Sara Alexandra Cardoso Caldeirinha

Development of purple sea urchin (*Paracentrotus lividus,* Lamark 1816) cultivation in a marine lagoon production area

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2022

Sara Alexandra Cardoso Caldeirinha

Development of purple sea urchin (*Paracentrotus lividus,* Lamark 1816) cultivation in a marine lagoon production area

Mestrado em Aquacultura e Pescas

(especialidade em Aquacultura)

Orientadores:

Dr. Pedro Marques Pousão Ferreira,
Instituto Português do Mar e da Atmosfera

Dr. Sofia Alexandra Dias Engrola,
Centro de Ciências do Mar

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2022

Development of purple sea urchin (*Paracentrotus lividus,* Lamark 1816) cultivation in a marine lagoon production area

Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

_____

Sara Alexandra Cardoso Caldeirinha

Acknowledgments

I want to thank my coordinators Dr. Pedro Pousão and Dr. Sofia Engrola for this project opportunity, and for all their support and availability demonstrated throughout the project. A big thank you to the researchers João Araújo and Ana Mendes, from EPPO, that helped me with everything I needed during the whole project, from sea urchin feedings in the Ria Formosa to the samplings. Also, a big thank you to Margarida Gamboa from histology for producing my slides and the availability for helping me with the histologic analysis. I also want to thank to Sr. Nuno from EPPO for his help in the construction of the structures tested in the Ria Formosa and during feedings. Equally want to thank to my college and friend Ioannis Choulis for all the help and support along the project and availability to help me with feeding and samplings in the ria Formosa and at EPPO.

I want to thank to my parents, for giving me all the support and encouragement to pursue my master's degree. I also want to thank to my boyfriend for all his support, love and good advice, helping me deal with all the stressful situations and encouragement to continue pursuing my dreams and objectives. A big thank you to my friends for always being there, supporting and encouraging me, even from hundreds of kilometers apart.

Abstract

Sea urchin are marine and free-living animals from class Echinoidea and phylum Echinodermata. Sea urchin are one of the most commercially captured and overexploited echinoderms, their gonads, referred as “roe”, are considered a delicacy in many parts of the world. With the increase in demand, and the overexploitation of the natural stocks, sea urchin aquaculture turned out to be the best solution to supply demand. In this study, two experiments were made to evaluate the production of the purple sea urchin, *Paracentrotus lividus* (Lamark, 1816) in a marine lagoon environment, by testing different cultivation conditions. Two experimental systems were tested, separately, in an offshore cultivation condition, in the Ria Formosa, as well as the incorporation of an inert feed into the sea urchin diet, by using treatments with different diet compositions. These were compared with an onshore cultivation condition, at the Aquaculture Research Center (EPPO) in Olhão (Portugal). Samplings were made during the experiments, with the aim to assess the effects of location, diet and system performance, in the sea urchin’s development. A good sea urchin development was observed along the experiments, occurring weight increase of the sea urchin and its gonads, and gonads maturation.

Key-words: Purple Sea urchin, *Paracentrotus lividus,* Echinoculture, Gonad quality, Nutrition

Resumo

Os ouriços do mar são animais marinhos, pertencentes à classe Echinoidea e o filo Echinodermata. Estes animais podem ter entre três a dez centímetros de comprimento, dependendo da espécie, zona geográfica e tipo de dieta, e podem ter diversas cores como, preto, verde, castanho e roxo. Estes animais são nutricionalmente ricos em ácidos gordos poli insaturados, e são conhecidos pelo seu efeito positivo na prevenção de diversos problemas de saúde, como doenças cardiovasculares e doenças cancerígenas. Os ouriços do mar são dos echinodermes mais capturados e sobreexplorados para comércio em todo o mundo. Diferentes espécies de ouriços do mar são capturadas desde o ínicio do século XVII, ao longo das costas do Oceano Atlântico e do mar mediterrâneo na Europa, no norte da Ásia, Nova Zelândia e Chile. As suas gónadas, referidas comercialmente como “roe” ou “uni”, são consideradas uma iguaria gastronómica em diversas zonas do mundo, como no Japão, França, Itália e Espanha. A qualidade das gónadas depende de diversos fatores como o seu tamanho, a sua textura, a sua cor, o seu cheiro, a sua consistência e paladar, sendo que estes fatores de qualidade podem variar de acordo com a espécie de ouriço do mar, fatores ambientais, ciclo reprodutivo, sexo e ingestão de nutrientes. Os preços desta iguaria gastronómica podem também variar de acordo com diversos fatores como, a cor e aparência geral dos ouriços do mar, a espécie, região de captura, forma de processamento, nível de procura e distribuição. A espécie *Paracentrotus lividus* (Lamark, 1816) é considerada a espécie de ouriço do mar mais valiosa na Europa. A sua distribuição pode ser bastante variável no meio natural, sendo esta influenciada por diversos fatores como a temperatura da água, a salinidade, a competição e predação. Com o aumento da procura desta iguaria gastronómica nos últimos anos, e a consequente sobreexploração dos stocks naturais de ouriços do mar, foi necessário encontrar uma solução que suprisse a procura atual e futura, sem continuar a comprometer os stocks naturais. A aquacultura de ouriços do mar demonstrou ser a melhor solução para tal, sendo foco de investigação nos últimos anos. Neste estudo, tem-se como objetivo a produção de ouriços do mar, da espécie *P. lividus* na Ria Formosa, testando dois sistemas de cultivo diferentes e os efeitos da incorporação de uma dieta

inerte no crescimento dos ouriços do mar. Este estudo teve lugar na Ria Formosa, situada no Algarve, no sul de Portugal e na Estação Piloto de Piscicultura em Olhão, Portugal. A Ria Formosa é formada por um conjunto de ilhas barreira e um sistema mesotidal lagunar, que se extende por aproximadamente 55 km. As características naturais da Ria Formosa fazem da região um local de grandes oportunidades para a produção em aquacultura de diversas espécies, como por exemplo a produção de bivalves, que é a mais comum na região, ocupando uma grande porção da ria. Para a execução do estudo, dois ensaios foram feitos entre Outubro de 2021 e Agosto de 2022, com o objetivo de avaliar a produção de ouriços do mar, da espécie *P. lividus* em ambiente lagunar, através do teste de diferentes condições de cultivo, offshore (Zona de produção de ostras, Ria Formosa) e onshore (Estação Pilo de Piscicultura em Olhão- EPPO), entre as quais foram testados diferentes tratamentos (com quatro replicados cada), que diferiam na dieta. No primeiro ensaio, foi testado o primeiro sistema de cultivo de ouriços do mar, implementado na condição de cultivo offshore, na Ria Formosa. No mesmo ensaio foi testada a performance e qualidade de uma dieta inerte no crescimento dos ouriços do mar. Para tal, os ouriços do mar utilizados na condição de cultivo offshore foram divididos em dois tratamentos, os quais foram alimentados com diferentes dietas. Aos ouriços do mar do primeiro tratamento, foi fornecida uma alimentação somente à base de Alga, *Ulva* spp*.,* enquanto aos ouriços do mar do segundo tratamento foi-lhes fornecida uma alimentação composta por alga, *Ulva* spp. e uma dieta inerte. Os dois tratamentos do sistema de cultivo offshore foram comparados com um terceiro tratamento no sistema de cultivo onshore, na Estação Piloto de Piscicultura, em Olhão, Portugal. Ao longo do ensaio sete (0-6) amostragens foram feitas, entre outubro de 2021 e abril de 2022, com o objetivo de avaliar o peso, diâmetro (sem os espinhos), peso das gónadas, maturação das gónadas e sobrevivência dos ouriços do mar. No segundo ensaio, foi somente testado o segundo sistema de cultivo de ouriços do mar na condição de cultivo offshore, na Ria Formosa, que foi comparado com o sistema de cultivo onshore, presente na Estação Piloto de Piscicultura em Olhão. Cada condição de cultivo teve um tratamento, que por sua vez foi composta por quatro replicados. Os ouriços do mar de ambos os tratamentos tiveram uma alimentação igual, sendo esta composta por alga, *Ulva* spp. e uma dieta inerte. Ao longo do período de ensaio, três (0-2) amostragens foram feitas entre abril de 2022 e agosto de 2022, com o objetivo de avaliar o peso, diâmetro (sem os espinhos) e sobrevivência dos ouriços do mar. Ao longo do estudo houve alguns problemas relacionados com a estrutura, influenciando os resultados. Os resultados do primeiro ensaio demonstraram um crescimento dos ouriços do mar na condição de cultivo offshore, onde os ouriços do mar do tratamento com *Ulva* spp. e dieta inerte se destacou, obtendo um maior aumento de peso. Neste primeiro ensaio, ambas as dietas obtiveram resultados semelhantes de performance. Ambos os tratamentos na condição de cultivo offshore apresentam resultados de desenvolvimento e maturação das gónadas. O sistema experimental demonstrou necessitar de algumas melhorias de modo a aumentar a sua eficácia. Os resultados obtidos no segundo ensaio demonstraram um crescimento dos ouriços do mar, destacando-se os ouriços do mar na condição de cultivo offshore. O sistema experimental usado no segundo ensaio demonstrou uma melhor performance, sendo mais indicado para a produção de ouriços do mar. O presente estudo demonstra que a Ria Formosa apresenta condições para a produção de ouriços do mar da espécie *P. lividus*, com boas condições ambientais, adequadas ao crescimento desta espécie.

Termos- chave: Ouriço-do-mar, *Paracentrotus lividus,* Echinocultura, Gónadas, Nutrição

Index

1. Introduction

1.1. Sea urchin

Sea urchin are marine and free-living animals from class Echinoidea and phylum Echinodermata. Sea urchin are small, spiny, and globular, being adapted for life on benthic surfaces. Can have between 3 to 10 cm and depending on the species, geographic zone, and diet, can be black, green, brown, and purple (Baião et al., 2021; Lawrence, 2020). Despite all sea urchin can be considered edible (Lawrence, 2020), only a few species are harvested. The most worldwide harvested species are *Loxechinus albus* and *Strongylocentrotus* spp., while *Paracentrotus lividus* (Lamark, 1816) is the most exploited species in the Mediterranean coasts.

All sea urchin are dioecious, and in sea urchin, of both sexes, the reproductive system consists of five separate gonads, each connected to the upper aboral surface by individual gonadopores. Sea urchin gonads have a double function, as they function as reproductive organs and nutrient stores (Hughes et al., 2006).

Nutritionally, sea urchin gonads are very rich in polyunsaturated fatty acids (PUFA) (Dincer and Cakli, 2007), especially arachidonic acid (ARA) and eicosapentaenoic acid (EPA). These are known to positively prevent health problems such as cardiovascular diseases, hypertension, inflammation arrhythmias, and cancer (Fetterman and Zdanowicz, 2009). The presence of antioxidants has the potential to be exploited as bioactive compounds with anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic activities (Mamelona and Peltetier, 2010; Soobrattee et al., 2005).

The distinct color of the sea urchin gonads results from the accumulation of carotenoid pigments within its tissue (Kelly and Symonds, 2013; Lourenço et al., 2022). Echonenone accounts for up to 85% of the carotenoids in the gonad, but others, such as ß- and a-carotenes, lutein, and other xanthophylls can be accumulated in minor concentrations (Borisovets et al., 2002; Liyana-Pathirana' et al., 2002; Plank et al., 2002; Rocha et al., 2019; Symonds et al., 2009). These pigments and the balance between them are responsible for the variation in gonad color attributes. Environmental factors, such as diet and season, and physiological factors, such as the sea urchin's sex and gonadosomatic stage, directly affect the balance of these pigments in the sea urchin (Hagen et al., 2008; Liyana-Pathirana' et al., 2002; Lourenço et al., 2022; Plank et al., 2002; Symonds et al., 2009, 2007).

1.2. Economic Importance

Different species of sea urchin have been fished as a feed resource since the beginning of XVII in the Atlantic and Mediterranean coasts of Europe, North Asia, New Zealand, and Chile (Andrew et al., 2002; Sartori et al., 2016). Sea urchin roe is considered a gastronomic delicacy, being appreciated worldwide. Sea urchin' gonads, both male and female, are referred to as roe or uni. Marketable roe has a bright orange color, slimy texture, and sweet-salty flavor (Lourenço et al., 2021; Sun and Chiang, 2015). The request for gonads has significantly increased since the early 70s, reaching its peak in 1995 with a landing of 113,654 tons, slightly declining until nowadays (FAO, 2011; Sartori et al., 2016). Every year, 70 000 tons of sea urchin are traded worldwide (Lourenço et al., 2021; Stefánsson et al., 2017), with the Japanese being the most important market, accounting for around 90% of the international demand (Lourenço et al., 2021; Sun and Chiang, 2015). In the European market, the French, Italian and Spanish markets are the most important (Lourenço et al., 2021; Monfort, 2002; Stefánsson et al., 2017). The increase in the global demand for this delicacy, and consequently increase in fishing efforts, led to instability of the stocks, with several cases of overexploitation, and annual recruitment indicated by a decrease in the abundance of biomass (Andrew et al., 2002; Candeias-Mendes et al., 2019; Micael et al., 2016). According to FAO (2020), worldwide sea urchin capture decreased by almost 50% from the peak in 1995, leading to a collapse in native stocks.

Roe's market value is quite variable. Prices depend on numerous factors, such as appearance and color, species and region of harvest, flavor and textures, form and processing, and demand and distribution, with prices ranging between 60€/Kg and 221€/Kg. (James and Samuelsen, 2017; Lourenço et al., 2021; Monfort, 2002; Sun and Chiang, 2015). Live sea urchin' prices in the French and Spanish markets vary between 15 €/Kg and 35 €/Kg, depending on species, while fresh roe in jars can cost up to 120 €/Kg and canned roe between 10 €/unit and 30 €/unit (Lourenço et al., 2021).

High-quality sea urchin gonads are described as being of large size coupled with a desirable combination of sensory characteristics, such as taste, texture, color, smell, and firmness (Candeias- Mendes et al., 2020; Stefánsson et al., 2017). These characteristics vary according to the species and depend on environmental cues, annual reproductive cycle, sex, and nutritional input (Baião et al., 2021).

1.3. Paracentrotus lividus

P. lividus is considered the most valuable species in Europe, being distributed along the Mediterranean and Northeast Atlantic coasts, from Ireland to Morocco, including the Canary Islands and Azores islands (Bertocci et al., 2014; Boudouresque and Verlaque, 2013a; Lourenço et al., 2021; Machado et al., 2019; Pais et al., 2012).

P. lividus is a large sea urchin, where the test diameter of the largest individuals can reach 7,5 cm. This species can have a wide variety of colors, such as black-purple, purple, red-brown, dark brown, yellow-brown, light brown, or olive green (Boudouresque and Verlaque, 2013). Their feed choice is frequency dependent on the relative abundance of available items, being more selective when there is a higher feed supply. Selectivity also depends on the overall morphology and texture of the feed (Boudouresque and Verlaque, 2013).

This species is highly variable in time and space (Boudouresque and Verlaque, 2013; Sala et al., 1998; Turon et al., 1995) and it is influenced by different factors such as water temperature, salinity, competition, predation, settlement, and habitat heterogeneity (Fernandez et al., 2006; Gianguzza et al., 2006; Guidetti, 2004; Hereu et al., 2005; Prado et al., 2012; Tomas et al., 2004).

P. lividus is a subtidal species, living from the mean low-water markdown to depths between 10m to 20m, and intertidal rock pools (Boudouresque and Verlaque, 2013; Crook et al., 2000). This species can live in lagoons, on coarse sand, or even on mud substrata (Boudouresque and Verlaque, 2013; Crook et al., 2000). According to Boudouresque and Verlaque, 2013, *P. lividus* lives in regions with temperatures between 10 ºC to 15 Cº in winter and 18 ºC to 25 ºC in the summer, and it is sensitive to low and high salinities, with lethal salinity values in order of 15 to 20 psu and 39 to 40 psu, respectively, for long term exposure.

P. lividus plays an important ecological role as a grazer and bioengineer. Grazers and seaweeds are important biological components in most temperate and shallow rocky coasts where their biological interactions play a key role in the stability, biodiversity, and production of marine ecosystems (Duffy and Hay, 1990; Korpinen et al., 2010; Kraufvelin, 2007). *P. lividus* is one of the most common benthic herbivore species on the continental Portuguese coast (Jacinto et al., 2013).

As consequence, in the last decade commercial fisheries intensified harvesting and improved their harvesting methods, which ended up leading to the overexploitation of populations of this species (Fernández-Boán et al., 2012).

The commercial harvesting of *P. lividus* in Portugal has increased significantly in the last years, where official landings have increased from 2 metric tons in 2010 to 298 metric tons in 2019 (INE, 2020). The harvested sea urchin in Portugal is mainly for exportation to nearby regions, in order to fulfill its demand. In the north-western Iberian Peninsula, harvesting of *P. lividus* occurs typically from October to April, corresponding to the period of maturity of the gonads, and when it is at its highest price (Bertocci et al., 2014; Fernández-Boán et al., 2012; Montero and Garcia, 2003).

1.4. Sea urchin aquaculture

Over the past decades, the wild sea urchin population decrease, combined with the increase in the demand for sea urchin products stimulated scientific and commercial interest in aquaculture, increasing the number of studies published aiming to improve the culturing of sea urchin (Cook and Kelly, 2007; le Gall et al., 1989; Spirlet et al., 1998) for commercial and preservation/restoration purposes (Carboni et al., 2014; Cook et al., 2007; Couvray et al., 2015; Prato et al., 2018a; Spirlet et al., 2000). To be economically practical, sea urchin aquaculture must maximize yield and quality to match market requirements (Mos and Dworjanyn, 2019a). Although there is substantial research done to improve gonad yield by manipulating diet and culture methods, reliable production of high-quality roe remains a challenge for the industry (Eddy et al., 2012; Heflin et al., 2016; Mos and Dworjanyn, 2019a; Shpigel et al., 2018).

There are two forms of sea urchin aquaculture: the first one involves gonad enhancement (an increase of gonad size and improvement of gonad quality) of wild-caught adults fed with prepared diets in captivity for a short period of time (Cook et al., 1998; James, 2007; Lawrence et al., 1997; Siikavuopio et al., 2006). A wide variety of grow-out systems have been tested for juveniles and adults, ranging from relocating from poor to good feeding grounds to the ranching of sea urchin caged on the seafloor. The time taken for juveniles to reach market size is between 1 to 3 years (Kelly, 2005; Moylan, 1997) . The second form is in a closed cycle culture, involving the spawning of adult broodstock, rearing larvae, and juveniles until they reach market size (Lawrence, 2007; Spirlet et al., 1998). The eggs develop to form pluteus larvae, and then, after a period of planktonic development, settle to a substrate and undergo metamorphosis to form juvenile sea urchin (Araújo et al., 2020).

Although the sea urchin life cycle is already controlled under captivity, echinoculture is still largely dependent on a wild-caught sea urchin, as growing out from small-sized juveniles in aquaculture is faster and less cost-intensive (Phillips et al., 2009). Different factors influence both yield and quality of roe, such as biotic factors (temperature, salinity, and photoperiod), nutritional factors (feed ingredients and chemical composition), and rearing conditions (stock density and water circulation) (Baião et al., 2019a; Lourenço et al., 2020; Rocha et al., 2019). In the absence of feed constraints, temperature represents the single most important abiotic factor for sea urchin (Catarino et al., 2012; Santos et al., 2020a; Shpigel et al., 2004; Spirlet et al., 2000; Yeruham et al., 2019, 2015). It controls the physiological performance of sea urchin, such as feeding rates and metabolic processes, including respiratory rates, and therefore, somatic and gonad growth rates (Brockington and Clarke, 2001; Brown et al., 2004; Byrne et al., 2009; Lawrence et al., 2009; Santos et al., 2020a; Siikavuopio et al., 2008; Watts et al., 2011).

One of the key factors that have been identified for the development of gonad enhancement and sea urchin on-growing is the availability of an effective diet, suitable, cost-effective, and nutritionally balanced (Eddy et al., 2012; James, 2007; Pearce et al., 2002; Woods et al., 2008). Feed type, feed quality, and feeding regimes used will affect feed intake, digestion, and eventual nutrient availability, impacting gametogenesis and reproductive success of animals (Azad et al., 2011). Acceptability, selectivity, and preference are the most important components that can influence the consumption pattern of feed resources (Cardoso et al., 2020; Jackson and Underwood, 2007). In echinoculture, formulated feeds, *Ulva* spp., and combination diets are commonly used. *Ulva* spp. has a high nutritive content and is rich in essential amino acids, lipids, proteins, minerals, and fatty acids (linolenic and palmitic acid) (Cyrus et al., 2015; Kabeya et al., 2017). These are frequently grown in effluent water to reduce nutrient release from aquaculture farms, resulting in improved growth and nutritional content (Jimenez et al., 1996; Mata et al., 2010). *Ulva* spp. has also known anti-microbial roles and are feeding stimulant (Cyrus et al., 2015; Ismail et al., 2018).

In the last decades, formulated feeds have been studied in order to reduce the dependency on wild macroalgae, which exhibits strong fluctuations in terms of availability and quality (Baião et al., 2019; Basuyaux and Blin, 1998; Castilla-Gavilán et al., 2019; Eddy et al., 2012; Prato et al., 2018; Santos et al., 2020; Sartori et al., 2016; Schiener et al., 2015; Shpigel et al., 2005; Woods et al., 2008). The development of artificial diets represents one of the main challenges of viable aquaculture (Pearce et al., 2002).

The optimal diet should be able to promote good somatic and gonad growth with long shelf-life (Pearce et al., 2002), but also produce gonads with a suitable color, taste, and texture, suitable for the market (McBride et al., 2004; Shpigel et al., 2005b). Previous studies have shown good results with formulated diets, promoting faster gonad growth in comparison with natural algal feeding(Siikavuopio et al., 2012), especially in *P. lividus*, where formulated diets have proven to also promote gonad production (Fernandez and Boudouresque, 2000; Prato et al., 2018; Spirlet et al., 2000) In addition, formulated feeds are typically more suitable than natural feeds as they can be optimized for maximal production and have a consistent nutrient composition (Lawrence et al., 1997; Warren-Myers et al., 2022).

Studies suggest that, even though single diets have advantages in aquaculture, conditioning broodstock on combination diets consisting of a number of feeds could be beneficial for somatic growth, gametogenesis, and subsequent reproductive performance (Beddingfield and McClintock, 1998; Vadas et al., 2000). Studies also suggest that different feeding strategies for adult sea urchin should be used for different purposes, such as market acceptance and reproductive success, as it is likely that sea urchin have different nutrient requirements during different development stages (Cyrus et al., 2015; Heflin et al., 2012).

Most inert diets contain a selection of soybean meal and cereals, either with or without animal-origin protein and lipids (Cook et al., 1998; Fernandez and Boudouresque, 1998; Spirlet et al., 2001). These diets can be wet, moist, or extruded in commercial processing equipment (Goebel and Barker, 1998; Klinger et al., 1994; Lawrence et al., 1997; Olave et al., 2001; Pantazis et al., 2000).

Dietary protein is an important macronutrient that provides essential amino acids for several biological processes, including maintenance, growth, and reproduction (Hammer et al., 2012). Studies observed that a 30% DM dietary protein with 7% lipids could induce high nutrient utilization and promote a higher gonadosomatic index (GSI) (Baião et al., 2019).

One of the conditioning factors to consistent production of high-quality sea urchin gonads is that producers cannot assess the size or quality of the gonads prior to harvest. Between 10% to 100% of sea urchin within a cohort may be harvested at an inopportune time, with roe obtained being low grade or unsaleable due to poor color, and small size (Azad et al., 2011; James, 2006; Mos and Dworjanyn, 2019; Shpigel et al., 2006; Spirlet et al., 1998; Woods et al., 2008). Until today, the used option to estimate harvest readiness with accuracy is by sacrificing a sample group. This imposes time and economic costs on producers and might be

a poor predictor of the quality of the individuals in the remainder of the cohort if culture conditions across a farm are variable (Mos and Dworjanyn, 2019).

1.5. Diseases and mortality

Microbial pathogens affecting echinoids such as sea urchin have been widely described to be responsible for several sea urchin mass mortality events (Bower et al., 1994; Girard et al., 2012b; Lafferty, 2004). High population densities have been described as a factor that increases susceptibility to infectious diseases (Behrens and Lafferty, 2004; Lafferty, 2004; Lafferty and Gerber, 2002). Environmental factors have also been demonstrated to be correlated with the incidence of diseases. High temperatures promote the occurrence of diseases in the oceans (Harvell et al., 1999; Lafferty, 2004) and reduce pathogen resistance in echinoids (Girard et al., 2012). Studies have shown that the development of diseases was positively correlated with temperature, and temperature may act as a disease activator. One of the most incident diseases is bald head disease. It is a widespread infection of the sea urchin body wall, caused by many different opportunistic bacteria (Becker et al., 2008). When the infection is limited, diseased individuals can recover by regenerating their body wall tissues and outer appendages(Girard et al., 2012; Jangoux, 1986), but if lesions extend over large areas of the body wall or perforate the test, individuals cannot recover (Girard et al., 2012b; Maes and Jangoux, 1984).

1.6. Ria Formosa

The Ria Formosa is a small barrier chain and mesotidal shallow lagoon in the central and eastern coast of the Algarve, in Portugal, extending approximately 55 Km (Andrade et al., 2004). The Ria Formosa lagoon is formed by five sand barrier inlands and six inlets, with a wet area of about 100 Km2, including tidal channels and an extensive intertidal area constructed by salt marshes, mud and muddy sand flats, sandy sediments, and macrophytes beds (Gamito, 2008). Intertidal features of the Ria Formosa system occupy almost 90% of its total area, of which only 14% are permanently flooded (Andrade et al., 2004).

The average depth relative to sea level is 2m (Andrade et al., 2004). The tidal amplitude ranges from 3.3 m on spring tides to 1.0 m on neap tides, causing important diurnal and fortnightly tidal amplitude variations (Saraiva et al., 2007). Tidal currents are responsible for water circulation inside the lagoon, with only a very small influence of wind (Salles et al., 2005).

Salinity ranges from 13 psu to 36.5 psu and temperature from 12 ºC to 27 ºC (Newton and Mudge, 2003). Ria Formosa is therefore classified as a vertically well-mixed system, with no persistent haline or thermal stratification, where salinity values are usually similar to those in open ocean waters (Newton and Mudge, 2003).

The characteristics of Ria Formosa give place to the development of significant aquaculture production. Bivalve production is one of the most common, occupying nearly 400 ha of the area (Amaral, 2008; Guimarães et al., 2012).

1.7. Objectives

This study aimed to develop a new sea urchin fattening system in the Ria Formosa lagoon. Two trials were conducted in this study, comparing two different cultivation condition systems with an onshore sea urchin production in the Aquaculture Research Center (EPPO) in Olhão (Portugal), to assess the more suitable cultivation system for sea urchin production in the Ria Formosa. The incorporation of an inert feed into the sea urchin diet was also analyzed. Biometric growth and gonad development were monitored to understand the best time for harvesting.

2. Materials and Methods

2.1. Location

The study was carried out in the Ria Formosa lagoon, between Faro and Olhão. In figure 1.1 the exact location where the study took place is marked in orange.

Figure 2.1: Geographical location of the study in the Ria Formosa lagoon, market in orange.

2.2. Sea urchin Selection

In this study, were made two sea urchin selection samplings, to test two different experimental systems. Each selection sampling was made for different experiments.

2.2.1 First experiment

The first sampling selection, used in the first experiment, occurred on the 6th of October 2021 at EPPO facilities. Sea urchin from the species *P. lividus* were chosen from an existing F1 sea urchin culture (with F0 being wild sea urchin collected in the nearby areas) at EPPO facilities. From this culture, 550 sea urchin were selected through a sampling process, according to their test diameter, weight, and general appearance of the individuals. Individuals were weighted and measured, and those with a weight between 8.4g and 37.4g and a test diameter between 2.45cm and 5.17cm were selected. Individuals with unhealthy appearances were not selected. To assess the initial state of the gonads, six more individuals were selected from the culture. These were measured, weighed individually, and had their gonads removed and weighted.

2.2.2 Second experiment

The second sampling selection, used in the second experiment, occurred on the 4th of April 2022 at EPPO facilities. Sea urchin from the species *P. lividus* were selected from the same existing F1 sea urchin culture held at EPPO. Similarly, to the first sampling selection process, 450 sea urchin were selected from the previous F1 sea urchin culture, according to their test diameter, weight, and general appearance. For this part of the study, all sea urchin were weighed and measured individually, where 300 individuals were selected with a weight between 3.41g and 41.11g and a test diameter between 2.27cm and 6.11cm. The remaining 150 sea urchin were chosen as smaller individuals, with weights between 2.44g and 12.78g and a test diameter between 1.58cm and 4.09 cm. Sea urchin with unhealthy appearances were not selected.

2.3. Experimental System

As this study was divided into two experiments, the previously selected sea urchin were used in different systems that were tested in different periods.

For the first experiment of the study, were used the first 550 selected sea urchin, distributed in Hexcyl ™ baskets, and divided into eleven groups of sea urchin, with 50 individuals per basket.

The first experimental fattening system was implemented in an offshore cultivation condition, in the Ria Formosa between the 22nd of October and the 8th of April. It was composed of eight Hexcyl ™ baskets, numbered from 1 to 8, with a 25L capacity and 3mm mesh, suspended on an iron structure with a 2.10m length, 0.80m height, and 0.90 m width (Fig.2.2). The base of the structure was improved with two PVC tubes of the same width of the structure to facilitate the movement of the system when needed. The system was suspended in place using ropes and weights, which were adjusted during the trial when necessary. The system was implemented in the Ria Formosa, near the shoreline in a bivalve production area, being permanently under water, with a 2.0m depth in the high tide.

The experimental system was compared with an onshore production system held, in an onshore cultivation condition, at EPPO. This system was composed of the remaining three Hexcyl ™ baskets, kept in an exterior fiberglass tank with a 3800 L capacity (Fig.2.3).

Figure 2.2: Experimental structure in the Ria Formosa of the first experiment, composed of height Hexcyl™ baskets with sea urchin, and an iron structure. Dimensions are represented in yellow.

Figure 2.3: Experimental system composed of height Hexcyl™ baskets holding sea urchin and an iron structure installed in the Ria Formosa (offshore cultivation condition - left), and onshore production system composed of three Hexcyl™ baskets holding sea urchin in a fiberglass tank at the Aquaculture Research Center in Olhão (EPPO) (onshore cultivation condition - right).

In the second experiment of the study were used the 450 sea urchin from the second sampling selection. These were distributed by 8 Hexcyl ™ baskets, being divided into 6 groups of 50 sea urchin each, and 2 groups, formed by the smaller individuals, with 75 sea urchin each.

The second experimental fattening system was implemented in the Ria Formosa between the 1st of May and the 10th of August. It is a fluctuant system composed of 4 Hexcyl ™ baskets (3 baskets with 50 sea urchin and 1 with 75 sea urchin) suspended in a rope above the water line. Each Hexcyl ™ basket has a 25L capacity and a 3mm mesh and was suspended between 2 buoys for them to float (Fig.2.4). Each cage required four fishing sinkers affixed to the bottom to enhance the weight of the baskets for them to be below the water line. The system was set perpendicularly to the shoreline, moored at one end, and fixed to a solid structure at the other end, assuring the entire emersion during all tide stages. It was implemented with a 1.3m depth.

This second experimental system was compared with an onshore production system composed of the remaining 4 groups of sea urchin (3 groups with 50 sea urchin and 1 with 75 sea urchin), kept in the same facilities as the onshore system from the first experimental system.

Figure 2.4: Second experimental system in the Ria Formosa, composed of four Hexcyl™ baskets holding sea urchin and a rope system (offshore cultivation condition).

The systems in both experiments need maintenance and cleaning. Every month, baskets were cleaned to remove all algae and other biofilm covering their surface and blocking water to flow through. Baskets were also replaced with new ones in both systems, in the middle of the trial periods.

2.4. Cultivation conditions and Diet

During the study, different cultivation conditions were tested, with the aim to assess the performance of two different cultivation structures and the incorporation of an inert feed into the sea urchin diet. These were assessed through the biometric growth of the sea urchin and the development of its gonads.

2.4.1. First experiment

In the first part of the study, two different cultivation conditions were tested, one Offshore in the Ria Formosa, and one onshore at EPPO. In the offshore cultivation condition, two different diets were tested. These two treatments were compared with a third treatment in the onshore cultivation condition at EPPO. The treatments were designed as A, B and C:

A. Ria Formosa experimental system: Diet composed exclusively by *Ulva* spp..
B. Ria Formosa experimental system: Diet composed by *Ulva* spp.+ Formulated feed.
C. Onshore production system at EPPO: Diet composed by *Ulva* spp. + Formulated feed.

In the Offshore cultivation condition, sea urchin from baskets 1-4 were fed exclusively with *Ulva spp.* (treatment A) and sea urchin from baskets 5-8 were fed with *Ulva* spp. and a

formulated inert feed (treatment B) (Fig.2.5). Sea urchin from both treatments were first fed on the 6th of October 2021 and continued to be fed simultaneously until the end of the experiment on the 8th of April 2022. Feeding was made at the site, with the support of a semi-rigid motorboat. As feeding site visits were subjected to weather conditions, it was tried for feedings to occur every 15 days, between the 6th of October and 26th of February, and every 10 days from the 4th of March until the 8th of April.

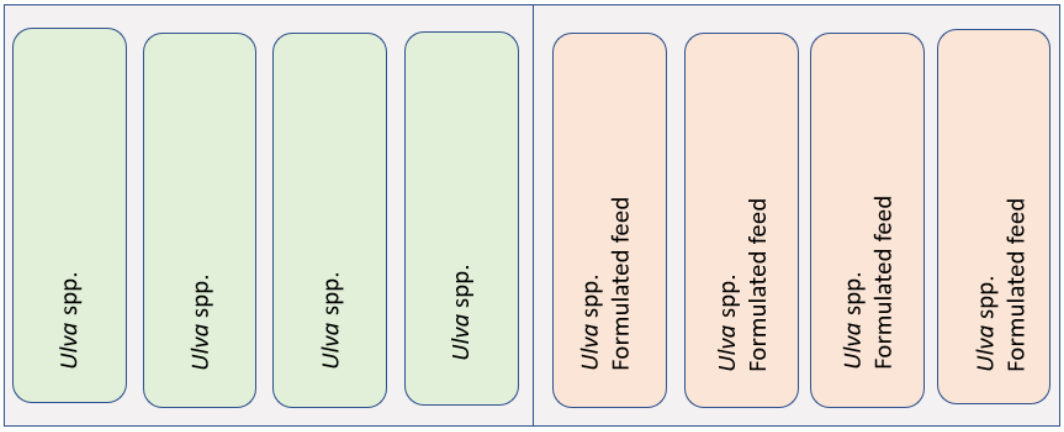

Figure 2.5: Experimental design of treatments tested in the Ria Formosa, in the offshore cultivation condition. Treatment A (left), composed of four Hexcyl™ baskets with 50 sea urchin each (n=50), fed with *Ulva* spp. and treatment B (right), composed of four Hexcyl™ baskets with 50 sea urchin each (n=50) fed with *Ulva* spp. and formulated feed.

For treatment C, sea urchin in the onshore production were fed with the same diet as sea urchin in treatment B, composed of *Ulva* spp. and a formulated inert feed (Fig.2.6). Sea urchin were fed during the same period as the ones in the Ria Formosa and on the same days.

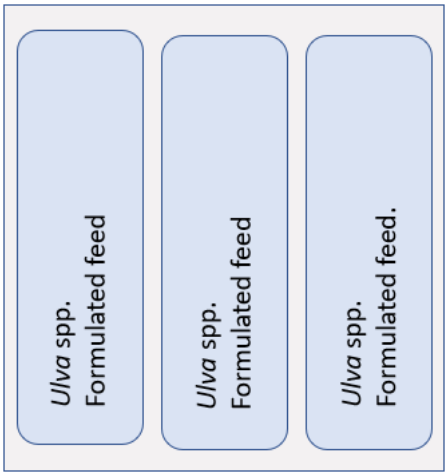

Figure 2.6: Experimental design of control treatment tested at EPPO, Olhão. Treatment C was composed of three Hexcyl™ baskets with 50 sea urchin each (n=50), fed with a diet composed of *Ulva* spp. and a formulated feed.

Throughout the experiment, the amount of feed provided varied according to the necessities of sea urchin. Thus, the feeding dates and the amount of feed per basket provided to the sea urchin during this experiment are represented in the following table (Table 2.1):

Table 2.1: Amount of feed provided to sea urchin per basket (g), during the first experiment, for treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp. + inert feed), and treatment C (onshore, *Ulva* spp. + inert feed):

Feeding Days	Treatment A		Treatment B		Treatment C	
	***Ulva* spp.**	**Inert Feed**	***Ulva* spp.**	**Inert Feed**	***Ulva* spp.**	**Inert Feed**
06/10/2021	450	-	400	50	400	50
22/10/2021	400	-	450	50	450	50
05/11/2021	650	-	600	50	600	50
19/11/2021	400	-	400	-	400	50
06/12/2021	700	-	650	50	650	50
21/12/2021	750	-	700	50	700	50
12/01/2022	900	-	850	50	850	50
16/02/2022	900	-	850	50	850	50
26/02/2022	-	-	-	50	-	50
04/03/2022	400	-	500	25	500	25
10/03/2022	350	-	300	25	300	25
18/03/2022	350	-	300	25	300	25
08/04/2022	350	-	300	25	300	25

2.4.2. Second experiment

In the second part of the study, two cultivation conditions were tested, one offshore cultivation condition in the Ria Formosa, compared with the onshore production system at EPPO. Both had the same conditions and only differed in location. Treatments D and E:

D. Ria Formosa second experimental system: Diet composed by *Ulva* spp. + Formulated feed.
E. Onshore production system at EPPO: Diet composed by *Ulva* spp. + Formulated feed.

Both systems were fed with *Ulva* spp. and a formulated feed (Fig.2.7). As in the first part of the trial, feeding was performed at the site, with the support of a semi-rigid motorboat to feed sea urchin from treatment D. Sea urchin from both cultivation regimes were fed every 15 to 20 days, between 1st of May until 10th of August.

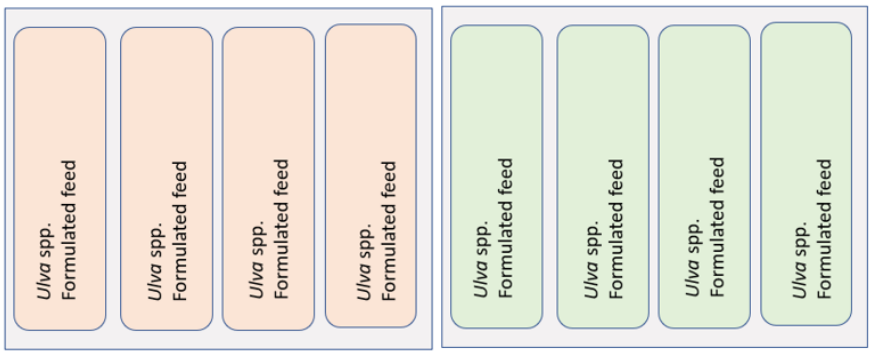

Figure 2.7: Experimental design of the treatment tested (treatment D) in the second experiment, in Ria Formosa, in the offshore cultivation condition and the control treatment (treatment E), at EPPO, Olhão. Treatment D (left), composed of three Hexcyl™ baskets with 50 sea urchin each (n=50) and one Hexcyl™ basket with 75 sea urchin each (n=75), fed with *Ulva* spp., and treatment E (right),), composed of three Hexcyl™ baskets with 50 sea urchin each (n=50) and one Hexcyl™ basket with 75 sea urchin each (n=75), fed with *Ulva* spp..

The amount of feed provided was adjusted according to the necessities of sea urchin. The feeding dates and the amount of feed per basket provided to the sea urchin during this trial are represented in the following table (Table.2.2):

Table 2.2: Amount of feed provided to sea urchin, per basket (g), during the second trial, in treatment D (*Ulva* spp.+ inert feed) and treatment E (*Ulva* spp. + inert feed):

Feeding Days	Treatment D		Treatment E	
	***Ulva* spp.**	**Inert Feed**	***Ulva* spp.**	**Inert Feed**
29/05/2022	600	25	600	25
06/06/2022	550	25	550	25
28/06/2022	600	25	600	25
07/07/2022	500	25	500	25
18/07/2022	500	25	500	25
11/08/2022	250	50	250	50

The formulated feed used was the same in both experiments and produced by SPAROS Lda. (Olhão, Portugal). It is mainly composed of macroalgae (*Ascophyllum nodosum*, 20% and *Ulva* spp., 20%) and corn gluten meal (17%) (Table.2.3). Proximate composition of both feed sources is described in Table.2.4.

Table 2.3: Formulation of inert feed used in the diets fed to sea urchin during the first and second experiments:

Ingredients, %	**Diet**
Fish gelatine	5.00
Macroalgae (*Ascophyllum nodosum*)	20.00
Macroalgae (*Ulva* spp. supplied by IPMA)	20.00
Wheat gluten	7.50
Corn gluten meal	17.00
Wheat meal	10.00
Potato starch (gelatinized)	
Sorbitol	
Vitamin and mineral premix	2.00
Antioxidant	0.40
Monocalcium phosphate	3.00
Calcium carbonate	5.00
Binder (sodium alginate)	
Beta-carotene 10%	0.50
Algae biomass (Schizochytrium 16%DHA)	9.60
Total	**100.00**

Table 2.4: Proximate composition of the inert feed and *Ulva* spp. used in the diets fed to sea urchin during first and second experiments:

Proximate Composition	**Inert feed**	***Ulva* spp.**
Protein, %	32.62	22.13
Fat, %	5.19	1.63
Ash %	21	30.34
Gross energy, kJ g-1	15.75	11.03

2.5. Data collection

For the study of *P. lividus* development in a marine lagoon production area, the first and second experiments had continuous sampling moments.

In the first experiment, samplings took place on a monthly basis between October 2021 and April 2022, for a total of six sampling moments: 1st sampling- on 5 November, 2nd sampling- on 21 December, 3rd sampling- on 12 January, 4th sampling- on 16 February, 5th sampling – on 18 March and 6th sampling- on 8 April.

During samplings, biometric growth, gonadosomatic index, state of development of the gonads, and different water parameters were assessed. For that, the same procedures were performed in each sampling for every treatment, in each basket individually.

In each sampling, all sea urchin from the basket being sampled were counted to check for mortality and apparent diseases and the total weight of the individuals was recorded using a bench scale from all individuals, 25 were selected randomly and were individually weighted using an analytical scale. These were then ordered and photographed for biometric measurement using the computer program ImageJ. Of the 25 sea urchin, 3 were selected for histologic analysis. Sea urchin were sacrificed first by placing them on ice for 3 to 4 minutes, and then were opened transversally, with the help of scissors and a scalpel, to reach the gonads. The gonads from each sea urchin were collected using tweezers and weighed on an analytical scale to assess the total weight of the gonads in each sea urchin (Fig.2.8). A sample of the gonads was removed and processed for histologic examination in histology cassettes.

Figure 2.7: Sampling procedures done in the first experiment for all sampling moments (Image 1- counting of sea urchin, Image 2- measurement of sea urchin, Image 3- gonad removal, Image 4- gonad weight).

Water parameters were assessed regularly. Water temperature was registered daily, every two hours, with the use of an IBCod temperature logger inside one of the baskets in the experimental system in the Ria Formosa and with a HANNA multi-parameter probe in the onshore cultivation system at EPPO. Dissolved oxygen values remained between 96% and 99% throughout the trial.

In the second experiment, there were three samplings overall, conducted monthly from May 29 to August 10: 1st sampling- on 29 May, 2nd sampling- on 28 and 29 June, and 3rd sampling- on 10 August. As in the first trial, the same procedures were performed in each sampling for every treatment, in each basket individually. The sampling procedures performed were equal to the first experiment except for gonad extraction, where biometric growth parameters and water parameters were assessed.

2.6. Histological analysis

Histological analysis of the sea urchin' gonads was performed to assess the sex of the sea urchin and the different development stages of the individuals throughout the first experiment.

During the first experiment, a total of 204 gonads have been sampled: 47 gonads in the 1st sampling, 31 gonads in the 2nd sampling, 33 gonads in the 3rd and 4th samplings, and 30 gonads in the 5th and 6th samplings. In each sampling, gonad samples were collected from three sea urchin per basket in each treatment and stored in formol at 4%. After 24 h, gonads were transferred to ethanol at 70%, and after, were processed using a standard histologic technique (Martoja & Martoja-Pearson, 1970). Slides were obtained using a tissue processor (Model Citadel 2000, Thermo Scientific, China), and tissue sections with a thickness of 4 µm

were prepared with a microtome (Model Jung RM 2035, Leica Instruments mb, Germany). Tissues were then stained with hematoxylin and eosin using an automatic slide Stainer (Model Shandon Varistain 24-4, Thermo Scientific, China). Mounted slides were scanned with a Hamamatsu NanoZoomer C13140-01 and images were visualized with the NDP for sex and stage gonadal development observation.

The gametogenic stages of both ovary and testes were identified according to Byrne (1990). Recovery stage (I), with primary gametes and nutritive phagocytes; growing stage (II), with clusters of primary gametes and packed nutritive phagocytes; premature stage (III), with gametes at all stages of development and reduced amount of nutritive phagocytes; mature stage (IV), with mature gametes and few nutritive phagocytes; partly spawned stage (V), with loosely packed gametes and depletion of nutritive phagocytes, and spent stage (VI), with gonads empty of gametes (Baiao et al, 2022).

2.7. Data analysis

Data collected during samplings allowed the calculation of growth performance parameters and gonadosomatic index. Parameters were calculated as follows (Loureiro, 2021):

$$Weight\ Gained\ (WG\ \%) = \left[\frac{(Final\ weight - Initial\ weight)}{Initial\ weight}\right] \times 100$$

$$Specific\ Growth\ Rate\ (SGR\ \%) = \left[\frac{(\ln Final\ weight - \ln Initial\ weight)}{T\ (days)}\right] \times 100$$

$$Survival\ rate\ (\%) = \frac{Final\ number\ of\ \ sea\ urchins}{Initial\ number\ of\ sea\ urchins} \times 100$$

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Total\ feed\ intake\ (g)}{Total\ wet\ weight\ gained\ (g)}$$

$$Gonadosomatic\ Index\ (GSI) = \frac{Gonads\ wet\ weight\ (g)}{Total\ wet\ weight\ (g)} \times 100$$

With the results from biometric growth and the index of condition and maturation of the gonads, it was intended to create a new index to understand the state of development of the sea urchin without the need for sacrifice.

2.8. Statistical analysis

All statistical tests were performed using R software and a significance level of $\alpha = 0.05$ was used. Results are expressed with mean ± standard deviation (SD). Feed intake and FCR were expressed as mean ± SD of the replicate baskets in each cultivation condition. Test diameter, wet weight, and GSI were expressed as mean ± SD for the group of individuals in each basket in each cultivation condition. Tests for normality and homogeneity of variances were performed by Shapiro-wilk and Levene´s tests, respectively. To assess differences between treatments, and types of diet, all results (Wet Weight, test diameter, mortality, feed intake, Feed Conversion Rate, and Specific Growth Rate) were analyzed through a t-test and one-way Anova,. The Kruskal-Wallis test was used when data did not fulfill the assumptions for the application of parametric tests. Tests were also applied to assess if there was any relationship between Wet Weight and gametogenic development, using the frequency of gametogenic stages in the group of individuals per basket from each treatment.

3. Results

3.1. First experiment

3.1.1. Water parameters

Water temperature was assessed in treatments A, B, and C daily in the Ria Formosa and EPPO, between the 8th of October 2021 and the 8th of April 2022. Results show temperature oscillations throughout the whole experiment, in both offshore and onshore cultivation conditions. In Fig.3.1 it is possible to observe the month's little low-temperature peaks, followed by an increase in temperature. Some of the low-temperature peaks can be observed in the offshore cultivation condition, between 12th of January when temperature decreased and 21st of January when temperature raised and between 18th of February when temperature decreased and 25th of February when the temperature raised, and in the onshore cultivation condition, between 30th of January when temperature decreased, and 31st of January when temperature raised (Fig.3.1). The opposite also happened, with temperature rise peaks, in the onshore cultivation condition, between 26th of

September and 29th of September, and in the offshore cultivation condition between 23rd of March and 24th of March (Fig.3.1).

Results show a decrease in the temperature between the end of October and the beginning of November. In the Ria Formosa (offshore cultivation condition), water temperature remained between 10 Cº and 20 Cº during most of the trial with some exceptions when the minimum temperature registered reached 10.64 Cº on the 21st of January, and the maximum temperature of 22.17 Cº reached on the 7th of April and 21.67 Cº on the 8th of April (Fig.3.1). Water temperature at EPPO declined between October and December, except for a temperature rise on October 27th, hitting 28.2 Cº. During the rest of the experiment, the temperature remained between 10Cº and 20Cº, similar to the Ria Formosa conditions (Fig.3.1).

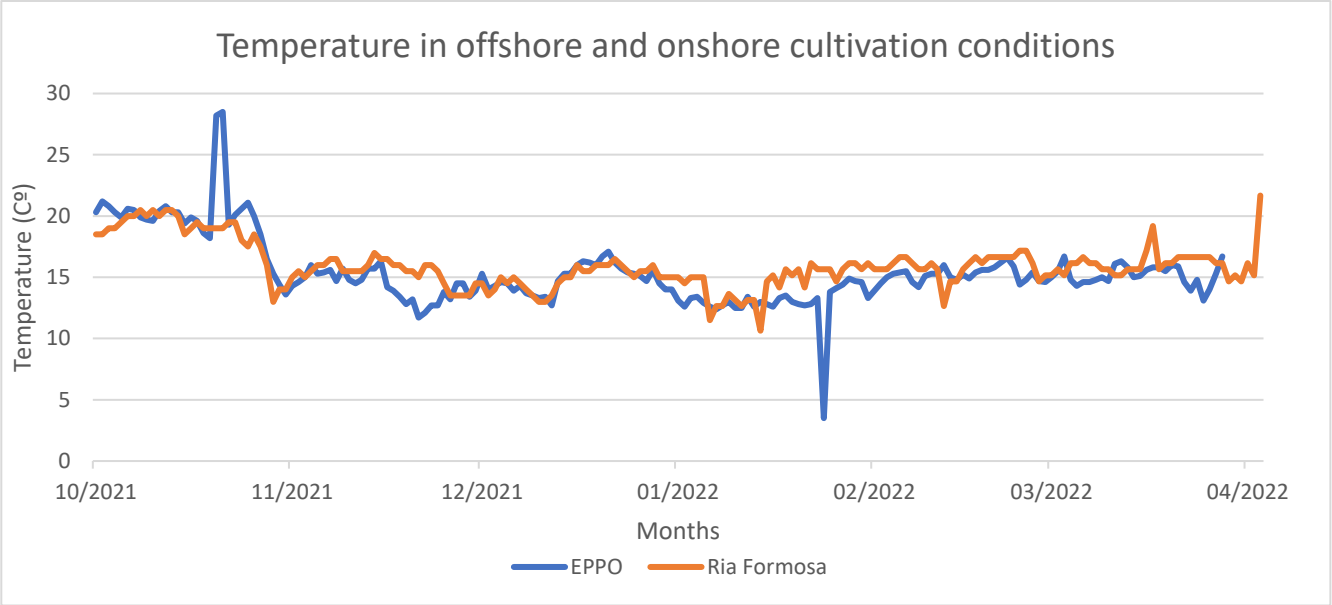

Figure 3.1: Water temperature in the offshore cultivation condition (treatment A, *Ulva* spp. and treatment B, *Ulva* spp.+ inert feed) and onshore cultivation condition (treatment C, *Ulva* spp.+ inert feed)

3.1.2. Structure

The initial structure had four long feet that would fix into the substrate not allowing the structure to move. Although it was effectively fixed to the substrate, it was found to be very difficult to handle the structure during feeding and samplings. Due to this issue, it was impossible to submerge the structure further and it was difficult to handle by boat. On the 12th of January, in sampling 3, the structure was found damaged, with its legs bent. Overall, the performance of the tested structure demonstrated that was not ideal, with the occurred problems

influencing sea urchin's health and development. To overcome these problems, the structure was taken to EPPO to be fixed and improved. A remake structure with the sea urchin was returned to the Ria Formosa on the 16th of February, with improved feet made of a PVC base to allow better handling of the structure, and with a new system of ropes that allowed the structure to be pushed into deeper water and pulled easily when necessary. On the 4th of March, after the implementation of the new system, the structure was found dislocated and completely off the water. It was presumed that it had happened days before and that with the low tides the structure was off the water for several hours a day. This situation led to the death of several individuals both treatments A and B.

Hexcyl™ baskets demonstrated an overall good performance for sea urchin cultivation, as they had enough space for all individuals and a good surface for them to attach. When the primary production is higher, the fast growth of the natural biofilm promotes the incrustation of a wide variety of invertebrates in the basket's nets, seriously affecting the water flow inside, and consequently the water quality available for the cultured sea urchin. During the trial baskets needed to be cleaned frequently and replaced with new ones when necessary.

3.1.3. Survival Rate

Along the study, high numbers of sea urchin survivals were registered in both cultivation conditions. A decrease in the number of sea urchin was expected to be observed in every sampling, as three sea urchin per basket were taken in each sampling for gonad development analysis. After the beginning of 2022, lower survival values of sea urchin were registered in all treatments (Fig.3.1). In the offshore cultivation condition, was observed in treatments A and B, a big reduction in the average number of sea urchin per basket between sampling 4, on the 16th of February and the 4th of March, indicating increased mortality in both treatments. On the 4th of March, basket 3 from treatment A was found with not enough sea urchin to continue the experiment, and as so, the basket was removed from the experiment. With the removal of basket 3, the average number of sea urchin per basket raised again.

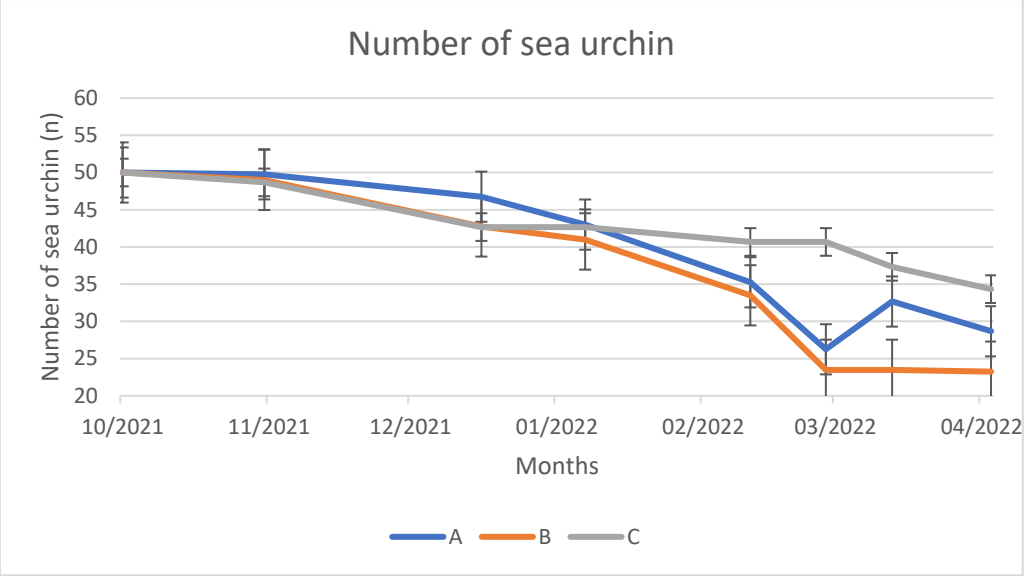

Figure 3.1: Average number of sea urchin (*Paracentrotus lividus*), per basket in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed), along the first experiment.

Results show significant differences between treatments A (offshore, *Ulva* spp.), B (Offshore, *Ulva* spp. + inert feed), and C (Onshore, *Ulva* spp. + inert feed) (p-value = 0,0296). Sea urchin in treatment A show a higher survival rate (Fig.3.2), when compared with sea urchin in treatment B. When compared, sea urchin in treatments A and B in the Ria Formosa, with sea urchin from treatment C, the ones in treatment C, demonstrated a higher survival rate (68,6%).

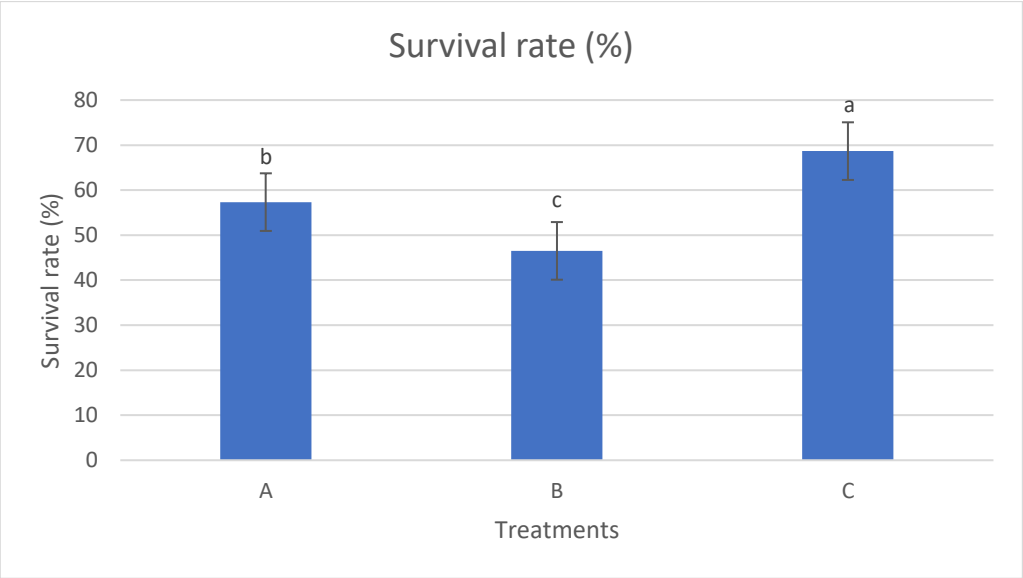

Figure 3.2: Sea urchin (*Paracentrotus lividus*) survival rate in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed), at the end of the first experiment. Significant differences were found between treatments (ANOVA, p-value ≤ 0.05). Different letters a, b, and c mean significant differences.

In the first experiment, sea urchin were found sick in different baskets. The number of sick sea urchin did not increase during the experiment, but on the 16th of February (sampling 4), baskets 1, 3, 5, 6 and 7 registered numerous sick sea urchin. After 16th of February, the average number of sea urchin per basket continued to gradually decrease along the rest of the experiment (Fig. 3.2), indicating the possible death of the sick ones. The death of the sick sea urchin possibly led to an increased mortality between 16th of March (sampling 4) and 4th of March.

3.1.4. Feed intake

Along the first experiment, the feed provided was not consumed in totality and equally among the cultivation systems. From the first feeding, on the 6th of October, until the 16th of February, all feed provided (*Ulva* spp. and inert feed) was consumed. On 26th of February, after sampling 4 (16th of February), there was a decrease in the amount of *Ulva* spp. consumed. Treatment C increased again after and maintained the total consumption of *Ulva* spp. and inert feed until the end of the experiment. Sea urchin from treatments B and C also increased *Ulva* spp. consumption after the 26th of February but continued to not consume the total amount of

algae provided. The amount of inert feed provided to treatments B and C was reduced from 50g to 25g on the 4th of March as the number of feeding days increased (Table. 3.1).

Table 3.1: Feed intake of sea urchin per basket, during the first experiment, in cultivation condition A (offshore, *Ulva* spp.), cultivation condition B (offshore, *Ulva* spp.+ inert feed), and C (onshore, *Ulva* spp. + inert feed):

	06/out		22/out		05/nov		19/nov		06/dez		21/dez		12/jan		16/fev		26/fev		04/mar		10/mar		18/mar		08/abr	
Ulva spp. /Inert feed																										
Treatment A	450	-	450	-	650	-	368	-	700	-	750	-	900	-	900	-	-	-	256	-	191	-	197	-	350	-
Treatment B	400	50	400	50	600	50	189	-	650	50	700	50	850	50	850	50	-	50	148	25	210	25	95	25	292	25
Treatment C	400	50	400	50	600	50	400	50	650	50	700	50	850	50	850	50	-	50	500	25	300	25	300	25	300	25

3.1.5. Biometric parameters

3.1.5.1. Growth

In the offshore cultivation condition, treatment A demonstrated an increase in the mean weight values in all baskets, with basket 2 having the highest weight values, reaching 19.21±3.23 g. After sampling 5 (T5), basket 3 was removed, having a number of individuals too small to continue in the experiment. This led to a big decrease in the weight of the sea urchin in treatment A. In treatment B, in the same cultivation condition, baskets 5 and 6 also demonstrated an increase in the weight of the sea urchin, with basket 6 reaching 19.44±4.32g. In baskets 7 and 8 was observed an increase in the mean weight only until sampling 4 (T4). From sampling 5 (T5), all baskets registered a decrease in the mean weight of the sea urchin. In the onshore cultivation condition, results show an increase in the mean weight values of all baskets in treatment C. Basket 3 obtained the highest mean weight value, reaching 21.12±4.95g.

When observing the mean weight of sea urchin per treatment, sea urchin from treatment A, had an initial weight decrease from 16.7±5.24g to 16.07±0.61g, between sampling 0 (6th of October) and sampling 1 (5th of November). Sea urchin started increasing after that, between every sampling, until sampling 3 (12 of January), reaching a mean weight of 19.54±1.30g. Between sampling 3 and sampling 5 (18th of March), sea urchin mean weight decreased reaching 17.80±1.01g, increasing again in the last sampling (8th of April), reaching a mean weight of 18.2±0.77g (Fig.3.3). Sea urchin in treatment B demonstrated a continuous weight increase from initial sampling 0 until sampling 4 (16th of February), reaching a mean weight of 20.89±1.92g. From sampling 5, the mean weight decreased in all sampling until the end of the experiment, reaching 18±1.44g (Fig.3.3). Treatment C demonstrated an increase in the mean

weight values between the 21st of December and 18th of March, reaching 20.43 ± 0.79g. Between the 18th of March and the last sampling, 8th of April, mean weight values slightly decreased to 20.26 ±0.61g (Fig.3.3).

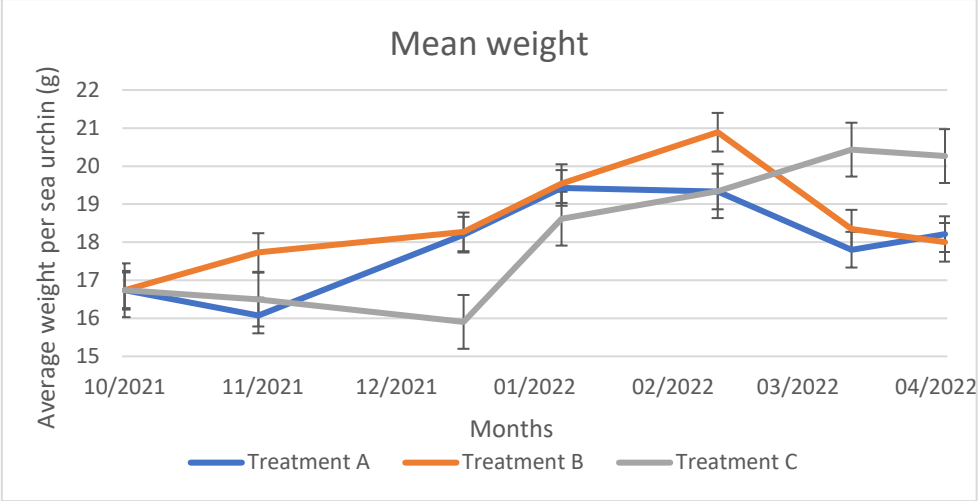

Figure 3.3: Mean weight of sea urchin (*Paracentrotus lividus*), in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp.+ inert feed), and treatment C (onshore, *Ulva* spp.+ inert feed).

Sea urchin from treatment A presents a higher weight gain (WG%) and specific growth rate (SGR) than those from treatment B. Compared with treatment C, sea urchin from both treatments in the offshore cultivation condition showed smaller weight gain and specific growth rate values. Feed conversion rate (FCR) was lower for sea urchin from treatment A, with 17.1. When comparing treatments, A and B from the offshore cultivation condition with treatment C from the onshore cultivation condition, both had higher FCR values (Table.3.4). Results show that there are no statistically significant differences of the growth parameters between treatments A, B and C (WG%- p-value = 0.113, SGR%- p-value = 0.075, FCR- p-value = 0.097).

Table 3.4: Growth parameters (WG% - Weight Gained, SGR%-Specific Growth Rate and FCR- Feed Conversion Rate) for treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp.+ inert feed) and treatment C (onshore, *Ulva* spp. + inert feed). Significant differences were not found between treatments (ANOVA, p-value ≤ 0.05). Letters a mean no significant differences:

Growth Parameters			
	WG%	SGR%	FCR
Treatment A	8.816 ± 6.9^{a}	0.068^{a}	17.1^{a}
Treatment B	7.534 ± 6.8^{a}	0.008^{a}	20.7^{a}
Treatment C	21.076 ± 6.8^{a}	0.112^{a}	7.36^{a}

When comparing the average weight of sea urchin, per sampling, between treatments A and B, there were no statistical significant differences (p-value=0.116). Comparing treatment B (offshore, *Ulva* spp. + inert feed) with treatment C (onshore, *Ulva* spp. + inert feed), results show that there are no statistical significant differences between them (p-value= 0.731).

Weight increase of sea urchin was not influenced by the type of diet (*Ulva* spp. and *Ulva* spp.+ inert feed) (p-value= 0.423) or location (p-value= 0.319), as there were no significant statistical differences between values from treatments A and B and treatment C.

3.1.5.2 Test diameter

Results show variations in the average diameter values of the sea urchin from all treatments. In the offshore cultivation condition, treatments A and B demonstrate an average test diameter increase at the beginning of December 2021, reaching 4.01 ± 0.17 cm in treatment A and 3.99 ± 0.2 cm in treatment B. In the onshore cultivation condition, results show a big increase of the average test diameter in February 2022, increasing from 3.74 ± 0.16 cm to 5.18 ± 0.20 cm (Fig. 3.4). All treatments show decreases of the average weight values along the experiment, possibly caused by sampling errors due to the big size dispersion between the sea urchin from the same treatments. The bigger individuals from each treatment were probably the ones selected for gonad removal, which also might have influenced the decrease of the average test diameter in all treatments.

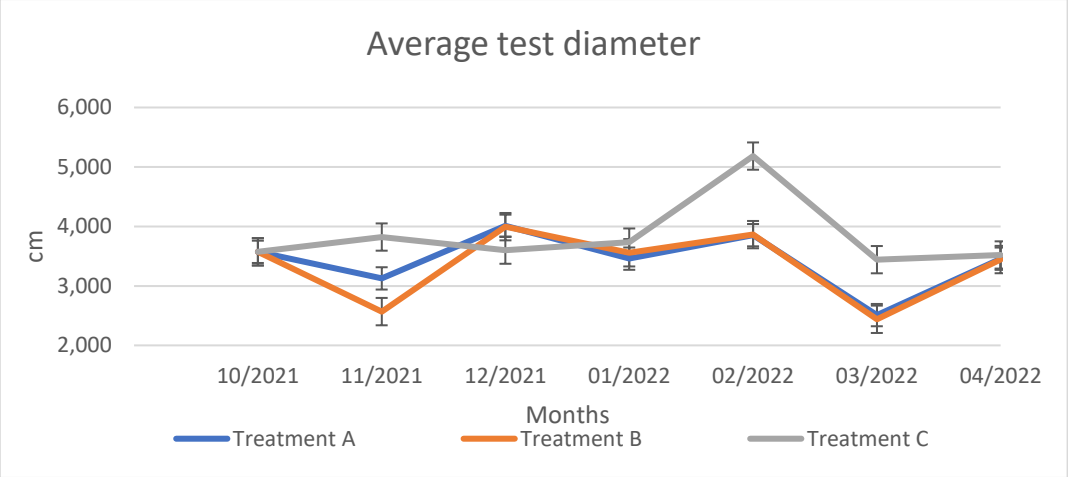

Figure 3.4: Average test diameter of sea urchin (*Paracentrotus lividus*) in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp.+ inert feed), and treatment C (onshore, *Ulva* spp.+ inert feed).

Even though the results show decreases in the average test diameter of the sea urchin along the experiment, when observing the average test diameter values per basket of each treatment, it is possible to observe that sea urchin from almost all baskets had increased values along the experiment. It is possible to observe that at the end of the experiment, in almost all baskets, the average test diameter value was bigger than in the beginning of the experiment (Table. 3.5). Only basket C1 from treatment C (Onshore, *Ulva* spp. + inert feed) demonstrated a smaller average test diameter value in the end of the experiment, decreasing from 3.65±0.46 cm to 3.52±0.63 cm.

Both treatments A and B had similar values along the experiment, as there were no significant statistical differences between them (p-value =0.3768).

Offshore cultivation condition (treatments A and B) did not demonstrate significant statistical differences when comparing each treatment with the onshore cultivation condition (treatment C) (p-value = 0.2496 and p-value = 0.2321, respectively).

3.1.5.3. Gonad development

Along the experiment, gonads from all treatments show a weight increase. Sea urchin from both treatments in the offshore cultivation condition, in Ria Formosa showed the highest average gonad weight in sampling 4 (16th of February), with 1.70 ±0.38 g for treatment A and

2.28 ± 0.33 g for treatment B. From sampling 5, the average gonad weight in all baskets from treatments A and B decreased every sampling while in treatment C continued to increase. In the last sampling (8th of April), treatments A and B reached an average gonad weight of 0.96 ± 0.02g and 1.33 ± 0.10g, respectively, while treatment C reached its highest value with 2.44 ± 0.15g (Fig: 3.5).

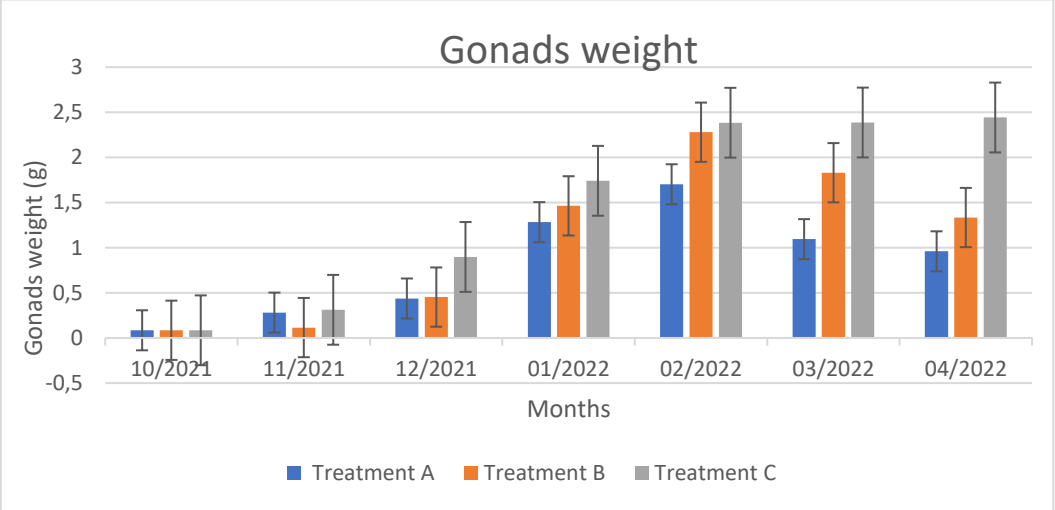

Figure 3.5: Average weight of sea urchin (*Paracentrotus lividus*) gonads in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed).

Gonadosomatic index (GSI) increased along the trial until sampling 4, where were registered the highest values for all treatments. After sampling 4, GSI values started to decrease until the end of the experiment. Treatment B, on average, registered higher values than treatment A, after sampling 2 (21st of December) and until the end of the experiment. Treatment B registered 10.94 ± 1.46 as its highest value and treatment A registered 8.86 ± 2.10. Both treatments A and B registered lower GSI values than treatment C (Fig.3.6). Differences between GSI values according to type of diet are not statistically significant (p-value = 0.282).

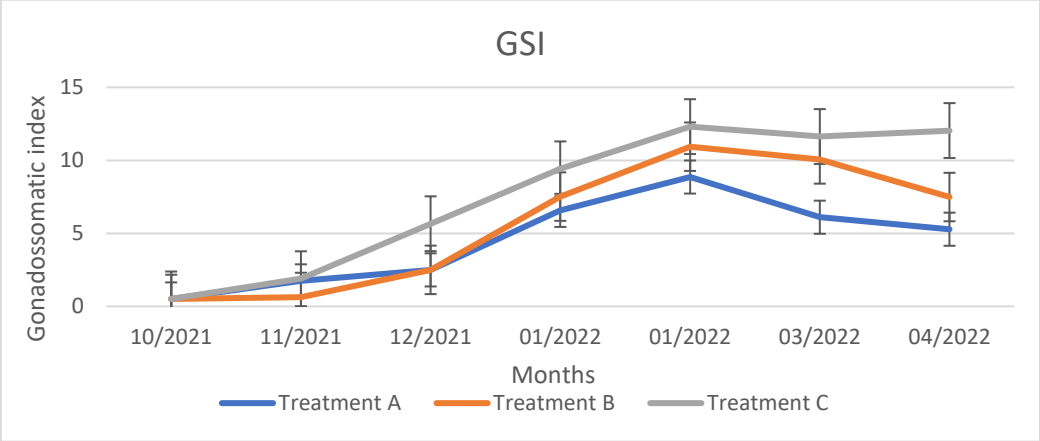

Figure 3.6: Gonadosomatic index (GSI) of sea urchin *(Paracenteotus lividus),* in treatment A (offshore, *Ulva* spp.*),* treatment B (offshore*, Ulva* spp. + inert feed) and treatment C (onshore*, Ulva* spp. + inert feed).

3.1.5.4 Histology

The different stages of gonad development along the experiment were assessed through microscopical analysis of the histological slides of the sampled gonads. Figures 3.7 and 3.8 show all phases of sea urchin growth for the female and male, respectively. It was possible to identify all six stages of development for both sexes along the experiment. As demonstrated in Figures 3.9, 3.10 and 3.11, in every sampling along the experiment, different gonad development stages were registered, as sampled sea urchin did not develop its gonads at the same time, although they were in similar culture conditions. Even so, it was possible to notice a continuous development of the gonads along the trial, evolving its gametogenic stages.

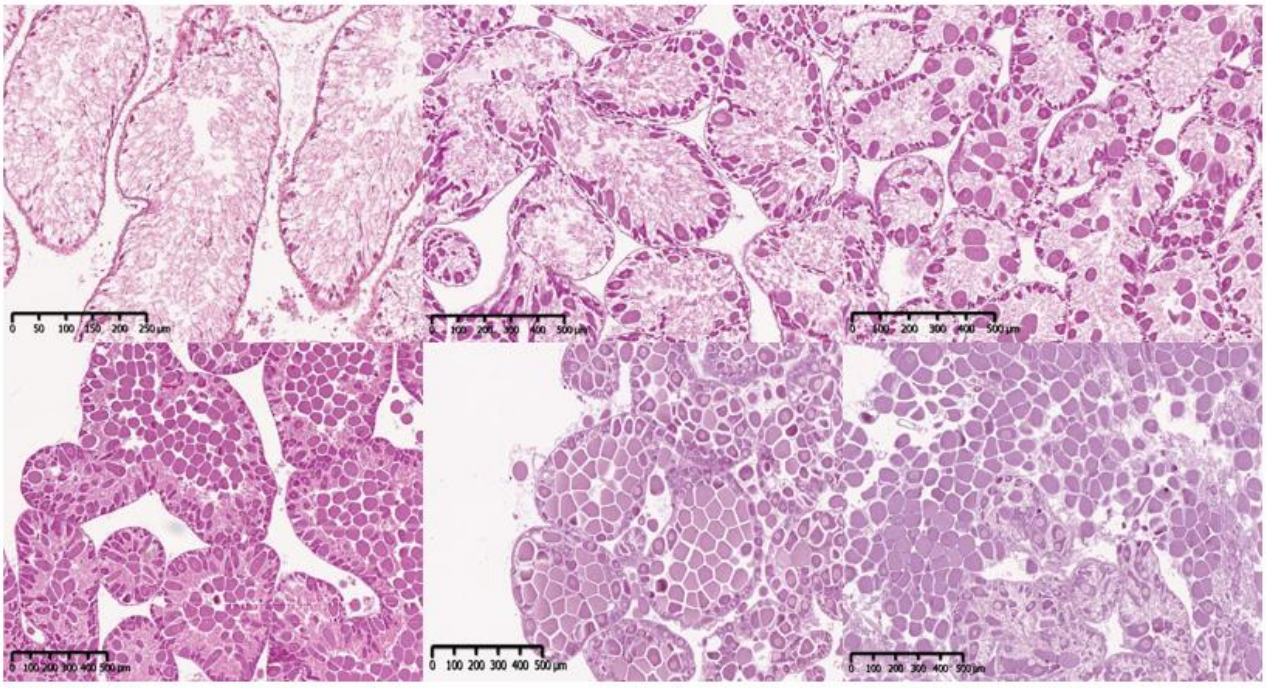

Figure 3.7: Development stages of the gonads from female sea urchin (*Paracentrotus lividus)* (1- stage i; 2- stage ii; 3-stage iii; 4- stage iv; 5- stage v; 6- stage vi).

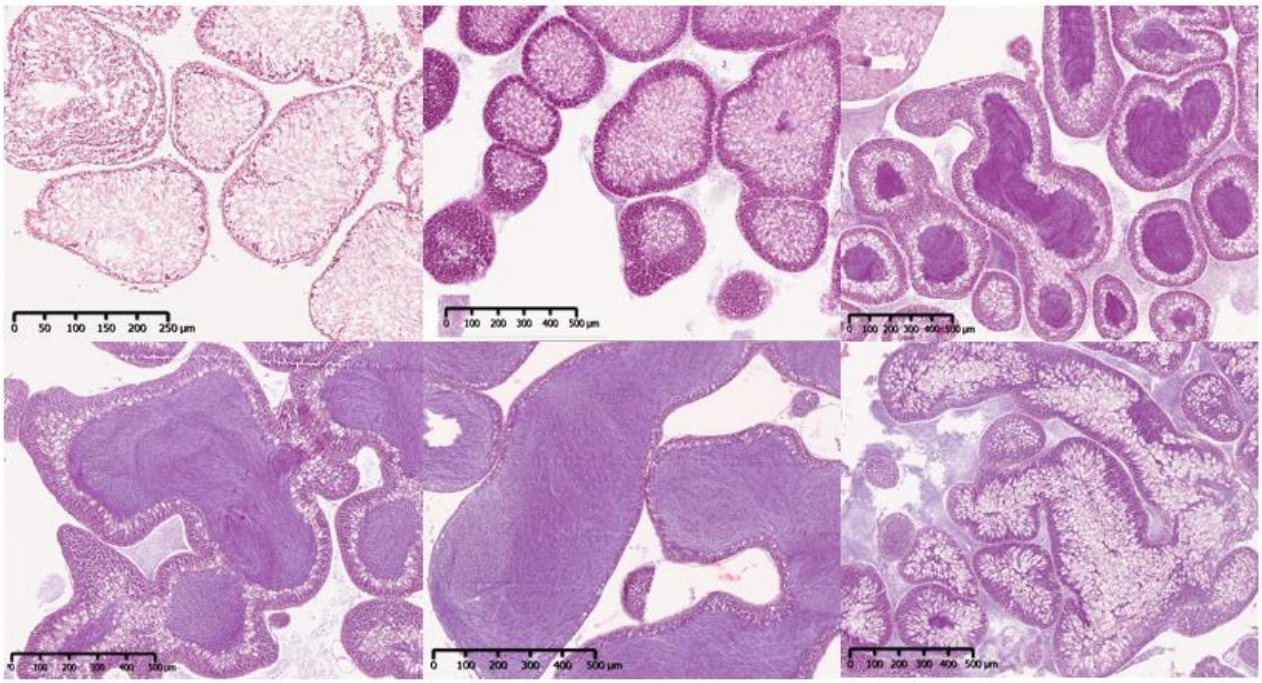

Figure 3.8: Development stages of the gonads from male sea urchin *(Paracentrotus lividus)* (1- stage i; 2- stage ii; 3-stage iii; 4- stage iv; 5- stage v; 6- stage vi).

As seen in Figures 3.9, 3.10 and 3.11, in the first sampling all sampled sea urchin, were in the first stage of development, except for one sampled male. Different development stages of the gonads were registered, changing, and increasing between samplings. Sea urchins in stage

IV were found mainly in treatments B and C, in sampling 4 (T4). In the last sampling, were registered in all treatments, gonads in the final stages of maturation (stages V and VI of gonad development). Results demonstrate that there are no statistically significant differences between gonad maturation in the different treatments (p-value =0.358).

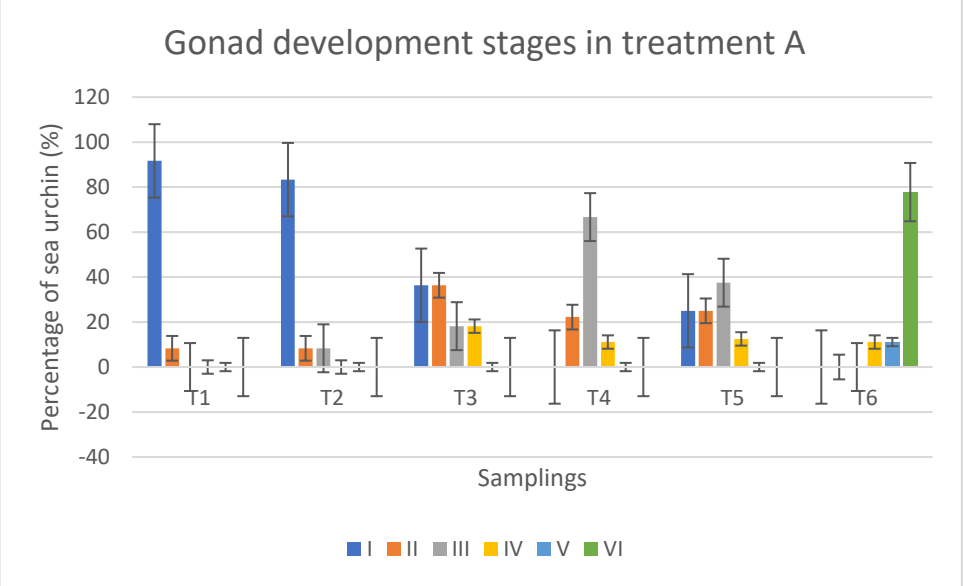

Figure 3.9: Percentage of sea urchin (*Paracentrotus lividus)* per stage of gonad development in treatment A (offshore, *Ulva* spp.*),* in the first experiment.

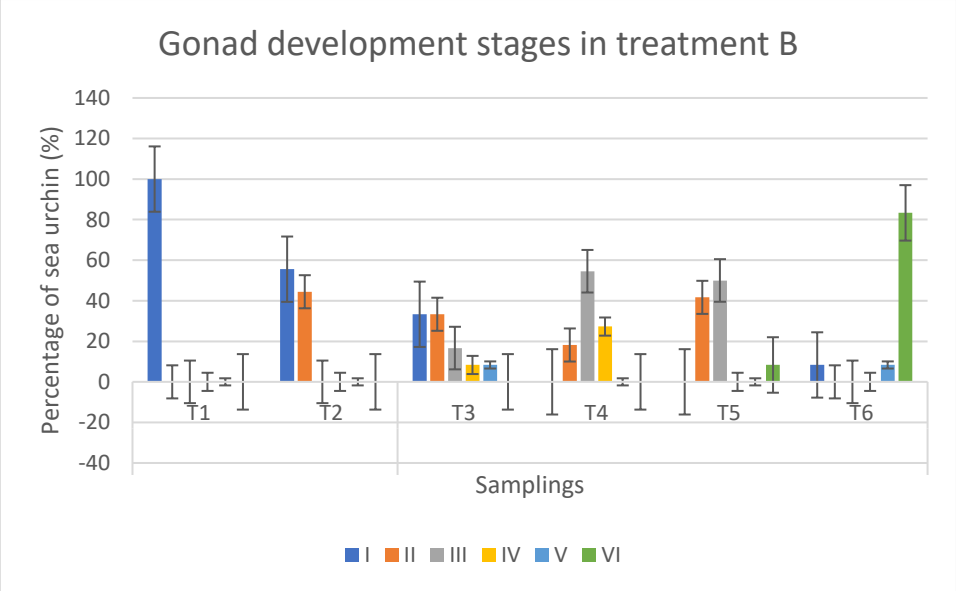

Figure 3.10: Percentage of sea urchin (*Paracentrotus lividus)* per stage of gonad development in treatment B (offshore, *Ulva* spp. + inert feed*),* in the first experiment.

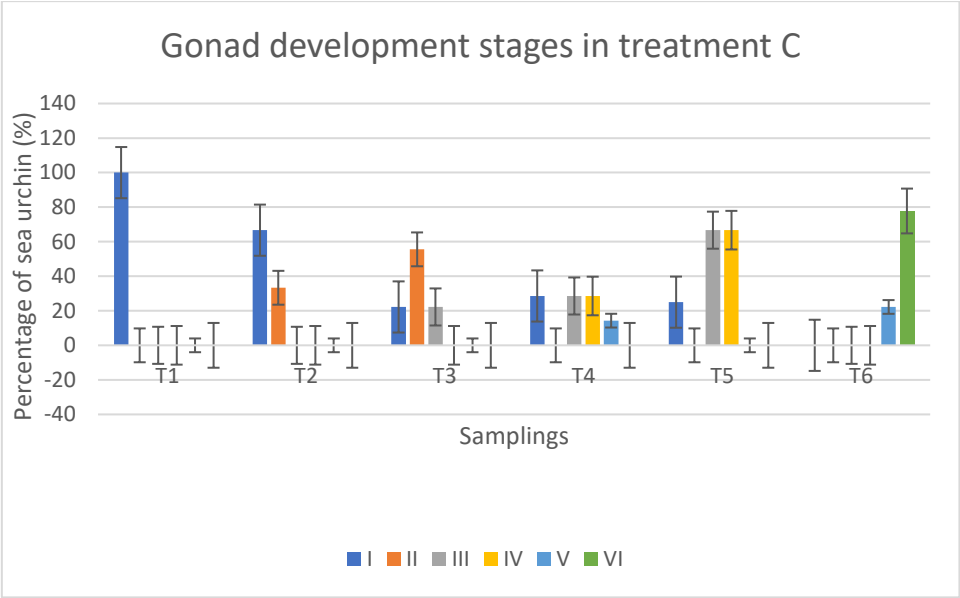

FIGURE 3.11: Percentage of sea urchin (*Paracentrotus lividus)* per stage of gonad development in treatment C (onshore, *Ulva* spp. + inert feed*),* in the first experiment.

Results show that female sea urchin reached more mature states of the gonads earlier in the experiment than males. Females started to register stages IV and V in sampling 3 (12th of January), while males only registered stage IV in sampling 4 (16th of February). Although the differences, both sexes registered the highest number of sea urchin in stage VI (spent stage) in sampling 6 (8th of April). Stage V (mature stage) was only registered in 6 females in samplings 3, 4 and 6 and in 3 males in sampling 6 (table.3.6).

Table 3.5: Number of female and male sea urchins (*Paracentrotus lividus*) per development stage along the experiment:

Development stages of sea urchin gonads												
Stages	T1		T2		T3		T4		T5		T6	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
I	27	18	12	9	2	8	-	2	1	3	1	-
II	1	-	3	5	9	4	5	2	4	4	-	-
III	-	-	-	1	1	-	5	5	6	6	-	-
IV	-	-	-	-	-	1	2	4	3	1	1	-
V	-	-	-	-	-	1	-	4	-	-	3	1
VI	-	-	-	-	-	-	-	-	1	1	13	11

Statistical analysis, testing for normality and using the non-parametric Kruskal Wallis test, demonstrate a relationship between the gametogenic stages and GSI (p-value < 2,2 e^{-16}).

Results also demonstrate a relationship between the gametogenic stages and wet weight of sea urchin (p-value < 2,0 e^{-6}).

3.2. Second Experiment

Treatment D (offshore, *Ulva* spp.+inert feed) in the Ria Formosa and treatment E (onshore, *Ulva* spp.+inert feed) at EPPO were tested in the second experiment.

3.2.1. Water Parameters

During the second experiment, water temperature was assessed daily for both treatments D and E, between 1st of May and 10th of August for treatment D in the Ria Formosa and between the 1st of May and 31st of July for treatment E, at EPPO. In treatment D, during May and June, water temperature ranged between 14.5 Cº and 22.5 Cº. Results show a temperature rise in the Ria Formosa, from June to August, with maximum temperature of the water reaching 25.5 Cº on the 24th and 25th of July. Between 26 th and 28th of July, results show a big decrease in water temperature, reaching 17.5Cº on day 28 (Fig.3.12). In treatment E, minimum water temperature was higher than in the Ria Formosa, with the lowest temperature being 18.2 Cº on the 4th of May. In the beginning of July temperature raised, reaching 25.6 Cº on days 24, 25 and 26 (Fig.3.12).

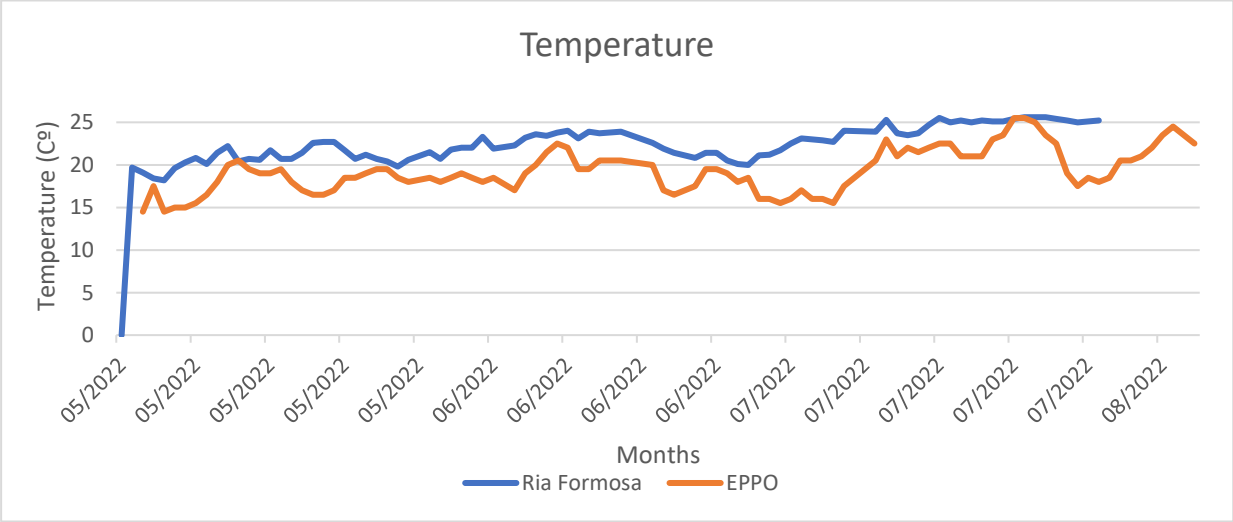

Figure 3.12: Daily water temperature inside the baskets containing sea urchin, in the offshore cultivation condition, in the Ria Formosa (treatment D) and onshore cultivation condition at EPPO (treatment E).

3.2.2. Structure

The improved structure for the second experiment had an overall good performance. During the experiment, the new structure was able to keep sea urchin permanently underwater. Baskets were well secured and were able to maintain the same position in the water, keeping always the same distance to the shore. Although the structure was well secured during the whole trial, on the 18th of July the structure was found damaged, as one of the ropes that held the structure was missing, entangling all baskets. The structure was put back in place in the same day, keeping the same position until the end of the experiment. Similarly, to what happened in the first experiment, Hexcyl™ baskets demonstrated a good performance for sea urchin cultivation. Baskets had to be cleaned along the experiment to remove attached algae and other biofilm and were replaced for new ones once when the used ones were not able to be well cleaned at the site.

3.2.3 Survival Rate

Treatment D registered a decrease in the average number of sea urchin per basket in the last sampling. The same happened in treatment E, where the decrease of the average number of sea urchin was only noticed in the last sampling (Table.3.6; Fig.3.13).

Table 3.6: Average number of sea urchin (*Paracentrotus lividus)* in treatment D (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed), along the second experiment:

Average number of sea urchin per basket			
Treatment	29/04/2022	28/06/2022	10/08/2022
D	56.25± 10.83	56.25± 10.83	54.31 ± 11.65
E	56.25± 10.83	56.25± 10.83	54.38 ± 9.34

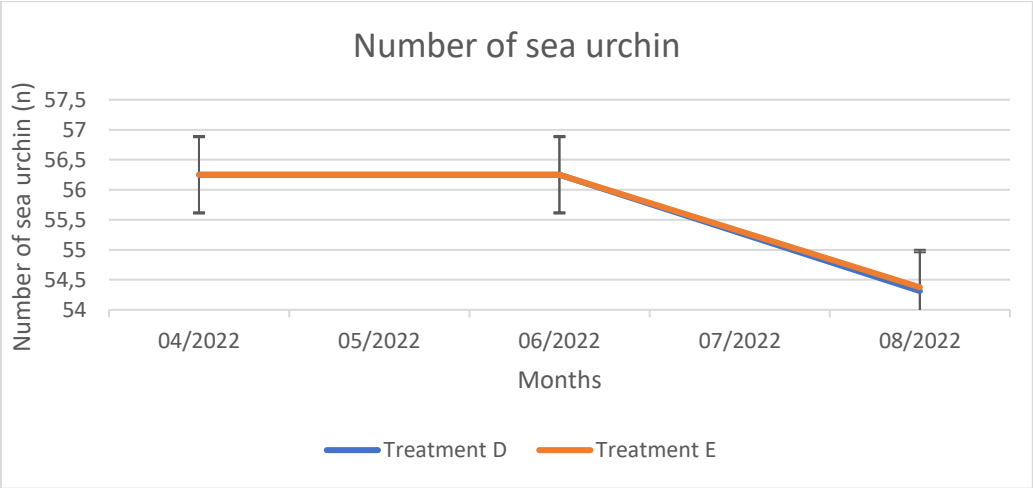

Figure 3.13: Average number of sea urchin (*Paracentrotus* lividus) per basket in treatment D (offshore, *Ulva* spp.) and treatment E (onshore, *Ulva* spp + inert feed), along the second experiment.

The same results can be observed on survival rate, where both treatments show a high survival rate of 96.25% for treatment D and 97% for treatment E (Fig.3.14).

Results demonstrate that there are no statistical significant differences between treatments D and E (p-value=1). Similarly, to the first experiment, in the last sampling, some sea urchin were found sick, with the same disease characteristics of green spots on the test and loss of spines in the affected zone.

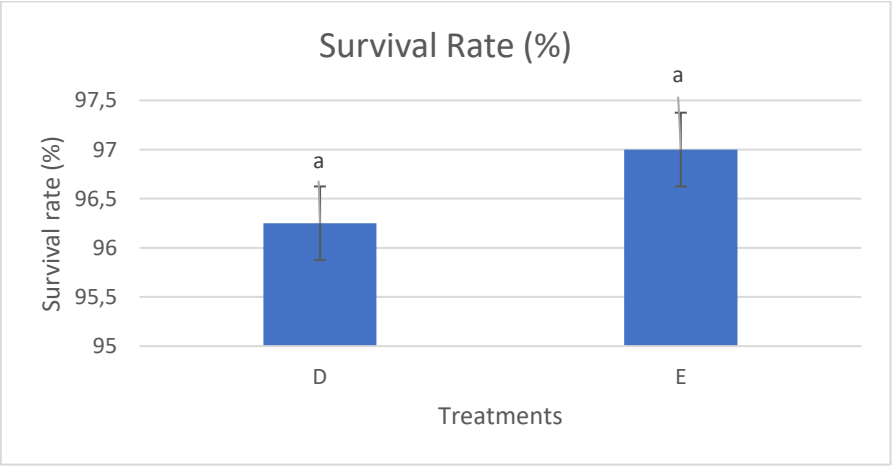

Figure 3.14: Sea urchin (*Paracentrotus lividus*) final survival rate (%), per basket, in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore, *Ulva* spp. + inert feed), in the second experiment. Significant differences were not found between treatments (ANOVA, p-value ≤ 0.05). Letters a mean no significant difference.

3.2.4. Feed intake

During the second experiment, the quantity of feed provided was not equal in every feeding. In the beginning of the experiment the quantity of provided feed increased according to the consumption and demand of sea urchin. On 7th of July, as the feed previously provided was not fully consumed, the quantity of feed was reduced, adjusting to the sea urchin needs (Table.3.7).

Table 3.7: Feed intake of sea urchin per basket, in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore, *Ulva* spp. + inert feed):

	29/abr		06/jun		28/jun		07/jul		18/jul		11/ago	
	Ulva spp.	Inert feed	*Ulva spp.*	Inert feed	*Ulva spp.*	Inert feed	*Ulva spp.*	Inert feed	*Ulva spp.*	Inert feed	*Ulva spp.*	Inert feed
Treatment D												
R1	600	25	550	25	600	25	500	25	500	25	250	50
R2	600	25	550	25	600	25	500	25	500	25	250	50
R3	600	25	550	25	600	25	500	25	500	25	250	50
R4	600	25	550	25	600	25	500	25	500	25	250	50
Treatment E												
C1	600	25	550	25	600	25	500	25	500	25	250	50
C2	600	25	550	25	600	25	500	25	500	25	250	50
C3	600	25	550	25	600	25	500	25	500	25	250	50
C4	600	25	550	25	600	25	500	25	500	25	250	50

3.2.5. Biometric parameters

3.2.5.1. Growth

Results demonstrate an increase in the average weight of the sea urchin along the experiment. In treatment D, sea urchin from baskets 1 and 4 had the highest average weight increase, where sea urchin from basket 1 increased from 15.05 ± 4.3g to 18.89 ± 4.6g and sea urchin from basket 4 increased from 6.95 ± 2.2g to 9.50 ± 2.34. In treatment E, all baskets demonstrated a continuous increase in average weight except for basket 3, that presented a weight decrease between sampling 1(29th of May) and sampling 2 (28th of June). Sea urchin from basket 1 showed the highest average weight increase from 15.44 ± 6.0g to 19.38 ± 6.8g.

When observing the mean weight of sea urchin per treatment, results show an increase of the mean weight values in both treatments. In the end of the experiment treatment D from the offshore cultivation condition obtained a higher average weight value (Fig.3.15).

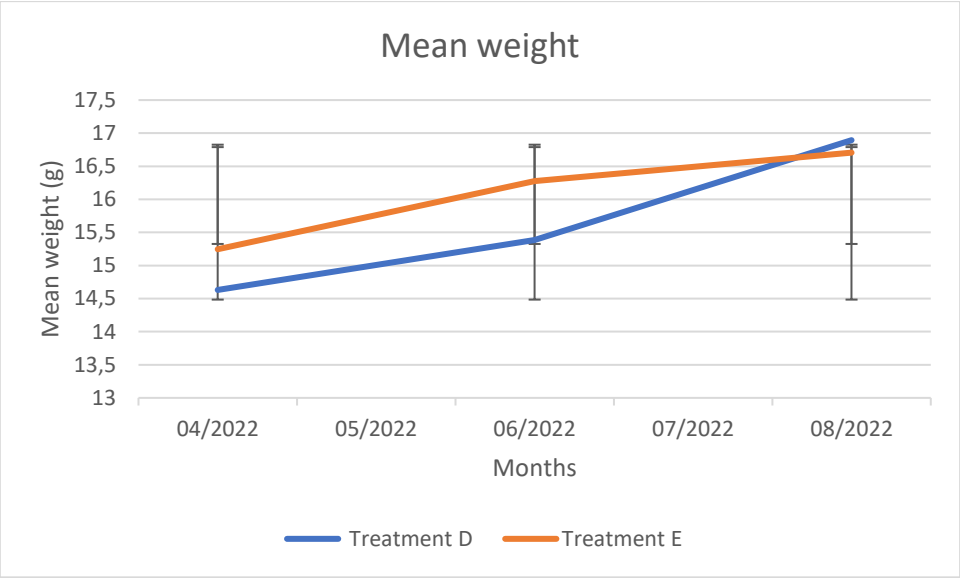

Figure 3.15: Mean weight of sea urchin *(Paracentrotus lividus*), in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore, *Ulva* spp. + inert feed), in the second experiment.

Sea urchin from treatment D showed a higher weight Gain (WG%) and specific growth rate (SGR%), with 18.12% and 0.17%, respectively. The lowest feed conversion rate (FCR) was also presented in treatment D, with 31.03 (Table.3.8).

When compared the mean weight of the sea urchin from treatment D and treatment E, there were no statistical significant differences (p-value= 0.3076) between them. Weight increase was not influenced by location of the cultivation conditions, as there was no statistical significant evidence (p-value= 0.408).

Table 3.8: Growth parameters (WG- Weight gained, SGR - Specific Growth Rate and FCR- Feed Conversion Rate) in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore*, Ulva* spp. + inert feed) Significant differences were not found between treatments (ANOVA, p-value ≤ 0.05). Letters a mean no significant differences:

Growth Parameters			
	WG%	SGR%	FCR
Treatment D	18.118 ± 12.95^{a}	0.166658^{a}	31.02822^{a}
Treatment E	7.291 ± 10.60^{a}	0.085679^{a}	78.33101^{a}

3.2.5.2. Test diameter

. In treatment D, almost all baskets had an increase in the mean test diameter, where basket 1 registered the highest value with 3.84 ± 0.5 cm. Basket 3 registered an increase of the

mean test diameter between sampling 0 (T0) and sampling (T1), reaching 3.88 ± 0.3cm. Baskets 1 and 2 demonstrated an initial decrease in the mean test diameter and an increase between sampling 1 (T1) and the last sampling (T2). In basket 3 the opposite happens, with an initial increase in the mean test diameter, followed by a decrease in these values between samplings 1 and 2, ending the experiment with an average test diameter value of 3.64 ± 0.4cm. Sea urchin from basket 4 demonstrated a continuous increase of test diameter values along the experiment, increasing from 2.45 ± 0.5 cm to 3.15 ± 0.4 cm.

In treatment E, all baskets, with exception of basket 3, show an increase in the average test diameter values. Basket 2 registered the highest value, with 3.96 ± 0.4 cm. Basket 3 registered a decrease in the average test diameter values in all samplings, ending the experiment with an average value of 3.91 ± 0.5cm.

Results show in treatment D an increase of the average test diameter of the sea urchin between June 2022 and August 2022, reaching an average value of 3.60 ± 0.27 cm. In treatment E results show an increase of the average test diameter between April 2022 and June 2022, reaching an average test diameter value of 3.55 ± 0.41cm (Fig.3.18).

Statistical results, when comparing treatments D and E, show that there are no statistical significant differences (p-value= 0.6661) between them.

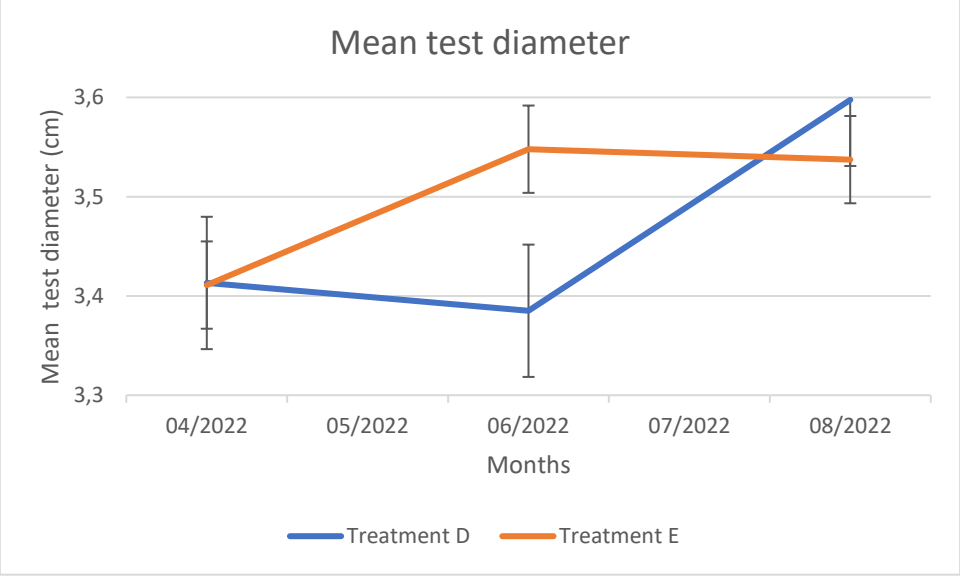

Figure 3.18: Average test diameter of sea urchin (*Paracentrotus lividus*), in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore, *Ulva* spp. + inert feed), in the second experiment.

4. Discussion

The results on the present study show a successful growth of the sea urchin in the Ria Formosa. In both trials done, sea urchin increased its weight and test diameter, indicating a successful growth in the Ria Formosa conditions.

In the first trial, sea urchins from treatment B (offshore, *Ulva* spp. + inert feed) reached the highest average weight, registered on sampling 4 (16th of February). Both treatments in the Ria Formosa, treatments A and B, registered a decrease in feed ingestion after the 4th of March and, consequently, a big average weight decrease on sampling 5 (18th of March). This weight decrease might be correlated with the problem faced on the 4th of March, when the structure was found to be completely out of the water for several hours, probably been happening in the past few days. Treatment C corroborates this hypothesis, as there is no decrease in its average weight values. In this same period, mortality increased between sampling 4 and the 4th of March, indicating that it was caused by the situation previously described. Changes in the parameters can also be seen within this period in water temperature, where a temperature rise was registered on the 25th of February, after sampling 4, decreasing again on the 4th of March when the structure was put back in place. The temperature raise inside the baskets during this period can be associated with the fact of the atmospheric temperature is higher than the temperature in the water, so in periods when the structure was out of the water, temperature inside the baskets increased.

Values obtained for the test diameter of sea urchin studied in the first experiment fluctuated between samplings for all treatments. Even though, for each treatment it was registered a decrease of the obtained values of average test diameter, when analyzing the obtained values assessed per basket for each treatment, it is possible to see an increase of these values in most of the baskets. This could have happened because sea urchin from the different baskets within the same treatments did not grow equally (possibly due to competition and variation of water quality in the replicate baskets), causing a big population dispersion. Samplings might have also influenced the sea urchin population. When choosing individuals for gonads removal, selecting the bigger individuals might have influenced the average test diameter values obtained in the next samplings.

As for the second experiment, sea urchin from both treatments increased its average weight, with sea urchin from treatment D, in the offshore cultivation condition, in the Ria

Formosa, registering a higher weight increase than the ones from treatment E at EPPO.

Mortality was only registered in the last sampling in both treatments. This mortality increase can be associated with the increase of temperature occurred in the beginning of July and extended until the end of the experiment. According to Boudouresque & Verlaque (2013), *P.lividus* sea urchin live in regions with summer temperatures between 18 ºC and 25 ºC. Temperatures reached between July and the beginning of August surpassed these values. Although populations from the south may have better temperature adaptation strategies, the temperature increase above these values may have caused this mortality increase.

Sea urchin from the second experiment had an overall increase of test diameter. In both treatments there were some exceptions, with one basket in each treatment registering in the last sampling lower average test diameters than in the first sampling. This could have been caused in sea urchin' measurement during samplings, by randomly choosing for sampling smaller sea urchin than the ones chosen previously.

Feed intake was not equal along the first experiment, drastically decreasing the quantity of feed ingested after the replacement of the damaged structure for the improved one, not recovering during the rest of the experiment. A possible explanation could be the change of environment, from EPPO back to the Ria, with more harsh water conditions from the wind, temperature, and tides, being stressful conditions for the sea urchin. This hypothesis is supported by the fact that in this same period were found sick sea urchin in most of the baskets of both treatments.

As for the second experiment, all sea urchin were fed with the same diet, with just small adjustments of the amount of feed provided, according to their needs. After 7th of July the amount of feed provided on each feeding was slightly smaller, but the frequency of feeding increased, as with a smaller period between feedings, the amount of feed provided needed to be adjusted so feed would not be wasted. This could have been caused by the water temperature rise faced in the same period, in which and according to IPCC (2014) and Lemoine and Burkepile (2012), at higher water temperatures marine ectotherms face reduced oxygen concentrations, which limits their capacity to feed.

First experiment showed similar results on growth performance among different diets. Diet composed by an inert feed and *Ulva* spp. had a good performance, but it did not stand out from the natural diet composed only with *Ulva* spp.. Even though it did not stand out, it is still

a good result, as formulated feeds are important to reduce the dependency of fresh macroalgae, which exhibit strong fluctuations in availability and quality. Similar results are shown in previous literature. According to Lourenço et al.(2018), protein content between 20% and 45% with a fixed lipid level does not affect *P. lividus* growth. Spirlet et al. (2001), also observed that *P. lividus* fed with high protein formulated diets, with soya-beans, ingested lower feed quantities than the ones only fed with algal diets. Results might also be influenced by the loss of some of the inert feed, as it can dissolve into the water, not being consumed by the sea urchins. In addition, sea urchin from Treatment A, (offshore, *Ulva* spp.) had a lower FCR and a higher WG% than treatment B (offshore, *Ulva* spp. + inert feed). When compared with treatment C (onshore, *Ulva* spp. + inert feed), results show that it has the lowest FCR and higher WG% and SGR% from the three, indicating that both diet and location influenced sea urchin' growth.

Results show a similar gonad development between the tested treatments A and B, in the Ria Formosa, with both average gonad weight and gonadosomatic index increasing continuously until sampling 4 and then decreasing the rest of the experiment. Such thing did not happen to treatment C at EPPO, indicating that conditions in the Ria Formosa influenced gonad development of sea urchin. Equally to what is observed in all results from the first experiment, all parameters demonstrated positive results until sampling 4. The decrease of gonads weight and GSI might have also been caused by the decrease of feed intake. Azad et al. (2011), refers that sea urchin' gonads act as the main organ of nutrient storage, and the amount of nutrient intake and subsequent gonad growth depends on feed quantity/quality and rate of consumption, digestion, and absorption. Also, Baião et al., (2019) observed in his studies that 30% DM dietary protein with 7% lipids, could induce high nutrient utilization and promote a higher GSI.

All development stages were observed for both female and male sea urchin, in all treatments. The optimal stage for gonads to be consumed (stage IV- Mature stage) was registered in treatments B and C in a higher number of individuals at the same time. Sea urchin in mature stage was also registered in treatment A but dispersed along the samplings. It was not possible to register of a well defined period for optimal gonad consumption, as all stages were found in a big number of sea urchin along the whole experiment. Gonad weight and GSI results support this information, as their highest values were also registered on sampling 4. Few individuals of stage V were registered, being most individuals registered after in stage VI, already in the last sampling, indicating that the optimal stage for consumption on most of the sampled sea urchin was between samplings.

With all problems occurred between samplings 4 and 5, and the inability to obtain sea urchin in its optimal consumption period, running organoleptic tests was no feasible, as gonads were not proper for consumption.

Results demonstrate a relationship between the wet weight of the sea urchin and the gametogenic stages, but, even with this relation, the creation of an index of gonad maturation would not be completely accurate by just relying on the wet weigh of the sea urchins. Although most of the weight of the sea urchin is due to the gonads, this can also be related with nutrient storage and not only with gonad maturation. Also, sea urchin with different diameters and the same weight do not necessarily are in the same gonad development stage. In previous studies, Matsuno and Tsushima (2011), referred that for sea urchin, carotenoids are primarily stored in the gonads, but are also found in external structures including the test and spines. Mos et al. (2019), also found a positive relationship between spine color and color and size of the gonads in sea urchin from the species *Tripneustes gratilla*, indicating that spine color is a suitable proxy for determining the harvest readiness of the species. With further studies, the relationship between the sea urchin weight with the maturation stages, allied with the relationship between color of the spines and color and size of the gonads, will allow the possibility to create a reliable gonad maturation index, allowing a non-lethal and practical method for harvest readiness of sea urchin.

The structure used in the first experiment presented some problems that difficulted structure handling and affected sea urchin development. It was difficult to find a way to combine an easy way to move the structure and at the same time a solution to maintain the structure in the same place when is not being handled. The first structure was able to be kept in place , but because it had too narrow feet, stuck in the sand making it very difficult to move the structure in order to feed the sea urchin. The developed version of the structure, with two PVC tubes at the end of the feet, prevented the structures feet to be stuck in the sand, but it was still difficult to handle it from its position to a shallow water zone for feeding and samplings. There was also the inconvenient of the feeding and samplings needed to be always during low tide, in order to be able to get access to the structure. All problems relative to the structure promoted the increase of stressful factors, such the temperature peaks registered along the experiment, and the feed intake decrease, explaining the sea urchin growth decrease, disease situations and mortality

observed in the results. For sea urchin production several improvements should be done from the experimental structure used in this experiment.

The structure used for the second experiment had a better performance. Because it was permanently floating it was of easy access, allowing feedings to be made from the boat, which would not happen in the first experiment. There was no time restriction, as the access to the structure was not determined by the tides. The attaching method of the structure to the shore should be improved with a more stable and secured method, as during the experiment the structure became loose and entangled. Overall, the structure used in the second experiment had a good performance and would be suitable for sea urchin production. Yet, also improvements should be done, as the poor results in July may be reflex of a higher insolation that the present study could not conclude once there was no sufficient time to verify it.

5. Conclusion

Ria Formosa lagoon demonstrated to have good conditions for sea urchin production, with good water and environmental conditions, optimal for sea urchin' growth.

The tested inert diet had a similar performance to *Ulva* spp. in the sea urchin' development. Having a similar performance to an algae diet means that it is equally effective and can and should be used on sea urchin aquaculture to reduce algae dependence in diets, as they are highly variable in quantity and quality along the year.

From the two structures tested, the second structure demonstrated to be suitable and effective for sea urchin aquaculture. Although it needs some improvements in its attachments on the coastline, the structure was easy to handle and to work with and, demonstrated to be effective and secure for the sea urchin. It would be interesting to study the effectiveness of the structure for a longer period and, as in the first experiment, analyze the development of the sea urchin and its gonads.

Overall, with this study was possible to conclude that the production of sea urchin in the Ria Formosa lagoon, through an innovative system, allied with an effective diet, it is possible and worthy of further studies.

6. Bibliography

Allain J.Y., 1975. Structure des populations de *Paracentrotus lividus* (Lamarck) (Echinodermata, Echinoidea) soumises, a la peche sur les cotes nord de Bretagne. Revue des Travaux de l'Institut des Pêches Maritimes, 39(2), 171-212. https://archimer.ifremer.fr/doc/00000/2013/.

Amaral, A., 2008. Management of Aquaculture of the Clam, *Ruditapes Decussatus* (Linnaeus, 1758) in the Ria Formosa lagoon (South of Portugal): Effects on the Ecosystem and Species Physiology (PhD thesis). Santiago de Compostela University.

Andrade, C., Freitas, M.C., Moreno, J., Craveiro, S.C., 2004. Stratigraphical evidence of Late Holocene barrier breaching and extreme storms in lagoonal sediments of Ria Formosa, Algarve, Portugal. Marine Geology 210, 339–362. https://doi.org/10.1016/j.margeo.2004.05.016

Andrew, N L, Agatsuma, Y., Ballesteros, E., Bazhin, A.G., Creaser, E.P., Barnes, D.K.A., Botsford, L.W., Bradbury, A., Campbell, A., Dixon, J.D., Einarsson, S., Gerring, P.K., Hebert, K., Hunter, M., Hur, S.B., Johnson, C.R., Juinio-Meñez, M.A., Kalvass, P., Miller, R.J., Moreno, C.A., Palleiro, J.S., Rivas, D., Robinson, S.M.L., Schroeter, S.C., Steneck, R.S., Vadas, R.L., Woodby, D.A., Xiaoqi, Z., Gibson, N., Barnes, M., Atkinson, R.J.A., 2002. Status and Management of World Sea Urchin Fisheries. Oceanography and Marine Biology. An Annual Review, 40. 343-425. Doi: 10.1201/9780203180594.ch7.

Araújo, J., Mendes, A., Monteiro, I., Teixeira, D., Soares, F., Pousão-Ferreira, P., 2020. The use of diatom *Skeletonema costatum* on aquaculture-produced purple sea urchin (*Paracentrotus lividus*) larvae and post-larvae diet. Aquaculture Reseacrch, 51, 2545–2554. https://doi.org/10.1111/are.14597

Azad, A.K., Pearce, C.M., McKinley, R.S., 2011. Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*). Aquaculture, 317, 187–196. https://doi.org/10.1016/j.aquaculture.2011.03.019

Baião, L. F. (2021). 'Characterization of the sensory profile of sea urchin aiming at the selection of premium gonads.' PhD thesis, University of Porto.

Baião, L.F., Rocha, F., Costa, M., Sá, T., Oliveira, A., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019. Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture 506, 127–138. https://doi.org/10.1016/j.aquaculture.2019.03.005

Basuyaux, O., Blin, JL., 1998. Use of maize as food source for sea urchins in a recirculating rearing system. Aquaculture International, 6, 233-247. https://doi.org/10.1023/A:1009206811475

Becker, P.T., Egea, E., Eeckhaut, I., 2008. Characterization of the bacterial communities associated with the bald sea urchin disease of the echinoid *Paracentrotus lividus*. Journal of Invertebrate Pathology 98, 136–147. https://doi.org/10.1016/j.jip.2007.12.002

Beddingfield, S.D., McClintock, J.B., 1998. Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid *Lytechinus variegatus* (Lamarck) fed natural diets. Journal of Experimental Marine Biology and Ecology 226, 195–215. https://doi.org/10.1016/S0022-0981(97)00247-5

Behrens, M.D., Lafferty, K.D., 2004. Effects of marine reserves and urchin disease on southern Californian rocky reef communities. Marine Ecology Progress Series 279, 129–139. https://doi.org/10.3354/meps279129

Bertocci, I., Dominguez, R., Machado, I., Freitas, C., Domínguez Godino, J., Sousa-Pinto, I., Gonçalves, M., Gaspar, M.B., 2014. Multiple effects of harvesting on populations of the purple sea urchin *Paracentrotus lividus* in north Portugal. Fisheries Research 150, 60–65. https://doi.org/10.1016/J.FISHRES.2013.10.010

Borisovets, E.E., Zadorozhny, P.A., Kalinina, M. V., Lepskaya, N. V., Yakush, E. V., 2002. Changes of major carotenoids in gonads of sea urchins (*Strongylocentrotus intermedius* and *S. nudus*) at maturation. Comparative Biochemistry and Physiology- B Biochemistry and Mollecular Biology 132, 779–790. https://doi.org/10.1016/S1096-4959(02)00099-4

Boudouresque, C.F., Verlaque, M., 2013. *Paracentrotus lividus*. Developments in Aquaculture and Fisheries Science 38, 297–327. https://doi.org/10.1016/B978-0-12-396491-5.00021-6

Bower, S.M., McGladdery, S.E., Price, I.M., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annual Review of Fish Diseases 4, 1–199. https://doi.org/10.1016/0959-8030(94)90028-0

Brockington, S., Clarke, A., 2001. The relative influence of temperature and food on the metabolism of a marine invertebrate. Journal of Experimental Marine Biology and Ecology 258, 87–99. https://doi.org/10.1016/S0022-0981(00)00347-6

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology, Ecology 85(7), 1771-1789. doi:10.1890/03-9000

Byrne, M., Ho, M., Selvakumaraswamy, P., Nguyen, H.D., Dworjanyn, S.A., Davis, A.R., 2009. Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. Proceedings of the Royal Society B: Biological Sciences 276, 1883–1888. https://doi.org/10.1098/rspb.2008.1935

Carboni, S., Kelly, M.S., Hughes, A.D., Vignier, J., Atack, T., Migaud, H., 2014. Evaluation of flow through culture technique for commercial production of sea urchin (*Paracentrotus lividus*) larvae. Aquaculture Research 45, 768–772. https://doi.org/10.1111/are.12019

Cardoso, A.C., Arenas, F., Sousa-Pinto, I., Barreiro, A., Franco, J.N., 2020. Sea urchin grazing preferences on native and non-native macroalgae. Ecology Indicators 111. https://doi.org/10.1016/j.ecolind.2019.106046

Castilla-Gavilán, M., Cognie, B., Ragueneau, E., Turpin, V., Decottignies, P., 2019. Evaluation of dried macrophytes as an alternative diet for the rearing of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture Research 50, 1762–1769. https://doi.org/10.1111/are.14045

Catarino, A.I., Bauwens, M., Dubois, P., 2012. Acid-base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. Environmental Science and Pollution Research 19, 2344–2353. https://doi.org/10.1007/s11356-012-0743-1

Cook, E., Kelly, M., Mackenzie, J., 1998. Gonad development and somatic growth in the green sea urchin, *Psammechinus miliaris* fed artifitial and natural diets. Journal of Shellfish Research 17, 1549–1555.

Cook, E.J., Hughes, A.D., Orr, H., Kelly, M.S., Black, K.D., 2007a. Influence of dietary protein on essential fatty acids in the gonadal tissue of the sea urchins *Psammechinus miliaris* and

Paracentrotus lividus (Echinodermata). Aquaculture 273, 586–594. https://doi.org/10.1016/j.aquaculture.2007.10.032

Cook, E.J., Kelly, M.S., 2007b. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic salmon *Salmo salar*. Aquaculture 273, 573–585. https://doi.org/10.1016/j.aquaculture.2007.10.038

Couvray, S., Miard, T., Bunet, R., Martin, Y., Grillasca, J.P., Bonnefont, J.L., Coupé, S., 2015. Experimental release of juvenile sea urchins *(Paracentrotus lividus*) in exploited sites along the French mediterranean coast. Journal of Shellfish Research 34, 555–563. https://doi.org/10.2983/035.034.0240

Crook, A.C., Long, M., Barnes, D.K.A., 2000. Quantifying daily migration in the sea urchin *Paracentrotus lividus*. Journal of the Marine Biological Association of the United Kingdom 80, 177–178. https://doi.org/10.1017/S0025315499001721

Cuthbert, F., Hooper, R., Mckeever, T., 1995. Sea urchin aquaculture phase I: sea urchin feeding and ranching experiments, Report of project AUI-503. Government of Newfoundland.

Cyrus, M.D., Bolton, J.J., Macey, B.M., 2015. The role of the green seaweed Ulva as a dietary supplement for full life-cycle grow-out of *Tripneustes gratilla*. Aquaculture 446, 187–197. https://doi.org/10.1016/j.aquaculture.2015.05.002

Dincer, T., Cakli, S., 2007. Chemical composition and biometrical measurements of the Turkish sea urchin (*Paracentrotus lividus,* Lamarck, 1816). Critical Reviews in Food Science and Nutrition 47, 21–26. https://doi.org/10.1080/10408390600550265

E. Tortonese, 1965. Echinodermata. Fauna d'Italia.Echinodermata Edizioni Calderini Bologna; Officine Grafiche Calderini. Bologna, Italy. 422p.

Eddy, S.D., Brown, N.P., Kling, A.L., Watts, S.A., Lawrence, A., 2012. Growth of Juvenile Green Sea Urchins, *Strongylocentrotus droebachiensis*, Fed Formulated Feeds with Varying Protein Levels Compared with a Macroalgal Diet and a Commercial Abalone Feed, Journal of the World Aquaculture Society.

Fernandez, C., Boudouresque, C.F., 2000. Nutrition of the sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) fed different artificial food. Marine Ecology Progress Series 204, 131–141. https://doi.org/10.3354/meps204131

Fernandez, C., Boudouresque, C.F., 1998. Evaluating artificial diets for small *Paracentrotus lividus* (Echinodermata : Echinoidea). Echinoderms 651–657.

Fernandez, C., Pasqualini, V., Boudouresque, C.F., Johnson, M., Ferrat, L., Caltagirone, A., Mouillot, D., 2006. Effect of an exceptional rainfall event on the sea urchin (*Paracentrotus lividus*) stock and seagrass distribution in a Mediterranean coastal lagoon. Estuarine, Coastal and Shelf Science 68, 259–270. https://doi.org/10.1016/j.ecss.2006.02.020

Fernández-Boán, M., Fernández, L., Freire, J., 2012. History and management strategies of the sea urchin *Paracentrotus lividus* fishery in Galicia (NW Spain). Ocean and Coastal Management 69, 265–272. https://doi.org/10.1016/j.ocecoaman.2012.07.032

Fetterman, J.W., Zdanowicz, M.M., 2009. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. American Journal of Health-System Pharmacy. https://doi.org/10.2146/ajhp080411

Gamito, S., 2008. Three main stressors acting on the Ria Formosa lagoonal system (Southern Portugal): Physical stress, organic matter pollution and the land-ocean gradient. Estuarine, Coastal and Shelf Science 77, 710–720. https://doi.org/10.1016/j.ecss.2007.11.013

Gianguzza, P., Chiantore, M., Bonaviri, C., Cattaneo-Vietti, R., Vielmini, I., Riggio, S., 2006. The effects of recreational *Paracentrotus lividus* fishing on distribution patterns of sea urchins at Ustica Island MPA (Western Mediterranean, Italy). Fisheries Research 81, 37–44. https://doi.org/10.1016/j.fishres.2006.06.002

Girard, D., Clemente, S., Toledo-Guedes, K., Brito, A., Hernández, J.C., 2012. A mass mortality of subtropical intertidal populations of the sea urchin *Paracentrotus lividus*: Analysis of potential links with environmental conditions. Marine Ecology 33, 377–385. https://doi.org/10.1111/j.1439-0485.2011.00491.x

Goebel, N., Barker, M.F., 1998. Artificial diets supplemented with carotenoid pigments as feeds for sea urchins, in: Echinoderms. San Francisco, Rotterdam, pp. 667–672.

Guidetti, P., 2004. Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in shallow Mediterranean rocky reefs. Helgoland Marine Research 58, 110–116. https://doi.org/10.1007/s10152-004-0176-4

Guimarães, M.H.M.E., Cunha, A.H., Nzinga, R.L., Marques, J.F., 2012. The distribution of seagrass (*Zostera noltii*) in the Ria Formosa lagoon system and the implications of clam farming on its conservation. Journal for Nature Conservation 20, 30–40. https://doi.org/10.1016/j.jnc.2011.07.005

Hagen, N.T., Jørgensen, I., Egeland, E.S., 2008. Sex-specific seasonal variation in the carotenoid content of sea urchin gonads. Aquatic Biology 3, 227–235. https://doi.org/10.3354/ab00084

Hammer, H.S., Powell, M.L., Jones, W.T., Gibbs, V.K., Lawrence, A.L., Lawrence, J.M., Watts, S.A., 2012. Effect of Feed Protein and Carbohydrate Levels on Feed Intake, Growth, and Gonad Production of the Sea Urchin, *Lytechinus variegatus*, Journal of the World Aquaculture Society.

Harvell, C. D.,1999. Emerging Marine Diseases: Climate Links and Anthropogenic Factors. Science, 285(5433), 1505–1510. doi:10.1126/science.285.5433.1505

Heflin, L.E., Gibbs, V.K., Powell, M.L., Makowsky, R., Lawrence, J.M., Lawrence, A.L., Watts, S.A., 2012. Effect of dietary protein and carbohydrate levels on weight gain and gonad production in the sea urchin *Lytechinus variegatus*. Aquaculture 358–359, 253–261. https://doi.org/10.1016/J.AQUACULTURE.2012.06.009

Heflin, L.E., Makowsky, R., Taylor, J.C., Williams, M.B., Lawrence, A.L., Watts, S.A., 2016. Production and economic optimization of dietary protein and carbohydrate in the culture of Juvenile Sea Urchin *Lytechinus variegatus*. Aquaculture 463, 51–60. https://doi.org/10.1016/j.aquaculture.2016.05.023

Hereu, B., Zabala, M., Linares, C., Sala, E., 2005. The effects of predator abundance and habitat structural complexity on survival of juvenile sea urchins. Marine Biology 146, 293–299. https://doi.org/10.1007/s00227-004-1439-y

Hughes, A.D., Kelly, M.S., Barnes, D.K.A., Catarino, A.I., Black, K.D., 2006. The dual functions of sea urchin gonads are reflected in the temporal variations of their biochemistry. Marine Biology 148, 789–798. https://doi.org/10.1007/s00227-005-0124-0

Hur, S., Hunter, M., Hebert, K., Botsford, L., Johnson, C., Einarsson, S., Campbell, A., Ballesteros, E., Andrew, N., Gerring, P., Dixon, J., Bazhin, A., Juinio-Me√±ez, M., Barnes, D., Creaser, E., Bradbury, A., Agatsuma, Y., Kalvass, P., Miller, R., Moreno, C., Palleiro, J., Rivas, D., Robinson, S., Schroeter, S., Steneck, R., Vadas, R., Woodby, D., Xiaoqi, Z., 2002. Status and Management of World Sea Urchin Fisheries. https://doi.org/10.1201/9780203180594.ch7

Ismail, A., Ktari, L., ben Redjem Romdhane, Y., Aoun, B., Sadok, S., Boudabous, A., el Bour, M., 2018. Antimicrobial Fatty Acids from Green Alga *Ulva rigida* (Chlorophyta). BioMed Research International, 2018. https://doi.org/10.1155/2018/3069595

J. Emmett Duffy, Mark E. Hay, Seaweed Adaptations to Herbivory, BioScience, Volume 40, Issue 5, May 1990, Pages 368–375, https://doi.org/10.2307/1311214

J. G.Harmelinn, Bouchon, C., Duval, C., Hong, J.S., 1980. Les échinodermes des substrats durs de l'île de Port-Cros, Parc National (Méditerranée Nord-occidentale).

Jacinto, D., Bulleri, F., Benedetti-Cecchi, L., Cruz, T., 2013. Patterns of abundance, population size structure and microhabitat usage of *Paracentrotus lividus* (Echinodermata: Echinoidea) in SW Portugal and NW Italy. Marine Biology 160, 1135–1146. https://doi.org/10.1007/s00227-013-2166-z

Jackson, A.C., Underwood, A.J., 2007. Application of new techniques for the accurate analysis of choice of prey. Journal of Experimental Marine Biology and Ecology 341, 1–9. https://doi.org/10.1016/J.JEMBE.2006.11.002

James, P., 2007. 'The Effects of Environmental Factors and Husbandry Techniques on Roe Enhancement of the New Zealand Sea Urchin , *Evechinus chloroticus'*. PhD thesis, Victoria University of Wellington, New Zeland

James, P., Samuelsen, A., 2017. Commercial scale sea urchin roe enhancement in Norway: Enhancement, transport and market assessment. (No. 11259/20933.7), Nofima AS. http://hdl.handle.net/11250/2436979

James, P.J., 2006. A comparison of roe enhancement of the sea *urchin Evechinus chloroticus* in sea-based and land-based cages. Aquaculture 253, 290–300. https://doi.org/10.1016/j.aquaculture.2005.03.050

Jangoux, M., 1986. Diseases of Echinodermata. I. Agents microorganisms and protistans. Diseases of Aquatic Organisms 2, 147–162. https://doi.org/10.3354/dao002147

Jimenez, M., Rio, D., Ramazanov, Z., Garcia-Reina, G., 1996. *Ulva rigida* (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents, Hydrobiologia 326, 61–66 (1996). https://doi.org/10.1007/BF00047787

Kabeya, N., Sanz-Jorquera, A., Carboni, S., Davie, A., Oboh, A., Monroig, O., 2017. Biosynthesis of polyunsaturated fatty acids in sea urchins: Molecular and functional characterization of three fatty acyl desaturases from *Paracentrotus lividus* (Lamark 1816). PLoS One 12. https://doi.org/10.1371/journal.pone.0169374

Kelly, M.S., 2005. Echinoderms: their culture and bioactive compounds., Progress in Molecular and Subcellular Biology. pp. 139–165. https://doi.org/10.1007/3-540-27683-1_7

Kelly, M.S., Symonds, R.C., 2013. Carotenoids in Sea Urchins. Developments in Aquaculture and Fisheries Science 38, 171–177. https://doi.org/10.1016/B978-0-12-396491-5.00011-3

Klinger, T.S., Lawrence, J.M., Lawrence, A.L., 1994. Digestive characteristics of the sea-urchin *Lytechinus variegatus*. Journal of World Aquaculture Society 25, 489–496.

Korpinen, S., Jormalainen, V., Pettay, E., 2010. Nutrient availability modifies species abundance and community structure of Fucus-associated littoral benthic fauna. Marine Environmental Research 70, 283–292. https://doi.org/10.1016/j.marenvres.2010.05.010

Kraufvelin, P., 2007. Responses to nutrient enrichment, wave action and disturbance in rocky shore communities. Aquatic Botany 87, 262–274. https://doi.org/10.1016/j.aquabot.2007.06.011

Lafferty, K.D., 2004. Fishing for lobsters indirectly increases epidemics in sea urchins, Ecological Applications. 14: 1566-1573. https://doi.org/10.1890/03-5088

Lafferty, K.D., Gerber, L.R., 2002. Good Medicine for Conservation Biology: the Intersection of Epidemiology and Conservation Theory, *Conservation Biology*, *16*(3), 593-604. https://doi.org/10.1046/j.1523-1739.2002.00446.x

Lawrence, J.M., 2020. Sea urchin life-history strategies. Developments in Aquaculture and Fisheries Science 43, 19–28. https://doi.org/10.1016/B978-0-12-819570-3.00002-0

Lawrence, J.M., Cao, X., Chang, Y., Wang, P., Yu, Y., Lawrence, A.L., Watts, S.A., 2009. Temperature effect on feed consumption, absorption, and assimilation efficiencies and production of the sea urchin *Strongylocentrotus Intermedius*. Journal of Shellfish Research 28, 389–395. https://doi.org/10.2983/035.028.0223

Lawrence, J.M., Olave, S., Otaiza, R., Lawrence, A.L., Bustos, E., 1997. Enhancement of gonad production in the sea urchin *Loxechinus albus* in Chile fed extruded feeds. Journal of World Aquaculture Society 28, 91–96. https://doi.org/10.1111/j.1749-7345.1997.tb00966.x

le Gall, P., Bucaille, D., Dutot, P., 1989. Résistance aux variations de salinité chez *Paracentrotus* et *Psammechinus* 10, 83–84.

Liyana-Pathirana, C., Shahidi, F., Whittick, A. and Hooper, R., 2002. Lipid and lipid soluble components of gonads of green sea urchin (*strongylocentrotus droeba chiensis*). Journal of Food Lipids, 9: 105-126. https://doi.org/10.1111/j.1745-4522.2002.tb00213.x

Lourenço, S., José, R., Andrade, C., Valente, L.M.P., 2020. Growth performance and gonad yield of sea urchin *Paracentrotus lividus* (Lamarck, 1816) fed with diets of increasing protein: energy ratios. Animal Feed Science and Technology 270. https://doi.org/10.1016/j.anifeedsci.2020.114690

Lourenço, S., Mendes, S., Raposo, A., Santos, P.M., Gomes, A.S., Ganhão, R., Costa, J.L., Gil, M.M., Pombo, A., 2021. Motivation and preferences of Portuguese consumers' towards sea urchin roe. International Journal of Gastronomy and Food Science 24. https://doi.org/10.1016/j.ijgfs.2021.100312

Lourenço, S., Raposo, A., Cunha, B., Pinheiro, J., Santos, P.M., Gomes, A.S., Ferreira, S., Gil, M.M., Costa, J.L., Pombo, A., 2022. Temporal changes in sex-specific color attributes and carotenoid concentration in the gonads (roe) of the purple sea urchin (*Paracentrotus lividus*) provided dry feeds supplemented with β-carotene. Aquaculture 560. https://doi.org/10.1016/j.aquaculture.2022.738608

M Lawrence, J., 2007. Chapter 1 Edible sea urchins: Use and life-history strategies. Developments in Aquaculture and Fisheries Science 37, 1–9. https://doi.org/10.1016/S0167-9309(07)80065-2

Machado, I., Moura, P., Pereira, F., Vasconcelos, P., Gaspar, M.B., 2019. Reproductive cycle of the commercially harvested sea urchin (*Paracentrotus lividus*) along the western coast of Portugal. Invertebrate Biology 138, 40–54. https://doi.org/10.1111/ivb.12238

Maes, P., Jangoux, M., 1984. The bald-sea-urchin disease: a biopathological approach. Helgolander Meeresunters 37, 2017-224. https://doi.org/10.1007/BF01989306

Mamelona, J., Pelletuer, E., 2010. Producing high antioxidant activity extracts from echinoderm by products using pressured liquid extraction. Biotechnology, 9: 523-528. https://doi.org/10.3923/biotech.2010.523.528

Mata, L., Schuenhoff, A., Santos, R., 2010. A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. Journal of Applied Phycology 22, 639–644. https://doi.org/10.1007/s10811-010-9504-z

McBride, S.C., Price, R.J., Tom, P.D., Lawrence, J.M., Lawrence, A.L., 2004. Comparison of gonad quality factors: Color, hardness and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. Aquaculture 233, 405–422. https://doi.org/10.1016/j.aquaculture.2003.10.014

Mendes, A., Araújo, J., Soares, F., Pousão, P., Cardoso, C., Bandarra, N., Afonso, C., 2020. Growth, survival and fatty acids profile of sea urchins, *Paracentrotus lividus* juveniles fed with *Ulva spp.* and maize in aquaculture production. First results using G1 generation in Portugal. Journal of Aquaculture & Marine Biology 9, 208–214. https://doi.org/10.15406/jamb.2020.09.00297

Mendes, A., Araújo, J., Soares, F., Pousão, P.F., 2019. Production of Purple Sea Urchin *Paracentrotus lividus* in Portugal. Wold Aquaculture Society 50, 46-50.

Micael, J., Alves, M.J., Costa, A.C., Jones, M.B., 2016. Exploitation and Conservation of Echinoderms. Oceanography and Marine Biology: An Annual Review 47, 191–208. https://doi.org/10.1201/9781420094220.ch4

Monfort, M. C, 2002. Fish Roe in Europe: Supply and Demand Conditions. FAO/GLOBEFISH Research Programme, Vol. 72. Rome, FAO. 47p

Montero, T. M., Garcia. M. P., 2003. Seasonal changes in the biochemical composition of the body components of the sea urchin, *Paracentrotus lividus,* in Lorbé (Galicia, north-western Spain). Journal of the Marine Biological Association of the United Kingdom, 83 (3), 575-581.

Mos, B., Dworjanyn, S.A., 2019. Ready to harvest? Spine colour predicts gonad index and gonad colour rating of a commercially important sea urchin. Aquaculture 505, 510–516. https://doi.org/10.1016/j.aquaculture.2019.03.010

Moylan, E., 1997. Gonad conditioning and wild stock enhancement of the purple sea urchin *Paracentrotus lividus* on the west coast of Ireland. Bulletin of the Aquaculture Association of Canada. 97:38-45.

Newton, A., Mudge, S.M., 2003. Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal. Estuarine Coastal and Shelf Science 57, 73–85. https://doi.org/10.1016/S0272-7714(02)00332-3

Olave, S., Lawrence, M., Carcamo, P., 2001. The Effect of Size and Diet on Gonad Production by the Chilean Sea Urchin *Loxechinus albus*. Journal of the World Aquaculture Society, 32: 210-214. https://doi.org/10.1111/j.1749-7345.2001.tb01097.x

Organización de las Naciones Unidas para la Agricultura y la Alimentación., Organización de las Naciones Unidas para la Agricultura y la Alimentación., 2011. Fishery and aquaculture statistics : 2009. FAO.

Pais, A., Serra, S., Meloni, G., Saba, S., Ceccherelli, G., 2012. Harvesting effects *on Paracentrotus lividus* population structure: A case study from Northwestern Sardinia, Italy, before and after the fishing season. Journal of Coastal Research 28, 570–575. https://doi.org/10.2112/JCOASTRES-D-10-00119.1

Pantazis, P.A., Kelly, M., Black, K., Connolly, J.G., 2000. Effect of artifitial diets on growth, lipid utilization, and gonad biochemistry in the adult sea urchin *Psammechinus miliaris*. Journal of Shellfish Research 19, 995–1001.

Pearce, C.M., Daggett, T.L., Robinson, S.M.C., 2002. Optimizing prepared feed ration for gonad production of the green sea urchin *Strongylocentrotus droebachiensis*. Journal of the World Aquaculture Society 33, 268–277. https://doi.org/10.1111/j.1749-7345.2002.tb00503.x

Phillips, K., Bremer, P., Silcock, P., Hamid, N., Delahunty, C., Barker, M., Kissick, J., 2009. Effect of gender, diet and storage time on the physical properties and sensory quality of sea urchin (*Evechinus chloroticus*) gonads. Aquaculture 288, 205–215. https://doi.org/10.1016/j.aquaculture.2008.11.026

Plank, L.R., Lawrence, J.M., Lawrence, A.L., Montoya Olvera, C., 2002. The Effect of Dietary Carotenoids on Gonad Production and Carotenoid Profiles in the Sea Urchin *Lytechinus variegatus*, Journal of the World Aquaculture Society.

Prado, P., Tomas, F., Pinna, S., Farina, S., Roca, G., Ceccherelli, G., Romero, J., Alcoverro, T., 2012. Habitat and scale shape the demographic fate of the keystone sea *urchin Paracentrotus lividus* in mediterranean macrophyte communities. PLoS One 7, 1–10. https://doi.org/10.1371/journal.pone.0035170

Prato, E., Fanelli, G., Angioni, A., Biandolino, F., Parlapiano, I., Papa, L., Denti, G., Secci, M., Chiantore, M., Kelly, M.S., Ferranti, M.P., Addis, P., 2018. Influence of a prepared diet and a macroalga (*Ulva sp*.) on the growth, nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus*. Aquaculture 493, 240–250. https://doi.org/10.1016/j.aquaculture.2018.05.010

Régis. M. B, 1978. “Croissance De Deux Echinoides Du Golfe De Marseille (*Paracentrotus Lividus* (Lamark, 1816) et *Arbacia Lixula* L.). Aspects Ecologiques De La Microstructure Du Squelette Et De L'evolution Des Indices Physiologiques.” dissertation

Rocha, F., Baião, L.F., Moutinho, S., Reis, B., Oliveira, A., Arenas, F., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal. Scientific Reports 9. https://doi.org/10.1038/s41598-019-39912-w

Sala, E., Ribes, M., Hereu, B., Zabala, M., Alvà, V., Coma, R., Garrabou, J., 1998. Temporal variability in abundance of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* in the northwestern Mediterranean: Comparison between a marine reserve and an unprotected area. Marine Ecology Progress Series 168, 135–145. https://doi.org/10.3354/meps168135

Salles, P., Voulgaris, G., Aubrey, D.G., 2005. Contribution of nonlinear mechanisms in the persistence of multiple tidal inlet systems. Estuarine Coastal and Shelf Science 65, 475–491. https://doi.org/10.1016/j.ecss.2005.06.018

Santos, P.M., Albano, P., Raposo, A., Ferreira, S.M.F., Costa, J.L., Pombo, A., 2020. The effect of temperature on somatic and gonadal development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture 528. https://doi.org/10.1016/j.aquaculture.2020.735487

Saraiva, S., Pina, P., Martins, F., Santos, M., Braunschweig, F., Neves, R., 2007. Modelling the influence of nutrient loads on Portuguese estuaries. Hydrobiologia 587, 5–18. https://doi.org/10.1007/s10750-007-0675-9

Sartori, D., Pellegrin, D., Macchia, S., Gaion, A., 2016. Can echinoculture be a feasible and effective activity? Analysis of fast reliable breeding conditions to promote gonadal growth and sexual maturation in *Paracentrotus lividus*. Aquaculture 451, 39–46. https://doi.org/10.1016/j.aquaculture.2015.08.037

Schiener, P., Black, K.D., Stanley, M.S., Green, D.H., 2015. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 27, 363–373. https://doi.org/10.1007/s10811-014-0327-1

Shpigel, M., McBride, S.C., Marciano, S., Lupatsch, I., 2004. The effect of photoperiod and temperature on the reproduction of European sea urchin *Paracentrotus lividus*. Aquaculture 232, 343–355. https://doi.org/10.1016/S0044-8486(03)00539-8

Shpigel, M., McBride, S.C., Marciano, S., Ron, S., Ben-Amotz, A., 2005. Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*. Aquaculture 245, 101–109. https://doi.org/10.1016/j.aquaculture.2004.11.043

Shpigel, M., Schlosser, S.C., Ben-Amotz, A., Lawrence, A.L., Lawrence, J.M., 2006. Effects of dietary carotenoid on the gut and the gonad of the sea urchin *Paracentrotus lividus*. Aquaculture 261, 1269–1280. https://doi.org/10.1016/j.aquaculture.2006.08.029

Shpigel, M., Shauli, L., Odintsov, V., Ashkenazi, N., Ben-Ezra, D., 2018. *Ulva lactuca* biofilter from a land-based integrated multi trophic aquaculture (IMTA) system as a sole food source for the tropical sea urchin *Tripneustes gratilla elatensis*. Aquaculture 496, 221–231. https://doi.org/10.1016/J.AQUACULTURE.2018.06.038

Siikavuopio, S.I., Christiansen, J.S., Dale, T., 2006. Effects of temperature and season on gonad growth and feed intake in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 255, 389–394. https://doi.org/10.1016/j.aquaculture.2005.12.021

Siikavuopio, S.I., James, P., Lysne, H., Sæther, B.S., Samuelsen, T.A., Mortensen, A., 2012. Effects of size and temperature on growth and feed conversion of juvenile green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 354–355, 27–30. https://doi.org/10.1016/J.AQUACULTURE.2012.04.036

Siikavuopio, S.I., Mortensen, A., Christiansen, J.S., 2008. Effects of body weight and temperature on feed intake, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 281, 77–82. https://doi.org/10.1016/J.AQUACULTURE.2008.05.033

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I., Bahorun, T., 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 579, 200–213. https://doi.org/10.1016/j.mrfmmm.2005.03.023

Spirlet, C., Grisjean, P., Jangoux, M., 2001. Cultivation of *Paracentrotus lividus* on extruded feeds: digestive efficiency, somatic and gonadal growth. Aquaculture Nutrition 7, 91–99.

Spirlet, C., Grosjean, P., Jangoux, M., 2000. Optimization of gonad growth by manipulation of temperature and photoperiod in cultivated sea urchins, Paracentrotus *lividus* (Lamarck) (Echinodermata). Aquaculture 185, 85–99. https://doi.org/10.1016/S0044-8486(99)00340-3

Spirlet, C., Grosjean, P., Jangoux, M., 1998. Reproductive cycle of the echinoid *Paracentrotus lividus*: Analysis by means of the maturity index. Invertebrate Reproduction and Development 34, 69–81. https://doi.org/10.1080/07924259.1998.9652355

Stefánsson, G., Kristinsson, H., Ziemer, N., Hannon, C., James, P., 2017. Markets for sea urchins: A review of global supply and markets. Skýrsla Matís 45 pp. 10.13140/RG.2.2.12657.99683.

Sun, J., Chiang, F.-S., 2015. Use and Exploitation of Sea Urchins.10.1002/9781119005810.ch2.

Symonds, R.C., Kelly, M.S., Caris-Veyrat, C., Young, A.J., 2007. Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9′-cis-echinenone as the dominant carotenoid in gonad colour determination. Comparative Biochemistry Physiology -B Biochemical and Molecular Biology 148, 432–444. https://doi.org/10.1016/J.CBPB.2007.07.012

Symonds, R.C., Kelly, M.S., Suckling, C.C., Young, A.J., 2009. Carotenoids in the gonad and gut of the edible sea urchin *Psammechinus miliaris*. Aquaculture 288, 120–125. https://doi.org/10.1016/J.AQUACULTURE.2008.11.018

Tiara Dewi, Muhammad Amir Masruhim, R.S., 2016. Echinodermata, Laboratorium Penelitian dan Pengembangan Farmaka tropis Fakultas Farmasi Universitas Mualawarman, Samarinda, Kalimantan Timur. Springer, Berlin, Heidelbe. https://doi.org/https://doi.org/10.1007/3-540-27683-1

Tomas, F., Romero, J., Turon, X., 2004. Settlement and recruitment of the sea urchin *Paracentrotus lividus* in two contrasting habitats in the Mediterranean. Marine Ecolology Progress Series 282, 173–184. https://doi.org/10.3354/meps282173

Turon, X., Giribet, G., López, S., Palacín, C., 1995. Growth and population of *Paracentrotus lividus* (Echinodermata: Echinodea) in two contrasting habitats. Marine Ecology Progress Series 122, 193–204.

Vadas, R.L., Beal, B., Dowling, T., Fegley, J.C., 2000. Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. Aquaculture 182, 115–135. https://doi.org/10.1016/S0044-8486(99)00254-9

Warren-Myers, F., Swearer, S.E., Francis, D.S., Turchini, G.M., Overton, K., Dempster, T., 2022. Algal supplements in formulated feeds: Effects on sea urchin gonad quality. Aquaculture 548. https://doi.org/10.1016/j.aquaculture.2021.737673

Watts, S.A., Hofer, S.C., Desmond, R.A., Lawrence, A.L., Lawrence, J.M., 2011. The effect of temperature on feeding and growth characteristics of the sea urchin *Lytechinus variegatus* fed a formulated feed. Journal of Experimental Marine Biology and Ecology 397, 188–195. https://doi.org/10.1016/J.JEMBE.2010.10.007

Woods, C.M.C., James, P.J., Moss, G.A., Wright, J., Siikavuopio, S., 2008. A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus*

Valenciennes. Aquaculture International 16, 49–68. https://doi.org/10.1007/s10499-007-9124-z

Yeruham, E., Abelson, A., Rilov, G., ben Ezra, D., Shpigel, M., 2019. Energy budget of cultured *Paracentrotus lividus* under different temperatures. Aquaculture 501, 7–13. https://doi.org/10.1016/J.AQUACULTURE.2018.11.006

Yeruham, E., Rilov, G., Shpigel, M., Abelson, A., 2015. Collapse of the echinoid *Paracentrotus lividus* populations in the Eastern Mediterranean - Result of climate change?. Sci Rep 5. https://doi.org/10.1038/srep13479

7. Appendix

Table 7.1: Avergare number of sea urchin (*Paracentrotus lividus*) in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed), along the first experiment:

Average number of sea urchin per basket								
Treatment	06/10/2021	05/11/2021	21/12/2021	12/01/2022	16/02/2022	04/03/2022	18/03/2022	08/04/2022
A	50.0±0	49.75±0.43	46.75±0.43	43.0±0	35.25±5,97	26.25±11.34	32.67±2.6	28.67±2.62
B	50.0±0	49.0±1.73	42.75±3.42	41.0±2.45	33.50±4.50	23.5±4.3	23.50±4.33	23.25±4.76
C	50.0±0	48.67±0.94	42.67±2.36	42.67±0.94	40.67±2.49	40.67±2.5	37.33±1.89	34.33±1.89

Table 7.2: Individual mean weight of sea urchin (*Paracentrotus lividus*) of each basket, obtained from treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp.+ inert feed), and treatment C (onshore, *Ulva* spp.+ inert feed) (T1-T6: different samplings):

Mean weight per sea urchin (g)						
	T1	T2	T3	T4	T5	T6
Treatment A						
1	16.08 ± 4.75	22.44± 12.25	21.05±6.95	21.05±6.95	18.39±6.25	18.09±4.40
2	16.38± 4.46	18.70±4.52	20.26±4.78	20.60±3.24	18.64±4.02	19.21±3.23
3	15.09± 3.78	15.40±4.83	17.75±4.65	18.02±4.63	-	-
4	16.74±5.07	16.26±4.73	18.64±5.82	17.67±6.06	16.39±4.99	17.33±4.94
Treatment B						
5	16.74±4.88	20.65±6.78	19.44±5.20	24.00±6.79	19.13±4.24	19.35±4.37
6	18.67±5.88	18.45±4.00	20.43±4.55	20.49±5.45	19.64±4.55	19.44±4.32
7	17.95±7.44	16.14±5.90	18.97±6.83	20.35±7.23	17.96±5.71	17.07±4.66
8	17.51±4.55	17.85±5.23	19.32±4.95	18.72±4.32	16.64±3.20	16.11±3.03
Treatment C						
C1	15.37±3.81	16.31±3.96	17.87±4.19	19.45±4.61	20.45±5.52	19.81±2.63
C2	16.01±5.16	17.07±2.98	19.74±4.45	18.78±4.05	19.46±4.06	19.86±4.64
C3	18.09±5.03	14.34±4.04	18.24±4.73	19.79±6.07	21.39±6.07	21.12±4.95

Table 7.3: Average test diameter per sea urchin (*Paracentrotus lividus*), of each basket, in treatment A (offshore, *Ulva* spp.), treatment B (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed):

Mean test diameter per sea urchin (cm)						
	T1	T2	T3	T4	T5	T6
Treatment A						
1	3.42 ± 0.61	4.04± 0.43	3.32±0.86	4.02±1.39	2.48±0.83	3.50±0.62
2	3.02± 0.56	4.21±0.45	3.54±0.57	3.99±1.27	2.42±0.81	3.57±0.76
3	3.08±0.40	4.05±0.41	3.51±0.81	3.73±1.49	-	-
4	2.99±0.47	3.74±0.44	3.46±1.01	3.67±1.55	2.64±0.65	3.30±0.60
Treatment B						
5	2.95±0.32	3.98±0.78	3.57±0.82	3.75±1.54	2.24±0.61	3.48±0.65
6	3.08±0.41	4.16±0.62	3.65±0.73	3.93±1.28	2.18±0.73	3.53±0.53
7	2.70±0.93	4.16±0.56	3.38±0.78	4.06±1.53	2.58±0.65	3.38±0.71
8	1.55±0.22	3.68±0.38	3.63±0.99	3.70±1.69	2.75±0.72	3.38±0.47
Treatment C						
c1	3.65±0.46	4.02±0.47	3.51±0.82	4.94±1.09	3.55±0.53	3.52±0.63
c2	3.34±0.47	3.13±0.45	3.86±0.89	5.19±0.70	3.43±0.56	3.44±0.57
c3	4.47±1.71	3.65±0.28	3.85±0.96	5.42±0.93	3.35±0.44	3.61±0.44

Table 7.4: Average number of sea urchin (*Paracentrotus lividus*) in treatment D (offshore, *Ulva* spp. + inert feed) and treatment C (onshore, *Ulva* spp. + inert feed), along the second experiment:

Average number of sea urchin per basket			
Treatment	29/04/2022	28/06/2022	10/08/2022
D	56.25± 10.83	56.25± 10.83	54.31 ± 11.65
E	56.25± 10.83	56.25± 10.83	54.38 ± 9.34

Table 7.5: Mean weight per sea urchin (*Paracentrotus lividus*) of each cage, in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore, *Ulva* spp. + inert feed):

Mean weight per sea urchin (g)			
	T0	T1	T2
Treatment D			
1	15.05 ± 4.3	16.11 ± 4.0	18.89 ± 4.6
2	19.31± 5.9	19.48 ± 5.8	20.38 ± 4.5
3	16.96 ± 5.0	18.37 ± 3.6	18.82 ± 3.8
4	6.95 ± 2.2	7.59 ± 2.03	9.50 ± 2.34
Treatment E			
C1	15.44 ± 6.0	17.57 ± 4.2	19.38 ± 6.8
C2	19.77 ± 5.9	20.56 ± 4.5	20.67 ± 4.1
C3	18.98 ± 5.1	20.01 ± 4.7	19.71 ± 4.7
C4	6.78 ± 3.0	6.97 ± 1.6	7.07 ± 2.0

Table 7.6: Mean test diameter per sea urchin (*Paracentrotus lividus*) of each basket, in treatment D (offshore, *Ulva* spp. + inert feed) and treatment E (onshore, *Ulva* spp. + inert feed):

Average test diameter per sea urchin (cm)			
	T0	T1	T2
Treatment D			
1	3.81 ± 0.6	3.45 ± 0.4	3.84 ± 0.5
2	3.71 ± 0.5	3.32 ± 0.4	3.76 ± 0.4
3	3.68 ± 0.6	3.88 ± 0.3	3.64 ± 0.4
4	2.45 ± 0.5	2.89 ± 0.4	3.15 ± 0.4
Treatment E			
C1	3.56 ± 0.7	3.78 ± 0.4	3.64 ± 0.5
C2	3.66 ± 0.6	3.69 ± 0.4	3.96 ± 0.4
C3	4.15 ± 0.6	3.88 ± 0.4	3.91 ± 0.5
C4	2.28 ± 0.4	2.84 ± 0.4	2.64 ± 0.3