POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

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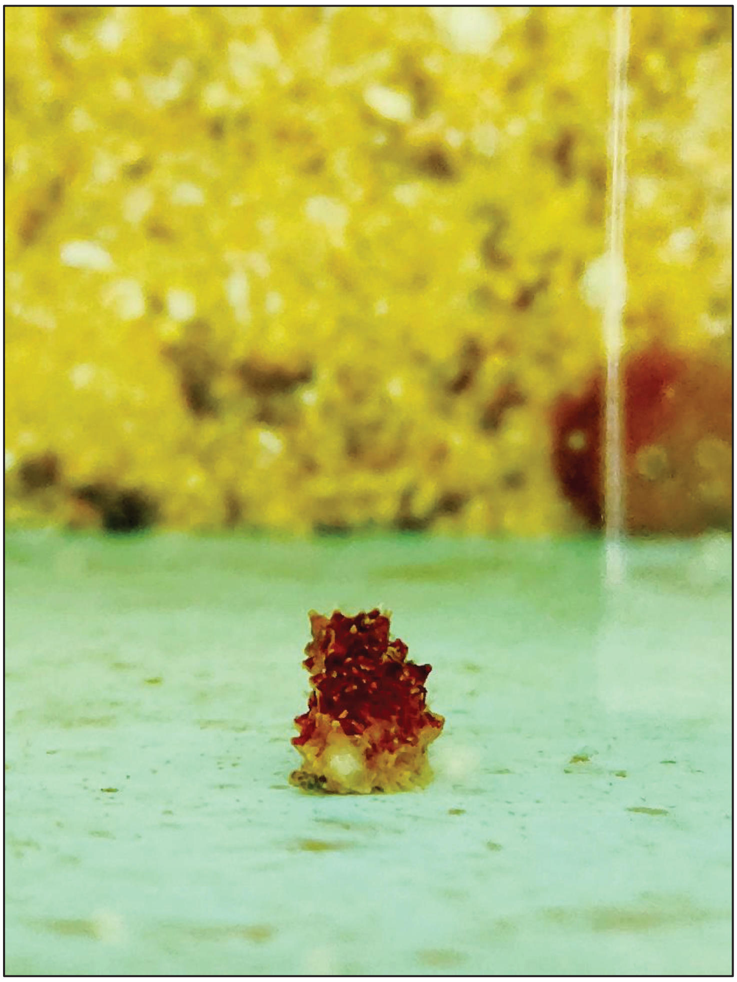

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Dissertação para obtenção do Grau de Mestre em Aquacultura

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Escola Superior de Turismo e Tecnologia do Mar – Peniche

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Resumo

Atualmente existe um problema emergente relativamente à disponibilidade de alimento e ao aumento da população global, esta tendência está a ser acompanhada pela sobre exploração dos stocks pesqueiros globais, que afeta a maioria dos organismos aquáticos, como os pepinos do mar. Na região do oceano pacífico, a Ásia colocou pressão excessiva no mercado de pepinos do mar, o que resultou na apanha intensa destes animais; muitas espécies já foram declaradas como ameaçadas com extinção pela IUCN. O aumento da demanda de pepinos do mar está relacionado com uma maior liberdade económica e com uma maior conscientização sobre o valor nutricional destes animais. Como resposta a esta procura, nas regiões asiáticas, desde a década de 1980, o desenvolvimento da aquacultura de *Apostichopus japonicus* (Selenka, 1867) tornou-se uma prioridade. Atualmente, a maioria dos produtos de *A. japonicus* que são vendidos nos mercados asiáticos vêm da aquacultura. Deste modo, e por haver uma maior procura no mercado na Europa os desembarques e as exportações de pepinos do mar aumentaram; no mar Mediterrâneo muitas operações ilegais ocorreram ao longo dos anos, devido à falta de legislação relativa à pesca do pepino do mar, alguns estudos já demonstraram a perda das maiores classes de tamanho de pepinos do mar em algumas áreas; deste modo, é necessário desenvolver técnicas de produção destas espécies de forma a salvaguardar a biodiversidade e dar lugar à valorização dos produtos europeus. Uma das espécies que apresenta um elevado valor comercial é a *Holothuria arguinensis; e*sta espécie pode ser encontrada no norte do Senegal, Mauritânia, Marrocos, Canárias, Argélia, Sul da Espanha e em Portugal. Considerando que um dos parâmetros físico-químicos mais importantes que afetam o crescimento dos pepinos do mar é a temperatura, este estudo foi direcionado a avaliar de que forma a temperatura pode influenciar o crescimento de juvenis de *H. arguinensis*. Foram utilizados quatro tratamentos de temperatura diferentes 18, 20, 22 e 24°C durante 4 meses. No final do ensaio os indivíduos que demonstraram o maior ganho de peso e comprimento foram os a 20°C com um ganho de peso de 2,79 g ±0,14 e um ganho de comprimento de 1,45 cm ± 0,16; da mesma forma, o tratamento a 20°C também produziu a maior taxa de crescimento específico (0,50%/dia) e a maior taxa de comprimento corporal diária (0,30%cm/dia). Por outro lado, ao considerar as relações alométricas dos juvenis, tanto o maior coeficiente alométrico como o melhor ajuste foram encontrados a 18°C (b = 1,93; R2 = 0,73); ao examinar o fator de condição de Fulton, o mais elevado foi encontrado a 24°C se considerarmos o crescimento; por outro lado, se considerarmos os tempos de amostragem, o fator de condição mais elevado foi encontrado a 22°C (T0 = 6,18, T4 = 7,68). A temperatura que demonstrou melhores resultados para o crescimento dos juvenis de *H. arguinensis* foi de 20°C, no entanto mais estudos futuros necessitam de ser considerados com um intervalo de tempo.

Palavras-chave: Holothuroidea, Equinoderme, Aquacultura, crescimento, comprimento, peso, relação alométrica, índice de condição.

Abstract

In the present day there is an ever-emerging problem concerning food security and global population increase, this trend is being accompanied with the overexploitation of global fisheries stocks, which is affecting not only fish but most aquatic organisms, with special remark of sea cucumbers. In the pacific region, Asia has pressured the sea cucumber market, which resulted in intense harvesting, with many species being declared as threatened with extinction by the IUCN. This increase in demand of sea cucumbers is related to a growth in wealth and to an increased awareness of the nutritional value of these animals. To provide a response to this demand, in the Asiatic regions and since the 1980's the development of *Apostichopus japonicus* (Selenka, 1867) culture became a priority. Presently most of the *A. japonicus* products that are sold on Asiatic markets come from aquaculture. Likewise, recently other species have started to demonstrate large scale production. In Europe, sea cucumber landings and exportations have increased; in the Mediterranean Sea many illegal operations have occurred through the years, due to the lack of legislation regarding sea cucumber fisheries, some studies have demonstrated already loss of the larger size classes of sea cucumbers in some areas; thus, sea cucumber culture techniques need to be developed to safeguard our biodiversity and to give way to the valorisation of European products. One species that demonstrated to have a high market value is *Holothuria arguinensis;* this species can be found in north Senegal, Mauritania, Morocco, Canary Islands, Algeria, South of Spain, and in Portugal. Considering that one of the most important physicochemical parameters that affect growth of sea cucumbers is temperature, this study was aimed at evaluating how temperature can influence the growth of *H. arguinensis* juveniles. As such, the juveniles were placed under four different temperature treatments 18, 20, 22 and 24°C during 4 months. The individuals that resulted in the higher weight and length gains were the ones at 20°C, with a weight increase of 2,79 g ± 0,14 and a length increase of 1,45 cm ± 0,16; Similarly, the 20°C treatment also produced the highest values for Specific growth rate (0.50 %/day) and the daily body length rate (0.30 %cm/day). Conversely, when considering the allometric relations of the juveniles, both the highest allometric coefficient and best fit was found at 18°C (b= 1.93; R^2= 0.73); When looking into the Fulton's condition factor, the highest values were found at 24°C if we consider growth; differently if we consider the sampling times the highest condition factor was found at 22°C (T0= 6.18, T4= 7.68). The best rearing temperature for *H. arguinensis* juveniles during this trial was 20°C, however future studies need to be done with a broader time span.

Keywords: Holothuroidea, Echinoderm, Aquaculture, growth, length, weight, allometric ratio, condition factor.

Contents

1. Introduction

1.1. Food production: Future Challenges

The fast-growing world population combined with a steady increase of wealth in low and middle-income countries, will present various new challenges to today's primary production sector. The global population is forecast to grow from 7.6 to 9.8 billion by 2050, which will increase the need for food by 60-100% above 2005 levels, hence, one of the challenges presented, is directly related to the increase in demand for high-quality protein, due to a larger population and a higher purchase power (Engle et al., 2017), leading to shifts from cereal-based to meat-based products. This can only emphasise the need to establish and develop the ever-expanding aquaculture sector, which could alleviate future protein shortages (Yarnold et al., 2019). Due to its rapid growth, aquaculture has been playing a significant role in global food security, revealing itself as one of the 21st century's critical challenges (Domínguez-Godino & González-Wangüemert, 2019b; Froehlich et al., 2018; Tilman et al., 2011; Tzounis et al., 2017).

The increasing consumption of marine products will not slow down in the coming years, with two main sectors responsible for responding to this demand, fisheries and aquaculture. Since 1990, there has been an increase of around 122% in total fish consumption, while in 2018, record values were reached both in consumption and in the sale of marine products. As such, the aquaculture sector has grown in the order of 527% during this period, when compared to the 14% of fisheries in that same interval (Boyd et al., 2020). From figure 1 it is possible to observe the total production in million tonnes for 2018. During this year, fisheries production reached 96 million tonnes, while the total aquaculture production was 82 million tonnes (FAO, 2020; Boyd et al., 2020).

The ever-increasing stock decline is directly related to unregulated capture or ineffective stock management measures, which are leading to the yield decrease observed in the fisheries sector. Additionally, the development of better fish capturing technologies, has only worsened the problem (Squires & Vestergaard, 2009, 2018). Conversely, technological development in aquaculture allowed lower production costs when compared to its counterpart, leading to an incremental growth over the years. Presently, aquaculture systems can be implemented in the most diverse locations with varying technological levels, whilst producing various species. Aquaculture products have proven themselves as a viable and competitive alternative within the fish market contributing to a greater availability of products, and thus reducing food scarcity and poverty through new job openings as well as new food and nutrient availability (Asche & Smith, 2018; Thilsted et al., 2016; Little et al., 2016).

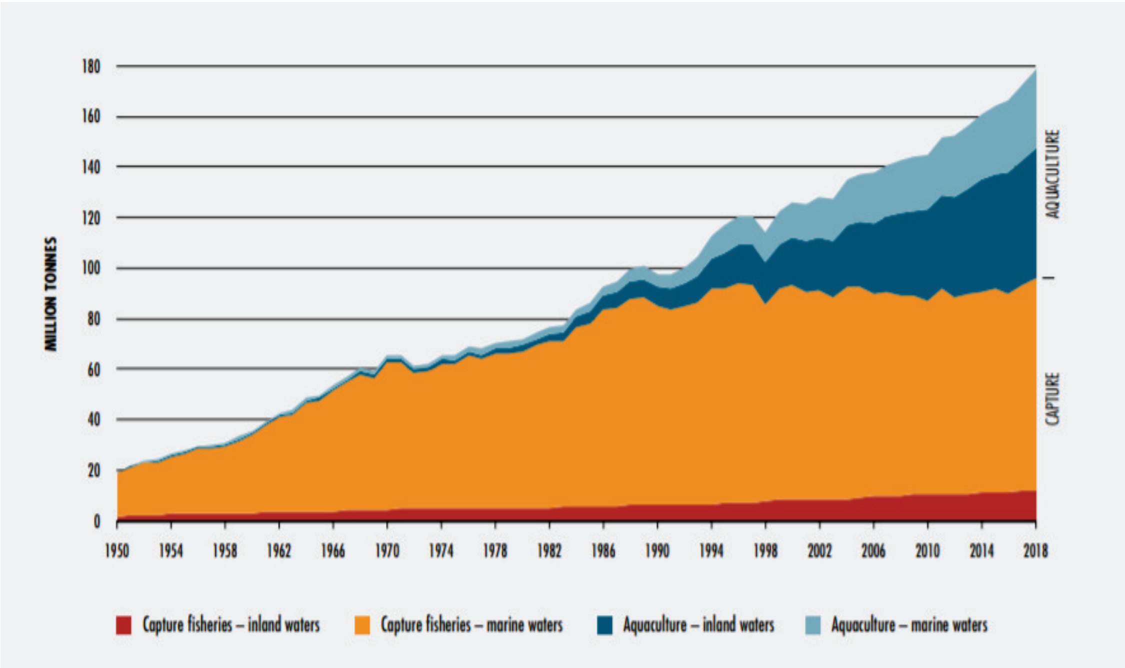

Figure 1. Global fisheries and aquaculture production (FAO, 2020).

According to FAO, (2020) the fraction of fish stocks that are within biologically sustainable levels has decreased from 90% in 1974, to 65.8% in 2017, and overfishing has grown from 33% in 2015, to 60% in 2018 of their maximum level. In areas where, fisheries management is lacking or ineffective the stocks tend to deteriorate rather quickly, these occurrences should be tended to, and successful policies should be replicated and adapted.

The increasing pressure that is being exerted on the world's fisheries is quickly being transferred to non-finfish resources. We are witnessing a decline in the mean trophic level of fisheries landings, as high-trophic-level fisheries are continuously replaced by lower-trophic-level fisheries, such as the expansion of invertebrate fisheries (Branch, 2015; Christensen, 2015). This is mainly caused by the need to find new resources and by growth in demand. However, the exploitation of lower-trophic invertebrates is occurring at a faster rate than its required management policies (Anderson et al., 2011a). 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. However, bust periods are linked to resource depletion, poor recruitment, or socioeconomic reasons (Anderson et al., 2008). For example, sea urchins (globally), oysters (coasts of the US and eastern Australia), shrimp and crabs (Greater Gulf of

Alaska) have shown severe signs of depletion because of increased demand which in turn lead to such exploitation patterns (Lotze et al., 2010).

1.2. Indo-pacific Sea cucumber exploitation

Sea cucumber fisheries is a paradigmatic example of a boom-and-bust exploitation pattern (Eriksson & Byrne, 2015; Hair et al., 2016; Lane & Limbong, 2015). Most sea cucumber species are deposit feeders, as such, they ingest large amounts of sediment from which they assimilate bacterial, fungal, and detrital organic matter (OM) (Navarro, García-Sanz, & Tuya, 2013; Navarro et al., 2013). Their feeding behaviour causes the bioturbation of the sediment, thus, promoting oxygenation and resuspension of nutrients in the water column, making these animals remarkably important for the ecosystem. The bioturbation rates of sea cucumbers are influenced by life-history patterns, age–size distribution, abundance, habitat type and environmental features and seasons (Hou et al., 2018; Williamson, 2021). In addition the amount of ingested sediment is influenced by the assimilation efficiency of the animal (Domínguez-Godino & González-Wangüemert, 2019b, 2020; Moriarty et al., 1985; Yang et al., 2015).

In the trade market these animals are commonly known as “*Bêche-de-mer*”, “*trepang*” or “*haisom*” (Sicuro & Levine, 2011), and have been exploited for over 400 years, mainly for Chinese import, and have been a very important resource in the economic development of the Western Pacific regions (Plagányi et al., 2020). For many coastal communities around the world, sea cucumbers are an important high value export product. In some cases, such as in New Caledonia and Tonga, it even surpassed the fin-fish earnings. In the 1980’s a few factors came into play that led to a considerable rise of the sea cucumber market (Purcell et al., 2013). Namely, because of global market liberalization, which eased trading activities around the globe (Sicuro & Levine, 2011), leading to an economic boom and sea cucumber price increases due to a growing demand from the People’s Republic of China and Chinese communities around the world (Anderson et al., 2011a; Friedman et al., 2011).

Nowadays, 66 species of sea cucumber are reported to be exploited in over 70 countries, around the globe (González-Wangüemertet al., 2018; Purcell, 2014; Purcell et al., 2010; Robinson & Lovatelli, 2015). In Asia, these animals are consumed as a luxury food in contemporary Chinese cuisine and traditional medicine. Sea cucumbers contain high levels of protein and certain bioactive compounds, such as mucopolysaccharides, chondroitin sulfate and antioxidants. Extracts help to heal wounds, have microbial, antioxidant and anticancer properties (Mondol et al., 2017; Santos et al., 2015, 2016; Zmemlia et al., 2020).

In 2011, over 70% of the species in tropical waters were maximally exploited, over-exploited or even depleted, from figure 2 it is possible to see where these stocks have been most affected (Eriksson & Byrne, 2015; González-Wangüemert, et al., 2018; Purcell, 2014; Wolfe & Byrne, 2017).

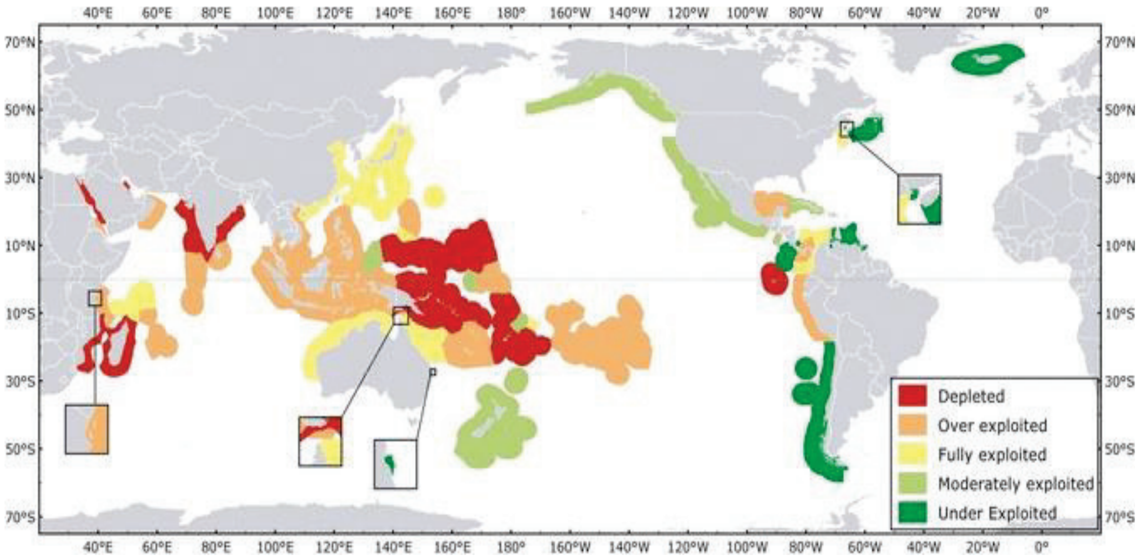

Figure 2. Sea cucumber fisheries status (Purcell, 2011).

About 16 species of sea cucumber have been deemed as threatened with extinction by the IUCN. Here, the same happens as in fin-fishing activities. As the abundance of high-value species decline they are replaced with lower value ones (Hair et al., 2016; Jontila et al., 2014; Lane & Limbong, 2015; Robinson & Lovatelli, 2015). Holothurians are particularly prone to overfishing. These animals exhibit low mobility, late sexual maturity, slow growth and low recruitment rates (that mostly depend on density), contributing to a slow population replenishment, which in turn leads to population collapses (Anderson et al., 2011b; Kevrekidis et al., 2003).

The Indo-Pacific regions are undeniably the larger exporters of these animals. Many of the exporters comprise small scale operations, as most of these providers do not have any other form of income, due to lack of alternatives. This results in fishing operations that are carried out even when there is not enough wild stock for population recovery, hence leaving the ecosystem damaged (Conand, 2018; González-Wangüemert, Domínguez-Godino, et al., 2018). Asia has encouraged the establishment of such operations, the intensive fishing lasts for short period of time, reaching peak catches 5-8 years after initiating the activity, afterwards it is possible to observe a steep decline in catches, which forces operations to halt, and move to other areas (Eriksson & Byrne, 2015). In the past several nations (e.g. Comoros, Costa Rica, Ecuador mainland, Egypt, India, Mauritius, Mayotte, Mexico, Oman, Palau, Panama, Papua New Guinea, Samoa, Solomon Islands, Tanzania mainland, Tonga, Vanuatu, Venezuela) had their sea cucumber fisheries closed due to overfishing (Eriksson & Byrne, 2015; Lane & Limbong, 2015).

1.3. European Sea cucumber exploitation

From, high market demands, overexploitation, depletion, or by moratoriums in most tropical countries, this fishery quickly developed in the Mediterranean and North-eastern Atlantic Ocean (Purcell et al., 2010). Here, Turkey, Spain, Greece and Italy, are the main explorers, with new target species such as, *Holothuria mammata* (Grube, 1840)*, H. arguinensis, Holothuria tubulosa* (Gmelin, 1791) and *Holothuria polii* (Chiaje, 1824) (Dereli & Aydin, 2021; González-Wangüemert et al., 2018; Kevrekidis et al., 2003). In Turkey, this activity began in 1996 and today it has a production of over 550 000 kg/year (80% being *H. polli*) (table 1). Most of the production in Turkey is exported to Asian countries. In Spain, the most exploited species are *H. tubulosa*, *Holothuria forskali* (Delle Chiaje, 1823), *H. mammata*, *H. arguinensis* and *Parastichopus regalis* (Cuvier, 1817). Even though *P. regalis* is consumed in some parts of Spain (Catalonia, Valencia, and Balearic Islands), most of its production is exported. At least 10 Spanish fishing companies exported sea cucumbers to Asia in the last decades. With some of them having a revenue of 1-2 millions $US (Domínguez-Godino & González-Wangüemert, 2019a). In Greece, *H. tubulosa* is harvested by hand or by using hooks and has been utilized as bait in longline fisheries for more than a century (Kazanidis et al., 2014). In Italy, some small scale operations have begun to take place (Sicuro & Levine, 2011). Differently in Portugal, several sea cucumber species, mainly *H. arguinensis*, *H. forskali* and *H. mammata*, are caught artisanally or illegally (Domínguez-Godino & González-Wangüemert, 2020; González-Wangüemert, Domínguez-Godino, et al., 2018; Siegenthaler et al., 2015). In Europe, Turkey remains the major exporter of sea cucumbers (Dereli & Aydin, 2021; González-Wangüemert et al., 2016; Purcell et al., 2013). Nevertheless, in Europe, inefficient stock management, overexploitation and illegal capture are an emerging topic. González-Wanguëmerte (2016) found, that in Turkey, *H. polli* is already showing signs of overexploitation, as larger classes of this species are much less frequent in explored sites, than in unexplored ones (González-Wangüemert et al., 2014).

Table 1. Total fishing production of sea cucumbers in Turkey by year and type of product (frozen, dried, pulverized and salted) (Dereli & Aydın, 2021).

Years	Total production (kg)	Processing method		Number of infringements	Confiscated sea cucumber (kg)	Fines (TL)
1996	19 868	Frozen				
1997	37 665	Frozen				
2002	172	Pulverulent				
2003	10 843	Dried and pulverulent				
2004	5 421	Dried				
2005	53 293	Dried and frozen				
2006	24 200	Dried and frozen				
2007	77 238	Dried and frozen and salted				
		Dried (kg)	Frozen (kg)			
2008	33 669	789	32 880			
2009	37 976	7 036	30 940			
2010	97 183	16 203	80 980			
2011	479 985	13 930	466 055			
2012	447 644	27 479	420 165			
2013	254 226	21 465	232 761			
2014	247 585	23 585	224 000			
2015	270 270	51 300	218 970	25	4 360	24 265
2016	378 883	72 654	306 229	48	7 050	57 890
2017	854 972	130 062	724 910	45	12 276	57 785
2018	943 585	141 841	801 744	134	32 093	191 690
2019	1 292 121	122 271	1 169 850	243	20 352	442 259
2020[a]	238 143	26 760	211 383	46	854	169 500
Total	**5 804 942**			**541**	**79 780**	**943 389** (=**134 769 dollars**)

[a]Includes the first 5 months.

In Turkey, some regulations have already been implemented to impede further misuse of this resource. In 2002, it was prohibited the capture of sea cucumbers during the reproduction period (1st of June –1st of November) (Aydin, 2017). Later in 2007, and mostly due to overfishing and illegal captures, more regulations were implemented; Licensing, market chain reporting, restrictions on fishing gears and quotas as well as rotational harvesting cites. The harvesting cites were divided into two areas in the northern Mediterranean region. The two areas would then alternate every four years, leaving one for stock recovery and the other open for exploitation. However, overexploitation and illegal capture were still observed (González-Wangüemert et al., 2014; Hasan, 2008; Roggatz et al., 2018), and more recently in 2020, the fishing was capped at 2500 tonnes annually (Dereli & Aydin, 2021).

In Italy, illegal fishing of sea cucumbers has been recorded with ever-increasing frequency in Sardinia and Puglia region. In 2015, a total of 29,030 kg of sea cucumbers were illegally captured which resulted in monetary penalties, seizure of vessels and dismantling of clandestine processing facilities (Meloni & Esposito, 2018). In 2016 and 2017 there was a total of 23,900 kg of illegal catches and several monetary penalties. More recently, in 2018 it was implemented a moratorium that prohibited the capture until 31st of December of 2019 (Dereli & Aydin, 2021).

In Spain, there is some legislation concerning *H. forskali* catches, which forbids the fishing activity in Galicia. Still, most of the Spanish communities do not have regulations regarding sea cucumbers, which allows the perpetuation of illegal catches. In Portugal, the scenario is similar. There is only legislation mentioning *H. forskali*, *P. regalis* and *Mesothuria intestinalis* (Ascanius, 1805) as possible fishing targets, but with no quotas or minimum catch size for licenced fishermen. For recreational fisheries the generic maximum fishing limit is 2 kg/day/person for unregulated species (Regulamento de Apanha, Portaria n.º 1228/2010, de 6 de dezembro; Portaria n.º 1228/2010, de 22 de novembro). Such gap leads to uncontrolled exploitation and illegal captures which are common in both Portugal, in the protected lagoon area of Ria Formosa, Portimão and Albufeira; and Spain, at the coast of Cadiz (South of Spain) (González-Wangüemertet al., 2018; Olaya-Restrepo et al., 2018).

We are already witnessing the beginning of the decline of European stocks, and we can expect the same scenario that unfolded in most tropical countries, to happen here in the Mediterranean Sea and NE Atlantic Ocean. Hereafter, the future of sea cucumbers all over the world could lie in aquaculture that could provide an alternative source of product and therefore support efforts to preserve natural populations (Sicuro & Levine, 2011).

1.4. Sea cucumber aquaculture: State of the art

Sea cucumbers have been a staple tradition to Asian communities; however, the aquaculture of sea cucumbers has only developed in the last century. In China during 1954, Zhang Fengying started to develop this technology with the temperate species *A. japonicus*. During this experiment it was attempted to rear *A. japonicus* on an artificial reef. Prior to 1980s, most of the Chinese populations lived in poor conditions and the nutritional levels of these animals were not widely acknowledged and so, market demand was limited. Consequently, Asian wild stocks could satisfy this need. However, over time, there was a shift in consumers purchase power. There was a considerable socioeconomic boom which caused an increased awareness of the health benefits that came with consuming *A. japonicus*. Thus, market demand grew dramatically, which caused the uncontrolled exploitation of wild stocks. During the 1960s the fishing effort produced about 260-280 tonnes whilst in 1970s the total production was only 60-80 tonnes of sea cucumber, as result of a population decrease (Yang et al., 2015). The overexploitation of wild stocks led to the near extinction of this species in some areas. Since natural populations could no longer satisfy market demands *A. japonicus* aquaculture became a priority. During the 1970s, hatchery protocols had been developed, and successfully produced juveniles. Soon after, in the 1980s, this activity prospered, and commercial scale production of *A. japonicus* began (Chen, 2004; Kinch et al., 2008; Purcell et al., 2012). This development allowed a steady supply of product to Asian markets and, also, allowed rehabilitation of depleted sea cucumber populations. This was done by enhancing stocks with hatchery-reared juveniles, by sea ranching or through pond cultures (Domínguez-Godino et al., 2015; Eriksson et al., 2012). In 2002, in China, the total sea cucumber aquaculture production was about 5865 fresh weight tonnes, when compared to the 470 fresh weight tonnes captured by fishing. Since the production of sea cucumbers was dominated by aquaculture, after 2003 most Chinese provinces saw a decline in fishing yield caused by overfishing. In 2014 the total broad aquaculture output of sea cucumbers in China reached 200,969 fresh weight tonnes (Han et al., 2016).

To successfully rear *A. japonicus*, the individuals go through several stages, from broodstock collection, reproduction, larval rearing to juvenile grow out. The reproduction stage happens naturally during the summer from June to the end of July, however, if they do not spawn naturally this process can be induced by desiccation (air exposure with evening type lighting conditions), thermal shock (temperature increase), running water (increased flow of seawater) or by a combination of all three (Yamano et al., 2015; Yang et al., 2015). During larval development stages these animals are fed with an increasing concentration of microalgae (i.e., *Dunaliella salina* (Teodoresco, 1905), *Phaeodactyl tricornutum* (Bohlin, 1897)), and 20 days, post fertilization, the first juveniles can be observed. At this stage they mostly feed on benthic diatoms and biofilm but can also be supplied with a macroalgae filtrate. After 100 days these individuals reach 1 cm and can be considered seedlings (Liu et al., 2015; Ru et al., 2019; Wang et al., 2015; Yang et al., 2015). Currently there are four ways that these juveniles can be reared: indoor facilities, pond cultures, sea ranching and pen cultures, all of these techniques depend on tide and hydrodynamism, except for indoor facilities (Chen, 2003, 2004; Yang et al., 2015). Indoor facilities rely on flow-through systems at a rate that ensures two complete water changes every day (Zhang et al., 2015). Pond cultures are usually carried out in old shrimp farms near shore so that water exchanges occur naturally (Chen, 2004). Conversely, sea ranching is done in near shore delimited areas, these areas should have sandy bottoms as well as shelters. Another difference is related to natural predators, so, for this method to work, studies must be carried out to infer the existence and number of predators. Lastly, pen cultures are done by means of floating cages that are placed near shore (Purcell et al., 2012). Of all these methods sea ranching comes as a more affordable way of rearing as it does not require any infrastructure (Bai et al., 2016; Chen, 2004;

Purcell et al., 2012). In order to successfully rear a species, studies must be done regarding sea cucumbers, ecology, reproductive cycles, life-history traits, 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 applied to other species with some adjustments, so efforts must be made in this direction. Species such as, *Holothuria scabra* (Jaeger, 1833), *Holothuria lessoni* (Massin, Uthicke, Purcell, Rowe & Samyn, 2009), *Parastichopus californicus* (Stimpson, 1857), and others, are reared today. Some of which have commercial scale production values, while others are simply cultured for stock enhancement and conservation purposes. These aquacultures can be found in different countries, such as, Australia, Canada, China, Japan, Philippines, Madagascar, and Mexico, among others (Conand, 2018; Han et al., 2016; Purcell et al., 2012, 2018).

Being a relatively recent technology, and without a sea cucumber exploitation culture, Europe still lags behind when it comes to sea cucumber production. Nonetheless over the years there have been an increasing number of papers published in this area (Domínguez-Godino et al., 2015; Eriksson et al., 2012; Mueller, 2017; Purcell et al., 2018; Rakaj et al., 2018, 2019; Robinson et al., 2019; Venâncio et al., 2021).

Some European sea cucumber species have demonstrated to be potential aquaculture candidates (Domínguez-Godino et al., 2015; Domínguez-Godino & González-Wangüemert, 2018a, 2019b; González-Wangüemert et al., 2016; Rakaj et al., 2018, 2019; Santos et al., 2016). Several of these temperate species are new targets for exploitation and have high market values (Hossain et al., 2020). The species *H. arguinensis* is one of those candidates. This species not only has high market value but shows a high potential for aquaculture production (Domínguez-Godino & González-Wangüemert, 2019b). Similarly to *A. japonicus*, this species is targeted not only for commercial scale production (Domínguez-Godino et al., 2015), but also for restocking natural populations (seacucumber.eu).

1.5. Biology of *Holothuria arguinensis*

H. arguinensis, can be found from the Berlengas Islands to Morocco and Mauritania including the Canary Islands, and more recently it was found colonizing the Mediterranean Sea (Alicante, Spain) and Algerian Waters. It can be found from the intertidal zone from 0 m to 52 m of depth and is frequently observed in sandy bottoms, macroalgal dominated beds and sea grass meadows, mainly due to its feeding habits as it prefers macroalgae (González-Wangüemert & Borrero-Pérez, 2014; Mezali & Thandar, 2014; Olaya-Restrepo et al., 2018; Rodrigues, 2012; Siegenthaler et al., 2015). Research on ecology and behavioural studies on *H. arguinensis* have shown that this species moves continuously during day and night without any form of sheltering behaviour, however, it is often found covered with seagrass leaves, algae, shells and rocks (Domínguez-Godino et al., 2015; Marquet et al., 2017; Navarro et al., 2014; Olaya-Restrepo et al., 2018). Adult individuals from this species commonly reach a mean size of 18.5 cm (Marquet et al., 2014). During Azevedo e Silva's (2021) study in Arrábida, the specimens in the area reached the a mean size of, 18.9 cm ± 2.49 and a mean weight of 469.28 g ± 9.91. In another assessment, in Ria Formosa, by González-Wangüemert (2013), found that in that area the most frequent size class was 19-20 cm. Conversely, another study done by, Domínguez-Godino & González-Wangüemert (2020), analysed the length and weight of 354 individuals from two separate locations, Praia de Faro and Ramalhete. The Praia de Faro's animal length ranged from 12 to 41 cm and the weight ranged from 104,6 g to 366,1 g; It was found that the individuals from Praia de Faro were larger and heavier reaching a mean length of 24.04 cm ± 5.62 and a mean weight of 213.08 g ± 51.23 ; whilst the animals from Ramalhete were smaller with a mean length of 21.87 cm ± 4.84 and a mean weight of 193.54 g ± 43.59 . In another experiment, the weight and length of *H. arguinensis* was evaluated in two separate locations in Morocco (Skhirat and Souiria k'dima); during this experiment it was found that the weight and length of these animals varied between 6 and 23 cm, whereas weight varied between 17.5 and 425.2 g; In Skhirat the mean for length and weight was, respectively, 144.6 g ± 60.6 and 13.0 cm ± 3.0 , and in Souiria k'dima the mean length and weight was, respectively, 13.8 cm ± 3.2 and 173.3 g ± 64.9 (Haddi et al., 2021).

Macroscopically, *H. arguinensis* has some particular features (figure 3). The ventral face (trivium) of these animals is moderately whitish and dotted with numerous light brown pedicels, that when retracted appear as punctures on the tegument. Differently, the dorsal face (bivium) is more convex, and exhibits wrinkles and cracks, that are uniformly coloured in a red brownish tone. The bivium also has pedicels, though these are not as frequent or as numerous as in the trivium. On the lateral faces the colouring is attenuated and imperceptibly blends into a more whitish colour. Another trait that is very characteristic of this species is the presence of several rows of blackish-brown papillae, with sometimes white tips, on the bivium (González-Wangüemert & Borrero-Pérez, 2014; Koehler & Vaney, 1906).

Figure 3. Adult individuals of *Holothuria arguinensis* (A – Trivium; B – Bivium).

1.6. *Holothuria arguinensis* larval development and juvenile rearing

Several techniques learned from other species can be applied in the culture of *H. arguinensis*, from broodstock maintenance, reproduction, larval development, and juvenile rearing (figure 4). Regardless of crucial differences (i.e., fecundity and duration of the larval development) spawn induction and larval development have been described by Domínguez-Godino (2015) and in Cetemares facilities by Sousa (2020). However, there is still a lack of studies pertaining to juvenile rearing. Rearing juveniles can be done almost in the same way as broodstock maintenance, however, optimal temperature conditions for the growth of this species are still unknown. Temperature is one of the most important abiotic parameters that influence aquatic species (Tepler et al., 2011). Echinoderms are ectoderms, as such, they depend on environmental temperatures as they are unable to regulate their own body temperature (Díaz et al., 2017). This characteristic can deeply influence the development of echinoderms, as higher temperatures tend to increase the rate at which they go through their developmental stages, this is mostly due to the increased speed of metabolic reactions (An et al., 2007; Dong et al., 2008; Dong & Dong, 2006; Ji et al., 2008; Meng et al., 2009). When in thermal stress, energy allocation can undergo some crucial changes and therefore affect normal activities such as, growth, reproduction, and foraging, leading to consequences in their performance and fitness (Mora & Ospína, 2001; Pörtner et al., 2008). The only available data for *H. arguinensis* describes how low temperatures can induce a period of reduced feeding, movements, absorption efficiency and growth (Domínguez-Godino & González-Wangüemert, 2019a) . For *A. japonicus* it is also know that temperatures above 25°C can induce an aestivation period, which result in lower activity and weight, non-feeding, and metabolic disturbances. However, for *H. arguinensis* and according to observations made by (Domínguez-Godino & González-Wangüemert, 2019a)) higher water temperatures do not seem to provoke such a response, as in summer months the individuals demonstrate feeding activity and movement in tanks (Domínguez-Godino & González-Wangüemert, 2019a).

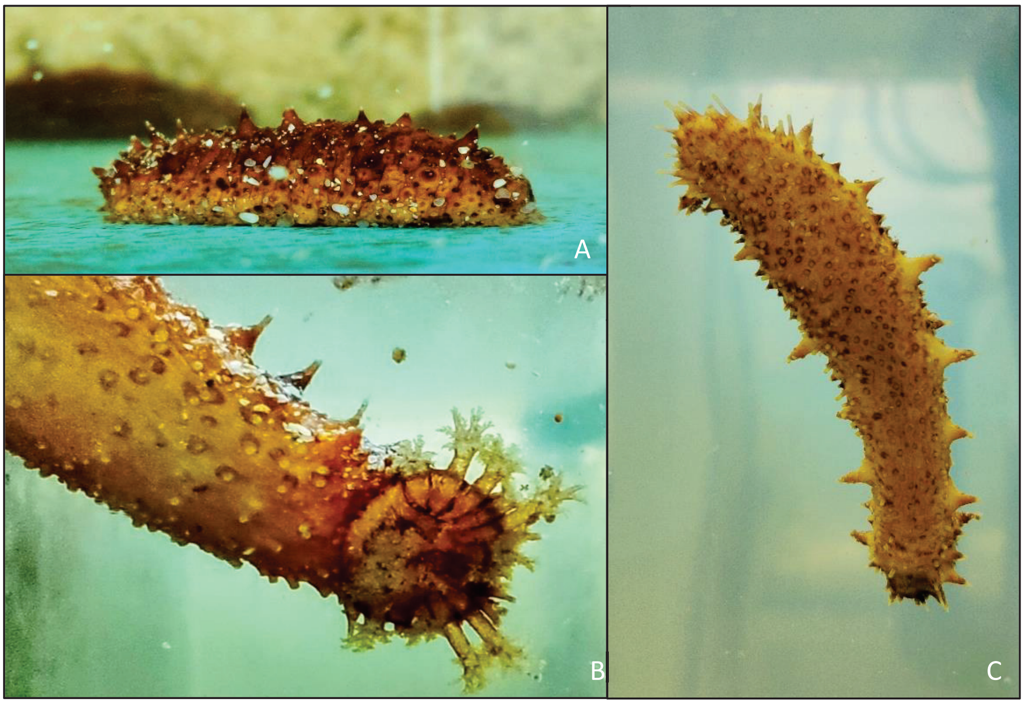

Figure 4. Juvenile individuals of *Holothuria arguinensis* (A –Lateral view; B – Buccal apparatus; C – Trivium).

2. Objectives

The development of sea cucumber aquaculture is still lagging with European species when compared with some of the Asiatic species (*A. japonicus*, *H. scabra*). Even though some studies have been conducted with various species and, concerning themes such as, broodstock conditioning, reproduction, substrate and feed experiments, as well as, water parameters (Domínguez-Godino et al., 2015; Domínguez-Godino & González-Wangüemert, 2018a, 2019a; Hongsheng et al., 2005; Venâncio et al., 2021), only a small percentage of these studies were conducted with juvenile specimens of European species (Domínguez-Godino & González-Wangüemert, 2018a). Advancements with this intent should become a priority to the domestication of a species. Two main points should be taken into consideration, the first is relative to the conservation of the species, as management will take longer to develop, the artificial culture of this species could alleviate some pressure of the wild stocks (Domínguez-Godino et al., 2015; González-Wangüemert et al., 2014), and the second being the high value that this species has on the market (Domínguez-Godino et al., 2015), future opportunities may arise, and it could allow the valorisation of undervalued species, in turn creating a whole new market.

Thus, this experiment aims to study the effect of temperature on the survival and growth of *H. arguinensis* juveniles. To achieve that, weight and length data, survival rates, allometric relations and Fulton's condition factor are analysed for individuals exposed to different environmental temperatures. The data obtained here will aid in with future experiments as the water temperature is one of the most important parameters to take into consideration when rearing aquatic species as physiological performances are crucial for animals to adapt to environmental variations.

3. Materials and Methods

2.1 RAS system preparation

The experimental design comprehended four recirculating aquaculture systems (RAS), each with three tanks with 60L (40x40x40 cm), that were used as replicates as seen in figure 5. Each system had its own sump with 100L (60x40x40 cm), that had three compartments. These compartments 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).

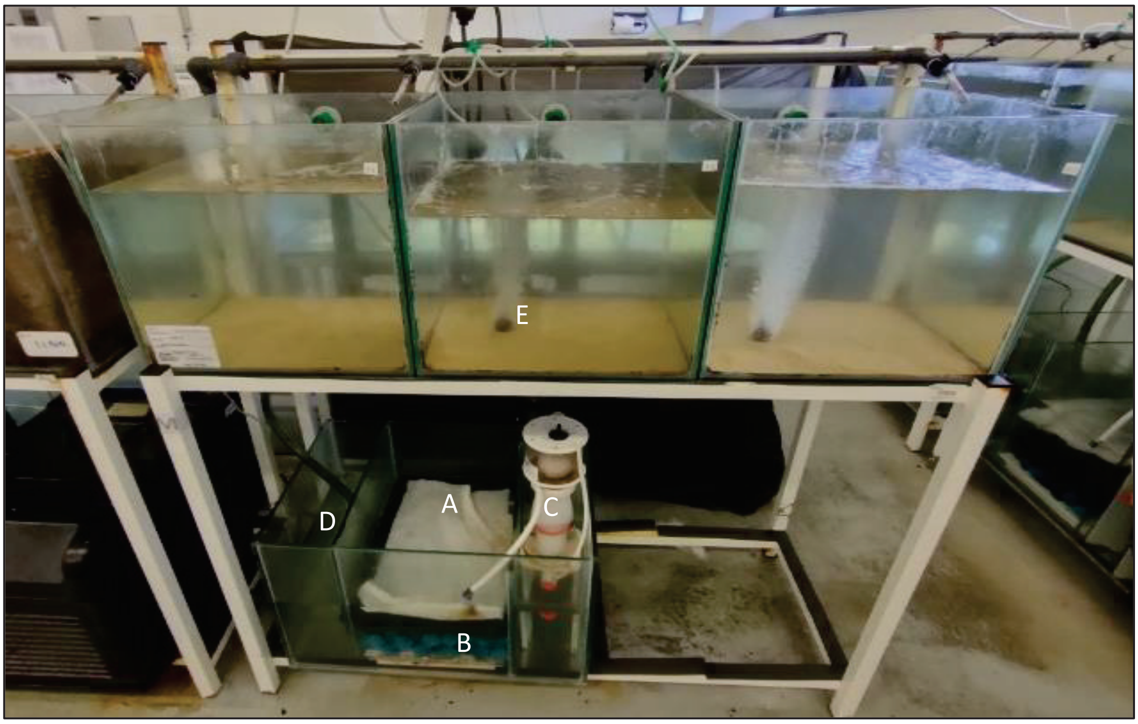

Figure 5. Experimental set-up used with the RAS system, where: A - sponges, B- bio-balls, C- skimmer, D- water pump, E- aeration stone.

Each tank had its own aeration, and it was regulated so that it was equal on all tanks. To disinfect the tanks, they were recirculated with fresh water and sodium hypochlorite (0.5%) for two days. After this period, they were refilled with fresh water to remove any hypochlorite residues, before being finally emptied and filled with saltwater for one week, to mature the biological filters. During this period, substrate was also added to the tanks. The sand used in the systems went through cleaning treatments before it was placed in the respective tanks. Beforehand, it was washed in a 400L (60x40x40 cm) vat. Saltwater was added to the sand, and it was stirred and aerated to remove any pockets of anoxia trapped in the sedimented layers, throughout this process the water was changed. This process was carried out several times, consecutively. Once the water appeared clear, aeration was placed, and sodium hypochlorite (0.01%) was added for a period of two days. Afterwards, the sand was stirred again, and the saltwater was renewed, aeration was set, and the sand was left in these conditions for two more days, to remove any hypochlorite residues. Finally, EHEIM thermocontrol 150 thermostats (EHEIM GmbH & Co. KG. Germany) were placed in the systems to regulate the temperature. The water was maintained at 20°C to avoid physiological stress after transferring the animals.

All juveniles of *H. arguinensis* in the present trial were hatchery-reared in MARE-Polytechnic of Leiria. These animals were in a tank with 550L (250x80x40cm), at 20°C. The animals were measured and randomly distributed throughout the 4 systems, with 14 specimens per tank (168 individuals). Total length was measured (± standard deviation) with the animals still submerged and without manipulation to avoid muscle contraction, whereas the weighing was done out of water (total wet weight ± 0.01 g) with a KERN PCB 2500-2 (KERN & SOHN GmbH Ziegelei 1.72336 Balingen, Germany) precision scale. After animal distribution, the temperature of each system was gradually readjusted, to obtain the four target temperatures of 18, 20, 22 and 24 °C. To access the weight and length condition of the animals, sampling was done once per month in almost regular intervals of 30 days (table 2), weighing and measuring was done with the same protocol as previously mentioned for animal distribution.

Table 2. *Holothuria arguinensis* sampling times and corresponding dates.

Date	Sampling time
05/12/2020	T0
07/01/2021	T1
11/02/2021	T2
13/03/2021	T3
13/04/2021	T4

One week before, and during the experiment before feeding, water parameters, temperature, pH and dissolved oxygen were measured with the multiparametric probe YSI-Pro Plus (YSI Incorporated, 1725 Brannum Lane, Yellow Springs, OH 45387, USA), salinity was measured with a Digital Refractometer for Seawater Analysis - HI96822 (Hanna Instruments Inc., Woonsocket, Rhode Island, 02895, USA), ammonia and nitrites (NH_3 and NO_2^- in $mg.L^{-1}$) were measured with API colour test kits (Mars fishcare, Waltham-on-the-Wolds, LE14 4RS, United Kingdom).

The feed was weighed with a precision scale (KERN PCB 2500-2). Before the feed was added to the systems, to allow a better intake of the feed and to not waste any particles, the water recirculation was stopped, and about 10-20% of water volume were removed from each tank. After a few hours, the water circulation was re-established. Saltwater was added to the sump to promote water exchanges and to return the missing water volume. Water exchanges were done whenever necessary, to maintain the water quality.

The diet used, included AlGAMAC 3050® and *Saccorhiza polyschides* (Batters, 1902) in the form of a dry powder as used by Sousa (2020). The animals were to be placed under a 3% biomass regime and fed three times a week; due to animal growth the amount of feed (g) was gradually adjusted throughout the experiment. The macroalgae used, *Saccorhiza polyschides,* was collected at Praia do Portinho da Areia Norte, Peniche (39°22'07.7"N 9°22'40.7"W). At the Aquaculture Lab of MARE-Polytechnic of Leiria, it was placed in a 400L vat (100x60x53.5 cm) with saltwater, aeration, and sodium hypochlorite (0.05%), for two hours, to disinfect it (Kerrison et al., 2016; Kerrison et al., 2015). Once disinfected, the water in the vat was completely replaced and the macroalgae was rinsed with saltwater to remove any remains of sodium hypochlorite. Afterwards, the macroalgae remained in this tank for two days with aeration. Following this treatment, the algae were separated and processed. Since *S. polyschides* was to be fed in the form of a dried powder this macroalgae was placed on trays and taken to the oven for two days at 25°C so that it could dry. After drying, it was crushed into fine powder, and then stored at 4°C (Rodrigues et al., 2015; Vanegas & Bartlett, 2013).

2.2 Data analysis

Growth performance and survival were assessed for each system by determination of daily body length rate (GR), Specific Growth Rate (SGR), Survival Rate (SR) and initial and final Condition Factor (K). Calculations were carried out using the following formulae:

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

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

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

4. $K = \frac{Weight}{Lenght^3} \times 100$

Where, *Wi* is the initial weight (g), *Wf* is the final weight (g), *Li* is the initial length (cm), *Lf* is the final length (cm), *T* is the experimental time (days) (Domínguez-Godino & González-Wangüemert, 2018b, 2019a; Nyadjeu & Eyango, 2020).

Statistical tests were performed using IBM SPSS™ Statistics for Windows, version 27 (IBM Corporation, Armonk, New York, U.S.). One-Way ANOVA were performed utilizing a value of $p < 0.05$ as level of significance. The Levene's test was used to test the homogeneity of variances. In the occurrence of statistically significant differences, a multiple pairwise comparisons were performed using the post-hoc parametric Fisher's Least Significant Difference (LSD).

The length-weight data were analysed through the power equation:

$$FW = a.FL^b$$

where FW is fresh weight and FL is fresh total length; a is the intercept and b is the allometric coefficient (Keys, 1928). It is to note that if b=3 then growth is considered isometric, meaning that, length increments are proportionate to weight increments. If b>3 then growth is no longer proportionate, and is in turn considered a positive allometry, where weight increments are more accentuated than length increments. Conversely a negative allometry occurs if b<3 then, length increments are larger than weight increments.

5. Results

3.1 Mortality rate

During the daily routines any casualties that occurred were registered. In table 3, it is possible to observe the survival rates for the different temperatures according to each sampling time. At the end of the experiment mortality rate values ranged from 2% (20°C) to 12% (22°C), being respectively the minimum and maximum mortality rates obtained. The other two mortality rates can be found within this interval, being, 7% (18°C) and 5% (24°C). Out of the initial 168 individuals, 157 successfully reached the end of the experiment (93%).

Table 3. Mortality rates (%) for *Holothuria arguinensis* at different temperatures (18, 20, 22 and 24°C) according to the sampling time (T0, T1, T2, T3 and T4).

	Mortality (%)			
	18°C	20°C	22°C	24°C
T0	0.0	0.0	0.0	0.0
T1	2.4	0.0	0.0	0.0
T2	2.4	0.0	0.0	0.0
T3	4.8	0.0	0.0	0.0
T4	7.1	2.4	11.9	4.8

3.2 Weight analysis

There was an increase in fresh weight across all temperature treatments after the 4-month period, in table 4 and figure 6, it is possible to find the corresponding values for the initial and final Fresh Weights (FW). The highest mean FW value found was at 20°C (5.90 g ±4.88) and the lowest at 18°C (4.11 g ±3.78). Nonetheless, there were no statistically significant differences found between each temperature treatment in any of the sampling times (T0, T1, T2, T3 and T4), according to a One-Way ANOVA.

It was found that the individuals at 20°C resulted in a higher SGR (0.50 %/day), whilst the ones at 18 had the lowest SGR (0.19 %/day). The other two temperatures resulted in 0.39 %/day and 0.35 %/day, respectively, 22 and 24°C (figure 7).

Table 4. Initial and final mean fresh weight (FW) for *Holothuria arguinensis* across temperatures (18, 20, 22 and 24°C) and sampling times (T0, T1, T2, T3 and T4).

	18°C	20°C	22°C	24°C
Initial FW (T0)	3.23±2.55	3.11±2.04	3.22±3.19	3.45±2.40
Final FW (T4)	4.11±3.78	5.90±4.88	5.37±5.28	5.45±4.10

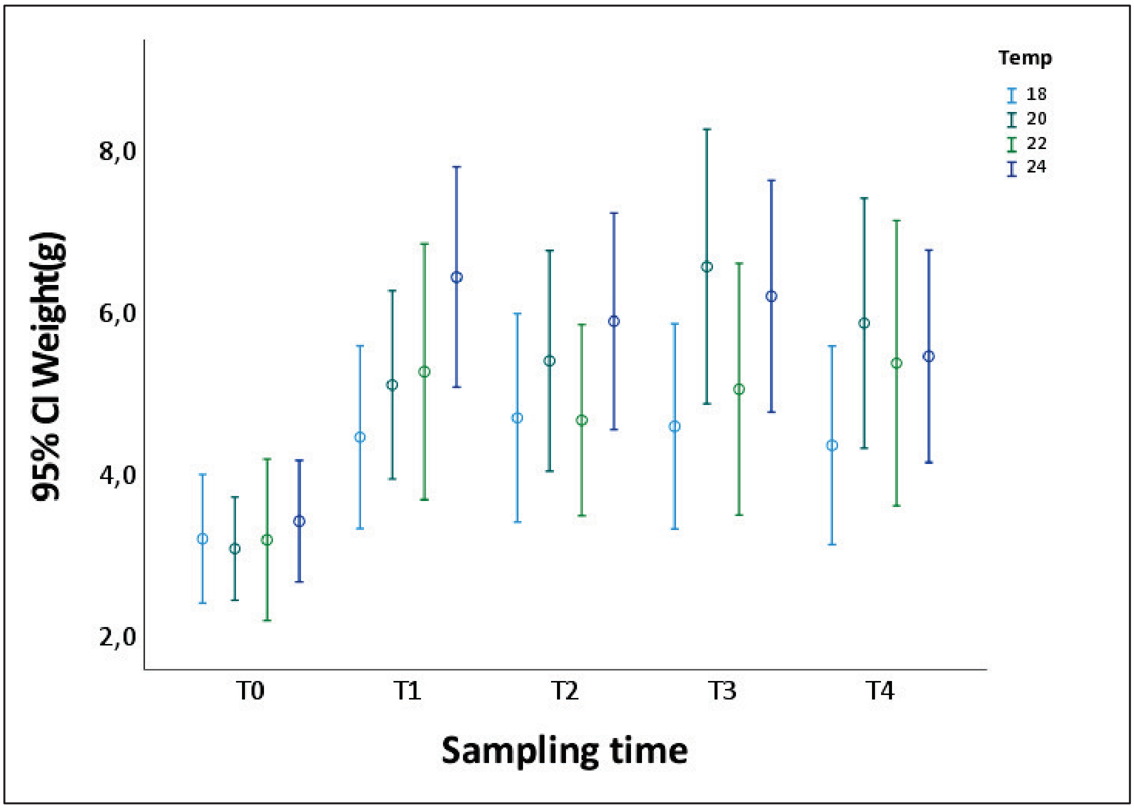

Figure 6. Fresh weight for *Holothuria arguinensis* across the different temperatures (18, 20, 22 and 24°C) in accordance with the sampling times (T0, T1, T2, T3 and T4).

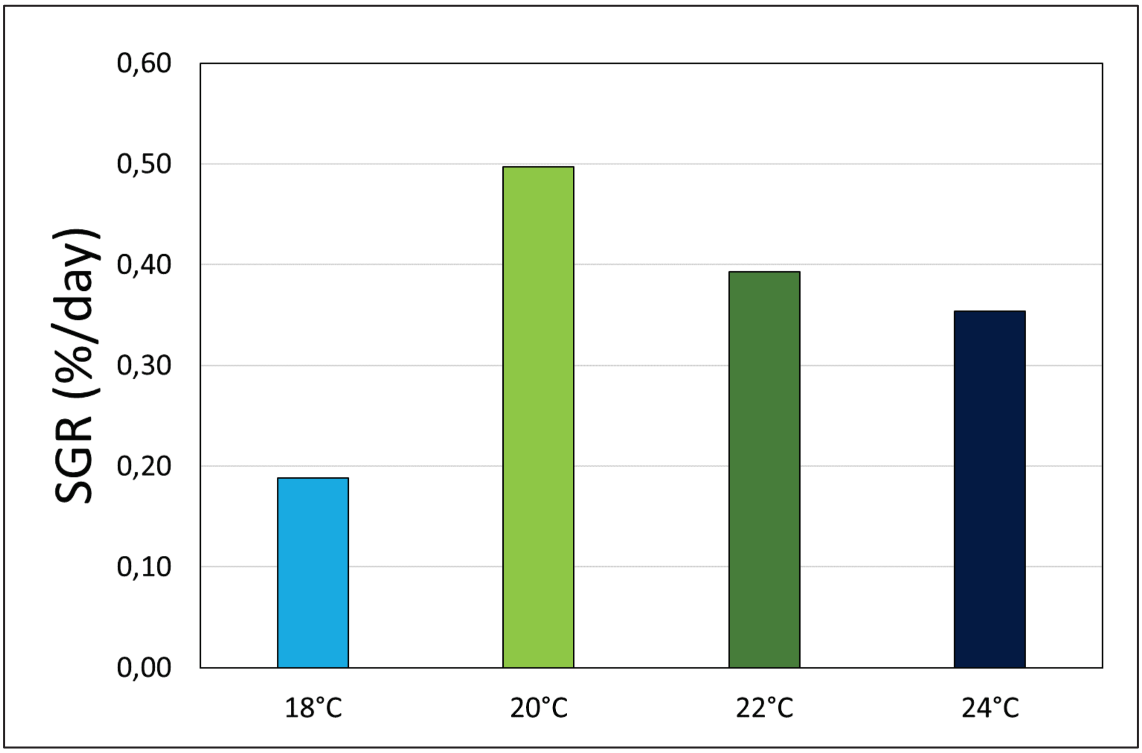

Figure 7. Specific growth rate (%/ day) for *Holothuria arguinensis*, relative to each temperature (18, 20, 22 and 24°C).

3.3 Length analysis

At the end of the trial, and similarly to the FW results, the highest Fresh Length (FL) found was at 20°C (5.15 cm ±1.74), however the lowest FL value found was at 22°C (4.13 cm ±1.71) (figure 8 and table 5). Posteriorly, the statistical evaluation indicated that at T0, T1, T2 and T3 there were no statistically significant differences between any of the different temperature treatments according to a One- Way ANOVA. However, in moment T4 statistically significant differences were found according to a One- Way ANOVA [$F4\ (3.\ 153) = 2.716$; $p = 0.047$]. The Post hoc Fisher's multiple comparison test (LSD) indicated that the individuals exposed to 20°C (5.15 cm ±1.74) had a significantly higher length than those in the 22°C treatment (4.14 cm ± 1.71).

Looking to the daily body length rate (%cm/day) the temperature treatment that produced the highest GR was the 20°C (0.30 %cm/day) and the lowest was found at 22°C (0.07 %cm/day), the other two temperatures resulted in similar GR values, being, 0.13 and 0.12 %cm/day, respectively, 18 and 24°C (figure 9).

Table 5. Initial and final fresh length (FL) for *Holothuria arguinensis*.

	18°C	20°C	22°C	24°C
Initial FL (T0)	3.93±1.30	3.70±1.26	3.81±1.70	3.86±1.30
Final FL (T4)	4.60±1.41	5.15±1.74	4.13±1.71	4.43±1.56

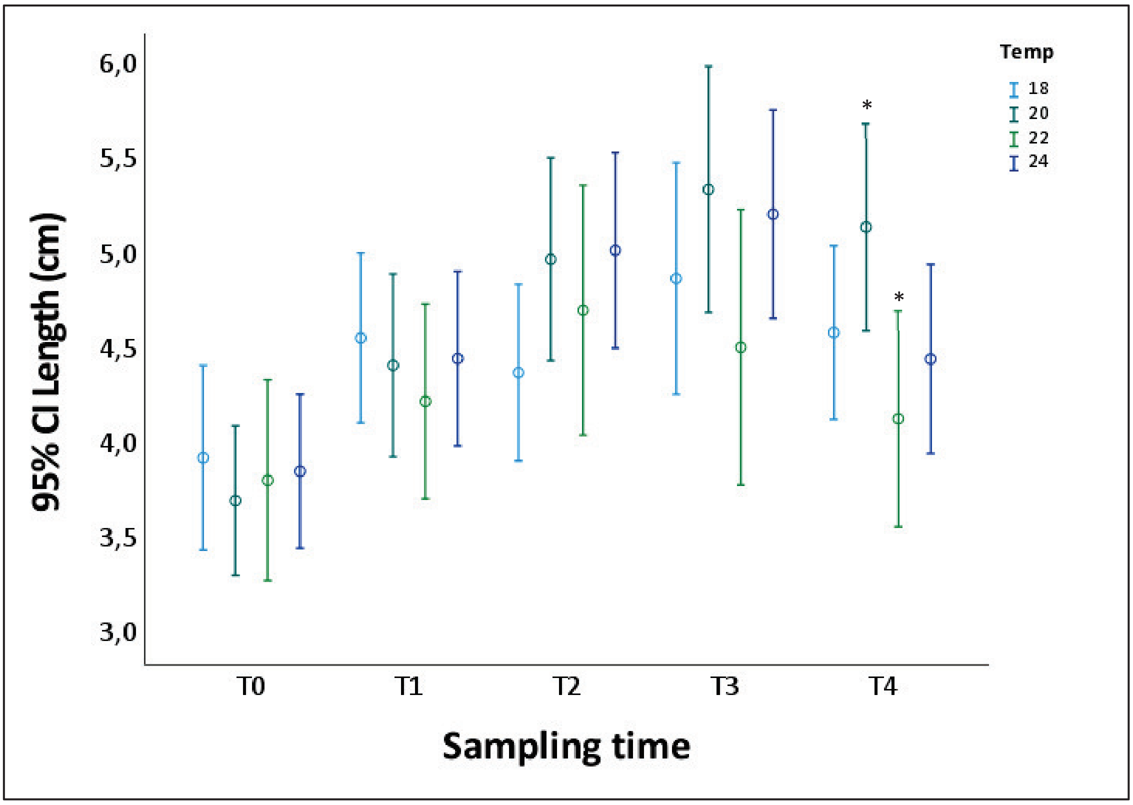

Figure 8. Mean fresh length for *Holothuria arguinensis* across the different temperatures (18, 20, 22 and 24°C) in accordance with the sampling times (T0, T1, T2, T3 and T4). Note: * represents statistically significant differences in sampling time T4, between 20 and 22°C

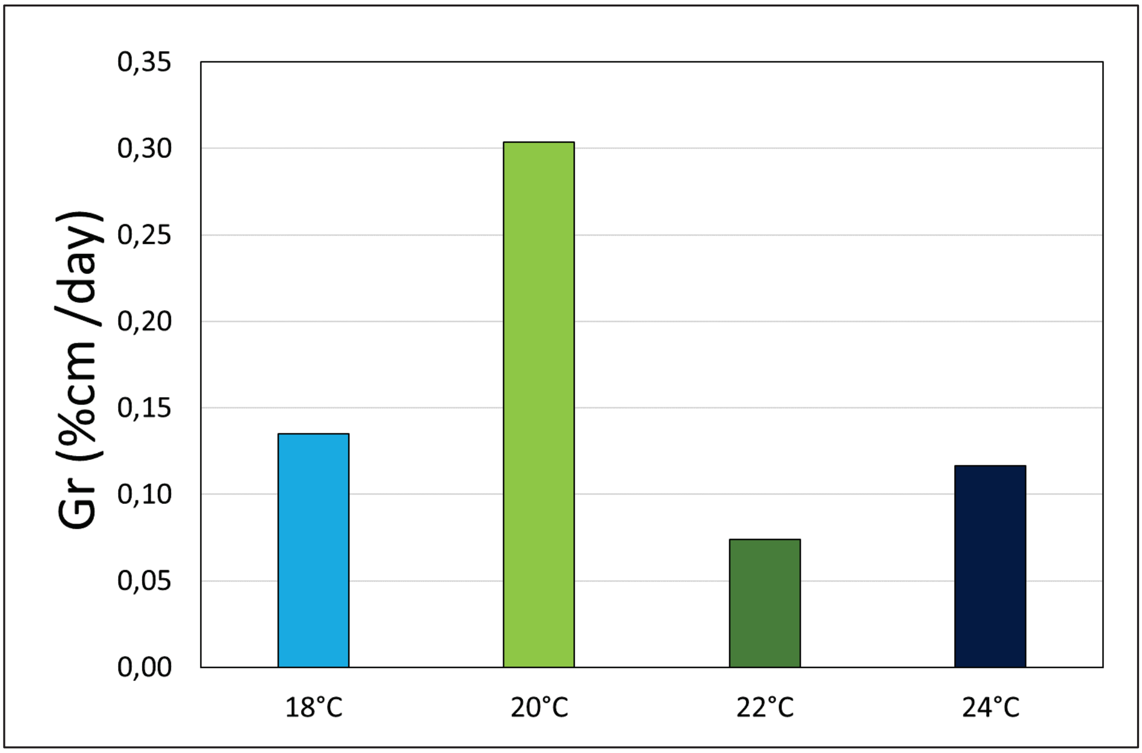

Figure 9. Growth rate (%cm/ day) for *Holothuria arguinensis*, relative to each temperature (18, 20, 22 and 24°C).

3.4 Condition analysis

Regressions were made to analyse the allometric relation between weight and length (figure 10). All the curves obtained translated into negative allometry (X<3). At 22°C it was obtained the worst fit of all temperatures ($R^2_{22°C}$ = 0.60), at 20°C and 24°C a slightly better fit was found ($R^2_{20°C}$ = 0.64; $R^2_{24°C}$ = 0.69), and at 18°C the model showed the best ($R^2_{18°C}$= 0.73).

The allometric coefficient demonstrated the lowest at 22°C ($b_{22°C}$ =1.82) and the highest at 18°C ($b_{18°C}$ =1.93), the other two values being within this interval ($b_{20°C}$=1.90; $b_{24°C}$ =1.91)

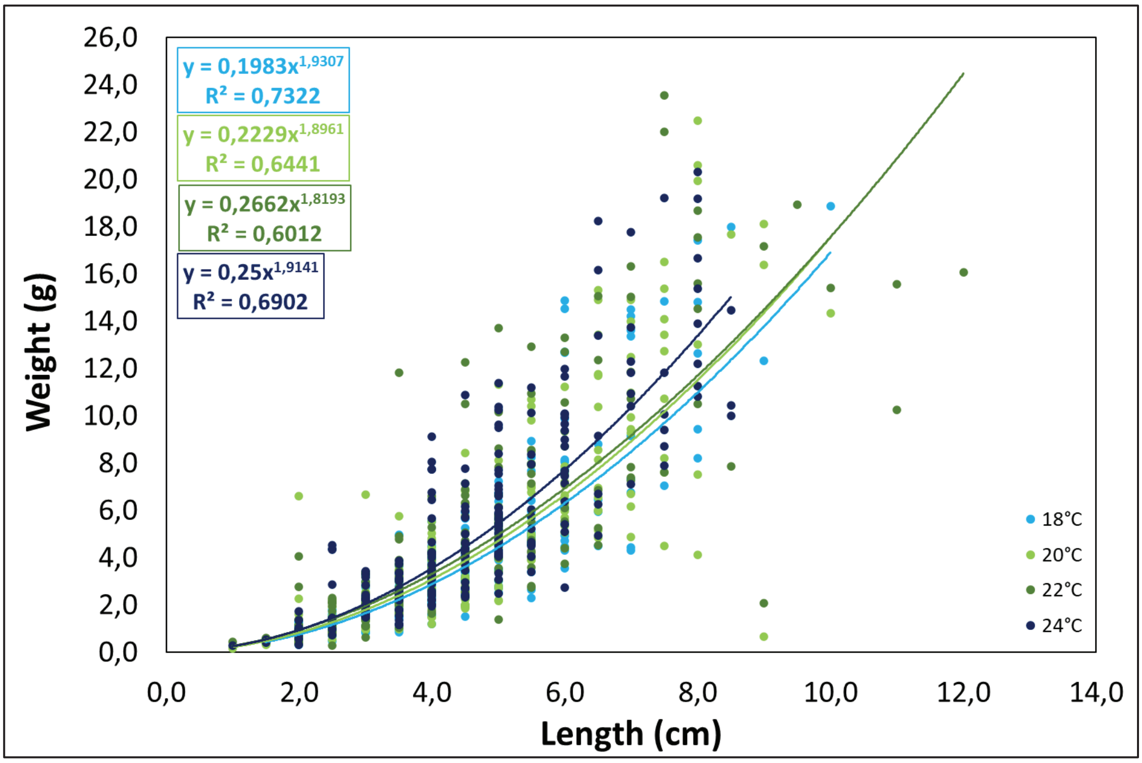

Figure 10. Allometric relationships for *Holothuria arguinensis* across all temperatures (18, 20, 22 and 24°C).

In order to analyse the development as well as the good condition of the animals during the experiment the Fulton's condition factor (K) was calculated. In the graph represented in figure 11 it is possible to see that the lowest line is relative to the 18°C treatment, and that the line that appears to have the highest frequency of values is the 24°C. Conversely according to the initial and final condition factor, between T0 and T4 (figure 12), there was a decrease at 18°C (T0=5.36; T4= 4.35) and at 20°C (T0=6.13; T4=4.33), whilst at

22 (T0= 6.18; T4= 7.68) and 24°C (T0= 6.18; T4= 6.41) there was an increase. Thus, according to the sampling times the highest condition factor that was found was at 22°C and the lowest at 18°C.

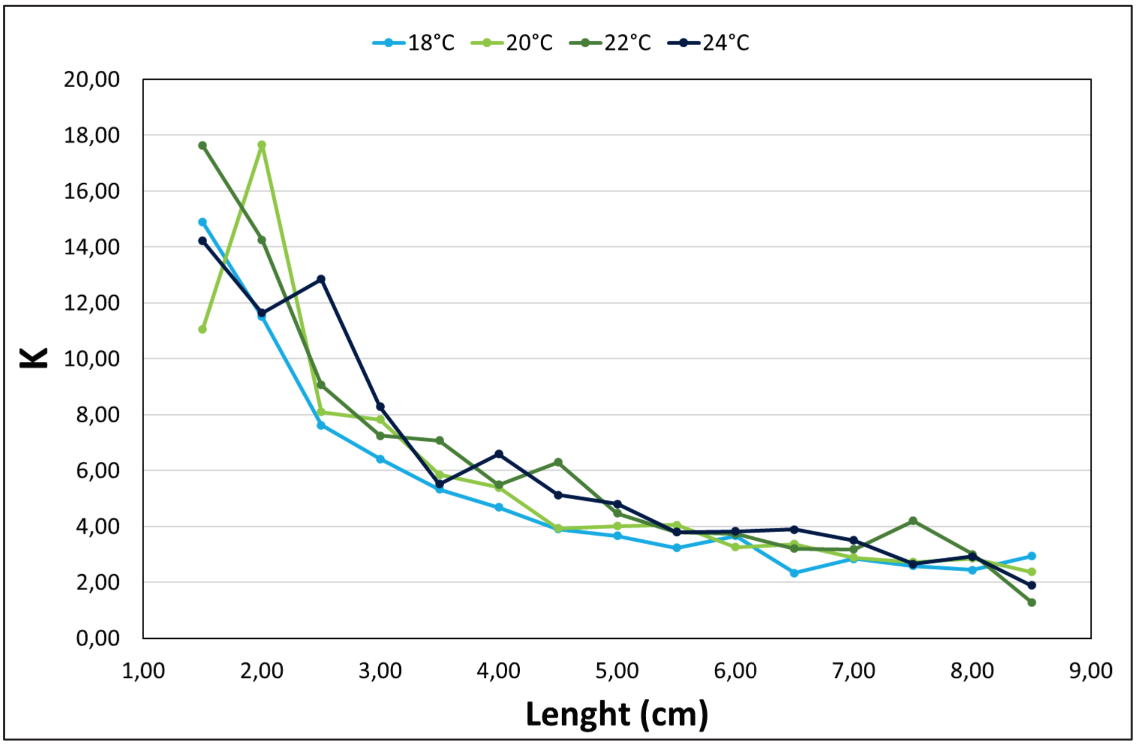

Figure 11. Fulton's condition factor (K) for *Holothuria arguinensis*, for all temperatures (18, 20, 22 and 24°C), according to each size class.

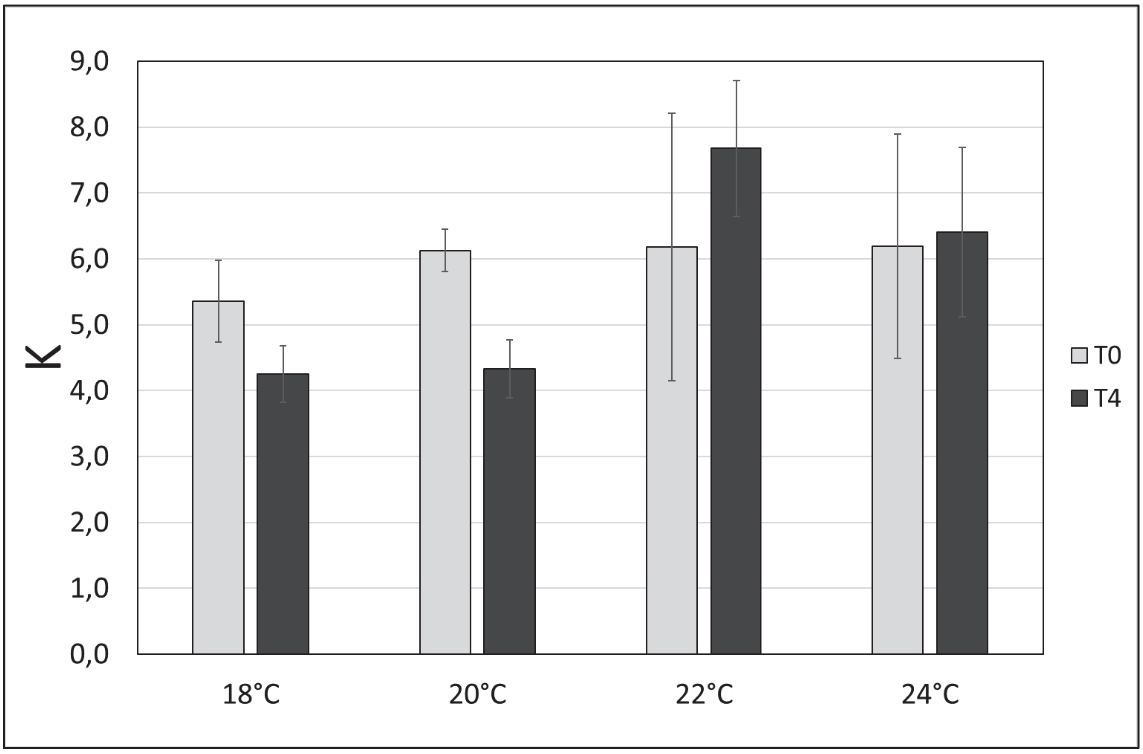

Figure 12. *Holothuria arguinensis* mean initial (T0) and final (T4) Fulton's condition factor (K) at different temperatures (18, 20, 22 and 24°C).

4. Discussion

As it is in most businesses, commercial profitability in aquaculture depends on production costs and profits (Engle, 2012). The viability of an aquaculture facility depends on various factors and, given that most of them are directly related to the organisms that are to be cultured, such as feed, oxygen, chemicals, and water, these need to be finely tuned so that waste is minimized, and profit maximized. Hence, investigation should be a priority when interest arises around a high value species (Domínguez-Godino & González-Wangüemert, 2019a; Hu et al., 2010; Naughton et al., 2020; Wang et al., 2013).

For aquatic organisms water temperature is without a doubt one of the most important parameters that needs to be taken into consideration, as it can dictate how feed intake and conversion occurs. Without proper control over the water temperature several problems may emerge, such as, feed waste and electricity waste, which alongside labour are the other two major production costs (Dong et al., 2008; Li et al., 2009). Some authors have described for various species the importance of temperature when considering sea cucumbers fitness and metabolism, and how non-optimal temperatures could reduce the growth rate and even possibly induce in aestivation when exposed to high temperatures and hibernation when exposed to low temperatures (Hongsheng et al., 2005; Huo et al., 2019). Thus, during this study important data relative to the rearing temperatures for *H. arguinensis* was analysed. When considering statistically significant differences these were only found at T4, between the 20 and 22°C lengths, these marginal differences were possibly the result of the mortality that was observed and because of length dispersion. The results obtained, suggest that the lowest temperature (18°C) might not be the best for rearing *H. arguinensis*, although some growth occurred, it resulted in the lowest weight and length increases, this has been described by other authors 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; Wang et al., 2013). Contrarily, for *H. arguinensis* better growth occurred at 20, 22 and 24°C. However, the 22 and 24°C treatments did not seem to be optimal. It would be expected that the higher the temperature the faster the growth due to metabolic rate increases, as these animals do not control their body temperature, however, as it was found in this study the 20°C treatment seems to be the best rearing temperature (González-Wangüemert & Borrero-Pérez, 2014; Mezali & Thandar, 2014; Olaya-Restrepo et al., 2018; Rodrigues, 2012; Siegenthaler et al., 2015). Similar studies have demonstrated this relation, with optimal and sub-optimal temperatures for different sea cucumber species; *Australostichopus mollis* (Hutton, 1872) juveniles presented growth in all temperature treatments however the optimal temperature was 15°C, with other temperatures presented as sub-optimal (Zamora & Jeffs, 2012); The same relationship has been described for *A. japonicus* juveniles (Wang et al., 2013) and for *H. tubulosa* juveniles (Günay et al., 2015).

Weight-length relationships are a common tool utilized in population dynamics and in fisheries management (Froese, 2006). When considering the morphometric structure of these animals the negative allometry (b<3) is naturally to be expected (Aydin, 2020; Kazanidis et al., 2010; Le Cren, 1951). The current study found that *H. arguinensis* juveniles demonstrated to have a negative allometric growth, with "b" values lower than 3; this was found to be the case in all four temperatures (b_{18}= 1.93; b_{20}= 1.90; b_{22}= 1.82; b_{24}= 1.91). The results presented are similar in all temperatures, except for the 22°C treatment. Here the allometric coefficient was lower and could have been the result of mortality. Furthermore, the allometric coefficients have higher values than adult specimens of the same species regardless of rearing temperature, suggesting that juveniles tend to gain more relative volume than adults. Azevedo e Silva (2021) carried out an experiment with adult individuals of *H. arguinensis* and used both the gutted weight (GW) as well as fresh weight (W) and fresh length (L), and found two allometric coefficients, b= 1.723 (W and L) and b=1,6983 (GW and L); In another study by Haddi (2021), *H. arguinensis* presented b= 1.610 (Skhirat) and b= 1.0025 (Souriria k'dima); Marquet (2017) investigated this relationship in two locations, Olhos de Água (b= 1.437; L and GW) and in Ria Formosa (0.980; L and GW). As such, the allometric coefficient values obtained by other authors are considerably lower than the minimum allometric coefficient value found in this experiment, this finding contrasts with two different life stages, as an increased "b" translates into a greater increment in length relative to weight. Similarly, some of the more economically important species of the Mediterranean Sea demonstrate lower allometric coefficients than *H. arguinensis* juveniles; the only exception would be *P. regalis* which does have a higher allometric coefficient than the juveniles and adults of *H. arguinensis* (Lleonart & Massutí, 2010). It is important to consider the different sampling methods applied by the different authors, as these can induce in some error.

The condition factor (K) can be used as a biological indicator that reflects both the living environment condition as well as the organism's condition (Nash et al., 2006; Pauly, 1983). This index measures various ecological and biological factors that depend on stress, sex, season, availability of feed and water quality parameters, which in turn are directly affected by the habitat's adequacy to the species. Therefore, when the value of K is higher, it is to be expected that a given organism of a given weight and length is in good condition (Kumara et al., 2021; Veronika et al., 2018). The lowest condition factor was found at 18°C, and is explained by the type of growth observed as the animals in this treatment demonstrated to be larger in length than in weight, thus resulting in a lower K. Conversely the similarly lower condition factor at 20°C is a result of the specimens being larger, and as expected the greater the length and weight the lower the K obtained is. Differently, the animals at 22°C and 24°C presented a higher K, this occurred because these treatments produced heavier and shorter animals. The variation observed in the condition factor could be explained by differential growth both in length and weight in the different temperature treatments. As such, these results are in agreement with what has already been described relatively to this species optimum temperature interval and with the same metabolic strategy that this species demonstrates, with increased movements and activity while under higher temperatures (Domínguez-Godino & González-Wangüemert, 2019a; Olaya-Restrepo et al., 2018).

5. Final Remarks

The absence of statistically significant differences between the temperature treatments is related to the high variability observed, which translated into a high heterogeneity in sizes. Nonetheless it is to note that, even though there were no statistically significant differences, differential growth was observed both in weight and length suggesting that when these animals are reared at 20°C they would grow to be larger and heavier than in other temperatures. As previous studies have mentioned and agreeing with what was observed in this experiment, this species does not develop as fast in lower temperatures, and instead preferring temperatures above 20°C. Future studies would have to access this species capacity to grow within a broader time span and broader temperature interval, while excluding temperatures lower than 20°C (Domínguez-Godino & González-Wangüemert, 2019a; Haddi et al., 2021; Marquet et al., 2017). Through this study, a deeper understanding of this species rearing temperature was reached, even though future studies are required this was a step in the right direction regarding the future of sea cucumber aquaculture in Europe, and especially in the domestication of H. arguinensis.

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