRO salt, Red Sea). The osmosis water was obtained by the circulation of freshwater through a reverse osmosis filtration system (V2 Pure 360, TMC).

2.2. Coral husbandry and fragmentation

The *M. digitata* husbandry colonies were obtained from the collection of marine invertebrates held at Ecomare, University of Aveiro. The selected colonies were fragmented using a sterilized cuticle scissors and immediately glued to a small marble base (Figure 1). A total of 80 fragments of 1 ± 0.05 cm were obtained, labelled and transferred to the experimental tanks.

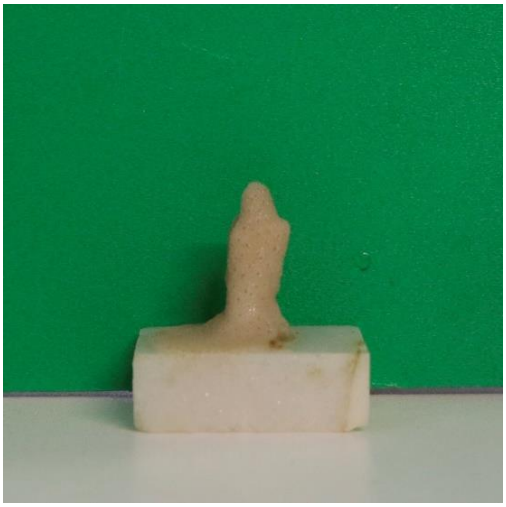

Figure 1 - Coral fragment of *M. digitata*.

2.3. Experimental design

After fragmentation the fragments were all transferred to the same experimental tank to acclimate before the feeding trials. The acclimatation period was supposed to be 3 months but due to the Covid pandemic and quarantine established in Portugal the coral fragments ended up in this acclimatation stage for 5 months. After this stage the fragments were

weighted in a precision scale and distributed in the 4 experimental tanks for the feeding trials (n=20). Three different microencapsulated diets, formulated by Sparos (Olhão, Portugal) were tested: a diet of phytoplankton, a diet of squid and fish meal and a diet of zooplankton, in this case *Artemia* sp.. In the control tank, no diet was given to the fragments, in order to test differences in growth, photobiology and energy allocation comparing autotrophic feeding and mixotrophic feeding. The coral fragments were fed twice per week (0,585 mg/L) during a total period of 80 days. After the feeding experiment, the corals were sampled to determine survival, growth (by weight, vertical length and base area), *in vivo* chlorophyll fluorescence and biochemical biomarkers of cellular energy allocation.

2.4. Growth measurements

In order to measure growth in coral fragments after the feeding period 3 types of biometrics were sampled before and after the experiment: weight (W_0 and W_f), vertical length (L_0 and L_f) and base area (A_0 and A_f). The specific growth rate (SGR) was calculated as: $SGR\ (\%growth\ per\ day) = \frac{\ln W_f - \ln W_0}{\Delta t} \times 100$ (Rocha *et al.*, 2013a; Costa *et al.*, 2016) The SGR was calculated for all growth measurements.

Vertical length and base area measurements were obtained by photography analysis using the software ImageJ. The initial vertical length (L_0) was considered to be 1 cm, as described before in the fragmentation process.

2.5. In vivo chlorophyll fluorescence

Chlorophyll fluorescence was measured *in vivo* using a pulse amplitude modulation (PAM) fluorometer, after the feeding trials. The coral fragments were acclimated in a dark room for 15 to 20 minutes before the measurements in order to have a full activation of the zooxanthellae photosynthetic system (Rocha *et al*., 2013a). After the acclimatation, a saturation pulse was applied for 0,8 seconds to determine the minimum level fluorescence (F_0) and the maximum fluorescence (F_m). To evaluate the photosynthetic efficiency of each

sample, the maximum quantum yield (F_v/F_m) of photosystem II (PSII) was calculated using the formula: $\frac{F_v}{F_m} = \frac{F_m - F_o}{F_m}$.

2.6. Biochemical biomarkers of cellular energy allocation

For the biochemical biomarkers analysis, *M.* digitata fragments were removed from the respective basis, frozen with liquid nitrogen and stored in a freezer with temperature of -80°C. At the laboratory the fragments were macerated, homogenized in 1000 µL of ultra-pure water on ice. After this procedure three aliquots were taken from each sample to measure the contents of sugars, lipids and proteins as well as the electron transport system (ETS) activity. The available energy (Ea) was calculated as: Ea = sugar + lipid + protein (mJ/mg of coral tissue). The energy consumption (Ec) was estimated by the ETS activity, cellular energy allocation (CEA) was calculated as: CEA = Ea/Ec.

The extraction and quantification of all energy biomarkers (sugars, lipids, proteins, ETS activity) was made following the methods of De Coen and Janssen (1997) with minor modifications described by Rodrigues *et al* (2015). All energy biomarkers measurements were determined spectrophotometrically, using the microplate reader MultiSkan Spectrum (Thermo Fisher Scientific, USA). Total lipid content was determined by measuring the absorbance at 375 nm with tripalmitin as a standard. Total protein content was determined by measuring the absorbance at 520 nm using bovine serum albumin as a standard. Total carbohydrate content was determined by measuring the absorbance at 492 nm using glucose as a standard. To determine the ETS activity, the absorbance was measured kinetically at 490 nm over a 3 minutes period (Rodrigues *et al*., 2015). The conversion into energetic values of the fractions of energy available was calculated using the corresponding energy of combustion: 39500 mJ/mg lipid, 17500 mJ/mg glycogen, 24000 mJ/mg protein (De Coen and Janssen, 1997).

2.7. Statistical analysis

The data and graphical illustrations of this thesis were obtained using different softwares. ImageJ (version 1.53) was used to analyse coral photographs and obtain base area and vertical length data. GraphPad Prism (version 9.2.0) was used to report specific growth rate data in graphical illustrations. IBM SPSS Statistics was used for all statistical tests. A test of data normality was performed for all datasets. When data normality wasn't achieved, a Kruskal-Wallis test was conducted. When data normality was achieved, a one-way ANOVA test was conducted with a Dunnet post hoc test to evaluate differences between groups for growth (in weight, base area and vertical length), photosynthetic efficiency and cellular energy allocation.

3. Results

3.1. Survival and growth

After the feeding trials, the corals were visually examined to check for coral mortality. In this experiment, out of 80 total fragments, none of the coral fragments was dead, so a survival percentage of 100% was accomplished. However, coral fragments showed high variability in terms of growth in weight (Figure 2). In the control tank corals showed, on average, a SGR of 0.021% per day, while the corals fed with phytoplankton diet and fish and squid meal diet showed, on average, SGR values of 0.022% and 0.023% per day, respectively. The corals fed with zooplankton diet showed the worst results with a SGR of 0.018% per day. No significant differences were found in terms of specific growth rate in weight (H=2.078; p=0.412). The results of weight measurements suggest that there was no significant effect of the microencapsulated diets on coral growth.

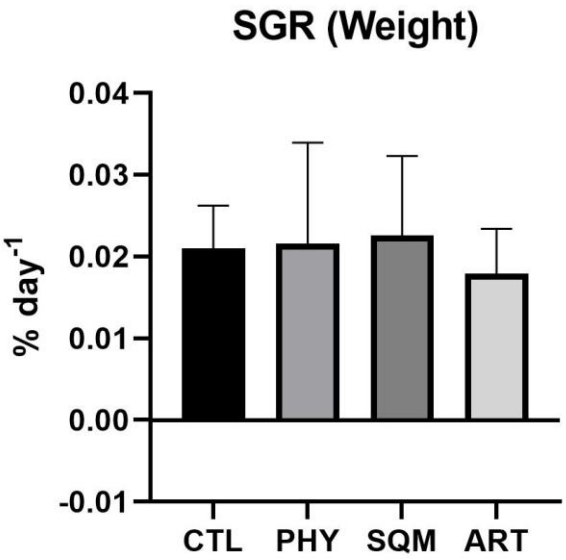

Figure 2 - Graphical illustration of the specific growth rate in weight (% day^{-1}, mean ± SD, n=20) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

Regarding base area growth, the results were similar in terms of the microencapsulated diets' effect on coral growth (Figure 3). Coral fragments showed an average specific growth rate in base area of 2.524% per day on autotrophic regime. Under the phytoplankton diet coral fragments showed a SGR of 2.202% per day and 2.421% per day under the fish and squid meal diet. Corals fed with *Artemia* diet showed a SGR of, on average, 2.362% per day. The Kruskal-Wallis test performed on SGR in base area data indicated that no significant differences were found between corals with autotrophic and mixotrophic feeding (H=5.471; p=0.140).

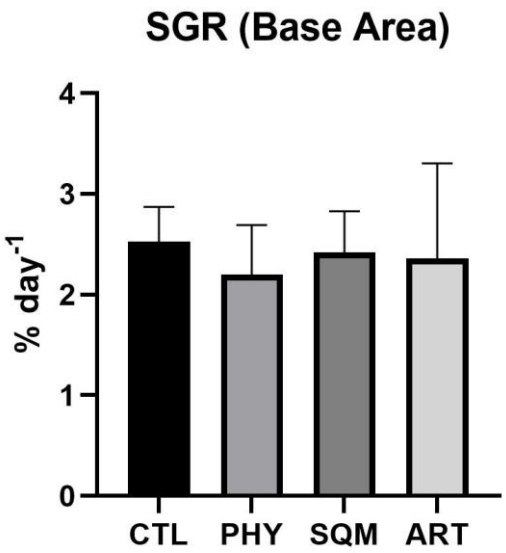

Figure 3 - Graphical illustration of the specific growth rate in base area (% day^{-1}, mean ± SD, n=20) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

In terms of vertical length, the coral fragments showed different rates according to each diet (Figure 4). On the autotrophic diet tank coral fragments showed, on average, a SGR of 0.853% per day. On the phytoplankton diet tank coral fragments showed, on average, a SGR of 0.751% per day. The corals fed with fish and squid meal diet showed the best results in vertical length growth with an average of SGR values of 0.886% per day. On the *Artemia* diet tank, corals showed the worst results with a SGR of 0.742% per day, on average. ANOVA results suggest that there are significant differences between non-fed and fed corals, regarding the specific growth rate in vertical length (F(3, 76)=3.211;

p=0.028). The post hoc Dunnett test didn't find significant differences when comparing corals from each diet to the control group (autotrophic diet).

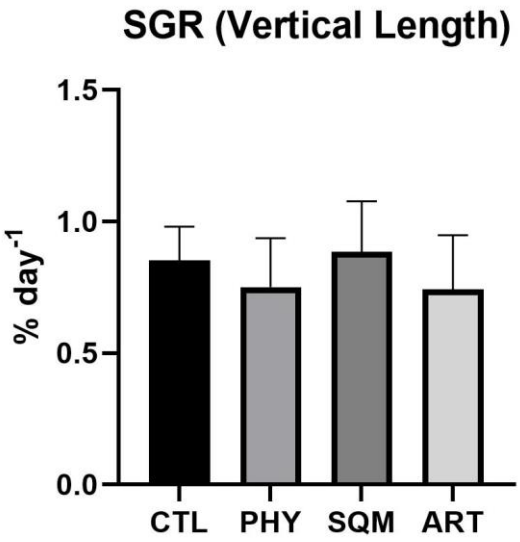

Figure 4 - Graphical illustration of the specific growth rate in vertical length (% day^{-1}, mean ± SD, n=20) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

3.2. *In vivo* chlorophyll fluorescence

Photosynthetic efficiency was measured as the maximum quantum yield of photosystem II (PSII) on every coral fragment (Figure 5). Results showed significant differences related to the type of diet of the coral fragments (H=12.930; p=0.005). The coral fragments that grew under autotrophic diet showed an average ratio Fv/Fm of 0.588. On the phytoplankton diet tank corals showed, on average, a ratio of efficiency of 0.586. The coral fragments that grew under fish and squid meal diet showed, on average, a ratio of 0.576. The best results in terms of photosynthetic efficiency were obtained by the coral fragments of the *Artemia* diet tank with an average ratio of 0.601.

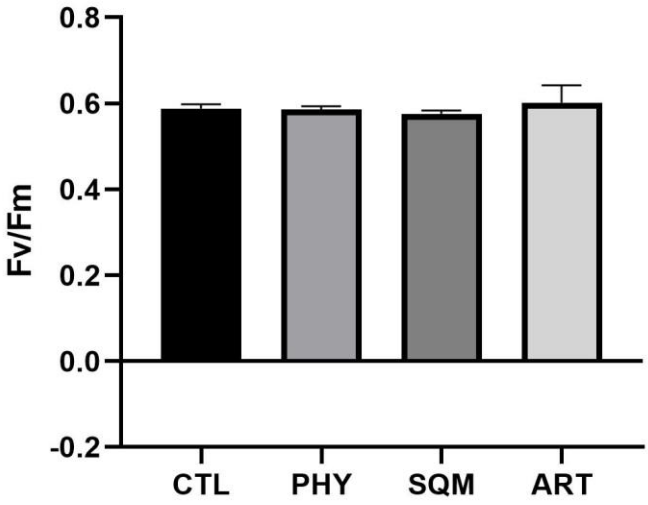

Figure 5 - Graphical illustration of the photosynthetic efficiency (Fv/Fm, mean ± SD, n=20) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

3.3. Biochemical biomarkers of cellular energy allocation

3.3.1. Sugars

Regarding the concentration of sugars in the coral samples, the results showed no statistically significant differences between treatments ($F(3, 24)=0.068$; $p=0.976$). All treatments showed similar amounts of sugars per mg of coral tissue (Figure 6).

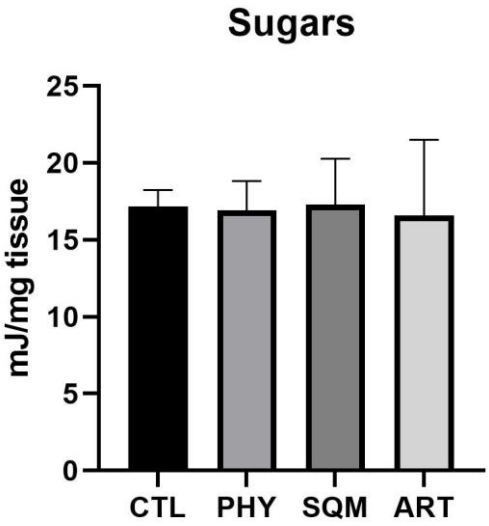

Figure 6 - Graphical illustration of the sugar's concentrations (mJ/ mg coral tissue, mean ± SD, n=7) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

3.3.2. Lipids

The lipids' concentration was not significantly different ($F(3, 24)=1.482$; $p=0.245$) among the different treatments but showed some variation (Figure 7). The corals fed with the *Artemia* microencapsulated diet showed the lowest mean concentration of lipids and the corals with no diet supplement showed the highest mean concentration of lipids.

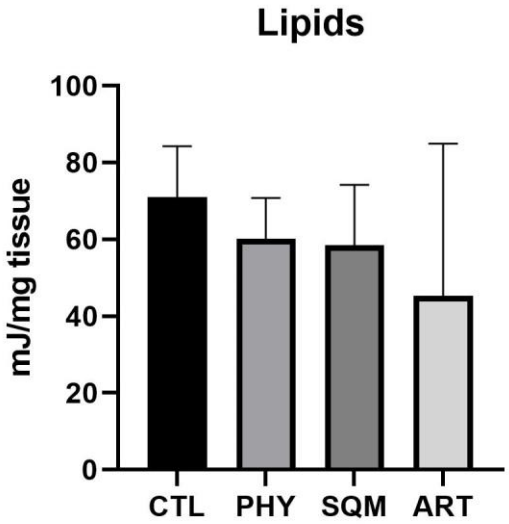

Figure 7 - Graphical illustration of the lipid's concentrations (mJ/ mg coral tissue, mean ± SD, n=7) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

3.3.3. Proteins

Regarding the proteins' concentration, the results showed a significant variation between autotrophic and mixotrophic diets ($F(3, 24)=7.930$; $p=0.0008$) (Figure 8). The corals fed with *Artemia* diet showed a big depletion in proteins' concentration, as the rest of the treatments showed similar concentrations, with the corals from the tank provided with phytoplankton diet being the ones with the best results. The post hoc Dunnett test found significative differences only for the corals fed with *Artemia* microencapsulated diet, when comparing with the non-fed corals ($p=0.002$).

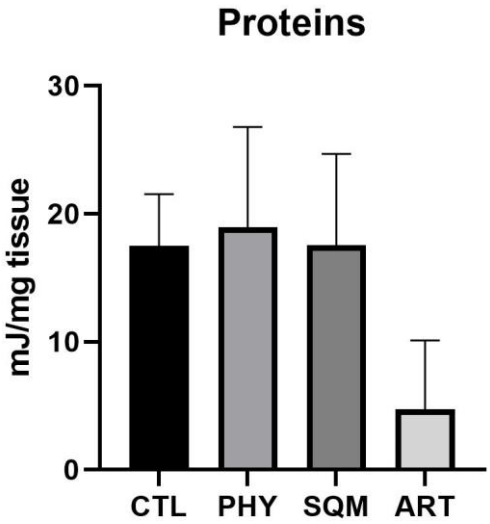

Figure 8 - Graphical illustration of the protein's concentrations (mJ/ mg coral tissue, mean ± SD, n=7) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

3.3.4. Energy consumption

The energy consumption was estimated by the electron system (ETS) activity. The results showed a significant variation between the non-fed corals and the fed corals ($F(3, 24)=5.674$; $p=0.0044$). The corals fed with fish and squid meal microencapsulated diet showed the highest mean energy consumption and the corals fed with *Artemia* microencapsulated diet showed the lowest mean energy consumption. The post hoc Dunnett test showed significant differences only for the corals fed with *Artemia* microencapsulated diet, when comparing with the non-fed corals ($p=0.004$).

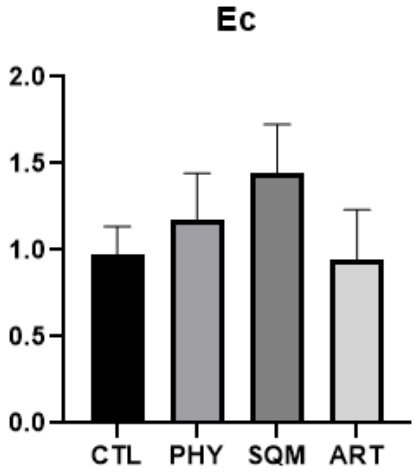

Figure 9 - Graphical illustration of the energy consumption (mJ/ h/ mg coral tissue, mean ± SD, n=7) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

3.3.5. Cellular energy allocation

The cellular energy allocation was obtained by the ratio between the available energy and the energy consumed. The results showed a significant variation between the coral fed by autotrophic diet and mixotrophic diet (H=13.09; p=0.0044). The corals fed with Artemia diet showed high variable results, as the non-fed corals (autotrophic diet) showed the better results for cellular energy allocation (Figure 9).

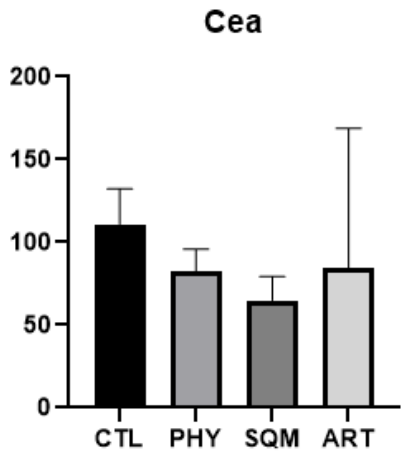

Figure 10 - Graphical illustration of the cellular energy allocation (Energy available/ Energy consumption, mean ± SD, n=7) for the four treatments: CTL – no food added; PHY – phytoplankton microencapsulated diet; SQM – fish and squid meal microencapsulated diet; ART – *Artemia* microencapsulated diet.

4. Discussion

The corals fed with fish and squid meal microencapsulated diet showed the best results in growth measurements, possibly indicating that this could be a suitable microencapsulated diet for this species in terms of growth promotion. However, these results must be addressed critically and with caution, since only vertical length growth showed statistically relevant differences between the coral fragments. Further research and longer duration trials are important to confirm these results, since corals are slow growing organisms (Costa *et al.,* 2019). The corals fed with phytoplankton microencapsulated diet also showed good results in all growth measurements, especially in weight growth, but no statistically relevant differences were found. Conlan *et al.* (2019) also found better results in growth and lipids profile with phytoplankton and autotrophic diets for *Acropora* species, indicating that Scleratinia corals seem to perform better in treatments close to the natural environment conditions. The corals fed with *Artemia* microencapsulated diet showed the less positive results in growth, suggesting that it may not be a suitable food supply in the culture of *Montipora digitata* fragments. Costa *et al* (2016) also reported a negative effect of live zooplankton food supply (rotifers) in the growth rate of *ex-situ* culture of soft coral *Sarcophyton cf. glaucum*. It's also worth mentioning that the non-fed corals (autotrophic diet) showed good growth rates, indicating that autotrophy plays a very important role in the nutrition process of *Montipora digitata*.

Regarding photosynthetic efficiency, corals cultivated under all treatments showed healthy results. Despite the statistically significant differences found in between the non-fed and fed corals, the results showed Fv/Fm ratios that are considered within the healthy range for corals that weren't exposed to stress and acclimated in a dark room for 15 minutes (Hoegh-Guldberg and Jones, 1999).

The biomarkers of cellular energy allocation were measured to have an insight on the metabolic condition of the coral fragments and investigate the effect of mixotrophy in their energy reserves. Lipids and sugars concentrations showed no statistically relevant differences between non-fed and fed corals. Since corals get sugars from zooxanthellae activity (Smith *et al.,* 2005), sugars' concentrations were expected to be similar, due to the healthy values of Fv/Fm for non-fed and fed corals. On the contrary, proteins' concentration was significantly different between non-fed and fed corals, with the corals

fed with *Artemia* microencapsulated diet showing very low concentrations of proteins per mg of coral tissue. This also indicates that the coral fragments reared with a zooplankton microencapsulated diet were not in optimum health conditions. The cellular energy allocation was calculated as the ratio between energy available and energy consumed. The results showed a significant effect between treatments and the autotrophic diet was the one that promoted better energetic balance in the fragments of *Montipora digitata*. Although the non-fed corals showed the best results in cellular energy allocation, corals fed with both phytoplankton and fish and squid meal microencapsulated diets showed positive results on this matter.

Microencapsulated diets have been widely used in aquaculture to promote growth and survival of bivalve shellfish and planktonic stages of fish species (Yúfera *et al.,* 1999; Willer and Aldridge, 2019), but information about its use on coral species is scarce (Costa *et al.,* 2019). In this study, different microencapsulated diets available as food were translated into differences on *Montipora digitata* fragments' growth rates, which may suggest that there are species-specific requirements that need to be considered in order to improve the *ex-situ* aquaculture of tropical mixotrophic corals.

The general conditions of this experiment (water temperature, salinity, light spectrum) proved to be adequate for the ex-situ aquaculture of this species since it was accomplished a 100% survival rate during the 5 months acclimatation stage and the 80 days period of the feeding experiment.

5. Conclusions

Considering all the data obtained in this study, the results showed a positive effect of microencapsulated diets food supply to *Montipora digitata* fragments. Both phytoplankton and fish and squid meal microencapsulated diets seem suitable to this species, as the *Artemia* diet showed not so positive results. Corals fed with fish and squid meal microencapsulated diet showed indications of better specific growth rate in weight and vertical length, although no statistically relevant differences were found, as well as healthy Fv/Fm values and balanced energetic concentrations. Non-fed corals also showed good results, indicating that autotrophic diet is very important for Scleratinia corals. Due to the lack of studies using microencapsulated diets on tropical corals, the results of this work must be addressed carefully and critically.

In order to standardise laboratory conditions, compare investigation studies and develop the feeding methodology more studies on these subjects must be done, both with hard and soft corals.

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