

**Effect of enriched substrate on the growth of the sea  
cucumber *Holothuria arguinensis* (Koehler & Vaney,  
1906) juveniles**

Mestrado em Aquacultura

Tiago Xavier Gonçalves Rodrigues

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Dissertação sob a orientação de:

Professora Doutora Ana Margarida Violante Pombo

Doutor Pedro Miguel Félix

Setembro de 2022

## **Originalidade e Direitos de Autor**

A presente dissertação de projeto é original, elaborada unicamente para este fim, tendo sido devidamente citados todos os autores cujos estudos e publicações contribuíram para a elaborar.

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## Resumo

Na Ásia, uma pressão excessiva por parte do mercado de pepinos do mar resultou na exploração intensiva e captura destes organismos, levando à depleção de muitas espécies. A redução da grande maioria de pepinos do mar na Ásia levou a uma procura de espécies provenientes de outras áreas fora do oceano Pacífico, por exemplo no oceano Índico e também, na Europa, no mar mediterrâneo e oceano Atlântico. De modo a salvaguardar a sobre exploração de pepinos do mar, diversos estudos têm sido efetuados, desenvolvendo técnicas para a sua produção, preservando a biodiversidade e valorizando os produtos europeus no mercado. Uma das espécies que apresenta um elevado valor comercial é a *Holothuria arguinensis*. Tem vindo a ser bastante valorizada no mercado, levando a diversos estudos com objetivo de produção. Até hoje, estudos relacionados com a alimentação e crescimento deste pepino do mar em cativeiro têm resultado em crescimentos lentos e heterogéneos, e por isso este estudo tem como foco, a avaliação do crescimento de juvenis de *H. arguinensis* através do enriquecimento do substrato – mistura direta de *Sargassum vulgare* com o sedimento. Foram usados sistemas RAS para o estudo do crescimento de *H. arguinensis* de diferentes classes de tamanho e durante 4 meses foi avaliado o seu crescimento, alimentados com e sem enriquecimento de *S. vulgare* no sedimento. Os indivíduos de uma das réplicas usadas para testar o efeito da adição de enriquecimento demonstrou o maior crescimento em todas as classes com uma diferença entre peso inicial e peso final de 14,6; 10,98 e 6,63 g, nas classes 1, 2 e 3, respetivamente, assim como um maior comprimento: 3,35; 3,75 e 5,2 cm pela mesma ordem de classes. Em relação ao controlo (sem enriquecimento), os pepinos do mar sujeitos a enriquecimento apresentaram ainda uma maior taxa de crescimento específico em todas as classes (0,59; 0,83; 1,13%/dia) e também a maior taxa de comprimento corporal diária (0,368; 0,546; 1,180%/cm/dia) correspondente às classes 1, 2 e 3. Os resultados obtidos neste estudo mostraram um crescimento favorável dos pepinos do mar quando na presença de um substrato enriquecido com a alga testada, sendo um avanço no que trata à alimentação de pepinos do mar em cativeiro e também à forma com que esta é feita.

**Palavras-chave:** holothuroidea, equinoderme, aquacultura, *Sargassum*, dieta, otimização de crescimento, cativeiro.

## Abstract

In Asia, excessive market pressure for sea cucumbers has resulted in the intensive exploitation and capture of these organisms, leading to the depletion of many species. The reduction of the vast majority of sea cucumbers with commercial interest in Asia has led to a demand for species from other areas outside the Pacific Ocean, for example in the Indian Ocean, and also in Europe in the Mediterranean Sea and Atlantic Ocean. In order to safeguard the overexploitation of sea cucumbers, several studies have been carried out, developing techniques for their production, preserving biodiversity and adding value to European products on the market. One of the species that presents a high commercial value is *Holothuria arguinensis*. Its increasing market value, has been leading to several studies aimed at production. However, up until this date, studies related to the feeding and growth of this sea cucumber in captivity have resulted in slow and heterogeneous growth, and therefore this study focuses on the evaluation of the growth of juveniles of *H. arguinensis* through substrate enrichment – direct mixing of *Sargassum vulgare* with sediment. RAS systems were used to study the growth of *H. arguinensis* of different size classes and for 4 months their growth was evaluated, fed with and without *S. vulgare* enrichment in the sediment. The individuals from one of the replicates used to test the effect of the enrichment addition showed the highest growth in all size-classes with a difference between initial and final weight of 14.6; 10.98 and 6.63 g, in classes 1, 2 and 3, respectively, as well as a greater length: 3.35; 3.75 and 5.2 cm in the same order of classes. Compared to the control (no enrichment), sea cucumbers fed with enriched sediment also showed the highest specific growth rate in all classes (0.59; 0.83; 1.13%/day) and also the highest daily body length rate (0.368; 0.546; 1.180%/cm/day) corresponding to classes 1, 2 and 3. The results obtained in this study showed a favourable growth of sea cucumbers when in the presence of a substrate enriched with the tested algae, representing an advance concerning the feeding of sea cucumbers in captivity and also the way in which it is done.

**Keywords:** holothuroidea, echinoderm, aquaculture, *Sargassum*, diet, growth optimization, captivity.



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# 1. Introduction

The constant need for resources to keep pace with the global growing population, which leads to a vast demand for nutritional parameters, has been, and will always be, one of the priorities of humankind. The global population is estimated to grow from 7.6 to 9.8 billion by 2050, which will, assuredly, increase the demand for high-quality protein food (Engle et al., 2017). This fact highlights the need to develop the ever-expanding aquaculture sector, which can sustain some future protein deficiencies (Yarnold et al., 2019). Aquaculture has been seen as the fastest-growing animal industry, worldwide. This accelerated growth is reflected by the fact that its contribution to aquatic supplies has increased from 1 million tonnes *per* year in the early 1950s, to 178 million tonnes in 2020 (FAO, 2022) (Fig. 1). In 2018, the global fish production reached about 179 million tonnes (FAO, 2022) more than 100 million tonnes of difference from 2014. Of this overall total, 156 million tonnes were used for human consumption, giving an annual supply of 20.5 kg *per* capita worldwide. As such, the aquaculture sector has grown in the order of 527% during this period, when compared to the 14% growth of fisheries in that same interval (Boyd et al., 2020). Nowadays, aquaculture systems can be executed in numerous locations using different technologies, producing a vast number of different species. Aquaculture has been positively seen as an alternative in the fish market for the decreased rentability in the fisheries sector.

Unregulated captures and ineffective stock management measures are some of the reasons for the observed decrease in the fisheries sector. The fish stocks that are supposedly biologically sustainable have decreased from 90% in 1974, to 65,8% in 2017, and overfishing species has increased from 33% in 2015 to 60% in 2018 (FAO, 2020). More than half of the world's finfish fisheries are at their maximum sustainable limit (FAO, 2010a), where the progress of improved fish capturing technologies has worsened the problem of overfishing (Squires & Vestergaard 2018). Caused by the need to find new resources and growing demand, there has been an increased pressure on non-fish resources, but this exploitation of low-trophic-level invertebrates is occurring at a faster rate than management can evolve (Anderson et al. 2011a, b). Many fisheries have followed a boom-and-bust cycle and have shown severe depletion or even collapse. These are considered periods of intense exploitation (boom) that are followed by a period of inactivity (bust) where the population is supposed to recover. For example, sea urchins

(globally), oysters (coasts of the US and eastern Australia), shrimps, and crabs (Greater Gulf of Alaska) have shown severe signs of depletion because of increased demand which, in turn, leads to such exploitation patterns (Lotze et al., 2010). Not only the pressure on high-trophic levels leads to the exploitation of lower-trophic levels, but the use of invertebrates as medicine and cuisine delicacies are also some reasons for the search and use of low trophic levels.

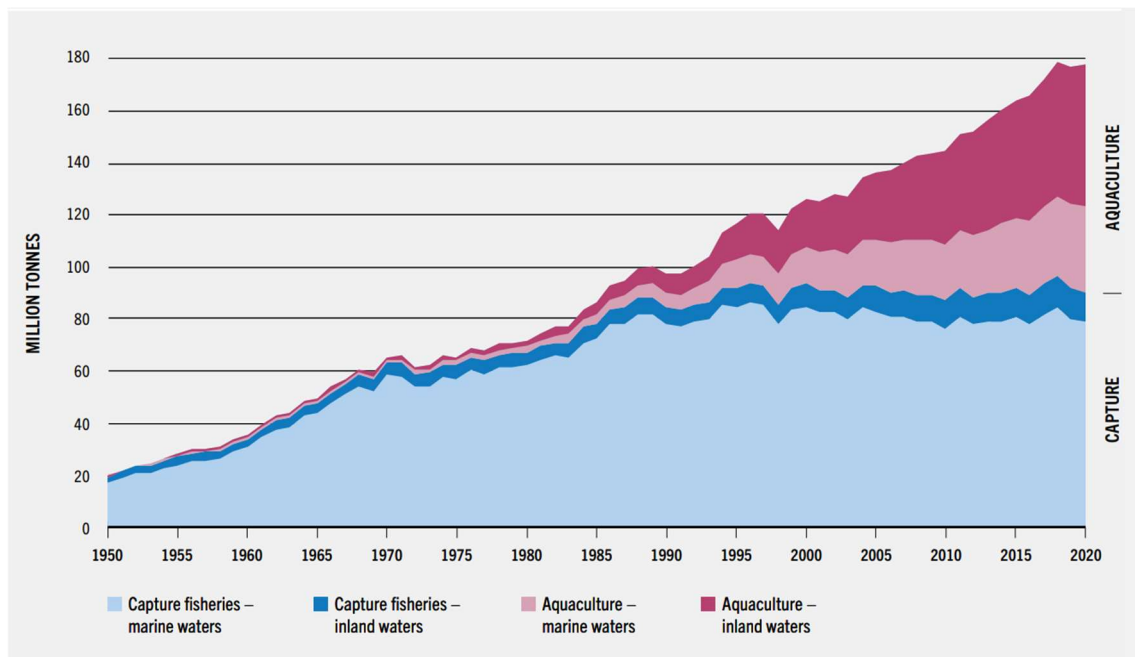


Figure 1-. Global fisheries and aquaculture production (FAO, 2022).

## 1.1 Sea cucumber exploitation

The use of sea cucumbers as food started to grow in China around the Ming Dynasty (1386-1644 AD), and their capture have been developing in the region, since then. Sea cucumbers are echinoderms of the class Holothuroidea, with high economic interest. These invertebrates are an important fishery resource with high export value, especially to Asian markets. They are harvested and traded in more than 70 countries worldwide, where, after being processed (cooked and dried), are often called *bêche-de-mer* or trepang. Sea cucumbers contain high levels of protein and bioactive components (Santos et al., 2015), such as bioactive peptides, carotenoids, triterpene glycosides, minerals (for instance, zinc, calcium, iron, magnesium), vitamins (for instance, A, B1, B2, B3), fatty acids, protein, and polysaccharides, which make them very attractive for consumption (Santos et al., 2017). Sea cucumbers have a vast quality profile making them

a very desirable product in the market, from a nutritional point of view to the medical aspect. But, due to their low motility, sea cucumbers are extremely susceptible to overfishing, with high production cycles being closely followed by abrupt dips in population numbers, also known as pulse fishing, representing most of these captures (Zamora et al., 2016). This, combined with the high values that this product can reach in the Asian markets and the inadequate and non-controllable harvesting of these species, led to an overexploitation scenario of different species.

At the start of the new millennium, the total global capture of sea cucumbers was around 100,000 tonnes of live animals each year, when included the countries where they are consumed (Purcell et al., 2010, 2012). This fishery started around 1700 in central Indo-pacific, but the continuous stock decline led the exploitation to new regions (Eriksson et al., 2012; Lovatelli et al., 2003; Toral-Granda et al., 2008). More than 66 species were captured worldwide and exported to the Asian markets (Choo, 2008; Conand, 2008; Kinch et al., 2008; Purcell et al., 2010). The W-Pacific region catches between 20,000 tonnes and 40,000 tonnes per year, exporting it to China and other Asian markets, with most of the products captured in Indonesia, Papua New Guinea, and the Philippines (Tuwo et al. 2015). This overfishing has affected the sustainability of sea cucumbers in these specific regions. In the Mediterranean Sea, species like *Parastichopus regalis* are commercially fished for human consumption in Spain, which is considered a delicacy (Ramón et al., 2010). In Greece, the *Holothuria tubulosa* has been captured for more than one century to be used as bait (Antoniadou et al, 2011). Although, Turkey is the principal Mediterranean country that captures and actively exports, species like *Holothuria mammata*, *Holothuria polii*, and *Holothuria tubulosa*, to Asian markets (Dereli & Aydin, 2021; González-Wangüemert et al., 2014, 2015).

The rapid decline of sea cucumber populations worldwide has triggered fluctuations in market prices, rapid expansion and spread of fisheries around the globe, severe overfishing, and poaching by nationals and foreign fishermen (Kinch, 2008). Due to this rapid exploitation of the most valuable species to the market, the devastation of natural organisms led to a blast in the search for smaller individuals and new target species. After many years of overexploitation, the sea cucumber price is currently affected by the reduced size of the animal and poor quality, hence the differences in export earnings (Mmbaga & Mgaya 2004).

The decimation of stocks has forced a moratoria in the Commonwealth of the Northern Mariana Islands, Costa Rica, mainland Ecuador, Egypt, India, Mauritius, Mayotte (France), Panama, Papua New Guinea, Solomon Islands, mainland Tanzania, Tonga, Vanuatu, and Venezuela (Purcell 2010). Small-scale fisheries cover 66% (Fig 2) of total sea cucumber fisheries, from which 27% were operating in an illegal way, where the captured organisms would be exported despite the national moratoria (Purcell et al, 2013). In Papua New Guinea size limits for dried and fresh animals were implanted, as the prohibition of the use of scuba-diving, season closures, and no-take zones (Kinch et al. 2008). On one hand, the moratoria saved stocks from disappearing by monitoring the catches at the point of export, but, on the other, illegal fishing still happens, since a centralized management system was not so effective at this point, needing wider enforcement.

In the Mediterranean, illegal fisheries of mainly, *H. tubulosa* and *H. polii* have been reported on Italian coasts, specifically in Sardinia and Puglia regions, describing around 52,930 kg of sea cucumbers captured between 2015 and 2017 (Meloni et al, 2018). In 2015 a penalty of 31,500 EUR was applied for the illegal harvesting of 29,030 kg of sea cucumbers, leading to a rupture of clandestine facilities and storage rooms. Between 2016 and 2017 the illegal harvesting was about 23,900 kg. In recent years, the capture of sea cucumbers has increased due to the strong Asian market demand, which led the Italian authorities to decide to apply the precautionary principle under art. 174 of the Treaty of Amsterdam (amending art. 130 of the Maastricht treaty) (Meloni et al, 2018). In Andalusia, Spain, *H. arguinensis* has been caught illegally and exported to China by 10 companies, as well in Portugal besides *H. arguinensis*, also *H. forskali*, *H. mammata* with variable prices from 70 to 350 € per kg (González-Wangüemert et al, 2016, 2018). Meanwhile in Portugal, even with no regulation on sea cucumbers fisheries, the holothurian catches have been forbidden by the law, in Dec Lei 38/2021.

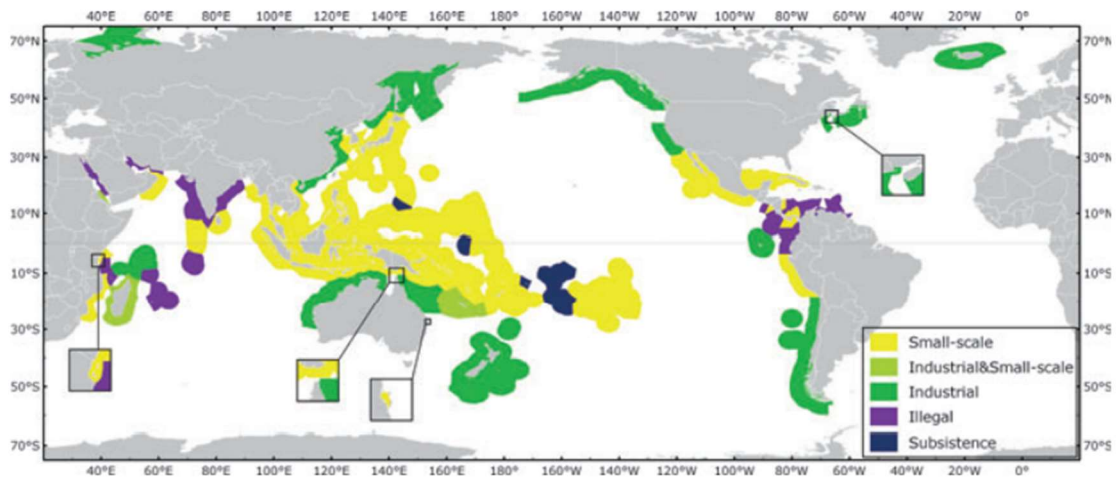


Figure 2- Scale of different fishing in global sea cucumber fisheries (Purcell et al, 2013)

From a nutritional point of view, sea cucumbers have been seen as a high nutritional product, reaching almost 70% of protein when dried (Chen, 2003). In traditional Chinese medicine, it is believed that sea cucumbers have the capacity to treat disorders and pathologies such as cancer and arthritis, as well as intestinal and urinary dysfunctions (Purcell, 2010). Sea cucumbers have been consumed, as a food source but also as a tonic and traditional treatment with similar properties to Ginseng (Fabinyi, 2014). Acting as medicine for fatigue, impotence, flu, urinary incontinence, and even frailty from old age (Huizeng, 2001). Recent studies have determined and shown the health benefits of the use of sea cucumbers as medicine, mostly because of the high content of polyunsaturated fatty acids (PUFA), mostly eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (Santos et al., 2015; Sales et al., 2021). Besides the rich protein content, high-value medicinal toxins are produced by some species of sea cucumbers. Some other species produce compounds that, if isolated, show anti-microbial, anti-inflammatory, antioxidant (Ketharani & Sivashanthini, 2016), anti-coagulant, and immune-modulatory activities due to the presence of specific substances such as sulphonamides and diketones (Aminin et al., 2001). Looking at the nutritional benefits and the history as a medicinal supplement (Zamora et al, 2016; Zhang et al, 2015), it is understandable the high value of the many different species of sea cucumbers, and why they reach such high prices in the market.

## 1.2 Evolution of sea cucumber aquaculture

In a context of increasing demand for sea cucumbers and decreasing ability for fisheries to supply the markets, the aquaculture production of these species offers a solution to minimize the depletion of natural stocks, while offering alternatives to meet demand, where, in the last years, the success of the existent production of sea cucumbers had surpassed its fisheries globally (Fig. 3). The growing aquaculture of sea cucumbers (*Apostichopus japonicus* and *Holothuria scabra*) has become a profitable industry while helping the conservation of species (Polidoro et al, 2011). Sea cucumbers aquaculture can be not only an alternative supply source, but also have the potential to replenish natural populations. The stocking of juveniles in depleted populations has been tested and showed a promising effectiveness (Bell et al, 2008; Hasan & Johnson 2019).

Starting around 1954, in China, Zhang Fengying developed a temperate species aquaculture, *A. japonicus*, using an artificial reef. But only in '80s, China started to produce in large scale, conducting to a market value exceeding the US\$ 5 billion (Zhang et al, 2015). Having Japan and China as main producers, the sea cucumber *A. japonicus* aquaculture expanded to a commercial scale, being also developed in North and South Korea, while in more tropical and sub-tropical regions, such as Madagascar and New Caledonia, *H. scabra* has been produced (Mohsen & Yang, 2021). In 2002 the aquaculture expanded gradually, and in 2003 the total production reached 3000 tonnes, increasing to 102 200 tonnes in 2009. Over the past decades, the culture of *A. japonicus* has become one of the most important aquaculture industries in China, and using the same rearing and cultivation techniques, the species *H. scabra* has also started to be produced on a commercial scale.

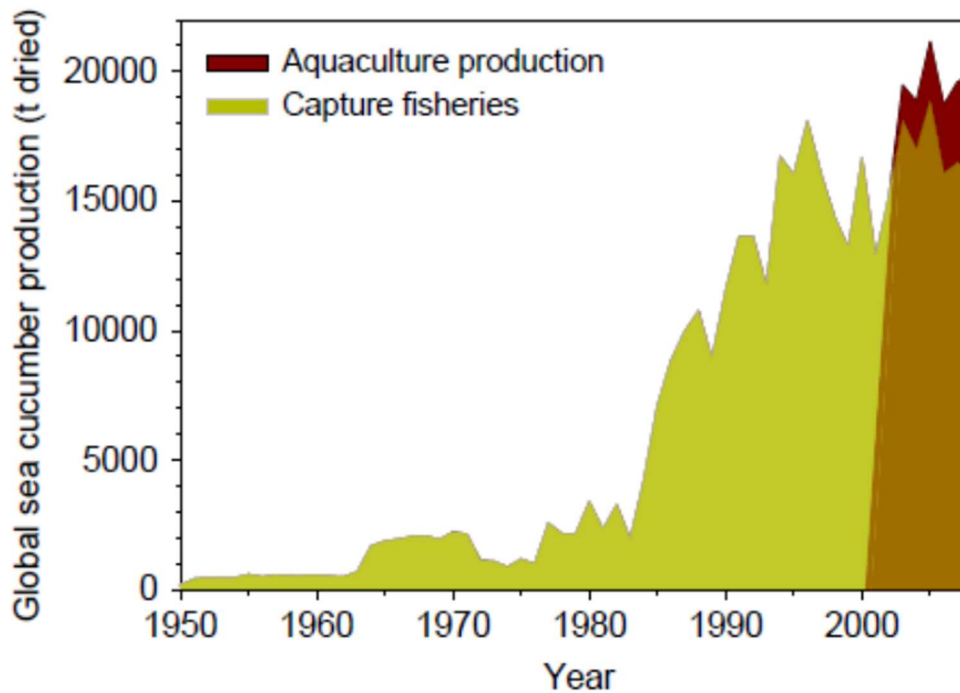


Figure 3- Global wild captures and aquaculture production of sea cucumbers over time; in metric tonnes (t), (Adapted from Polidoro et al, 2011).

Despite this big evolution in aquaculture and artificial breeding, all the other target species remain largely understudied and without means for production. As in *A. japonicus* and *H. scabra*, in order to effectively rear a species, studies must be done regarding sea cucumbers ecology, life-history traits, reproductive cycles, as well as their abundance in the wild (González-Wangüemert, et al., 2013; Sicuro & Levine, 2011; Venâncio et al., 2021). There are rearing techniques used with *A. japonicus* that can be adapted to other species. Species such as *Holothuria lessoni* (Massin, Uthicke, Purcell, Rowe & Samyn, 2009), *Parastichopus californicus* (Stimpson, 1857) have been tested in diverse trials for application in commercial size scale and stock enhancement and conservation purposes.

When it comes to sea cucumber production, Europe is still behind when compared to Asia. Nevertheless, over the years there has been an increasing number of papers published in this area (Domínguez-Godino et al., 2015; Eriksson et al., 2012; Purcell et al., 2018; Venâncio et al., 2021; Madruga et al., 2022). Some European sea cucumber species have demonstrated to be potential aquaculture candidates (Domínguez-Godino et al., 2015; Domínguez-Godino & González-Wangüemert, 2018, 2019; González-Wangüemert et al., 2016; Santos et al., 2015; Venâncio et al., 2021; Madruga et al., 2022). Several of these temperate species are new targets for exploitation and have high market

values (Hossain et al., 2020). The sea cucumber *H. arguinensis* is among those candidates. This species not only has high market value but shows a high potential for aquaculture production (Domínguez-Godino & González-Wangüemert, 2019b).

### **1.3 *Holothuria arguinensis***

*Holothuria* (Roweothuria) *arguinensis* Koehler & Vaney, 1906 belongs to the Class Holothuroidea. Living in depths of 0-50 meters, it can be associated with sand bottoms, seagrass and macroalgal beds and rocky reefs (González-Wangüemert & Borrero-Pérez, 2012; Navarro, 2012; Azevedo e Silva et al., 2021, 2022). This species is geographically found from Portugal (Berlengas Islands) to Morocco and Mauritania reaching the Canary Islands (Costello, 2001; Rodrigues, 2012; Thandar, 1988). It has also been found colonizing the western Mediterranean Sea, with occurrences in the South-eastern Spanish coast and the Algerian coast (Mezali and Thandar, 2014). Usually covered in sand, seagrass, and macroalgae (due to the feeding habits) on rocky reefs (Navarro, 2012), *H. arguinensis* does not show a sheltering behaviour (González-Wangüemert and Borrero-Pérez, 2012; Navarro et al., 2014; Azevedo e Silva et al., 2022), unlike other sympatric species (e.g. Félix et al., 2021).

As a new target species, *H. arguinensis* has been exploited in the Atlantic and Mediterranean for exportation to Asian markets. The catches driven by its high commercial value are growing (González-Wangüemert and Godino, 2016; González-Wangüemert et al., 2016; Roggatz et al., 2016). The illegal harvest of this species in the south of Portugal has been an increasing threat to the sustainability of this population, especially in the Ria Formosa lagoon (González-Wangüemert and Godino, 2016). Domínguez-Godino & González-Wangüemert (2020), measured the length and weight of 354 individuals in two different locations, being them Praia de Faro and Ramalhete. The mean length was 24.04 cm, with a mean weight of 213 g in Praia de Faro, while in Ramalhete lower results were obtain with a mean length of 21.87 cm and a mean weight of 193.54 g. Another study, conducted in Morocco by Haddi (2022), evaluated the weight and length of *H. arguinensis* in two separated places, Skhirat and Souiria k'dma. In Skhirat the mean for length and weight was, respectively, 144.6 g and 13.0 cm, and in Souiria k'dima the mean length and weight was, respectively, 13.8 cm and 173.3 g (Haddi et al., 2022). In all cases, the frequency of larger size classes was much lower than the

smaller ones, mainly due to harvesting activities of this new target species that can peak at 350 €/kg (Pires, 2016). In Algarve (Portugal) at Ria Formosa, the population shows multimodal size-frequency distribution, from 7 to 33 cm (González-Wangüemert et al., 2013) contrasting with the unimodal size-frequency shown in Azevedo e Silva et al. (2022) study in Arrábida (Portugal) where they had a mean size of 31,2 cm and a mean weight of 469.28 g. This size distribution in exploited zones reduces the spawning biomass, which will have consequences for the population.

Under this framework, the development of *H. arguinensis* aquaculture is crucial to face the overexploitation and high fishery pressure on this species, but the lack of knowledge on aquaculture of this species is hindering the progression. From broodstock maintenance to reproduction, larval development, and juvenile rearing, the studies with *H. arguinensis* have also been increasing. Spawning induction and larval development have been described by Sousa et al. (2022). However, there is still a lack of studies pertaining to juvenile rearing. Being similar to broodstock maintenance, the optimal temperature for rearing juveniles is still unknown, with this aspect being the most important parameter influencing aquatic species (Tepler et al., 2011). Being unable to regulate their body temperature (Díaz et al., 2017), the high temperatures tend to increase the rate at which they go through their development stages (Meng et al., 2009), on the other hand, when under thermal stress, energy allocation can affect normal activities such as growth, reproduction, and foraging, which can negatively impact their performance and fitness (Pörtner et al., 2008). Domínguez-Godino & González-Wangüemert (2019a) described how low temperatures can reduce feeding, movements, absorption efficiency and growth. A study performed by Madruga et al. (2022) tested the effect of rearing temperature on the growth of *Holothuria arguinensis* juveniles, using temperatures from 18, 20, 22 and 24°C. In this study temperatures under 20°C were not suitable for growing this species, showing a slow development when compared the different temperatures, and an optimal growth at 20°C, as shown in some other studies both in captivity and in the wild, as lower temperatures do seem to make this species reduce movement and in turn reducing its feed intakes (Olaya-Restrepo et al., 2018). Stocking density is also one of the main parameters affecting the growth, feeding, and survival of the sea cucumbers, where the best growth performance was seen with a density of 5 juvenile individuals.m<sup>-2</sup>, whereas with higher density, the growth would decrease significantly (Domínguez Godino & González-Wangüemert, 2018). From a nutritional point of view, authors have

been approaching a feeding method consisting in allocating fine algae powder in the trial tanks and letting them sink into the substrate. Using this method Domínguez-Godino et al., (2018) tested the use of seagrass *Zostera noltii* and *Cymodocea nodosa* as food source for. It was found that the use of *C. nodosa* provoked a negative growth in *H. arguinensis* with a reduced feeding rate, while the individuals fed with *Z. noltii* showed the highest growth (specific growth rate =  $0.09 \pm 0.06\%/day$ , absolute growth rate =  $0.11 \pm 0.07$  g/day). The same author tested in 2019 the growth of *H. arguinensis* under an aquaculture tank of sea bream (*S. aurata*) with negative results obtained in weight change and SGR, hypothesising that the main problem must have been the water temperature, set at 12.6°C. A study about optimization of growth and rearing techniques from larvae to juvenile for the sea cucumber *H. arguinensis* have been conducted by Sousa et al. (2022) where 3 diets were tested. At the end of the larval growth phase, diet A (*Rhodomonas baltica* + *Phaeodactylum tricornutum*) showed individuals with larger mean width, while diet C (*Rhodomonas baltica* + *Chaetoceros calcitrans*) presented an overall higher survival rate, being more suitable for that phase. Furthermore, there has not been any difference in the food application method, where the main focus of most of the studies is the different algae and different types of diets to give in order to improve the growth of sea cucumbers. Another key point is the substrate used to produce sea cucumbers. There are some studies focused on different substrates, especially with *Holothuria scabra*, from shape to mean sizes of the substrate particles. From an aquaculture perception, the feeding process for *A. japonicus* and *H. scabra* has been done by simply providing fine algae powder to the production tanks letting it sink, enriching the sediment, but there is yet to show any study that focuses on a different feeding method using the substrate as a via to provide the food. Sea cucumbers, as organisms that feed on the substrate, need this sediment to be enriched. This enrichment sediment is described as algae mixed in the substrate where the sea cucumbers will move and feed. With this method, the loss of food in the column of water is reduced, and the accessibility to food is done in a natural way while the sea cucumbers ingest the substrate.

## 2. Objectives

This study aimed to evaluate the effects the substrate mixed with algae (*Sargassum vulgare*) may have on the growth of *H. arguinensis* juveniles. For that, the difference between an enriched substrate and a non-enriched substrate was evaluated for a period of four months, in an experimental design comprising individuals from different size classes. During this period, all individuals were systematically assessed using growth metrics, as a proxy of condition.

### 3. Materials and Methods

#### 3.1 RAS system preparation

The experiment was conducted in 3 recirculating aquaculture systems (RAS), in 3 tanks (A, B, C) with 550 L (2.3 x 0.6 x 0.4 m), each divided into 3 sections, using PVC planks (n=9): A1, A2, A3; B1, B2, B3; C1, C2, C3, with 183 L each (0.76 x 0.2 cm) (Fig. 4). Holes were made in the planks allowing the water to flow between divisions of each RAS. Preventing the escape of the sea cucumbers from the different divisions, a plastic net was used to cover all the different holes in the PVC plank. To assure that the water is reaching the sump equally from each division, a perforated 1.70 m tube crossed all tanks to ensure homogeneity of water quality and filtration.



Figure 4- Tank division for the feeding trials with *Holothuria arguinensis* juveniles. Three tanks (A, B, C) divided in three equal spaces.

Each system with a tank was connected to its own sump that included mechanical filtration (wool and sponge filters), biological filtration (plastic bio-balls), a Bubble Magus C3.5 Needle Wheel Protein Skimmer (Jiyang Aquarium Equipment Co., Ltd., Jiangmen, China), and a Hailea HX-6530 water pump (Guangdong Hailea Group Co., Ltd., Guangdong, China) (Fig. 5)



Figure 5- Sump with mechanical and biological filtration from each RAS system where: A- skimmer, B- water exit tube, C- water compartment, D-sponges and bio-balls, E- aeration stone, F- water entrance tube.

Each tank division was provided with its own aeration, with a similar flow in all divisions. Before the preparation of the systems for the trials, a disinfection was performed, running freshwater with 0.25 mL of sodium hypochlorite (0.5%) per litre in the systems for 2 days. Afterward, the tanks were refilled with freshwater to clean any residues that could have remained and drained again after 24 hours. After this process, saltwater (with salinity 32) was added to the 3 systems, before the trial started, for one week to mature the biological filters. To test eventual escapes and issues related to the water quality and homogeneity of the systems, 45 sea cucumbers were distributed in the different divisions, 5 *per* division. This “escape test” was conducted for 3 days and was an important step to find some escape routes between divisions, that could risk the experiment or create any bias in the results. During this period the water parameters like pH (7-8), temperature (19-21 C°), salinity (31-33), dissolved oxygen (80-90%), ammonia ( $0.03 \pm 0.02 \text{ mg. L}^{-1}$ ), nitrites and nitrates ( $0.00 \pm 0.00 \text{ mg. L}^{-1}$ ) were monitored to test the system. When the system was shown without any problems the water used in this test was renewed and the sand was removed so the fasting period of all individuals could start, which is the starting point of the experiment.

### 3.2 Experimental design and preparation

The hatchery-reared juveniles used in the trial were previously grown in a RAS system with 0.4m<sup>3</sup> tanks with sediment (Fig. 6) with one sump. Here, each tank had its own aeration system with sand in the bottom and the sea cucumbers were feeding equally with the macroalgae *Ulva lactuta*.



Figure 6- Tanks with 0,4m<sup>3</sup> where the hatchery-reared sea cucumbers juveniles were grown.

The 4-month experiment was performed from January 2022 to May 2022. After all the procedures to prepare the experiment systems the fasting period started. A total of 108 sea cucumbers were translocated to the experiment systems (A, B and C) without any sand, at  $19 \pm 1$  °C, where they spent 2 days removing all the gut contents in the digestive system. After these 2 days all individuals were measured and weighed and assigned to 3 different weight classes: Class 1 (>8 g, N=33); Class 2 (4-8 g, N=36); Class 3 (<4 g; N=39). Weight class replicates were assigned to each compartment of different tanks (each tank with the filtration system and recirculation represents one RAS). Thus, each system contained all three weight classes (Fig. 7). Using the available hatchery reared sea cucumbers and in order to have an equal distribution of the different size classes in the different tank divisions, the sea cucumbers were distributed as follows: 11 individuals of

Class 1 in each division A1, B1, C1; 12 individuals of Class 2 in each division A2, B2, C2; and lastly 13 individuals of Class 3 in each division A3, B3, C3. Individuals distributed by the experimental tanks of the trial presented a non-normal distribution, thus according to a Kruskal-Wallis test, no statistically significant differences in the same size classes ( $p = 0.988$ ) were found, which allowed to begin the trial and perform viable comparisons between groups at the end of the trial.

To test the influence of the enriched sediment in juvenile development against a control group, without enrichment, systems A and B (6 compartments) were prepared with the enriched sediment (*S. vulgare*) and system C (3 compartments) without enriched sediment (Fig. 7). The used sand, collected from an area of occurrence of *H. arguinensis*, at Peniche, went through a cleaning process. This cleaning process involved sodium hypochlorite (0.5%), which was added with water to the sand container. After this first step, the water on the container was renewed every single day to clean every residual of sodium hypochlorite present in the sand, guaranteeing a cleaned and usable sand. When needed for use, a proportion of sand would be taken from the container and dried in the oven at 50°C for one day. The enriched sediment was formulated by mixing 8% of algal powder with 92% of sand, defined to represent a percentage of organic content similar to the maximum value in the natural environment of this population (Félix et al., 2021), and placing it in a total of 6 out of 9 recipients where 3 had no presence of algal powder. The macroalgae was previously grinded into fine fragments, allowing a better mix with the sand. Both recipients, with and without the sand-algae mix, were filled with water, which would soak the algae and sand allowing an easier settlement in the bottom by preventing algae from floating. As a baseline, every group was also fed with *Saccorhiza polyschides* three times per week during the experiment (Sousa et al., 2022). The daily feeding dose was calculated based on the body weight of sea cucumbers in each division separately, rating 3% of body weight (Wang et al., 2009). This value was readjusted accordingly to the weight of the animals every 14 days during the 4 months of the experiment.

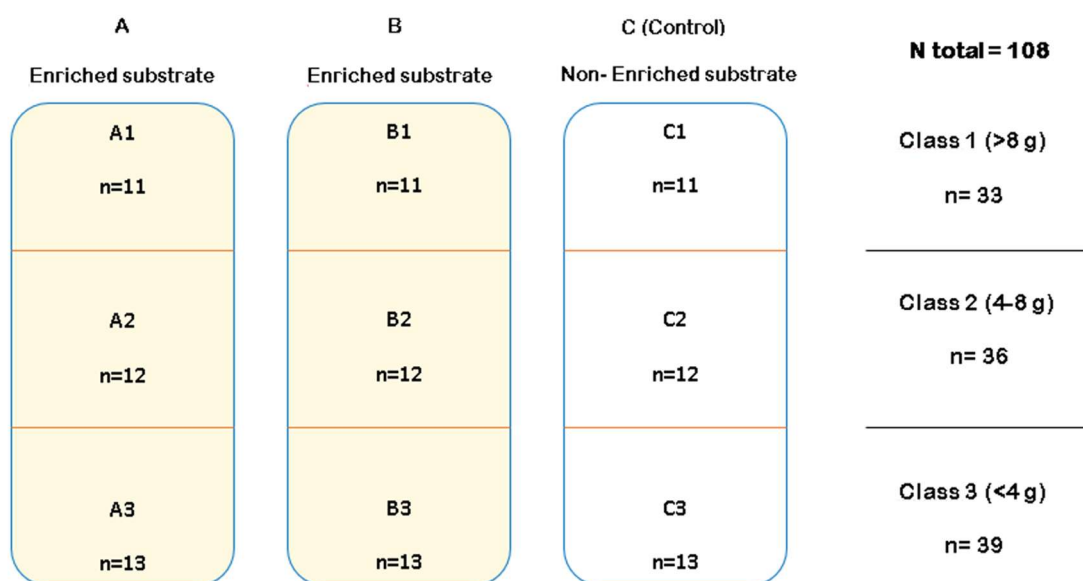


Figure 7- Three different RAS systems (A, B, C) with three divisions in each tank (A1, A2, A3, B1, B2, B3, C1, C2, C3). Each division from each tank received one of the three weight classes of *Holothuria arguinensis* juveniles. System A and B got the enriched substrate mix (sand + macroalgae) while the system C got only sand. A baseline diet of *Saccorhiza polyschides* was provided to all systems.

The entire experiment was conducted in a controlled environment, with a photoperiod 12hL:12hD. The seawater used in the experiment was previously filtered using mechanical and biological filtration. The measurements of temperature ( $^{\circ}\text{C}$ ), dissolved oxygen (%), pH,  $\text{NH}_3$  ( $\text{mg. L}^{-1}$ ),  $\text{NH}_4^+$  ( $\text{mg. L}^{-1}$ ), and  $\text{NO}_2^-$  ( $\text{mg. L}^{-1}$ ) were done daily during the trial. Water exchanges were done whenever necessary to maintain the water quality. The temperature of the water was  $20^{\circ}\text{C} \pm 1$ . Salinity was maintained in a range of  $33.34 \pm 0.12$  and dissolved oxygen around  $81.55 \pm 0.3$  %, while pH was kept  $8.06 \pm 0.02$ . Ammonia and nitrites ( $\text{NH}_3$  and  $\text{NO}_2^-$  in  $\text{mg. L}^{-1}$ ) were measured with API colour testing kits (Mars fishcare) and maintained at  $0.00 \text{ mg. L}^{-1} \pm 0.02$  and at  $0.00 \text{ mg. L}^{-1} \pm 0.03$  respectively. Every 14 days, matching with the sampling for weighing and measuring of the individuals (subchapter 3.3), the tanks were emptied. The sediment was totally removed from the tanks, as well around 90% of the water, with both parts being renew for a new cycle.

### 3.3 Sampling

Every 14 days (T0 to T9) all sea cucumbers were measured, using a flexible measuring tape, for total length ( $\pm 0.1$  cm), underwater without any manipulation avoiding muscular contractions and any bias in this metric. Using a digital scale (ADAM PGL Precision Balance NDPGL4001, EUA) with a precision of 0.01 g, the sea cucumbers were also weighted, and the data registered. The sea cucumbers were placed into the different recipients (one for each of the 9 groups) filled with water from the system, with presence of constant aeration. Without the sea cucumbers in the tanks, and after all the cleaning and renovation process, a new mix of sand and *S. vulgare* was placed in the tanks of the systems A and B, and new sand in the C. After this renew cycle, measurements (temperature, salinity, pH, ammonia, nitrites and nitrates) were done to the water to guarantee no differences between systems. Afterward, the sea cucumbers were placed back in the compartments of the tanks in the correct class divisions.

### 3.4 Data analysis

Growth performance and survival rate were evaluated by determination of survival rate (SR), specific growth rate (SGR), body length rate (GR). These calculations were supported using the following formulae:

$$SR (\%) = \frac{\text{final number of animals}}{\text{initial number of animals}} \times 100$$

$$SGR (\%/day) = \frac{\ln(Wf) - \ln(Wi)}{T} \times 100$$

$$GR (\%/cm/day) = \frac{Lf - Li}{Li \times T} \times 100$$

Where  $W_i$  is the initial weight (g) and  $W_f$  is the final weight (g).  $L_i$  is the initial length (cm), and  $L_f$  is the final length (cm).  $T$  is the experiment time in days.

The obtained data were used to perform a one-way analysis of variance Kruskal-Wallis, due to the non-normality of the data, in order to accurately determine any differences in the individuals before and after the trial. Whenever any statistically significant difference occurred ( $p < 0.05$ ), it was immediately followed by a Pairwise Multiple Comparison Procedure (Mann-Whitney) method for non-parametric tests. All statistical analyses were performed with the software IBM SPSS Statistics 28.0.0.1. All data were presented, whenever possible, as mean  $\pm$  standard deviation (SD). Relative standard deviation (RSD) was also performed using the weight of the sea cucumbers in the different classes, to determine the evolution of the sample unit's heterogeneity.

## 4. Results

### 4.1 Survival Rate

During the daily routine or sampling moments, any casualties that occurred were registered. In table 1, it is possible to observe the mortality rate for the different classes in the different systems for all duration of the experiment. At the end of the experiment, the survival rate ranged from 91% to 97.4%, showing an overall 93% of survival, as 101 out of the initial 108 individuals reached the end of the experiment successfully. The mortalities were mostly found in system C (5 in total), the control, followed by system A (2 in total). System B showed no casualties in any weight class. Although, no significant differences were found between systems ( $p > 0.05$ ).

Table 1-. Mortality rate (%) for the different weight classes (1, 2 and 3) and system (A, B – treatment – and C – control) for feeding trials with enriched sediment with *Holothuria arguinensis*.

	Class 1 (>8 g)	Class 2 (4-8 g)	Class 3 (<4 g)	Total %
<b>System A</b>	3.03	2.7	0	5.73
<b>System B</b>	0	0	0	0
<b>System C</b>	0	5.55	7.69	13.24

## 4.2 Weight analysis

At the end of the trial there was an increase in fresh weight (FW) in most of the treatments (table 2). In terms of classes, the highest mean FW was always found in system B (27.77 ±1.76 g for class 1; 16.96 ±1.33 g for class 2; 8.76 ±1.22 g for class 3). A growth in every class from T0 to T1 can be observed in figure 8, but from T1 to T2, classes 2 and 3 from the systems A and C decreased, while all classes present in the system B increased. In general, all classes from system B had a continuous growth until T5 when it started to stabilize. In system C, there was a decrease trend in weight after T1.

In class 1, at T0, no statistical differences were found between systems ( $p=0.988$ ), but at T9 there was a statistical difference ( $p= .015$ ) between the weight in the same class in different tanks. The pairwise tests showed a significant difference between each treatment group (A1, B1) and the control group (C1) (M-W:  $p <.001$ ), and no difference ( $p=.418$ ) between the two experimental groups (A1-B1). Class 2 showed a significant difference between tanks at T9 ( $p= <.001$ ). The Mann-Whitney test showed a statistical difference between all systems (M-W: A2-C2,  $p= <.001$ ; B2-C2,  $p= <.001$ ; A2-B2,  $p= <.001$ ). In class 3, at T9 there was a statistical difference between systems ( $p<.001$ ), for all pairs (M-W: A3-C3,  $p=.008$ ; B3-C3,  $p<.001$ ; and A3-B3,  $p=.003$ ).

Table 2- Initial and final mean fresh weight (FW) (g ±SD) for the different weight classes (1, 2 and 3) of *Holothuria arguinensis* in the different systems (A and B for treatment and C for control)

<b>System A</b>	<b>Class 1 (&gt;8g)</b>	<b>Class 2 (4-8g)</b>	<b>Class 3 (&lt;4g)</b>
<b>Initial FW (T0) (g)</b>	13.20 ± 4.58	6.04 ± 1.41	2.16 ± 0.91
<b>Final FW (T9) (g)</b>	22.52 ± 1.50	8.76 ± 0.84 g	3.95 ± 0.90
<b>System B</b>	<b>Class 1 (&gt;8g)</b>	<b>Class 2 (4-8g)</b>	<b>Class 3 (&lt;4g)</b>
<b>Initial FW (T0) (g)</b>	13.17 ± 4.38	5.98 ± 1.07	2.12 ± 1.10
<b>Final FW (T9) (g)</b>	27.78 ± 1.76	16.96 ± 1.33	8.76 ± 1.22
<b>System C</b>	<b>Class 1 (&gt;8g)</b>	<b>Class 2 (4-8g)</b>	<b>Class 3 (&lt;4g)</b>
<b>Initial FW (T0) (g)</b>	13.05 ± 5.74	6.05 ± 1.21	2.23 ± 1.06
<b>Final FW (T9) (g)</b>	15.04 ± 6.40	4.29 ± 1.94	2.12 ± 3.95

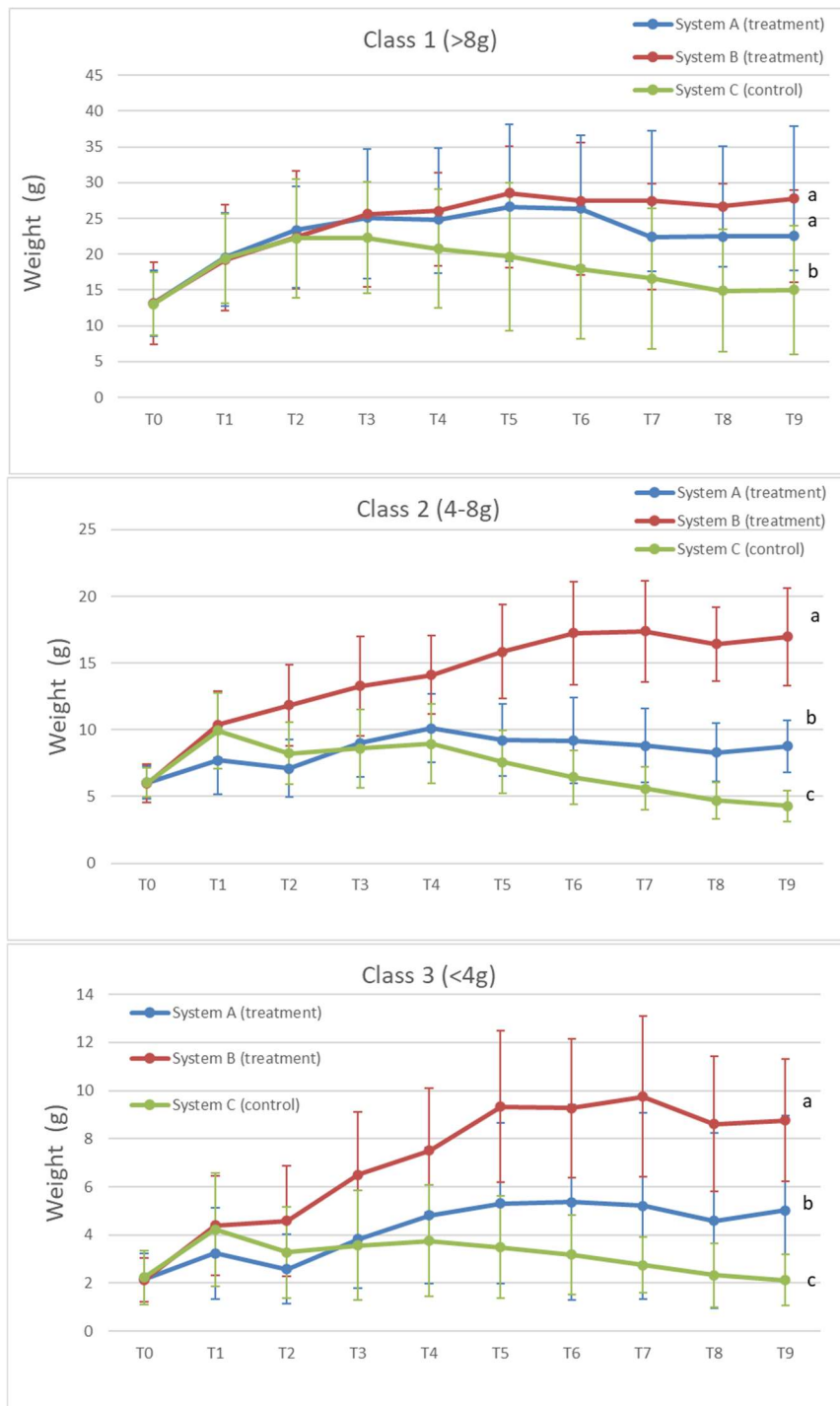


Figure 8- Fresh weight (FW) variation for the different weight classes (1, 2 and 3) of *Holothuria arguinensis* in the different systems (A and B for treatment and C for control) with fortnightly sampling times (T0, T1, T2, T3, T4, T5, T6, T7, T8, T9). Different letters indicate significant differences between systems.

In general, the sea cucumbers of system B showed a higher growth in each different class. Individuals from this system showed, in general, a higher SGR for all size-classes (Fig 9), whilst individuals from system C presented the lowest SGR, reaching even negative values. The sea cucumbers of the system B showed the highest SGR (1.13%) in class 3, while the lowest (-0.27%) was shown in class 2 in the system C.

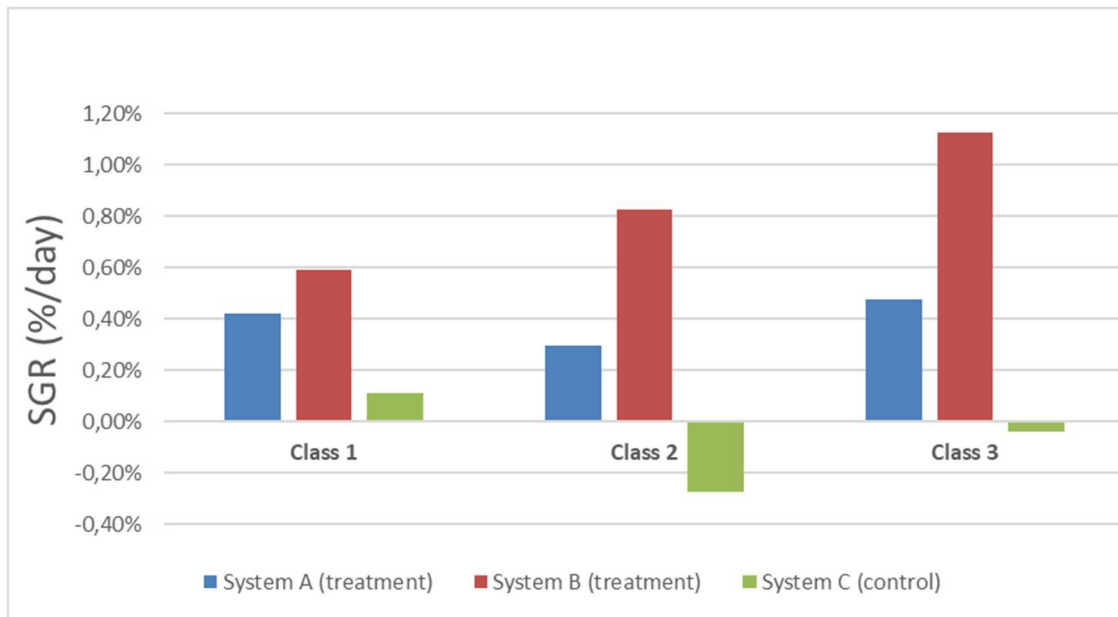


Figure 9.- Specific growth rate (%/day) for the different weight classes (class 1 (>8 grams), class 2 (4-8 grams) and class 3 (<4 grams)) of *Holothuria arguinensis* in the different systems (A and B for treatment and C for control)

### 4.3 Length analysis

At the end of the trial, similarly to the final FW, the higher results for fresh length (FL) in each class were obtained in the system B ( $10.58 \pm 1.76$  cm for class 1;  $9.23 \pm 1.33$  cm for class 2;  $8.76 \pm 1.22$  cm for class 3), represented in table 3 and figure 10.

The statistical evaluation at T9 indicated significant differences in the final fresh length between different classes in the different systems ( $p= .003$ ). The Mann-Whitney test showed a statistical difference between systems A-C ( $p= .016$ ) and B-C ( $p= .001$ ) while between the sea cucumbers of systems A-B no differences were found ( $p= .099$ ). Individuals in class 2 at T9 showed a statistical significance ( $p<.001$ ) between the final fresh length of the sea cucumbers of all systems (M-W: A2-C2,  $p= <.001$ ; B2-C2,  $p= <.001$ ; and A2-B2,  $p= <.001$ ). Individuals in the class 3 showed significant differences in the final fresh length between systems at T9 ( $p= <.001$ ). Using Mann-Whitney's test, a statistical difference between all tanks was found (M-W: A3-C3,  $p= <.001$ ; B3-C3,  $p= .003$ ; and A3-B3,  $p= <.001$ ).

Table 3- Initial and final mean fresh length (FL) (cm  $\pm$ SD) for the different weight classes (1, 2 and 3) of *Holothuria arguinensis* in the different systems (A and B for treatment and C for control)

<b>System A (treatment)</b>	<b>Class 1</b>	<b>Class 2</b>	<b>Class 3</b>
<b>Initial FL (T0) (cm)</b>	7.39 $\pm$ 1.40	5.71 $\pm$ 0.98	3.34 $\pm$ 0.64
<b>Final FL (T9) (cm)</b>	9.40 $\pm$ 1.71	6.05 $\pm$ 0.91	5.02 $\pm$ 1.21
<b>System B (treatment)</b>	<b>Class 1</b>	<b>Class 2</b>	<b>Class 3</b>
<b>Initial FL (T0) (cm)</b>	7.23 $\pm$ 1.95	5.47 $\pm$ 1.34	3.52 $\pm$ 0.98
<b>Final FL (T9) (cm)</b>	10.58 $\pm$ 1.76	9.23 $\pm$ 1.33	8.76 $\pm$ 1.22
<b>System Tank C (control)</b>	<b>Class 1</b>	<b>Class 2</b>	<b>Class 3</b>
<b>Initial FL (T0) (cm)</b>	6.68 $\pm$ 1.15	5.81 $\pm$ 1.25	3.73 $\pm$ 1.19
<b>Final FL (T9) (cm)</b>	7.59 $\pm$ 1.50	4.26 $\pm$ 0.84	3.24 $\pm$ 0.90

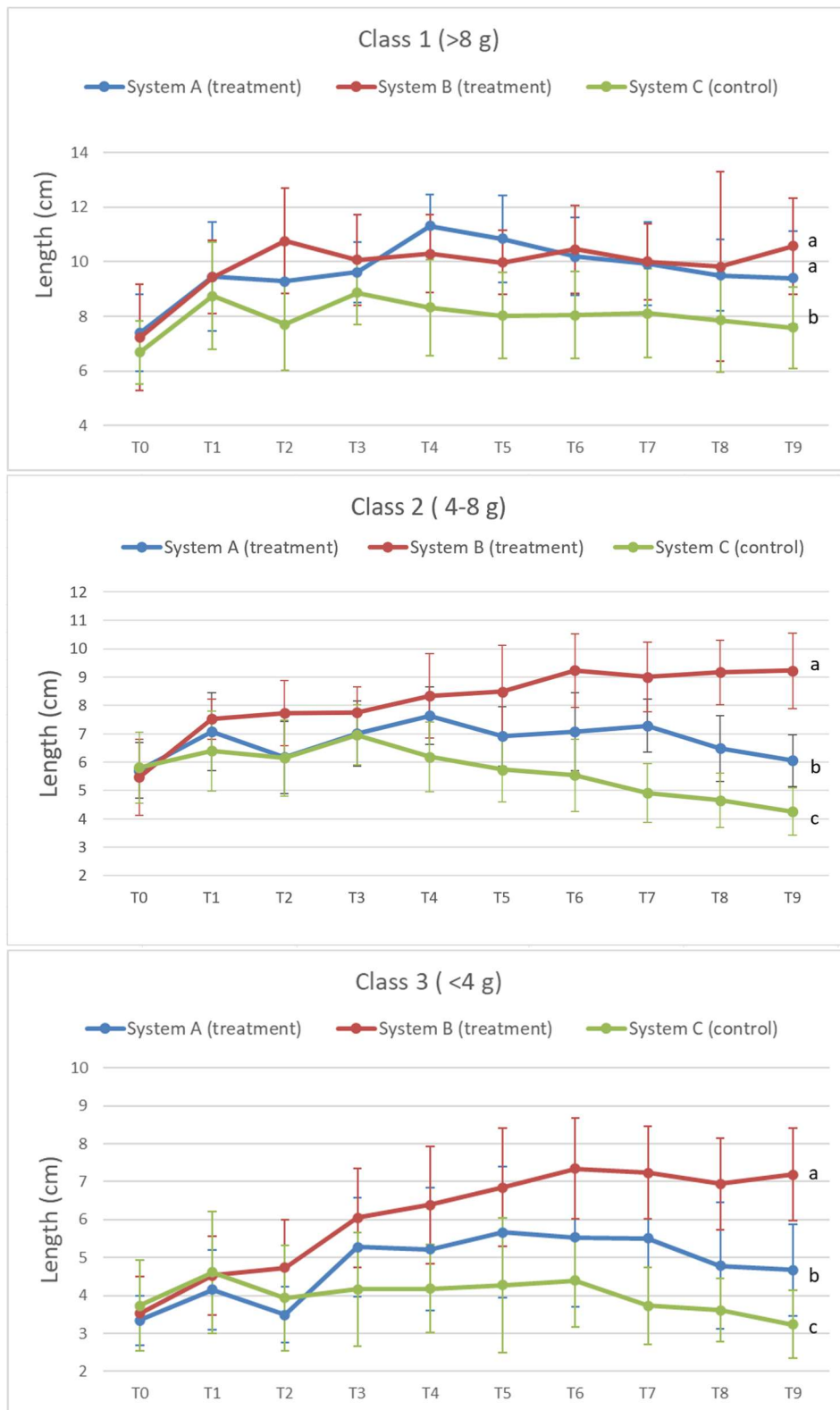


Figure 10- Fresh length (FL) variation for the different weight classes (1, 2 and 3) of *Holothuria arguinensis* in the different systems (A and B for treatment and C for control) with fortnightly sampling times (T0, T1, T2, T3, T4, T5, T6, T7, T8, T9). Different letters indicate significant differences between systems.

Regarding the body length rate (%cm/day) system B had the highest Gr in every class (Fig. 11). In tanks A and B, with the enriched sediment, the results were noticeable, especially in class 3, 0.399 and 1.180 %cm/day respectively. While in the control tank, the results were low and even negative, showing a minimum value of -0.212 %cm/day in class 2.

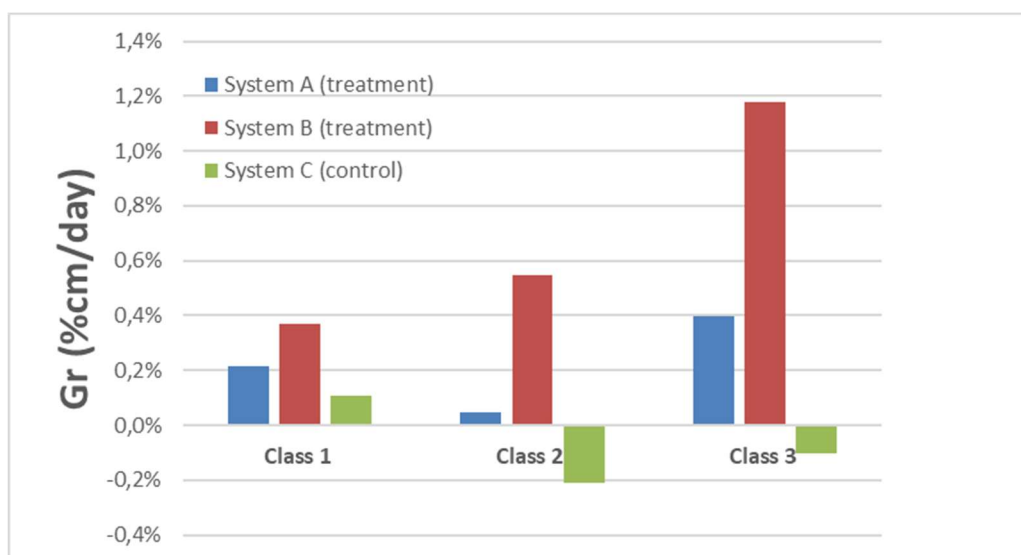


Figure 11- Growth rate (%/day) for *Holothuria arguinensis*, relative to each class (1, 2 and 3) in the different systems (A, B and C)

#### 4.4 RSD analyses

Relative Standard Deviation (RSD), with the measured weight, was performed to represent the evolution of size heterogeneity. It revealed the overall best results in system B, for all classes, but particularly in class 3, where overtime the individual sizes were becoming more homogeneous (Fig. 12). An increase in heterogeneity was found in the control group for the larger individuals (class 1) and in system A for the smaller size-class.

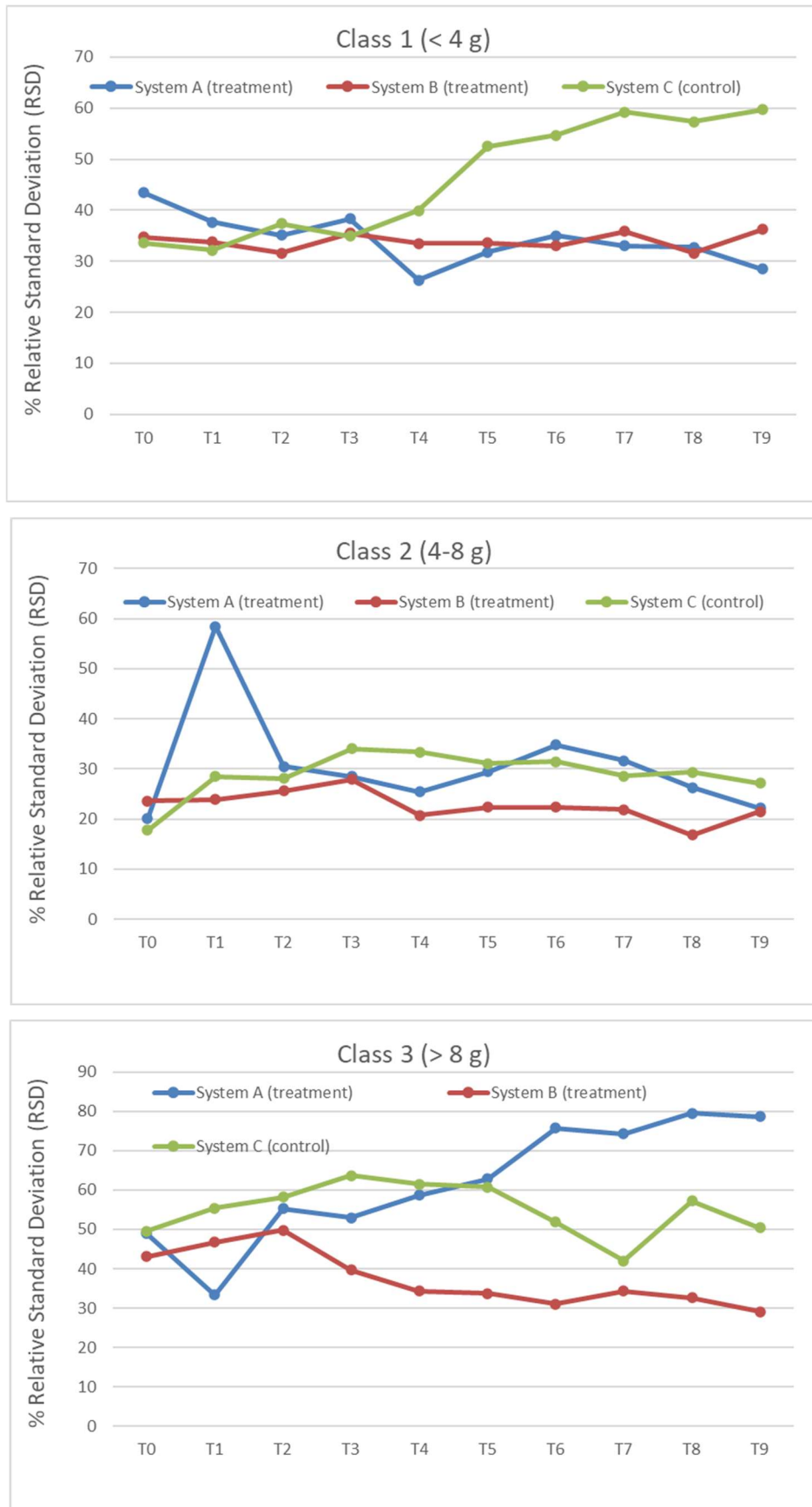


Figure 12- Relative Standard Deviation of *Holothuria arguinensis*, relative to the weight of each size-class (1, 2 and 3) in the different systems (A, B and C) and sampling times (T0 to T9).

## 5. Discussion

Growth and feeding methods of sea cucumbers are well-known for cultivated species but remains vague for *Holothuria arguinensis*. Different settings have been tested for the growth of sea cucumbers in recent years, from different temperatures (Madruga et al., 2022), different salinities (Xing et al., 2019) to different substrate size (Sabilu et al., 2022) and different diets (Venâncio et al., 2021; Sousa et al., 2022), but never a different feeding method was tested, while in all these cases the food was placed in the water column to sink to the bottom of the tank.

Regarding the present study, the results obtained showed that an enriched substrate influences the growth of *H. arguinensis* in a positive way. No significant differences were found in the mortality between all systems, showing that there were good conditions in terms of water and food parameters for the sea cucumbers physiology. The fatalities noticed in the control system were not expected, even though the feeding method, based only on the addition of microalgae, could be the justification for the casualties. As described in Sinsona & Juinio-Meñez (2018) study with *Holothuria scabra*, the survival of the largest juveniles in presence of an enriched sediment with *Sargassum* sp. was significantly higher than the smaller individuals with no enriched sediment. In the present study, the highest survival rate was obtained in the biggest size-class, and the most mortalities, with a lower survival rate in the smallest size-class, especially in the control system (without enrichment). But in the 60-days experiment with the enriched sediment with *Sargassum* sp. Sinsona & Juinio-Meñez (2018), showed a lower survival rate than the present study, with 63.3%. The high survival rate when using *Sargassum* sp. as a diet was also demonstrated in a study perform by Magcanta et al., (2021) where the sea cucumbers, *H. scabra*, fed with *Sargassum* showed a 92.67% of survival rate within the ideal range of salinity (32-35), showing similar results to those obtained in this study. One justification could be due to their low mobility. As Olaya- Restrepo (2018) showed, sea cucumbers move in order to find a suitable place to feed, this, with the smaller size of the sea cucumbers found in class 2 and 3 in system C, can express that the process to move for obtain food would require more energy than the energy obtained during the feeding process. This way, an enriched sediment would provide higher homogeneity in the food placement, allowing the sea cucumbers to find food more easily without

spending so much energy moving. Whereas sea cucumbers with a non-enriched sediment had the necessity to move to find a suitable place to feed, spending more energy.

The differences in weight found between the control and treatment are clear. As described in most of studies involving these organisms with different diets (e.g. Altamirano et al., 2017; Juinio-Meñez et al., 2014, 2017; Hair, 2012), a higher growth is noticed in the first sampling moments in all tanks, followed by a stabilization period. For systems A and B, the highest value before the stabilization of growth was noticed at T5 (70 days into the experiment), showing an improvement in the growth of the *H. arguinensis*, where in the previous mentioned studies the stabilization would happen after 30 days of experiment. While for tank C, at T2 (28 days into the experiment) the weight values start to get lower, similarly to the previous mentioned studies. The higher values of final weight obtained for tanks A and B comparing to tank C can be justified by the higher food availability in the tank with the enriched sediment. In Ria Formosa, higher growth rates of *H. arguinensis* were observed, having as possible justification the high productivity of the ecosystem from rich phyto-benthos of macrophytic and microphytic organisms (seagrass and diatoms) (Olaya-Restrepo et al., 2018; Brito et al., 2009). This is in agreement with the difference found in the results between the sea cucumbers present in the control system (C) and the test systems (A and B), as expected. Thus, an enriched sediment provides a higher percentage and quality of food in a way that a simple method of feeding with microalgae by deposition in the sediment cannot. So, an enriched sediment will not only provide an easy access to food, but also an easy way to fixate diatoms and other benthic species in the sediment, species that provide a complex set of nutritional elements for the diet of deposit-feeders, like sea cucumbers (Feng et al. 2016a, 2016b). Sinsona & Juinio-Meñez (2018), when testing an enriched sediment in *H. scabra*, obtained a higher weight of  $38.69 \pm 8.0\text{g}$  in sea cucumbers placed with the presence of an enriched sediment, showing similar results to those obtained in the present study, where the highest value of weight was obtained in sea cucumbers with the presence of enriched sediment. The SGR analyses showed a difference between the treatment systems and the control, where the SGR from sea cucumbers with enriched sediment was higher, whereas the control reached negative values. This type of conclusion was found in Sinsona & Juinio-Meñez (2018) study where the sea cucumbers fed with enriched sediment of *Saragssum* sp. shown the best values in terms of SGR after 30 days of the experiment (4.63 %/day). Contradicting our expectations was the difference found between the sea

cucumbers of system A and B in terms of SGR. This difference has no direct explanation since the food distribution, sand placement, and all the parameters were kept at the same levels. In the other hand, system A was the system that needed the most maintenance, since the pvc planks that made the separations got detached from the tank walls at the middle of the experiment. To prevent any escapes from the sea cucumbers to the other divisions in the tank it had to be emptied and the pvc planks had to be glued back to the tank walls in the same day. This manipulation could have induced additional stress on the sea cucumbers leading to lower growth, justifying the difference between systems A and B. The results of Xia et al. (2012) support the results of the present study, where *A. japonicus* exposed to sediment enriched with *Sargassum polycystum* showed more favourable growth when compared with the other diets, using different algae. The macroalgae *Sargassum* sp. has been used as diet in different studies, showing promising results. Magcanta et al. (2021) used *Sargassum* spp. and *Navicula* sp. as main diets, where *Sargassum*, extract or powder, showed the best results in survival, length, width and GR, concluding that *Sargassum* spp. is suitable for the diet of *H. scabra*. The present study, using *S. vulgare* in the enriched sediment, also obtained noticeable results in terms of growth, survival, SGR and GR for *H. arguinensis*.

Relative standard deviation described the size heterogeneity present in each class, with system B showing the highest overall values. System A represented some discrepancy between values along the study interval, especially in class 3, whereas in the other classes some stable period started to be noticed. Higher differences between the initial and final RSD are found in the biggest size class. As showed in class 3, the system C showed the most discrepancy in results, suggesting that a lack of food availability can cause differential growth between individuals. Sea cucumbers maintained in the system B showed the best results with the minor changes between values, showing a more homogenic growth, and the lowest values obtained during the study period. For individuals maintained in the system C, high differences were noticed, especially in class 1, showing that, in one hand, low food availability may aggravate size heterogeneity, but in the other hand, that an enriched sediment may invert the trend. High heterogeneity in the growth of sea cucumbers when in captivity is a difficult obstacle to overcome, like demonstrated in Laguerre et al. (2020) and Sousa et al. (2022) and can be a production bottleneck in aquaculture. Dietary studies should assess this issue systematically to determine all potential causes.

## 6. Conclusion

In this study has been demonstrated that an enriched sediment with *S. vulgare* improves the growth of *H. arguinensis*. Combined with the optimal temperature, the enriched sediment can provide an advantage in the production of *H. arguinensis*. Through this study, a deeper and positive understanding of this species feeding method was reached. This study was the first report of this feeding method for this sea cucumber species, and a step in the right direction regarding the future of sea cucumber aquaculture in Europe, and especially in the domestication of *H. arguinensis*. Knowing that the growth is improved by an enriched substrate, future studies should take into consideration the nutritional quality of the enriched sediment, understanding if the nutritional aspect can be a key point, or if the quantity of food is the reason why an enriched sediment showed positive results in terms of growth.

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