POLITÉCNICO DE LEIRIA
ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Growth analysis in the sea urchin *Paracentrotus lividus* (Lamarck, 1816): impact of feed and seawater sources in juvenile growth rate and morphometric relationships

David Chilra Abraços Teixeira

2022

Growth analysis in the sea urchin *Paracentrotus lividus* (Lamarck, 1816): impact of feed and seawater sources in juvenile growth rate and morphometric relationships

David Chilra Abraços Teixeira

Dissertation for obtaining the master’s degree in Aquaculture

Masters project carried out under the supervision of Doctor Sílvia Lourenço and Doctor Ana Pombo

2022

Title*:* Growth analysis in the sea urchin *Paracentrotus lividus* (Lamarck, 1816): impact of feed and seawater sources in juvenile growth rate and morphometric relationships

Acknowledgements

I would first like to sincerely thank my thesis advisors Doctor Ana Margarida Paulino Violante Pombo and researcher Sílvia Alexandra Pereira Lourenço from CETEMARES - IPLeiria. Despite some obstacles along the way they always supported me, answered my questions, and guided me throughout this journey. This help was especially evident the statistical analysis where they guided and helped through the whole process.

A special mention to my master´s colleague Catarina Lapa who did her study next to mine and supported and shared ideas, information, and for the constant encouragement and good mood.

Finally, i would like to thank my family for providing this opportunity and working towards providing me everything I needed and Maria for pushing me in the last stretch of this paper and helping ensure its timely conclusion.

Resumo

O ouriço-do-mar comum europeu *Paracentrotus lividus* (Lamarck, 1816) é a espécie de equinoderme mais pescada e consumida na Europa. Devido à sua sobre-exploração, as capturas anuais diminuíram na Europa em países como França e Itália levando a uma redução das populações desta espécie em áreas onde antes eram abundantes. Este declínio de capturas levou a um interesse acrescido por parte de vários países no desenvolvimento da produção de juvenis de ouriços-do-mar e melhoramento da qualidade das sua gónadas, como ferramenta para repovoamento de populações naturais e para suprir as necessidades do mercado global.

Este estudo teve como objetivo principal avaliar o efeito de diferentes dietas de macroalgas e de águas salgada de diferentes origens (com diferentes características químicas no crescimento e condição de juvenis de *P. lividus*. Adicionalmente, estudou-se o efeito das condições de cativeiro (alimentação com dietas secas) nas relações morfométricas existentes entre o peso individual e o diâmetro de ouriços-do-mar juvenis e adultos.

Neste estudo foi testada a influência de três dietas de macroalgas compostas por *Ulva rigida* ,*Porphyra dioica* e uma mistura de ambas as algas no crescimento de juvenis de *P. lividus* mantidos num sistema RAS num ensaio de crescimento de quatro meses. Após este ensaio, foi avaliado o efeito da qualidade de água salgada na condição dos juvenis numa experiência de 30 dias.

O estudo das relações morfométricas entre o peso individual e o diâmetro de ouriços-do-mar juvenis e adultos foi realizado usando dados provenientes de ensaios nutricionais realizados anteriormente e comparados com conjuntos de dados semelhantes recolhidos de uma população selvagem.

Os resultados mostram um aumento não significativo no TD (diâmetro total) médio e um decréscimo no BW (peso corporal) médio durante os 4 meses do estudo, aumentando 0.11 ± 0,24 mm e diminuindo de 15,07 ± 1,61 mg, durante o estudo o TD dos ouriços apresentaram diferenças entre si em quando comparadas as amostras das dietas, mais especificamente na terceira e quarta amostragem, no fim deste estudo todos os ouriços demonstraram uma diminuição na sua condição com a maioria dos espinhos ausentes.

No estudo sobre o efeito da qualidade da água salgada na condição dos ouriços-do-mar, foram testadas quatro fontes de água salgada: F - água salgada captada de furo, FC - água salgada captada de furo enriquecida com bicarbonato de sódio, SA - água salgada artificial e D - água salgada do porto local. Os resultados mostraram que os ouriços-do-mar mantidos na

água do furo apresentaram uma menor taxa de sobrevivência, enquanto as águas do porto local e água salgada artificial apresentaram taxa de sobrevivência em todos os grupos e a água do furo apresentou a maior taxa de sobrevivência, todas as águas garantiram recuperação dos espinhos.

A análise das relações morfométricas mostrou que tanto os juvenis como os adultos mantidos em cativeiro e de populações selvagens apresentaram um crescimento alométrico negativo. Ou seja, estes aumentam mais rapidamente em diâmetro que em peso, mas diferenças estatisticamente significativas no parâmetro de alometria (*b*) mostraram que os ouriços-do-mar adultos alimentados com dietas artificiais pesam relativamente mais que animais do mesmo diâmetro das populações selvagens.

Com este estudo foi possível determinar que o crescimento e desenvolvimento dos juvenis de *P. lividus* depende não só da abundância de comida mas também da composição da água, e com a análise das relações morfométricas foi possível determinar que os juvenis e adultos de *P.lividus* criados aquacultura crescem e engordam a um ritmo superior aos selvagens.

Abstract

The European purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) is the most harvested and consumed species in Europe. Due to *P. lividus* overexploitation, the annual catches have been decreasing in Europe, in countries such as France and Italy and its wild populations have been decreasing in areas where they used to be abundant. The decline of sea urchins harvesting in the last decade led to the interest into developing sea urchin aquaculture to produce hatchery-juveniles and to enhancement gonad quality in several countries aiming to provide seed for restocking programs and to meet international market demand for sea urchin roe.

The main objective of this study was to observe and analyze the influence off different macroalgae diets and seawater sources in the growth and condition of post-settled P*. lividus* sea urchins. Additionally, the sea urchin diameter – weight relationship was determined to sub-adult, and adult sea urchins to determine if captivity feeding conditions modify the type of growth observed for sea urchins in relation to wild conditions.

In this study, the influence of three macroalgal diets composed of Ulva *rigida*, *Porphyra dioica* and a mixture of both algae on the growth and development of *P.lividus* juveniles was tested during 4 months in a RAS system, after the 4 months the remaining sea urchins were transferred to a climate chamber for a month to test the influence of different water sources on sea urchin condition.

Besides the growth study, an analysis of the morphometric relationship between the body weight (BW) and the test diameter (TD) of juvenile and adult sea urchins from a feeding trial performed previously were compared with a similar set of data from a wild population. This analysis allowed to ascertain if the usage of artificial diets and captivity conditions affect the allometry of the growth of sea urchins.

The results show a minimal increase in overall average TD and a decrease in average BW during the 4 month experiment increasing from 0.11 ± 0,24 mm and decreasing from 15,07 ± 1,61 mg. During the growth experiment, the TD of the sea urchins showed differences among themselves when comparing the samplings of each diet, with all diets showing a decrease in sea urchin condition with most spines having fallen off.

In the Water Source study, 4 water sources were tested: F - Borehole seawater, FC - Enriched borehole seawater with Sodium bicarbonate, SA - Artificial seawater, and D - Water from the local port. With the sea urchins maintained in the borehole seawater showing the lowest survival rate and the seawater from the local port and artificial seawater showed survival rates

in all groups and the enriched borehole seawater having the highest survival rate, all waters ensured the recovery of the spines.

The analysis of morphometric relationships showed that both juveniles and adults kept in captivity and from wild populations showed negative allometric growth. That is, they increase more rapidly in diameter than in weight, but statistically significant differences in the allometry parameter (b) show that adult sea urchins fed artificial diets weigh relatively more than animals of the same diameter in wild populations.

With this study it was possible to determine that the growth and development of *P.lividus* juveniles depends not only on the abundance of food but also on the composition of the water, and with the analysis of the morphometric relationships it was possible to determine that the juveniles and adults of *P.lividus* bred in captivity grow and gain weight at a faster rate than wild animals.

Table of contents

List of figures

List of tables

Introduction

1.1 Fisheries and aquaculture

Many species of sea urchins are explored in several countries due to its roe or *uni*, considered a delicacy in many gastronomies (Carboni et al., 2013). The roe owes the high commercial value to its attractive organoleptic and nutritional characteristics like their reddish orange color, a unique sweet tropical flavor (Phillips et al., 2010; Baião et al., 2020), excellent source of carotenoids and proteins, low in fat, high in carbohydrate content and a high content of polyunsaturated fatty acids (PUFAs) mainly Eicosatetraenoic (EPA) and Arachidonic (ARA) acids (Siliani et al., 2016). These factors have contributed to the increasing demand for sea urchin roe and a steady rise in price, leading to a worldwide intensification of sea urchin fisheries (Conand and Sloan 1989, Le Gall 1990). In fact, the demand for roe has significantly increased since the early 1970s, reaching its peak in 1995 with a landing of 113 654 t, three times higher than what was captured in 1970, declining gradually to approximately 100 000 t per year in recent years (Williams, 2002; FAO, 2011). The most important market is the Japanese, representing 90% of the world trade followed by the French and Korean markets (Lawrence, 2013; Prato et al., 2018b).

The European purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) is the most harvested and consumed species in Europe (Stefánsson et al., 2017), mainly in France Ireland, Italy, Spain, and Portugal and across the Mediterranean for example in Egypt (Sloan, 1985; Stefánsson et al., 2017). These fisheries used to yield a yearly 1000 t of *P. lividus* but due to their overexploitation the annual catches decreased to around 250 – 300 t per year, leading to the decrease of *P. lividus* populations in areas where they used to be abundant (FAO, 2016; Sartori et al., 2016).

In Italy, the wild stocks of *P. lividus* are particularly overexploited due to the uncontrolled collection of sea urchins in the shallow subtidal rocky reef (Sartori et al, 2016), despite the national best efforts to regulate sea urchin fisheries (Pais et al., 2012).

In Portugal, the harvesting of *P. lividus* is conducted all over the coast mainly between December and April. In the northwestern coast, *P. lividus* is harvested intensively and in the southwestern coast its harvested over a shorter period, mainly to export to Spain and France. Specific legislations and fishing limitations are imposed aiming to maintain the local *P. lividus* populations, even though these are often biased and are not able to consider unregistered catches and sales (Machado et al., 2018)

In addition to overexploitation, other environmental stressors have been causing the depletion of sea urchin populations. These stressors include ocean warming and increasing pollution by cumulative effects from inorganic and organic pollutants, nutrient runoff and hypoxia, ocean-based pollution from shipping traffic, deposition of heavy metals and inorganic nitrogen (Coll, et al., 2012). This scenario is further exacerbated by the slow growth rates of sea urchins (Sartori et al., 2016). Consequently, sea urchin populations, particularly *P. lividus*, have not been able to properly recover their stocks. To enable the stocks to recover we need to adopt management strategies. These will not only remove pressure from the natural stocks but also create a more regular market supply with a higher quality control and providing seed (post-settled juveniles) to enhance depleted stocks. These strategies involve the development of aquaculture projects able to produce seed for species of echinoderms with economic interest like *P. lividus* and to implement a feasible full-cycle production of sea urchin roe to fill the market demand (Sun and Chiang, 2015; Sartori et al., 2016; Zupo et al., 2019)

The decline of sea urchins harvesting led to the interest into developing sea urchin gonad enhancement in several countries in this last decade (Carboni et al, 2012). Also led to the adaption other kinds of culture to sea urchins, for example long line culture. This method was adapted from methods of abalone or scallop rearing, where special cage nets are used that can hold up to 20000 juveniles at 10 mm for posterior growth (Brown and Eddy, 2015). Even though this industry has only seen an increase in commercial investment and development in recent years. Currently, echinoculture targets more than 20 different species, with the main selection factors being regional factors and financial support available for the research in this area (Andrew et al., 2002; McBride, 2005; Lourenço et al., 2019). In the 1980s to 1990s the most researched species were *Pseudocentrotus depressus* and *Strongylocentrotus intermedius* common in the Japanese coast (McBride, 2005). More recently the focus of this research went to species found in the Atlantic and Pacific coasts, namely *P. lividus*, the green sea urchin *Strongylocentrotus droebachiensis* and *Lytechinus variegatus* (Lourenco et al., 2019).

Sea urchin aquaculture can also be used to help sustain and recuperate the overexploited natural stocks by helping better understand how several diets influence *P. lividus* gonads characteristics such as color, quantity, and quality, and how it affects reproduction and larval development (Spirlet et al., 2000; Shpigel et al., 2006; Carboni et al.,2012; 2013; Vizzini et al., 2015; Brundu et al., 2016; Castilla-Gavillán et al., 2018a; Zuppo et al.,2018). This can be achieved in two different ways. The most used method is the short-term aquaculture, where adults are captured from fisheries and grown in captivity in intense feeding regimes guaranteeing high gonad yield and fair quality (McBride 2005). Despite using natural stocks this method removes the pressure on bigger sea urchins, allowing them to breed and increase the natural stock numbers, by introducing produced sea urchins with a desirable size in the global market.

The second method is the long-term aquaculture, where adults are captured from natural stocks and used to breed larvae that will complete their life cycle in hatcheries to later be sold when they reach the desired characteristics in size and gonad quality (Unuma et al., 2015). Unfortunately, intensive sea urchin aquaculture still is not considered an economically viable option, due to the high larval mortality rates and the lackluster taste and appearance of the gonads from reared sea urchins when compared with the fisheries and therefore not attracting the main high-end consumers (Sun and Chiang, 2015).

Despite these setbacks this option can still help mitigate some of the fishing pressure on the natural stocks by using some strategies of habitat enhancement (Doherty, 1999). This focuses on expanding areas that are a suitable habitat for sea urchins, meaning that they are also a good local for algae colonization as food (Morikawa, 1999) and transplantation. This strategy is based on the relocation adult individuals from areas that have a lower gonad productivity to areas with the potential to obtain higher gonad sizes like, for example, kelp forests or macroalgal beds (Carboni, 2013).

1.2 *Paracentrotus lividus* ecology and feeding habits

The European purple sea urchin *P. lividus* inhabit the shallow coastal from Scotland to Southern Morocco, among other areas, such as the English Channel, the Macaronesia region and the Mediterranean Sea. In these areas the sea urchin endures their minimal and maximal temperature limits of 8 ºC in winter and 28 ºC during summer (Girard et al., 2012; Boudouresque and Verlaque, 2020). Their habitat includes intertidal and subtidal areas ranging up to 20 m below the tide limits, including solid rocks bottoms but also seagrass beds in their lower limits (Tortonese, 1965). In the areas more exposed to the waves and to the movements of the tides, the sea urchins burrow into the bedrock and fixate themselves to resist being swept away (McBride, 2005, 2012; Lawrence, 2013; Boudouresque and Verlaque, 2020). However, *P. lividus* can also be found in brackish coastal lagoons (Allain, 1975; San Martín, 1987). Their population densities range from 50 - 100 specimens per m^2 depending on the environment they inhabit (Boudouresque and Verlaque, 2007).

Despite being found in a large variety of environments with fluctuating temperatures and salinities, *P. lividus* is still quite sensible too both, either too high or too low, and fluctuations in salinity levels can cause mass mortality on the local populations (Fernandez et al., 2006). Apart from factors like temperature and salinity, several experimental studies showed that this species has a high sensitivity to ammonia (Basuyaux and Mathieu., 1999; Alessandra et al., 2003), although this factor is more relevant in an aquaculture setup (Lawrence, 2013; Boudouresque and Verlaque, 2020).

Based in both behavioral and gut content data, *P. lividus* is considered an herbivore species (Tomas et al., 2005a, b; Wangensteen et al., 2011). They normally feed on algae, seagrass, be it by grazing the seabed or by capturing fragments transported by the current. In case of food scarcity, the adults can become detritivores (Lawrence, 2013; Boudouresque and Verlaque, 2020). This species has preferences for particularly seaweeds, particularly *Rissoella verruculosa* (Rhodobionta, Archaeplastida), *Cymodocea nodosa* (seagrass: Magnoliophyta, Archaeplastida), *Cystoseira amentacea*, *Padina pavonica*, *Undaria pinnatifida* (photosynthetic Stramenopiles), *Porphyra* sp and *Ulva* sp (Lawrence, 2013; Boudouresque and Verlaque, 2020). However, these preferences are related to an array of factors, such as, physical properties, availability, and seaweed local composition (Hay and Steinberg, 1992; Wright et al., 2005).

1.3 Biology and life cycle

Paracentrotus lividus is a marine invertebrate belonging large phylum Echinodermata (Pawson, 2007), included in the class Echinoidea (Leske, 1778) and Order Camarodonta (Jackson, 1912), and the *Paracentrotus genus* belongs to the family Parechinidae (Mortensen,1903).

The *P. lividus* sea urchins can present a large diversity of colours including black, purple, to yellow, green and a few others (Tortonese, 1965). As other regular species, this has a radial form, with a soft portion of the body in the test, coated by the epithelium and the spines (Figure 1) (Guidetti and Mori, 2005), the tube feet and pedicellariae aid in the locomotion (Verling et al., 2002). The pedicellariae are small pincer shaped appendages these are scarcely scattered over the sea urchin’s body. These can all be regenerated in case of loss by predation, disease or due to poor water quality, acidification, and pollution (Emerson et al., 2017; Maes and Jangoux, 1984). The test is composed by ten double columns of plates, five interambulacral and five ambulacral zones linked together. The interambulacral plates host the spines, whereas one pair of podia emerge from each ambulacral plate (Carboni, 2013). In the oral region, the mouth or “Aristotle´s lantern” is composed by five mobile plates, interconnected by more than 40 ossicles and inside each plate there is a tooth (Tortonese, 1965). In the aboral region, there is a membrane named periproct which surrounds the anus. The anus is surrounded by five plates with gonopores, the holes from which sperms and eggs are released into water.

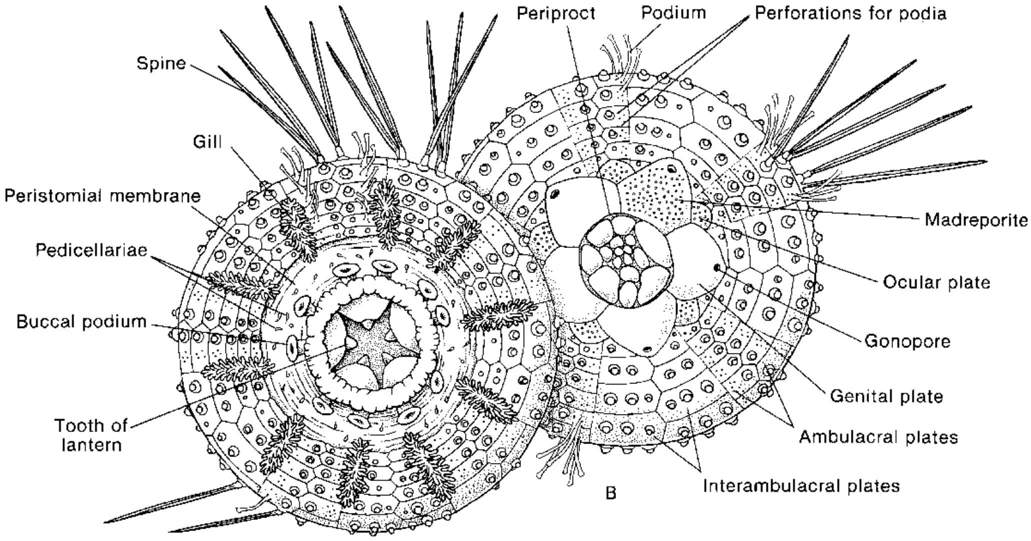

Figure.1. External anatomy of a regular sea urchin. A - oral view; B - aboral view (adapted from Grosjean, 2001).

The internal cavity, or coelom encloses the vascular, digestive, and reproductive systems (Figure 2). The vascular system is composed of canals connecting the numerous tube feet. The digestive system is a simple pipe or gut, attached to the inner wall of the body by means of a mesenteric membrane. The reproductive system is composed by five gonads, each one connected to gonopores in the aboral region (Pinna, 2014). This species is a gonochoric species (Byrne, 1990; Ouréns et al., 2011, 2013) with no external dimorphism between male and female. When mature, the gonads appear large, bright yellow, orange, or red colored and widespread from the aboral zone almost to the Aristotle's lantern (Pinna, 2014). Normally *P. lividus* reaches sexual maturity at 2 to 4 years old, or when they reach a size of - 2.0–2.8 cm (Ouréns et al., 2013).

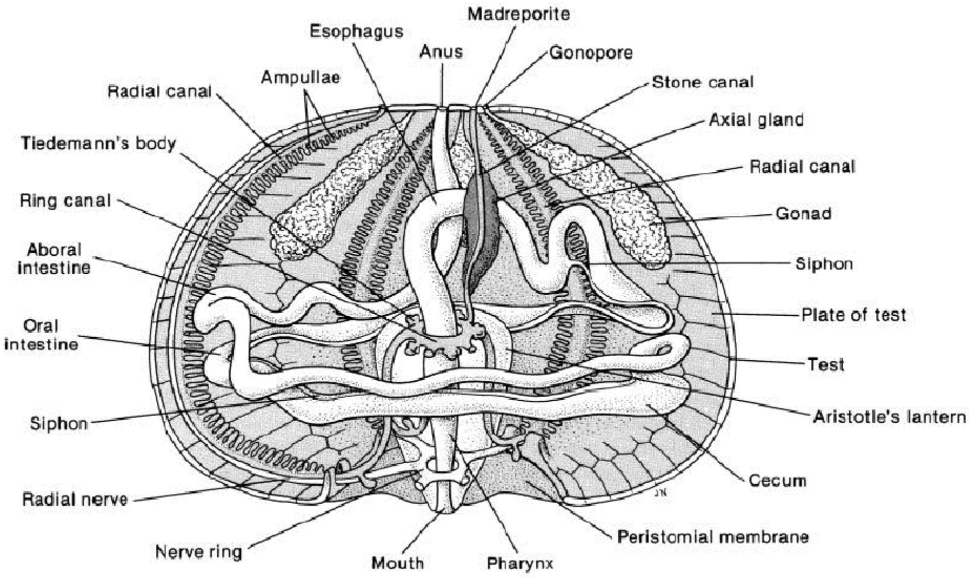

Figure.2. Side view of the Internal anatomy of a regular sea urchin (adapted from Grosjean, 2001).

In the northeast Atlantic coast, the reproductive cycle of the local *P. lividus* populations has two spawning peaks, usually between early spring and summer depending on local, population and conditions, (Pinna, 2014). In the Mediterranean Sea there is a shorter reproductive season in autumn (Pedrotti, 1993). These variations in reproductive activity can be explained by different environmental conditions that each population is subject to, such as seawater temperature and photoperiod (Spirlet et al., 1998; Siikavuopio et al., 2012). The reproductive period normally occurs when there is a steep increase in temperature followed by a sharp decrease (Byrne, 1990; Pedrotti, 1993; Sánchez-España et al., 2004; González-Irusta et al., 2010). However, the gametogenic cycle is annual meaning that each sea urchin only spawn once each year (Byrne, 1990; Spirlet et al., 1998; Martínez et al., 2003; Ouréns et al., 2011, 2013; Machado et al., 2019).

The gonadal growth of *Paracentrotus lividus* occurs in the winter months and shortest days, with water temperature possibly serving as exogenous cue for spawning in the following warmer months (Byrne, 1990). In fact, Walker et al. (1998, 2001) observed that gonads maturation occurs independently of water temperature, while release of gametes (spawning) would be initiated as a response to minimum temperature threshold. It was also observed a significant relationship between the interannual variation in the late winter phytoplankton bloom and the spring peak of larvae of this species, indicating that changes in larval abundance are coherent with changes in planktonic primary production (López et al., 1998)

Paracentrotus lividus has a complex life cycle including a larval planktonic stage followed by settlement to a benthic existence after a metamorphosis (Metaxas, 2013). The embryo develops larvae in a free-swimming planktotrophic larva that will develop several arms and a rudiment after a few weeks until it becomes competent. The larvae are considered competent when the rudiment is about the same length as the stomach. At this stage, if the larvae find an adequate surface to settle it starts the metamorphosis (Gosselin and Jangoux, 1996). This period of metamorphosis and settlement is called peri-metamorphic period and encompasses all the events involving the transformation of a pelagic organism into a benthic one (Gosselin and Jangoux,1998). The metamorphosis is composed by two major morphological and functional changes: the development of a functional digestive system and the regression of the primordial podia (Gosselin and Jangoux, 1998). The metamorphosis is influenced by several environmental signals/cues like microalgal and bacterial biofilms, specific chemicals, and proteins specific to the adults of the species, and turbulence (Gaylord et al., 2013; Mos et al., 2011). After settlement the post larvae resembles a miniaturized adult but has no mouth and no anus being endotrophic (Gosselin and Jangoux, 1998). After a week, the post larvae become an exotrophic juveniles with a fully developed and functional digestive tract (Gosselin and Jangoux, 1998).

The mouth becomes functional after opening of the oral orifice. The teeth are visible and mobile; at the same time the anal plate goes forth and resembles the anus. The regression of the primary podia is achieved by reducing their stem followed by the regression of the terminal disc and resorption of their respective skeletal structures (Carboni, 2013).

During this phase, the post-larvae suffer high mortality that can reach almost 99 % (Boudouresque and Verlaque, 2020), due to starvation, predation, and exposure to unfavorable conditions (Metaxas, 2013). Despite the laboratory research to develop successful rearing protocols, the intrinsic characteristics of the species has limited the production, mainly due to the unpredictability of the metamorphosis success and posterior fixation (Carboni, 2013; Zupo et al., 2018).

1.4 Larval development

From an aquaculture perspective the most critical stages for *P. lividus* aquaculture is the metamorphosis, settlement, and the survival of post-settlement juveniles (Grosjean et al., 1998; De La Uz et al., 2013). Despite the several studies conducted, there is still lack of stability in settlement and survival rates, with laboratory experiments reporting larval settlement and metamorphosis rates of 0 to 90% (Pearce and Scheibling, 1991; Gosselin and Jangoux, 1996; Grosjean et al., 1998; Rahim et al., 2004; Huggett et al., 2006) and mortality rates higher than 90% within the first weeks of *P. lividus* benthic life (Grosjean et al., 1998; Rahim et al., 2004;

Shimabukuro, 1991). In this sense, it is important to understand the effect of a diet to improve growth and survival of post-settlement juveniles (Rial et al., 2018). During settlement and post larvae growth, the most used farming technique involves producing a microbial film on a plastic support, that is used to induce settlement and growth of post settled larvae (Unuma et al., 2015). The microbial film must meet two requirements to be considered optimal, those requirements are offering the necessary nutritional value and enhance the metamorphosis success from planktonic to benthonic (Mos et al., 2011). Hopefully, by meeting these requirements, the biofilm should promote a high settlement rate of competent larvae, low mortality, and elevated growth of the post settlement juveniles (Grosjean et al., 1998; Azad et al., 2010; Mos et al., 2011).

To better understand how to improve *P. lividus* culture, Carboni et al. (2012) tested other live microalgae species to improve the survival and growth of *P. lividus* larvae, with normal growth being observed when the microalgae *Crisostera elongate, Pleurochrisys carterae* and *D. tertiolecta* were fed to the larvae, and the data gathered showed that a high dietary lipid content and n-3 long-chain polyunsaturated fatty acids, low DHA/EPA and high EPA/ARA showed the best results for fostering growth, development and survival of the larvae. After settling and becoming juveniles these started to ingest fragments of fresh brown algae, continuing to grow a few more millimeters which allowed to start feeding with a dry diet (Kelly 2002).

1.5 Growth and condition

The wild populations of *P. lividus* have a growth rate of around 1 cm per year meaning that individuals with 2 cm are usually 2 years old and individuals with 4 cm are around 4 to 5 years old (Sartori et al., 2015), decreasing after attaining maturity between two and four years old, reaching a maximum longevity of 11 years (Ouréns et al., 2013). The somatic growth of *P. lividus* are influenced by several factor, including water temperature, food availability and nutritional value and gonadal development (Fernandez, 1996). The highest records of growth rate for this species occur in the Mediterranean, especially in Spring and beginning of summer (Turon et al., 1995).

When echinoid growth surveys are performed at a population level, and the accession of sea urchin age in a direct manner is impossible, the growth rates are ascertained with the analysis of size frequencies. This methodology is based in the assumption that size distribution comprises a mixture of normal curves representing the different size/age cohorts (Ebert, 1973; Kenner, 1992; Guillou and Michel, 1993). On the other hand, direct analysis of growth can be supported by age studies based in the identification of growth rings in the ambulacral plates (Grosjean et al., 1995). However, both methods are still largely speculative.

To bypass this uncertainty, laboratory trials using animals reared under controlled conditions should allow a good monitorization of species growth through time (Grosjean et al., 1995).

Another growth-related analysis that can be conducted with a laboratory reared animal, is the evaluation of condition based on the morphometric relationships. The test diameter vs weight relationship in sea urchins is very useful to analyse both the condition of the sea urchins subject to different environmental conditions (wild populations vs captivity reared) as well as to determine if these present a negative or positive allometric growth (Nicolau et al., 2022).

1.6 Objectives

The main objective of this study was to observe and analyze the influence off different macroalgae diets and seawater sources in the growth and condition of post-settled *P. lividus* sea urchins. Additionally, the sea urchin diameter – weight relationship was determined to sub-adult, and adult sea urchins to determine if captivity feeding conditions modify the type of growth observed for sea urchins in relation to wild conditions.

2 Materials and Methods

2.1 Broodstock rearing and juveniles' production

Adult sea urchins *P. lividus* were collected in the intertidal zone of a rocky beach near Peniche (Porto Batel, 39° 19' N: 9° 21' W). The sea urchins were transported in isothermal box filled with aerated seawater from the collection site to the aquaculture laboratory at MARE-Politécnico de Leiria. In the laboratory, the sea urchins were induced to spawn by injection with a solution of potassium chloride 0.5 M. The fertilization was carried out in a beaker filled with sterilized seawater and a known concentration of eggs to which were added a small volume of sperm guaranteeing a spermatozoa: egg ratio of 500:1. Two hours after fertilization, the fertilized eggs were transferred to a plastic container filled with 17 liters of sterilized seawater and gentle aeration for 48 hours. When larvae attained the four-arm stage, they were transferred to 50 L cylindroconical tanks in closed system and maintain there for 30 days. During development, the larvae were fed with a mixture of microalgae *Dunaliella tertiolecta* and *Chaetoceros calcitrans*, which ration was adapted to larval development stage according to Gomes et al. (2021). When larvae attained competence (the larval rudiment attain a size equal to larval stomach), they were transferred to the rearing system consisted of three recirculating aquaculture systems (RAS) with three 60 L holding tanks, and a sump of 70 L. The walls of the glass tanks were covered with a *Ulvella viridis* filaments.

2.2 Microalgae biofilm production

Microalgae biofilm was produced by scaling up *Skeletonema costatum* 100 ml to 500 ml and 1000 mL culture, respectively. The cultures were maintained in sterilized seawater enriched with 10 ml per liter of nutrients solution Nutribloom Plus® (Necton, Portugal) enriched with 10 ml per liter of 40 % silicates solution. The *S. costatum* culture was maintained in batch lines for 30 days at 20 °C during the scale up, exposed to a continuous light and supplied with gentle aeration. The culture was transferred to the tanks containing the sea urchins' one week before the study started with the filtration turned off to promote the formation of microalgae biofilm in the tanks. This step was repeated weekly during the first month of growth experiment.

2.3 Growth experiment and diets

Juveniles (n = 90) (5/6 months after hatching) with test diameter (TD) between 1 and 4 mm were selected from the F1 generation bred as described previously, and randomly allocated to nine 20 L tanks with a density of 0.67 sea urchins. L^{-1} (n = 10 *per* tank) (Figure 3). The tanks were organized in a recirculatory aquatic system (RAS) including a 15 L sump tank equipped with mechanical and biological filtration, UV sterilization (EHEIM reflex UV 800, Germany), and an organic supplement of oyster shells to maintain pH and calcium carbonate levels in the system. The system seawater was maintained at an average temperature of 21 °C using two thermostats (Thermocontrol 50, EHEIM, Germany). Prior to the sea urchin transfer, the walls of the tanks were enriched with a biofilm of a diatom, *S. costatum* to facilitate the sea urchins' adaptation to the new environment.

For four months, the sea urchins were fed with three algal diets, a monospecific diet of *Ulva rigida* (diet Ulva), a monospecific diet of *Porphyra dioica* (diet Porph), and a mixed diet of equal proportion of both macroalgae (diet Mix, 1:1), in triplicate tanks. The proximate composition of macroalgae diets selected was calculated previously from different a different study and it is presented in table 1. The dried macroalgae were milled into small pieces and offered to the sea urchins each 48 hours in 10 mg portions per tank, this quantity was determined to ensure that when feeding the juveniles these would have access to the given diet independently of their position in the tanks. The feed was distributed through the whole tank to guarantee an even distribution in the water column. Before each feeding, the algae remaining from the previous feeding were removed to guarantee food and water quality.

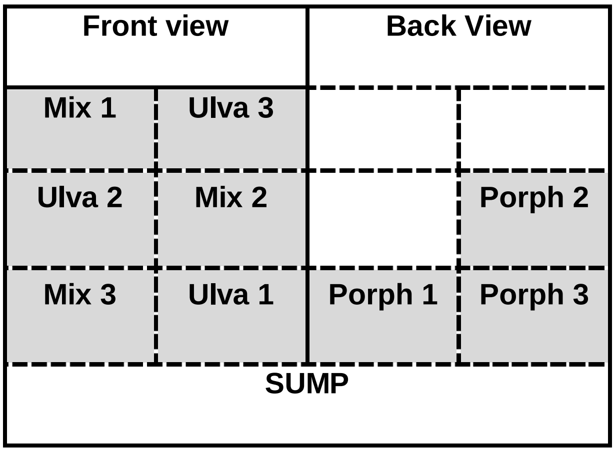

Figure 3. Experimental design of the layout of the tanks and monospecific diet of *Ulva rigida* (diet Ulva), monospecific diet of *Porphyra dioica* (diet Porph), and mixed diet of equal proportion of both macroalgae (diet Mix, 1:1) in triplicate.

Table 1 – Proximal composition of the three experimental algal diets used in the study of juvenile *Paracentrotus lividus* growth

	Protein (% DM)	Lipids (% DM)	Carbohydrates (% DM)	Dry matter (DM %)	Ashes (% DM)	Fibers (% DM)[1]
Ulva rigida	18.12 ± 0.1	1.93 ± 0.04	22.25 ± 0.67	91.85 ± 0.29	26.00 ± 0.29	31.70
Porphyra dioica	18.97 ± 0.39	2.27 ± 0.05	26.51 ± 0.28	95.60 ± 0.28	22.83 ± 0.62	29.42
Mix	19.08 ± 0.005	2.44 ± 0.05	26.15 ± 6.53	94.28 ± 0.48	24.53 ± 0.35	22.91

[1] % Fibres were indirectly determined as: Fibres (% DM) = 100 – (% Protein + % Lipids + % carbohydrates + % Ashes).

The physical parameters, temperature (T, ºC), salinity (S, ppm), pH and dissolved oxygen (DO %) were monitored daily with a handheld multiparameter probe (YSI Professional Plus, USA), the chemical parameters soluble ammonia (NH_3^+ mg/L) and nitrite (NO_2^- mg/L) were monitored every 48 hours by using commercial kits (Profi, Germany) and 30 % of system seawater was renewed every 48 h.

To determine the juvenile growth, information on their weight and test diameter was collected in monthly sampling events (T0 first sampling, T1 second sampling, T2 third sampling and T3 final sampling), with the first sampling event T0 starting a week after the sea urchins were transferred to the RAS system. In each sampling event, each sea urchin was photographed under stereo microscopes (Stemi 305 ZEISS, Germany) with a digital Axio camera MRc3 S/N 4524 with 5x magnification. Then, the TD was measured in the photograph by defining the longer line segment between test edges passing through the urchin mouth in the oral face of the urchin (Figure 4) using the image analysis system ZEN (version 2.3 ZEISS, Germany). After collecting the image for TD measurements, all the sea urchins were quickly weighed (TWW of the individual, ± 0.0001 g) with an analytical scale (Adam PGL 3002, Milton Keynes, UK) and put back in the tank.

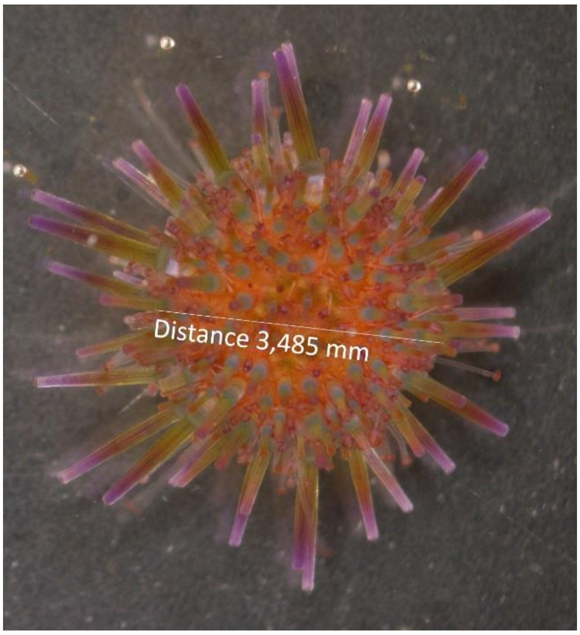

Figure 4. Digital measurement of the test diameter of a *Paracentrotus lividus* juvenile using image analysis system ZEN.

2.4 Impact of seawater sources in juvenile condition and survival

To access the impact of seawater type in juvenile condition and survival, it was conducted a thirty-day experiment where the sea urchins were reared with different seawater sources. For this experiment four seawater sources were selected: (F) Borehole seawater; (D) seawater from the local port; (FC) Borehole seawater enriched with 10 mg sodium bicarbonate; (SA) artificial seawater, with each presenting an average salinity of 35 S. The artificial seawater was made as a solution of deionized water to which was added 35 g of salt (Reef salt Aquaforest, Brzesko) per litter. For this experiment, five sea urchins were randomly collected from each dietary treatments and allocated in 1L plastic cups in triplicates. Each cup was filled with one of the seawater sources selected (Figure 5). All the cups were aerated and maintained at 21 °C in a climatic chamber (FITOCLIMA S600PLH, Aralab, Portugal) with a photoperiod of 8:16 h day: night. Water quality was maintained with daily renewals of 30% of the respective seawater type in each cup followed by the daily feeding of the Ulva diet. Survival, growth, and condition (presence or absence of spines) was assessed by measuring and weighing all the alive sea urchins the start and the end of the experiment (30 days after). Dead individuals were removed before each water exchange and counted to determine survival rate.

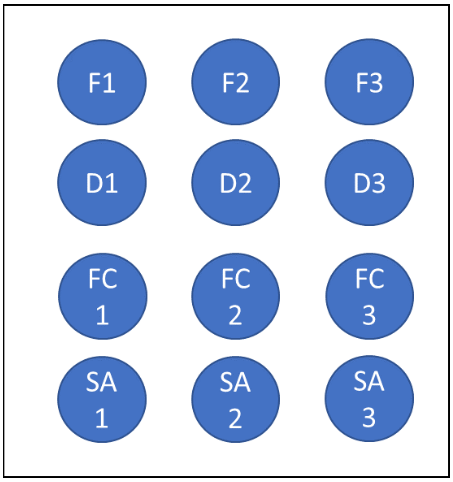

Figure 5. Experimental design of the experiment of seawater type impact in growth and condition of juvenile *Paracentrotus lividus*. Twelve plastic cups distributed by the four sources of seawater selected (F- Borehole seawater, D- seawater from the local port, FC- Enriched Borehole seawater, SA- Artificial seawater).

2.5 Morphometric relationships analysis

The morphometric relationships between TD (Total diameter) and BW (Body weight) were evaluated for juvenile and adult sea urchins reared in captivity (CJ and CA respectively) fed with 3 different dry diets formulated with animal, plant, and algae ingredients respectively in triplicate, and for sea urchins from wild populations (WJ and WA) as two different datasets. The paired data set of sea urchin TD and BW were collected opportunistically during the growth and nutritional trials conducted under the framework of Ouriceira AQUA project (16-02-01-FMP-0004) with different objectives. The data of both groups was sorted in two size groups identified as juvenile with a test diameter ranging between 14.5 and 25.5 mm and adult with a test diameter ranging between 37.5 and 40.5 mm.

Sea urchin TD and BW data were log transformed and a linear regression model was fit to the TD~BW data pairs. The obtained regression slope parameter (*b*) was used to evaluate growth isometry.

The growth can be either isometric (b = 3), meaning that the different body dimensions or structures, in this case diameter and weight, grow with an identical rate. If the selected body dimensions or structures grow at different rates, this is allometric. The growth rate is allometrically positive ($b > 3$) if, in this case, BW increases faster than TD. The growth rate is allometrically negative ($b < 3$) if TD increases faster than BW (Küçükdermenci and Lök, 2014).

2.6 Statistical analysis

The effect of diet in the juvenile's growth was evaluated by conducting one-way analysis of variance (ANOVA) to the juvenile TD and BW results by experimental diet (three levels: Mix, Ulva and Porph). The effect of the seawater type on the sea urchin survival and condition was conducted with a One-Way ANOVA, results by seawater sources selected (four levels: F, D, FC, and SA). The assumptions of variance homogeneity were evaluated by the Levene test. Whenever the variance of homogeneity was not verified, it was applied the Kruskal-Wallis non-parametric test to evaluate differences between experimental groups. In any case, when significant differences between experimental groups were identified, the HSD-Tukey multiple comparisons test was conducted to identify which groups were different.

Previously to determine the morphometric relationship between sea urchin test diameter and body weight, both data sets were separated in two size groups: sub-adults 14.5 to 25.5 mm; and adults 37.5 to 40.5 mm. Then, both parameters were log transformed to linearize the relationship between both parameters. For each data set, the assumptions of variance homogeneity were evaluated by applying the Levene tests of homogeneity. After verified this assumption, the parameters of *a* and *b* of the linear relationship between log (TD) and log (BW) were determined using the linear regression function where the data was sorted by juveniles and adults. Then, the similarity between linear regression parameters was assessed by analysis of co-variance (ANCOVA), using as co-variables the sea urchin life stage (sub-adult vs adult) and the sea urchins' origin (wild vs lab conditioned). For all statistical analysis, differences were considered statistically significant whenever p-value < 0.05. All analysis were performed using the IBM SPSS Statistics 28 statistical software.

3 Results

3.1 Juvenile growth experiment

3.1.1 Survival rate

By the end of the four months growth experiment, it was possible to observe that the group of sea urchins with the higher survival rate were those fed with the Mix diet (76%), followed by the group fed with Ulva (70%) and finally the group fed with Porph (60%) (Figure 6).

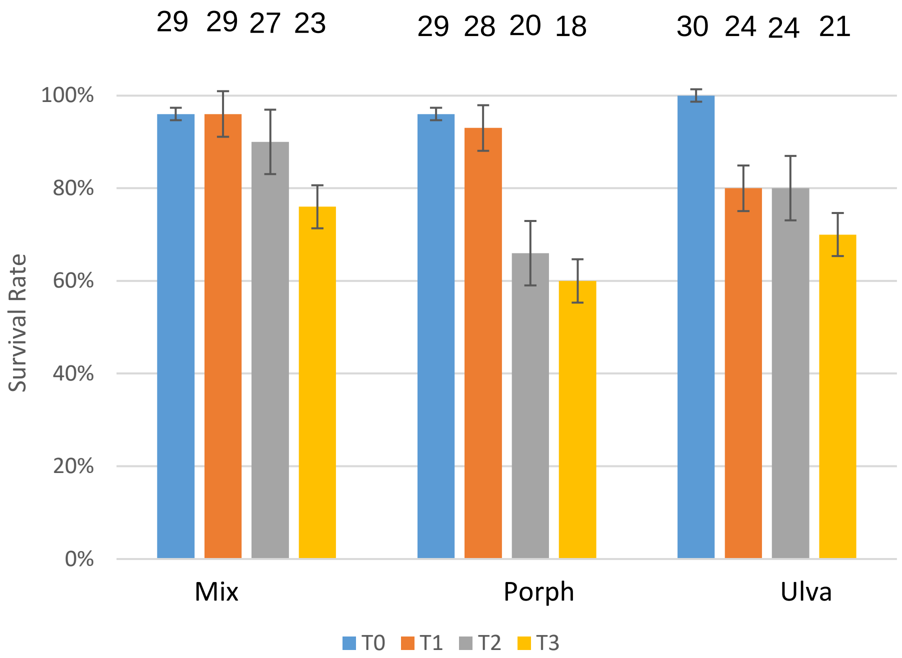

Figure 6 - Survival rate of *Paracentrotus lividus* sea urchins fed with the three experimental diets Mix diet (1:1 mixture of Porphyra and Ulva), *Porphyra dioica* (Porph) and *Ulva rigida* (Ulva) during the growth experiment. The numbers presented above each percentage bar indicates the number of alive sea urchins counted in each sampling event.

3.1.2 Test diameter and weight variation during growth trial

At the beginning of growth trial (T0), the average TD of the juveniles allocated to all diets was 3.54 mm, with differences between dietary groups (H_{KW} = 13.39. p-value < 0.05, n= 88). The average TD of the group fed with Mix diet was 4.02 mm, Mix presented a higher average TD than the group fed with Porph (average TD = 3.21 mm) and from the group fed with Ulva (average TD = 3.41 mm) (Figure 7).

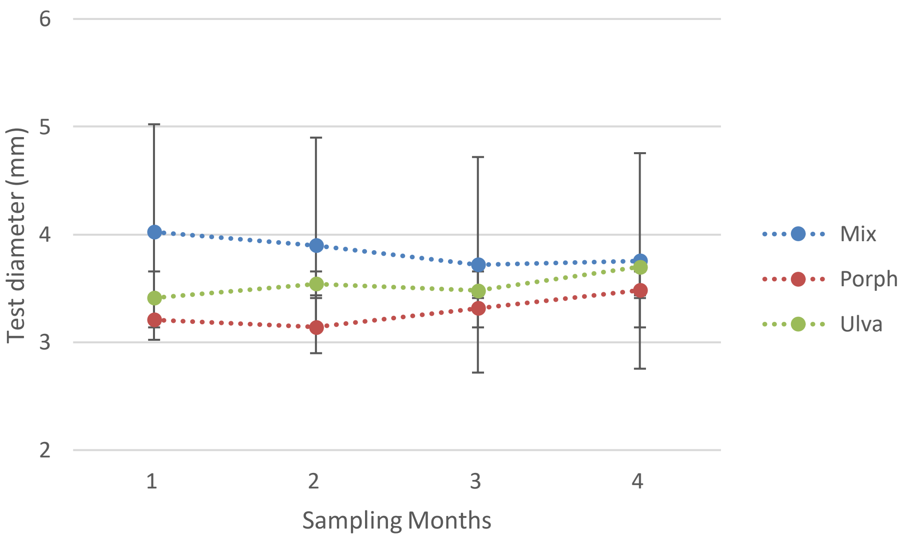

Figure 7 - Evolution of the mean diameter (± SD, mm) of *Paracentrotus lividus* juveniles fed with the different macroalgae diets during the growth experiment. Points and dotted line in blue represents the sea urchins fed with the mixed diet (Mix); points and dotted line in red represents the sea urchins fed with the *Porphyra dioica* (Porph); and points and dotted line in green represents the sea urchins fed with the *Ulva rigida* (Ulva).

The average TD of replicas by dietary treatment was analyzed at each sampling event and some differences were identified. In detail, for the juveniles fed with mix diet, differences in the average test diameter were identified at T2, with the juveniles in replica Mix1 being significantly lower than Mix3 (Table 2). For the juveniles fed with Porph, the sea urchins in the replica Porph1 were significantly smaller than from Porph2 in the sampling event T3 (Table 2). In the sea urchins fed with Ulva diet, the group of sea urchins in the replica Ulva2 was significantly lower than the sea urchins from Ulva3 in sampling event T2 (Table 2).

Table 2 - Statistical summary of the One-way ANOVA and Tukey HSD results on the average test diameter among replica of the *Paracentrotus lividus* juveniles fed the three experimental diets during the growth experiment (measurements collected in the sampling events T0, T1, T2, T3).

Sampling events	Homogeneity test	F	Df_{num}	Dfden	p-value	Tukey HSD
	Mix diet					
T0	0.22	2.98	2	21	0.72	
T1	0.48	3.02	2	12	0.87	
T2	0.77	8.65	2	24	0.001	M1 ≠ M3 (p-value = 0.001)
T3	0.85	4.02	2	18	0.36	
	Porph diet					
T0	0.19	4.06	2	22	0.32	
T1	0.17	1.93	2	24	0.17	
T2	0.81	2.57	2	16	0.11	
T3	0.76	0.16	2	15	0.02	P1 ≠ P2 (p-value =0.019)
	Ulva diet					
T0	0.44	1.01	2	27	0.38	
T1	0.002	0.20				
T2	0.14	4.65	2	17	0.025	U2 ≠ U3 (p-value= 0.019)
T3	0.92	2.92	2	17	0.81	

*Nonparametric Kruskal-wallis test output.

In the end of the growth trial, the juveniles fed with Mixed diet presented an average diameter of 3.74 mm, the juveniles fed with Porph presented an average diameter of 3.48 mm and the juveniles fed with Ulva presented an average diameter of 3.70 mm, with no differences between dietary groups ($F = 0.778$ $df_{num} = 2$, $df_{den} = 56$, p-value = 0.47, n= 65) (Figure 7).

At the end of the growth trial (T3), the average TD of the juveniles allocated to all diets was 3.66 mm. Across the growth experiment, there was no significant variation in the juveniles average TD between sampling events (p-value>0.05), therefore the diameter did not present statistical differences during the experiment (Table 3).

Table 3 - Statistical summary of One-Way ANOVA comparing average test diameters among sampling events across the growth experiment of the *Paracentrotus lividus* juveniles fed with the three experimental diets.

Diets	Homogeneity test	ANOVA output			
		F	Df_{num}	Dfden	p-value
Mix	0.39	0.53	3	90	0.66
Porph	0.74	1.05	3	85	0.37
Ulva	0.43	0.49	3	88	0.69

During the experiment the TD of the juveniles did not show statistical differences when comparing each diet in the full duration of the experiment, presenting an average TD of 3.66 mm. The sea urchins fed with Mix diet presented an initial average diameter of 4.02 ± 0.60 mm and a final average diameter of 3.74 ± 0.53 mm, corresponding to a decrease of 0.28 mm during the growth experiment. The juveniles fed with Porph presented an initial average diameter of 3.21 ± 0.83 mm and a final average diameter of 3.48 ± 0.73 mm (Figure 7, corresponding to an increase of 0.27 mm in four months. The juveniles fed with the Ulva presented an initial average diameter of 3.41 ± 1.08 and a final average diameter of 3.70 ± 0.77 (Figure 6), corresponding to an increase of 0.29 mm in four months. However, this increase in test diameter was not statistically significant.

At the beginning of growth trial (T0), the average BW of the juveniles allocated to all diets was 54.46 ± 0.03 mg. The group of sea urchins to be fed with the Mix diet presented an initial average BW of 64.57 ± 24.75 mg. The group to be fed with Porph presented an average initial BW of 47.18 ± 35.29 mg, and the group to be fed with Ulva presented an initial average body weight of 51.71 ± 36.94 mg. The initial average BW was identical between dietary groups ($F = 3.18$. $df_{num} = 2$, $df_{den} = 76$, *p*-value = 0.06).

In the end of the growth trial, the juveniles presented an average BW of 39.39 ± 1.64 mg independently of dietary group. In detail, the juveniles fed with Mixed diet presented an average weight of 41.82 ± 18.44 mg. The juveniles fed with Porph presented an average weight of 34.38 ± 0.73 mg and the juveniles fed with Ulva presented an average weight of 41.05 ± 20.18 mg, being identical between experimental groups ($F = 0.98$ $df_{num}= 2$, $df_{den}= 56$, p-value = 0.38) (Figure 8).

The results showed that independently of the given diet, all sea urchins decrease their weight during the growth experiment (Figure 8). The sea urchins fed with Mix diet showed a constant decrease in weight throughout the experiment (Figure 8). The sea urchins fed with Porph showed a strong decrease in weight during the first month and then stabilizing during the rest of the experiment and the sea urchins fed with Ulva have an increase in weight during the experiment between T0 and T1 (Figure 8), followed by a decrease during the remainder of the experiment having finished with a similar average weight than the sea urchins fed with Mix (Figure 8).

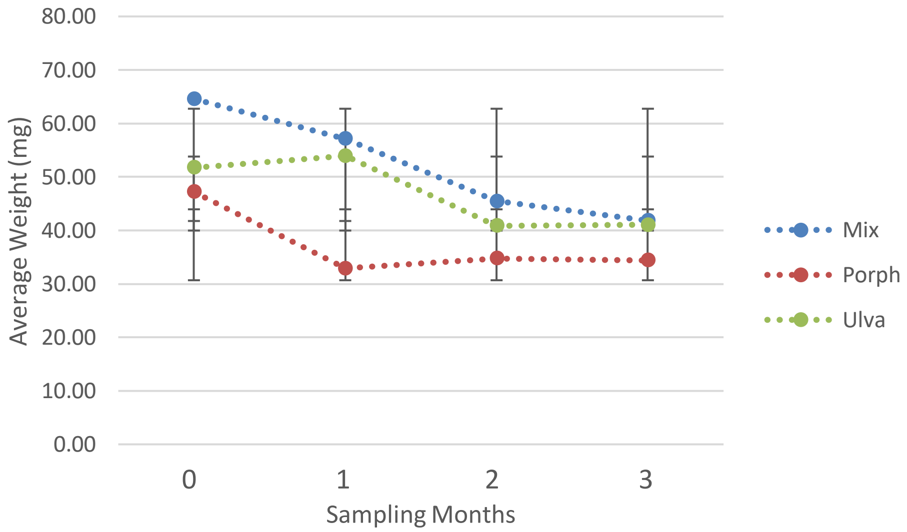

Figure 8. Evolution of the average body weight (±SD, mg) of the *Paracentrotus lividus* juvenile fed with the three macroalgae diets during the growth experiment. Points and dotted line in blue represents the sea urchins fed with the mixed diet; points and dotted line in red represents the sea urchins fed with the *Porphyra dioica* (Porph); and points and dotted line in green represents the sea urchins fed with the *Ulva rigida*.

Of all dietary treatments, only the weight of the juveniles fed with Mix remained homogeneous during the experiment, the One-way ANOVA analyses showed that there were statistical differences when comparing each of the weights of the juveniles fed with different diets during each month of the experiment p-value <0.05 (F = 8.40, df_{num} = 3, df_{den} = 83, *p*-value = <0.001) (Table 4).

The weight of the juveniles fed with the other 2 diets did not remain homogeneous during the experiment having both showed a p-value <0.05, therefore the Kruskal-Wallis nonparametric test was used which showed that there were no statistical differences, p-value >0.05, when comparing the differences between the medians (Porph - HKW= 1.41; p-value = 0.7 Ulva – HKW=1.94; p-value = 0.59) (Table 4).

Table 4. Statistical summary of the One-Way ANOVA comparing average body weight of the *Paracentrotus lividus* juveniles fed with the three macroalgae diets during the growth experiment (measurements collected in the sampling events T0, T1, T2, T3).

Diets	Homogeneity test	Anova Output				Kruskal-wallis	
		F	DF_{num}	DF_{den}	P-value	HKW	p-value
Mix	0.89	8.4	3	83	<0.001		
Porph	0.04					1.41	0.7
Ulva	<0.001					4.94	0.59

By the end of the experiment, it was also observed that the condition of all the sea urchins juveniles in the growth experiment suffered a loss of most of their spines and coloration as seen on figure 9.

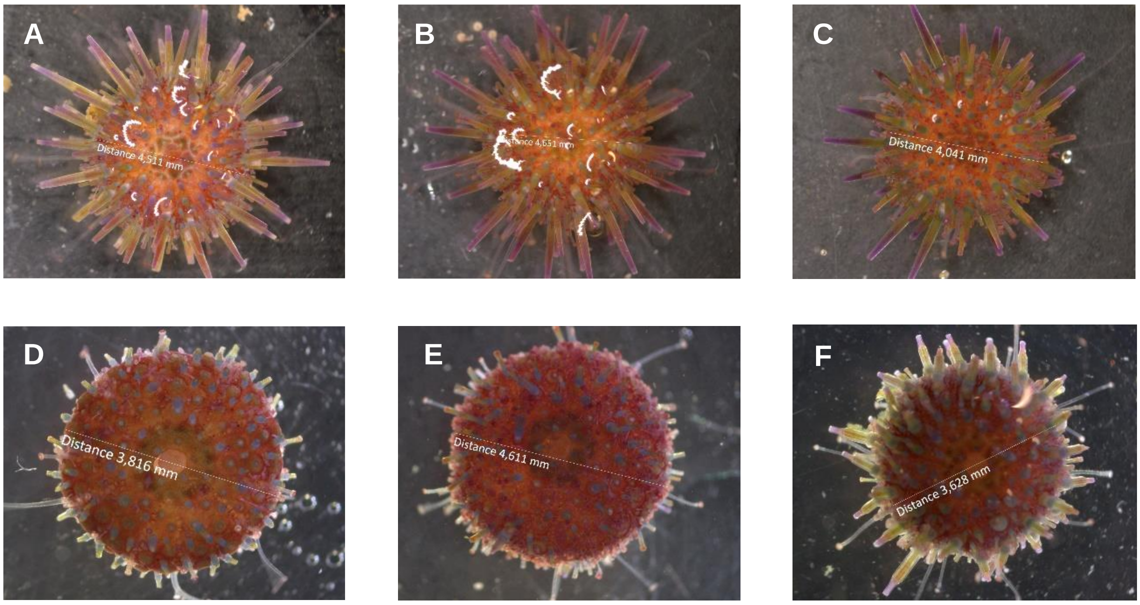

Figure 9. *P.lividus* sea urchin juveniles used in the study ant T0 and T3 (A - Mix diet at T0, B – Porph diet at T0, C – Ulva diet at T0, D – Mix diet at T3, E – Porph diet at T3 and F Ulva diet at T3).

3.2 Experiment of seawater sources effect on juvenile survival and condition

The average survival rate of the sea urchins during the experiment of seawater sources was 43%, with statistically significant differences between experimental groups (F=3.68. df_{num} = 3. df_{den} = 60, p-value = 0.02, n= 26). The group of juveniles maintained in FC water showed statistically higher survival rate that the group of juveniles maintained in the water F (FC ≠ F (p-value =0.032).

From the four sources tested, only D (Seawater from the local Port) and S (Artificial seawater) showed survival rate in all the juvenile groups used in this experiment, F (Borehole seawater) showed the worst results by presenting not only the least survival of all the waters but also by having the highest total mortality when observing the remaining sea urchins by the end of the experiment (Figure 10).

The only group presenting 100% of survival out of all the seawater sources used in this experiment was the group maintained in the seawater FC (Figure 10).

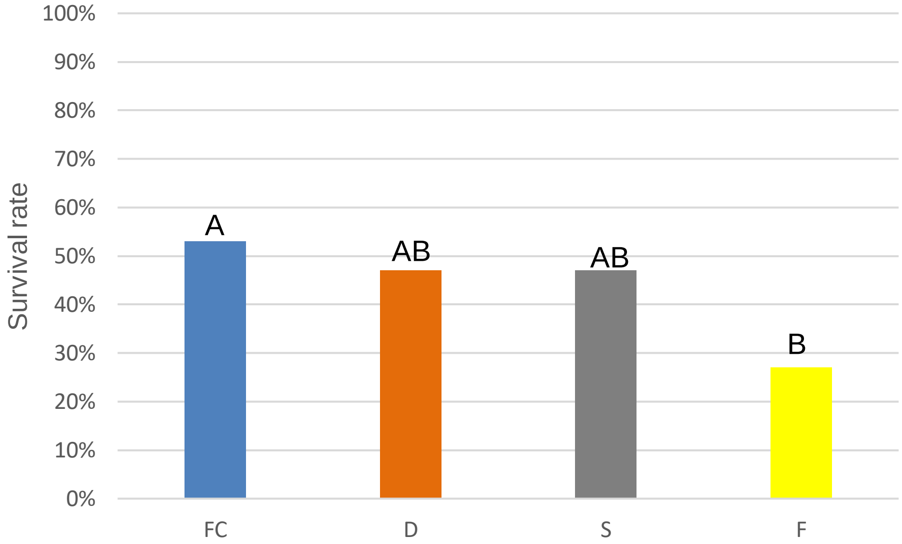

Figure 10. Survival rate of the *Paracentrotus lividus* sea urchins fed with Ulva at the end of the water comparison experiment (FC – Borehole seawater enriched with 10 mg sodium bicarbonate, D – Water from the local port, S – Artificial seawater, F – Borehole seawater).

By the of water comparison experiment we were to observe an improvement of the sea urchin juvenile conditions with the juveniles showing spines regeneration as seen in figure 11.

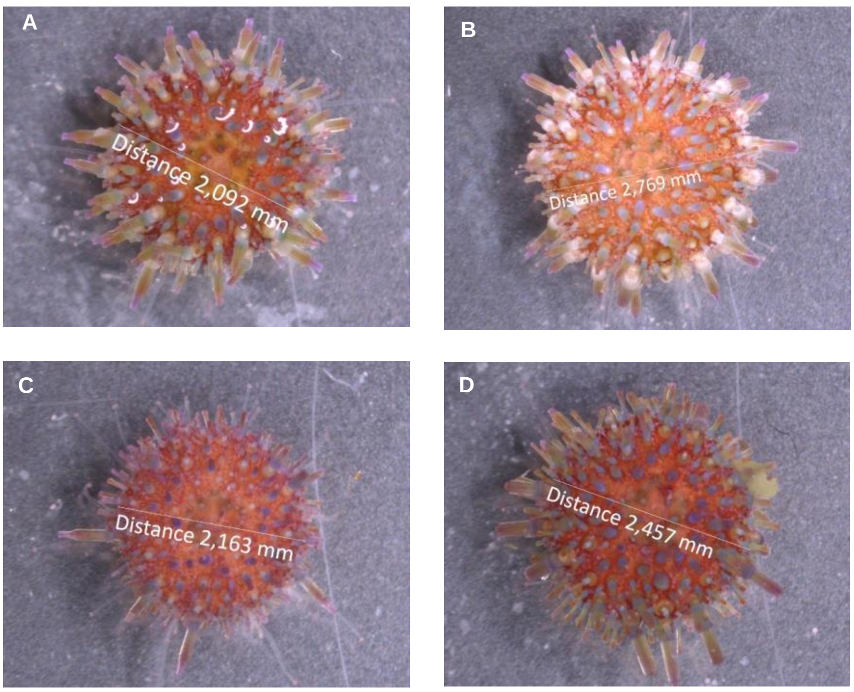

Figure 11. Sea urchin juveniles at the end of the water source experiment (A – Borehole seawater, B – Enriched borehole seawater, C – Seawater from the local port, D – Artificial seawater).

3.3 Test diameter vs body weight Morphometric relationships

The analysis of the feed trial juvenile and adult TD distributions (Juveniles [14.5 and 25.5 mm] with average TD = 24.33± 3,3 mm; adults [37.5 and 40.5 mm] with average TD = 37.15 ± 3,3 mm) showed that this dimension did not show statistical differences among diets during the experiment (*p*-value>0.05, Table 5).

For the wild sea urchins' dataset both juvenile ([17.20 to 31 mm], with average TD = 24.70 ± 3,8 mm) and adults ([32.00 – 48.00 mm] with average TD = 39.56 ± 2,6 mm) groups showed unpredictable variances, with the second replica of the wild sea urchin dataset (WA2) when comparing with the other replicas (Table 5). On average juvenile sea urchins from the feed trials and from wild studies presented a similar average TD (24.33 mm and 24.70 mm respectively) while the wild adult sea urchins from wild samples presented a larger average TD than the adult sea urchins from the feed trial adult sea urchins (39.56 mm and 37.35 mm respectively).

In the feed trials, when comparing the TD of both juvenile and adults' groups we can see that there are no statistical differences for each group (p-value= 0.36 and p-value= 0.21) but when comparing the TD of the juvenile and adult wild sea urchin trials we can see that there are statistical differences for each group (p-value <.001) (Table 5).

Table 5 - One-way ANOVA output comparing the test diameter (TD) distribution of juvenile and adult sea urchins by diet for the data collection from feeding trials sampling month for the data collected in the field.

Feeding trials sea urchin							
Size group	Homogeneity test	ANOVA Output					
		F	Dfnum	Dfden	p-value	Coeficient	
Juveniles	0.599	1.025	2	246	0.36	<.001	
Adults	0.469	1.573	2	222	0.21	<.001	
Wild Sea urchin							
Size Group	Homogeneity test	ANOVA Output					Tukey HSD
		F	Dfnum	Dfden	p-value	Coeficient	
Wild Juveniles	0.111	226.203	2	96	<.001	<.001	<.001
Wild Adults	0.155	4.875	2	101	<.001	<.001	* WA2 ≠ 1 & 3 (p-value = 0.022 & 0.012)

Figure 12 presents the graphical representation of the linear regression models of juvenile and adult *P. lividus* sea urchins from feed trial and wild populations.

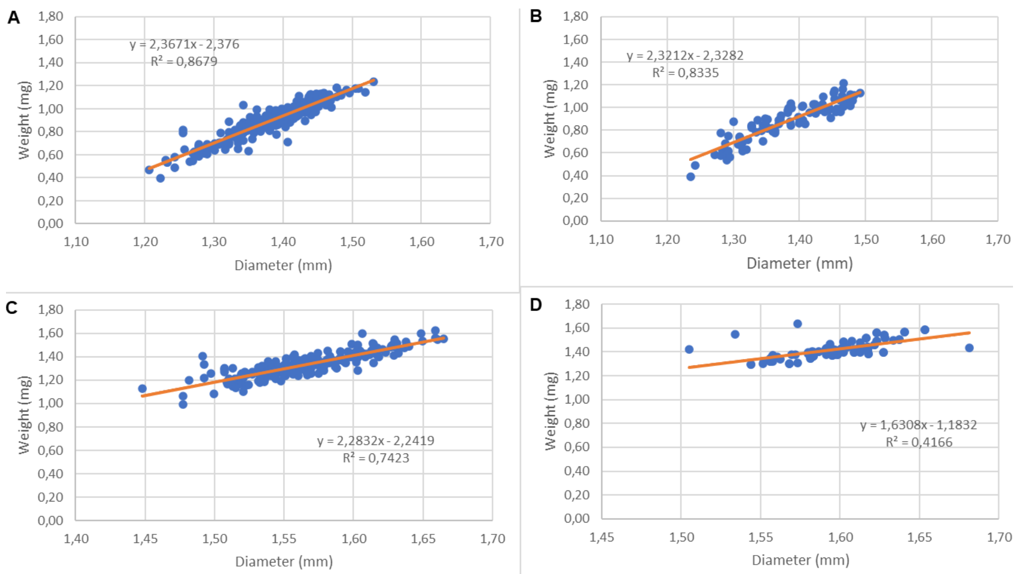

Figure 12. Linear regression models fitted to the data sets of log (weight) and log (diameter) of juvenile and adult *Paracentrotus lividus* from feed trial and wild sea urchins (A - juvenile from feeding trials; B – juvenile collected in the field; C – adults from feeding trials; D – adults collected in the field).

Feed trial sea urchins presented a much higher correlation coefficients between specimen TD (r=0.87 to 0.74) compared to the wild sea urchins (r=0.83 to 0.42) (Table 6 and 7).

Overall, the allometric coefficients showed that when comparing both feed trial and wild sea urchins we can observe that between juveniles there is a very similar allometric growth but between adults we can see that feed trial sea urchins present a higher allometric growth than wild sea urchins, with all groups presenting a negative allometry (Table 6 and 7).

Table 6 – Log-diameter vs log-weight linear relationship parameters and statistical significance for the *Paracentrotus lividus* juvenile and adults from feed trials

Size group	**N**	**Linear Regression parameters**			
		a	b	r2	t
Juveniles	248	-2.37 ± 0.08 (p-value < 0.001)	2.36 ± 0.06 (p-value < 0.001)	0.87	-29.053 (p-value < 0.001)
Adults	224	-2.24 ± 0.14 (p-value < 0.001)	2.28 ± 0.09 (p-value < 0.001)	0.74	-15.512 (p-value < 0.001)
Analysis of co-variance output					
		df	Mean square	F	*p*-value
Interaction effect Log_diameter * Size group		1	0.002	0.587	0.44

When comparing these regressions, we can see that they are similar, therefore both juveniles and adults present a similar diameter-weight relationship meaning that both juveniles and adults grow at similar rates with their TD increasing faster than their BW (Table 6).

Table 7. Log-diameter vs log-weight linear relationship parameters and statistical significance for the *Paracentrotus lividus* juvenile and adult sea urchins collected in the field.

Size group	n	Linear Regression parameters			
		a	b	r2	t
Juveniles	98	-2.33 ± 0.15 (p-value < 0.001)	2.32 ± 0.12 (p-value < 0.001)	0.83	-15.661 (p-value < 0.001)
Adults	103	-1.143 ± 0.28 (p-value < 0.001)	1.606 ± 0.17 (p-value < 0.001)	0.42	-3.706 (p-value < 0.001)
Analysis of co-variance output					
		df	Mean square	F	*p*-value
Interaction effect Log_diameter * Size group		1	0.034	8.49	0.004

When comparing these regressions, we can see that they aren't similar, despite that they both represent negative allometry therefore the growth rate is higher in their TD in comparison to their BW, with the feed trial juveniles showing a higher coefficient (0.83>0.42) and therefore presenting a higher growth rate in BW when comparing with the feed trial adult sea urchins (Table 7).

When comparing the regressions of feed trial and wild adult sea urchins, we can see that, even though both represent a negative allometry meaning they both grow their TD at a faster rate than their BW, the feed trial sea urchins have a higher coefficient than the wild adult sea urchins (0.74>0.42), therefore they gain BW at a faster rate than the wild adult sea urchins (Table 6 and 7).

4 Discussion

4.1 Impact of different macroalgae diets in the growth of *Paracentrotus lividus* juvenile

By the end of the growth trial, the average test diameter was 3.66 mm with no differences between dietary groups and in terms of body weight, the final average body weight was 39.39 ±1.64 mg, resulting in a decrease of 15.07 mg, showing that independently of the diet the juveniles lost weight despite of food availability, besides this lack of growth the juveniles also showed a lack of spines (Figure 11), when comparing these results with Mccarron et al. (2010)

and Mendes et al. (2019) we can observe that the sea urchins in this study are below what was to be expected in terms of growth.

In the study conducted by Mccarron et al. (2010), *P. lividus* juveniles reared with a diet of fresh algae with a photoperiod of 16 hours light and 8 dark in cages containing 24 sea urchins during a 6-month period attained an overall growth rate of 0.16 ± 0.11 mm/month^{-1}. In another study, Mendes et al. (2019) showed that the juveniles (210 days post hatching) raised in flow-through tanks with filtered seawater with a density of 10 g/L fed with *Ulva* spp. and the microalgae *I. galbana* increasing 35% of their initial size, attaining 2 cm in diameter at 7 months. The comparison of the present study results and those published showed that the somatic growth of *P. lividus* appears to be influenced mainly be water quality, temperature, food quality (Boudouresque et al., 2020). Therefore, the factors that could have affected our study was either the food used, or the seawater sources used to rear the animals. To improve the results obtained in the present study, the tanks used should have an adequate size/concentration ratio to ensure that the administered feed is accessible to all individuals and the water seawater used should come directly from the sea.

In this study the sea urchins were given three diets, two macroalgae of *Ulva rigida* and *Porphyra dioica* previously dried and rehydrated, and a third diet 1:1 mixture of both macroalgae. The selection of these diet was based in previous studies using these or similar species to feed juvenile sea urchins. Mendes et al. (2019) fed *P.lividus* juveniles until these reached maturity with *ulva.*sp, and Kelly et al. (2015) with *Psammechinus miliaris*.

In the study conducted by Mendes et al. (2019), *P. lividus* juveniles were fed with fresh *Ulva* spp until reaching maturity produced in earthen ponds. Additionally, Boudouresque et al., (2020) mentioned that that wild juvenile sea urchins ranging from 1 to 4 mm graze on a variety of seaweed with certain preferences to red algae like *Spermothamnion repens* and *Antithamnion cruciatum* with nutritional characteristics like *P. dioica.*

In terms of the algae given, the main difference that was observed regarding the interaction between the sea urchins and the diets selected. It was possible to observe that the sea urchins eat more Ulva then Porphyra. Moreover, in the tanks where the sea urchins were fed the mixed diet, most of the remaining pieces belonged to Porph showing a clear preference for Ulva. This behavior is referred by Boudouresque et al. (2020) which explained that depending on several factors like food availability and food combination the sea urchins of this species can select a preferred food. In general *P. lividus* show a preference towards green algae over red algae when both are available. This preference is also present in Mendes et al., (2019) where several macroalgae besides Ulva spp. where given, for example, *Asparagopsis armata*, *Cystoseira* *usneoides* and *Saccorhiza polyschides*. nonetheless *P. lividus* did not show any interest in consuming these macroalgae.

Another factor thar may have affected the algae consumption is related with the previous drying process suffered by the algae, that could have altered the algae consistency and flavor. Both algae present similar fiber content (an average of 40% fiber content, Echave et al., 2021, Thiviya et al., 2022) and one assumes that such drying process should have similar impact in both algae. Nevertheless, *P. dioica* was quite harder to shred and break down when comparing it to the *U. rigida* indicating that, despite the similar fibre content, *Porphyra* leafs are more resistant and tougher in the terms of texture when compared to Ulva. This may have affected the juvenile's capacity to ingest the former. These were very small (0.5 - 1mm) post-metamorphosed sea urchins. At this stage, these animals feed primarily on biofilm and only around 6-8 months are they able to ingest fresh brown algae (Kelly 2002). Most probably, the juveniles analyzed in this study were too small to be able to feed in the macroalgae diets selected.

4.2 Impact of seawater sources in the survival and condition of sea urchin juveniles

Besides the effect of diets, it was hypothesized that the chemical characteristics of the sea water used in the rearing system could have a negative impact in the juvenile survival and condition contributing to the absence of growth during the feeding experiment period.

The water used at the beginning of the study was collected from a borehole, which was usually used to keep adult sea urchins but not juvenile. During the growth experiment, the juvenile lost their spines and some even suffered a decrease in size after mid trial (results obtained at T2 sampling event). According to Ebert (2004) decrease in size can be related to unfavorable conditions or due to the tightening of the sutures that are relatively open during the rapid growth of the juvenile stage, when comparing this with our study we can assume that our decrease in size was due to that tightening of the sutures combined with the lack of growth which led to the shrinkage.

To verify this, the water composition of F (borehole seawater) and D (Seawater from the local port) were analyzed, and that analysis showed the only difference in both water compositions was Iodine, with F seawater showing a much lower Iodine concentration than D. Iodine's main function is to ensure proper thyroid function (Miller et al., 2013), meaning that in a low iodine environment thyroid function in sea urchins may be inhibited. Thyroid hormones accelerate skeletogenesis in larval and embryonic sea urchins (Taylor et al., 2018). This insufficiency in iodine in the borehole seawater may also constrain the skeletogenesis in the post-metamorphosed juveniles affecting their normal development.

Other possibility was the salinity levels in the different water sources, juvenile sea urchins have a very low tolerance to salinity variations (Goodbody, 1961; Lawrence 1975; Roller and Stickle, 1993), but salinity levels were monitored daily and kept at a constant level (35 ± 0.37 ppt) with regular water exchanges were regular to ensure this.

Recent work on a diverse array of echinoderm species has demonstrated, that thyroid hormone (TH) accelerates development to metamorphosis (Heyland et al., 2004). Larvae of several species of sea urchins, like *P. lividus* obtain, most likely their TH through their microalgae diet, therefore there is a possibility that the water used in combination with the diets used in their larval stage did not offer the proper environment for the best development of the larvae leading to a lower growth in juveniles.

By the end of this experiment the seawaters sources that showed the highest survival rate were the water FC, where one of the replicas completely died off followed by D and S. The sea urchins maintained in these seawaters showed spine regeneration when compared to the beginning of this study (Figure 12) indicating that sea urchins can regenerate their spines in case of loss due to being in an unfavorable environment (Tortonese, 1965). Several environmental factors can affect spine losses, pH, temperature or water condition and composition.

The sea urchin juveniles kept in D and S specifically showed survival among all replicas and good spine recovery, this may have been since the seawater D and S had higher iodine levels than F.

4.3 Test diameter vs body weight Morphometric relationships

Our study showed that both feed trial and wild *P.lividus* sea urchin juveniles had a similar negative allometric growth, meaning their TD grows at a faster rate than their BW, this kind of growth is in agreement with the exponential growth that juvenile sea urchins present during their first year of life (Sartori et al., 2015).

On the other hand, the adult sea urchins fed with dry diets in captivity show a different allometric growth when compared with those from wild populations. In both cases the allometry parameter (b) is inferior to 3 (isometry), indicative of negative allometric growth. Nevertheless, the sea urchins from the feeding trials show a higher allometry, meaning that for the same TD these urchins are heavier than those from wild populations. This is most likely since feeding trial sea urchins unlike wild sea urchins have the optimal conditions for growth with a constant water renewal and food intake, while the wild sea urchins need to expend energy finding their food. These differences can also be related with the fact that sea urchins have the capacity to alter the size and proportion of body components in response to varying environmental conditions (Küçükdermenci and Lök, 2014).

Another possible reason for the difference in the allometric growth in the adults is the fact that there is an inverse relation relationship between gonadal and somatic growth (Küçükdermenci and Lök, 2014), meaning that with the maturation of the gonads the somatic growth declines due to the allocation of nutrients (Beddingfield and McClintock., 1998), this change in nutrient allocation may also be a reason for the difference between wild and feed trial adult sea urchins in our study, since the feed trial sea urchins having a higher food intake will also have higher nutrient reserves which will allow for more constant somatic growth despite being mature.

In general, we can observe that the feeding trial sea urchins showed a higher allometric growth when compared with their wild counterparts, this difference in overall growth is not new from an aquaculture point of view as cultured *P.lividus* usually show a higher growth rate than wild ones, this is observed in Kelly (2002), Kelly et al. (2015) and Mendes et al. (2019).

5 Conclusion

Out of the 2 administered macroalgae the one that showed most promise was Ulva, with it being the most consumed among the 3 diets, and the easiest to prepare. Nevertheless, with the growth study we can ascertain that juvenile *P. lividus* growth and development are very dependent not only on food availability and quality but also on water quality and chemical composition.

In future studies, rearing conditions including the selection of appropriate relationship between rearing area/volume, animal density and amount of food provided should also be considered to improve the interaction between the small sea urchins with their food, and regarding the macroalgae used it would be pertinent to study how dry and fresh algae consumption varies among juveniles.

The morphometric relations study showed us that from a commercial point of view sea urchin aquaculture as a bright future because when compared to the wild sea urchins the feed trial sea urchins attained a higher diameter and weight in the same period.

6 Bibliographic references:

Agatsuma, Y., and Nakata, A. (2004). Age determination, reproduction, and growth of the sea urchin *Hemicentrotus pulcherrimus* in Oshoro Bay, Hokkaido, Japan. *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom*, *84*(2), 401.

Aihara, M., and Amemiya, S. (2001). Left-right positioning of the adult rudiment in sea urchin larvae is directed by the right side. *Development*, *128*(24), 4935-4948.

Allain, J. Y. (1975). Structure des populations de *Paracentrotus lividus* (Lamarck) (Echinodermata, Echinoidea) soumises à la pêche sur les côtes nord de Bretagne. *Revue des travaux de l'Institut des pêches maritimes*, *39*(2), 171-212.

Alessandra, A. N., Marco, P., Chiara, L., and Annamaria, V. G. (2003). Ammonia as confounding factor in toxicity tests with the sea urchin *Paracentrotus lividus* (Lmk). *Toxicology and Enviromental Chemistry*, *85*(4-6), 183-191.

Azad, A.K., McKinley, S. and Pearce, C.M., (2010). Factors influencing the growth and survival of larval and juvenile echinoids. *Reviews in Aquaculture*. 2, 121–137.

Baião, L. F., Rocha, F., Costa, M., Sá, T., Oliveira, A., Maia, M. R., ... and Valente, L. M. (2019). Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture*, *506*, 127-138.

Baião, L. F., Rocha, C., Lima, R. C., Marques, A., Valente, L. M., and Cunha, L. M. (2021). Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming future aquaculture applications. *Food Research International*, *140*, 109873.

Basuyaux, O., and Blin, J. L. (1998). Use of maize as a food source for sea urchins in a recirculating rearing system. *Aquaculture international*, 6(3), 233-247.

Basuyaux, O., and Mathieu, M. (1999). Inorganic nitrogen and its effect on growth of the abalone *Haliotis tuberculata* Linnaeus and the sea urchin *Paracentrotus lividus* Lamarck. *Aquaculture*, *174*(1-2), 95-107.

Benedetti-Cecchi, L., Bulleri, F. and Cinelli, F. (1998). Density dependent foraging of sea urchins in shallow subtidal reefs on the west coast of Italy (western Mediterranean). *Marine Ecology Progress Series, 163*, 203-211.

Boudouresque, C. F., and Verlaque, M. (2007). Ecology of *Paracentrotus lividus*. *In Developments in aquaculture and fisheries science* (Vol. 37, pp. 243-285). Elsevier.

Boudouresque, C. F. and Verlaque, M., (2020). Chapter 26 in *Sea urchins Biology and Ecology 4th edition*

Brundu, G., Monleon, L.V., Vallainc, D., A. and Carboni, S., (2016). Effects of larval diet and metamorphosis cue on survival and growth of sea urchin post-larvae (*Paracentrotus lividus*, Lamarck, 1816). *Aquaculture 465*, 265–271.

Byrne, M., (1990). Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Marine Biology. (Heidelberg, Ger.), 104*, 275–289.

Campbell, N. and Reece., J.B., (2009). Biology. In: Pearson Benjamin Cummings, *8th (ed) Biology*. Sansome St., San Francisco, USA.

Carbonara, S., D'Adamo, R., Novelli, A., Pelosi, S., and Fabbrocini, A. (2018). Ground Ulva solution (GUS): A promising metamorphosis cue for *Paracentrotus lividus* larviculture. *Aquaculture*, *491*, 289-294.

Carboni, S., (2013). Research and development of hatchery techniques to optimise juvenile production of the edible Sea Urchin, *Paracentrotus lividus.*

Carboni S., Addis P., Cau A., and Atack T., (2012). Aquaculture could enhance Mediterranean Sea urchin fishery, expand supply. *Global Aquaculture Advocate, 15*, 44-45.

Carboni, S., Hughes, A. D., Atack, T., Tocher, D. R., and Migaud, H. (2015). Influence of broodstock diet on somatic growth, fecundity, gonad carotenoids and larval survival of sea urchin. *Aquaculture Research*, *46*(4), 969-976.

Carboni, S., Vignier, J., Chiantore, M., Tocher, D.R and Migaud, H. (2012). Effects of dietary microalgae on growth, survival, and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development. *Aquaculture, 324*, 250.258.

Cárcamo, P. F., Candia, A. I., and Chaparro, O. R. (2005). Larval development and metamorphosis in the sea urchin *Loxechinus albus* (Echinodermata: Echinoidea): effects of diet type and feeding frequency. *Aquaculture*, *249*(1-4), 375-386.

Castilla-Gavillán, M., Buzin, F., Cognie, B., Dumay, J., Turpin, V. and Decottignies, P., (2018a). Optimising microalgae diets in sea urchin *Paracentrotus lividus* larviculture to promote aquaculture diversification. *Aquaculture 490*, 251-259.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W. W., Christensen, V., … and Pauly, D. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats, and marine reserves. *Global Ecology and Biogeography, 21*(4), 465-480

Conand, C. and N. A. Sloan. (1989). World fisheries for echinoderms. pp.647-663. In: J. F. Caddy (ed.). *Marine Invertebrate Fisheries: Their Assessment and Management*. J. Wiley and Sons, New York.

Coppard, S. E., and Campbell, A. C. (2005). Lunar periodicities of diadematid echinoids breeding in Fiji. *Coral Reefs*, *24*(2), 324-332.

Delmas, P., (1992). Etude des Populations de *Paracentrotus lividus* (Lam.) (Echinodermata: Echinoidea) Soumises à une Pollution Complexe en Provence Nord-occidentale: Densités, Structure, Processus de Détoxication (Zn, Cu, Pb, Cd, Fe). Thèse Doctorat, Université AixMarseille 3, Marseille

Delmas, P. and Régis, M.B., (1986). Données préliminaires sur le contenu digestif de l'oursin comestible *Paracentrotus lividus* (Lamarck) soumis à l'influence d'effluents domestiques. Marine Environment Research, 20, p.197–220.

De La Uz, S., Carrasco, J. F., Rodríguez, C., and Anadon, N. (2013). Metamorphosis, growth, and survival of early juveniles of *Paracentrotus lividus* (Echinodermata: Echinoidea): Effects of larval diet and settlement inducers. *Cah Biol Mar, 54*, 691-695.

Doherty, P. P. (1999). Recruitment-limitation is the theoretical basis for stock enhancement in marine populations. In Stock Enhancement and Sea Ranching. *First International Symposium on Stock Enhancement and Sea Ranching*, 8-11 September 1997. Bergen, Norway-pages: 9-21. Blackwell Science.

Dworjanyn, S. A., and Pirozzi, I. (2008). Induction of settlement in the sea urchin *Tripneustes gratilla* by macroalgae, biofilms and conspecifics: A role for bacteria? *Aquaculture*, *274*(2-4), 268-274.

Ebert, T. A. (1988). Allometry, design and constraint of body components and of shape in sea urchins. *Journal of Natural History*, *22*(5), 1407-1425.

Ebert, T. A. (1973). Estimating growth and mortality rates from size data. *Oecologia, 11(3)*, 281-298.

Echave, J., Lourenço-Lopes, C., Carreira-Casais, A., Chamorro, F., Fraga-Corral, M., Otero, P., ... and Simal-Gandara, J. (2021). Nutritional composition of the Atlantic seaweeds *Ulva rigida*, *Codium tomentosum, Palmaria palmata* and *Porphyra purpurea*. *Chemistry Proceedings*, *5*(1), 67.

FAO Fisheries and Aquaculture Information and Statistics Service (eds) (2011) FAO yearbook. *Fishery and aquaculture statistics. 2009/FAO annuaire.*

FAO, (2016). Food outlook, biannual report on global food markets. In: *Food and agriculture organization of the United Nations, 60-66.*

Fernandez, C. (1996). Croissance et nutrition de *Paracentrotus lividus* dans le cadre d'un projet aquacole avec alimentation artificielle *(Doctoral dissertation, Corte).*

Fernandez, C., Pasqualini, V., Boudouresque, C. F., Johnson, M., Ferrat, L., Caltagirone, A., and 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*(1-2), 259-270.

Fernandez, C., and Pergent, G. (1998). Effect of different formulated diets and rearing conditions on growth parameters in the sea urchin *Paracentrotus lividus*. *Journal of Shellfish research, 17*, 1571-1581.

Gago, J., Range, P., and Luis, O. J. (2001). Growth, reproductive biology, and habitat selection of the sea urchin *Paracentrotus lividus* in the coastal waters of Cascais, Portugal. *Echinoderm research*, 269-276.

Garaffo, M. A., Vassallo-Agius, R., Nengas, Y., Lembo, E., Rando, R., Maisano, R., ... and Giuffrida, D. (2011). Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid indices, of raw roe of blue fin tuna (Thunnus thynnus L.) and their salted product" Bottarga". *Food and Nutrition Sciences*, 2(7), 736.

Gaylord, B., Hodin, J., and Ferner, M. C. (2013). Turbulent shear spurs settlement in larval sea urchins. *Proceedings of the National Academy of Sciences*, *110*(17), 6901-6906.

Ghisaura, S., Loi, B., Biosa, G., Baroli, M., Pagnozzi, D., Roggio, T., ... and Addis, M. F. (2016). Proteomic changes occurring along gonad maturation in the edible sea urchin *Paracentrotus lividus*. *Journal of proteomics*, *144*, 63-72.

Gilbert, S.F. (2000). Developmental Biology. *6th edition. Sunderland (MA): Sinauer Associates;The Early Development of Sea Urchins.*

Girard, D., Clemente, S., Toledo-Guedes, K., Brito, A. and Hernández, J.C., (2012). A mass mortality of subtropical intertidal populations of the sea urchin *Paracentrotus lividus*: analyses of potential links with environmental conditions. *Marine Ecology* (Berlin, Ger.) 33, 377–385.

Giuseppe Pinna, M., (2013). The edible sea urchin *Paracentrotus lividus* (Lamarck, 1816): a valuable resource for fisheries and aquaculture purposes in Sardinia (Central western Mediterranean). *DR. UNIVERSITÀ DEGLI STUDI DI SASSARI.*

Gosselin, P., and Jangoux, M. (1996). Induction of metamorphosis in *Paracentrotus lividus* larvae (Echinodermata, Echinoidea). *Oceanologica acta, 19(3-4)*, 293-296.

Gosselin, P., and Jangoux, M. (1998). From competent larva to exotrophic juvenile: a morphofunctional study of the perimetamorphic period of *Paracentrotus lividus* (Echinodermata, Echinoida). *Zoomorphology*, *118*(1), 31-43.

González-Irusta, J. M., De Cerio, F. G., and Canteras, J. C. (2010). Reproductive cycle of the sea urchin *Paracentrotus lividus* in the Cantabrian Sea (northern Spain): environmental effects. *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom*, *90*(4), 699.

Guidetti, P., and Mori, M. (2005). Morpho-functional defences of Mediterranean Sea urchins, *Paracentrotus lividus* and Arbacia lixula, against fish predators. *Marine Biology*, *147*(3), 797-802.

Guillou, M., and Michel, C. (1993). Reproduction and growth of *Sphaerechinus granularis* (Echinodermata: Echinoidea) in southern Brittany. *Journal of the Marine Biological Association of the United Kingdom, 73(1)*, 179-192.

Gomes, A., Lourenço, S., Santos, P. M., Raposo, A., Mendes, S., Gonçalves, S. C., ... and Pombo, A. (2021). Effects of single and mixed-diatom diets on growth, condition, and survival of larvae of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture International*, *29*(3), 1069-1090.

Goodbody, I. (1961). Mass mortality of a marine fauna following tropical rains. *Ecology*, *42*(1), 150-155.

Grosjean, P., (2001). Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816), *PhD dissertation, Universite libre de Bruxelles.*

Grosjean, P., Spirlet, C., Gosselin, P., Vaitilingon, D. and Jangoux, M., (1998). Land-based, closed-cycle echiniculture of *Paracentrotus lividus* (Lamarck)

(EchinoIdea:Echinodermata): *a long-term experiment at a pilot scale. J. Shellfish Res.17*, 1523–1531.

Grosjean, P., Spirlet, C., and Jangoux, M. (1996). Experimental study of growth in the echinoid *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *Journal of Experimental Marine Biology and Ecology, 201(1-2)*, 173-184.

Hannon, C., Officer, R. A., and Chamberlain, J. (2017). Evaluation of the efficacy of algal-conditioned substrates for inducing settlement of *Paracentrotus lividus* larvae. *Aquaculture Research*, *48*(4), 1968-1973.

Hay, M.E., and Steinberg, P.D., (1992). The chemical ecology of plantherbivore interactions in marine versus terrestrial communities. In: *Rosenthal, J.A. and Berenbaum, M.R. (ed.) Herbivores: Their Interaction with Secondary Metabolites, Evolutionary and Ecological Processes. San Diego: Academic Press*, pp.371–413.

Huggett, M. J., Williamson, J. E., De Nys, R., Kjelleberg, S., and Steinberg, P. D. (2006). Larval settlement of the common Australian sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. *Oecologia*, *149*(4), 604-619.

Jackson, R. T. (1912). *Phylogeny of the echini: with a revision of palaeozoic species* (Vol. 7). The Society.

Kelly, M., Brodie, C. C., and McKenzie, J. D. (1998). Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) maintained in polyculture with the Atlantic salmon. *J Shellfish Res*, *17*(5), 1557-1562.

Kelly, M. S. (2002). Survivorship and growth rates of hatchery-reared sea urchins. *Aquaculture International*, *10*(4), 309-316.

Kelly, M., Carboni, S., Cook, E., and Hughes, A. (2015). Sea urchin aquaculture in Scotland. *Echinoderm Aquaculture*, 211-224.

Kenner, M. C. (1992). Population dynamics of the sea urchin *Strongylocentrotus purpuratus* in a Central California kelp forest: recruitment, mortality, growth, and diet. *Marine Biology, 112(1)*, 107-118.

Küçükdermenci, A., and Lök, A. (2014). Morphometric relationships and variability of annual body condition of sea urchin *Paracentrotus lividus* Echinodermata: Echinodermata at Foca coast in the South Aegean Sea. *Fresenius Environ Bull*, *23*(10), 1-9.

Lawrence, J. M. (1975). The effect of temperature-salinity combinations on the functional well-being of adult *Lytechinus variegatus* (Lamarck) (Echlnodermata, Echinoldea). *Journal of Experimental Marine Biology and Ecology*, *18*(3), 271-275.

Lawrence, J. M. (2013). *Sea urchins: biology and ecology*. Academic Press. Lawrence, J. M. (2013). *Sea urchin life history strategies. In Developments in Aquaculture and Fisheries Science* (Vol. 38, pp. 15-23). Elsevier.

Le Gall, P. (1990). Culture of echinoderms. pp. 443-462. In: *G. Barnabé (ed.). Aquaculture, vol. 1*. EIlis Horwood, New York.

Leske, N. G. (1778). Jacobi Theodori Klein naturalis dispositio echinodermatum, edita et descriptionibus novisque inventis et synonomis auctorem aucta. Addimenta ad I. T. Klein naturalem dispositionem Echinodermatum. G. E. Beer, Leipzig, pp. 278.

Liu, H., and Chang, Y., (2015). Sea urchin aquaculture in China. In N.P.Brown S.D.Eddy (Eds), *Echinoderm aquaculture*. Singapore: C.O.S. Printers Pte Ltd. pp.127-146.

López, S., Turon, X., Montero, E., Palacín, C., Duarte, C. M., and Tarjuelo, I. (1998). Larval abundance, recruitment, and early mortality in *Paracentrotus lividus* (Echinoidea). Interannual variability and plankton-benthos coupling. *Marine Ecology Progress Series*, *172*, 239-251.

Lourenco, S., Valente, L. M., and Andrade, C. (2019). Meta-analysis on nutrition studies modulating sea urchin roe growth, colour and taste. *Reviews in Aquaculture*, *11*(3), 766-781.

Lourenço, S., Mendes, S., Raposo, A., Santos, P. M., Gomes, A. S., Ganhão, R., ... and Pombo, A. (2021). Motivation and preferences of Portuguese consumers towards sea urchin roe. *International Journal of Gastronomy and Food Science*, *24*, 100312.

Lozano, J., Galera, J., López, S., Turon, X., and Palacin, C. (1995). Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series*, *122*, 179-191.

Luis, O., Delgado, F., and Gago, J. (2005). Year-round captive spawning performance of the sea urchin *Paracentrotus lividus*: relevance for the use of its larvae as live feed. *Aquatic Living Resources, 18(1)*, 45-54.

Machado, I., Moura, P., Pereira, F., Vasconcelos, P., and Gaspar, M. B. (2019). Reproductive cycle of the commercially harvested sea urchin (*Paracentrotus lividus*) along the western coast of Portugal. *Invertebrate Biology*, *138*(1), 40-54.

Martínez, I., García, F.J., Sánchez, A.I., et al., 2003. Biometric parameters and reproductive cycle of *Paracentrotus lividus* (Lamarck) in three habitats of southern Spain. In: Féral, J.P., David, B. (Eds.), *'Echinoderm Research 2001'*, Balkema, Lisse, pp. 281–287

McBride, S. C. (2005). Sea urchin aquaculture. In *American Fisheries Society Symposium* (Vol. 46, p. 179). American Fisheries Society.

McCarron, E., Burnell, G., Kerry, J., and Mouzakitis, G. (2010). An experimental assessment on the effects of photoperiod treatments on the somatic and gonadal growth of the juvenile European purple sea urchin *Paracentrotus lividus*. *Aquaculture research*, *41*(7), 1072-1081.

McClay, D. R. (2011). Evolutionary crossroads in developmental biology: sea urchins. *Development*, *138*(13), 2639-2648.

Mendes, A., Araújo, J., Soares, F., Bandarra, N., and Pousão-Ferreira, P. (2019). Production of Purple Sea Urchin *Paracentrotus lividus* in Portugal. *The World Aquaculture Society*, *50*(1), 46-50.

Metaxas A., (2013). Larval ecology of echinoids. In: Lawrence, J.M. (Ed) *Sea urchins: Biology and Ecology*. Elsevier, Amsterdam, Netherlands, pp 313-327.

Miller, A. E., and Heyland, A. (2013). Iodine accumulation in sea urchin larvae is dependent on peroxide. *Journal of Experimental Biology*, *216*(5), 915-926.

Morikawa, T. (1999). Status and prospects on the development and improvement of coastal fishing ground.

Mortensen, T. (1903). The Danish Ingolf Expedition. 4: Echinoidea. *Bianco Luno, Copenhagen*.

Mos, B., Cowden, K. L., Nielsen, S. J., and Dworjanyn, S. A. (2011). Do cues matter? Highly inductive settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. *PLoS One, 6(12)*, e28054.

Nicolau, L., Vasconcelos, P., Machado, I., Pereira, F., Moura, P., Carvalho, A. N., and Gaspar, M. B. (2022). Morphometric relationships, relative growth, and roe yield of the sea urchin (*Paracentrotus lividus*) from the Portuguese coast. *Regional Studies in Marine Science*, *52*, 102343.

Ouréns, R., Fernández, L., Fernández-Boán, M., Naya, I., and Freire, J. (2013). Reproductive dynamics of the sea urchin *Paracentrotus lividus* on the Galicia coast (NW Spain): effects of habitat and population density. *Marine biology, 160(9)*, 2413-2423.

Ouréns, R., Fernández, L., and Freire, J., (2011). Geographic, population, and seasonal patterns in the reproductive parameters of the sea urchin *Paracentrotus lividus. Marine Biology*. (Heidelberg, Ger.) 158, 793–804.

Ouréns, R., Flores, L., Fernández, L. and Freire, J., (2013). Habitat and density dependent growth of the sea urchin *Paracentrotus lividus* in Galicia (NW Spain). *Journal of Sea Research, 76*, 50–60.

Pais, A., Serra, S., Meloni, G., Saba, S., and 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(3),* 570-575.

Pawson, D. L. (2007). *Narcissia ahearnae*, a new species of sea star from the Western Atlantic (Echinodermata: Asteroidea: Valvatida). *Zootaxa*.

Pearce, C. M., and Scheibling, R. E. (1991). Effect of macroalgae, microbial films, and conspecifics on the induction of metamorphosis of the green sea urchin *Strongylocentrotus droebachiensis* (Mu¨ ller). *Journal of Experimental Marine Biology and Ecology, 147(2)*, 147-162.

Pearse, J. S. and Cameron, R. A. (1991). Echinodermata: Echinoidea. In: Reproduction of marine invertebrates. Vol 6: *Echinoderms and lophophorates*. Boxwood Press, Pacific Grove, CA, pp. 513-662.

Pedrotti, M. L. (1993). Spatial and temporal distribution and recruitment of echinoderm larvae in the Ligurian Sea. *Journal of the Marine Biological Association of the United Kingdom*, *73*(3), 513-530.

Phillips, K., Niimi, J., Hamid, N., Silcock, P., Delahunty, C., Barker, M., ... and Bremer, P. (2010). Sensory and volatile analysis of sea urchin roe from different geographical regions in New Zealand. *LWT-Food Science and Technology*, *43*(2), 202-213.

Pinna, M., G., (2014). The edible sea urchin *Paracentrotus lividus* (Lamarck, 1816): a valuable resource for fisheries and aquaculture purposes in Sardinia (Central western Mediterranean) *Tesi di dottorato in Scienze dei Sistemi Agrari e Forestali e delle Produzioni Alimentari, Indirizzo Scienze e Tecnologie Zootecniche*, Università degli Studi di Sassari.

Prato, E., Chiantore, M., Kelly, M. S., Hughes, A. D., James, P., Ferranti, M. P., ... and Fanelli, G. (2018). Effect of formulated diets on the proximate composition and fatty acid profiles of sea urchin *Paracentrotus lividus* gonad. *Aquaculture international*, *26*(1), 185-202.

Rahim, S. A. K. A., Li, J. Y., and Kitamura, H. (2004). Larval metamorphosis of the sea urchins, *Pseudocentrotus depressus* and *Anthocidaris crassispina* in response to microbial films. *Marine Biology, 144(1)*, 71-78.

Rahman, S., Tsuchiya, M., and Uehara, T. (2009). Effects of temperature on hatching rate, embryonic development, and early larval survival of the edible sea urchin, *Tripneustes gratilla*. *Biologia*, *64*(4), 768-775.

Régis, M.B., Pérès, J.M., and Gras, G. (1986). Données préliminaires sur l'exploitation de la ressource *Paracentrotus lividus* dans le quartier maritime de Marseille. *Vie Marine 7*,41–60.

Rial, D., Rial, P., Casal, A., Costoya, N., and Costas, D. (2018). Induction of settlement, growth, and survival of juveniles of *Paracentrotus lividus*. *Aquaculture*, *483*, 16-20.

Roller, R. A., and Stickle, W. B. (1993). Effects of temperature and salinity acclimation of adults on larval survival, physiology, and early development of *Lytechinus variegatus* (Echinodermata: Echinoidea). *Marine Biology*, *116*(4), 583-591.

Sánchez-España, A. I., Martínez-Pita, I., and García, F. J. (2004). Gonadal growth and reproduction in the commercial sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata: Echinoidea) from southern Spain. *Hydrobiologia*, *519*(1), 61-72.

Sartori, D., Pellegrini, D., Macchia, S., and 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.

Sartori, D., Scuderi, A., Sansone, G., and Gaion, A. (2015). Echinoculture: the rearing of *Paracentrotus lividus* in a recirculating aquaculture system—experiments of artificial diets for the maintenance of sexual maturation. *Aquaculture International*, *23*(1), 111-125.

Shimabukuro, S., (1991). Tripneustes gratilla, in: Shokita, S., Yamaguchi, M., Masashi, M. (Eds.), *Aquaculture in Tropical Areas*. Tokyo: Midori Shobo, pp. 313-328.

Shpigel, M., McBride, S. C., Marciano, S., and Lupatsch, I. (2004). The effect of photoperiod and temperature on the reproduction of European sea urchin *Paracentrotus lividus*. *Aquaculture*, *232*(1-4), 343-355.

Shpigel M., Schlosser S.C., Ben-Amotz A., Lawrence A.L., and Lawrence J.M., (2006). Effects of dietary carotenoid on the gut and the gonad of the sea urchin *Paracentrotus lividus*. Aquaculture, 261, 1269-1280. Sloan, N. A. (1985). *Echinoderm fisheries of the world: a review*. *AA Balkema, Rotterdam*, 109-124.

Siliani, S., Melis, R., Loi, B., Guala, I., Baroli, M., Sanna, R., ... and Anedda, R. (2016). Influence of seasonal and environmental patterns on the lipid content and fatty acid profiles in gonads of the edible sea urchin *Paracentrotus lividus* from Sardinia. *Marine environmental research*, *113*, 124-133.

Siikavuopio, S. I., James, P., Lysne, H., Sæther, B. S., Samuelsen, T. A., and Mortensen, A. (2012). Effects of size and temperature on growth and feed conversion of juvenile green sea urchin (*Strongylocentrotus droebachiensis*). *Aquaculture*, *354*, 27-30.

Spirlet, C., Grosjean, P., and Jangoux, M. (1998). Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. *Invertebrate reproduction and development*, *34*(1), 69-81.

Spirlet, C., Grosjean, P., and 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.

Starr, M., Himmelman, J. H., and Therriault, J. C. (1992). Isolation and properties of a substance from the diatom *Phaeodactylum tricornutum* which induces spawning in the sea urchin Strongylocentrotus droebachiensis. *Marine Ecology Progress Series*, 275-287.

Stefánsson, G., Kristinsson, H., Ziemer, N., Hannon, C., and James, P. (2017). Markets for sea urchins: a review of global supply and markets. *Internal Matis report: Skýrsla Matís*, 10-17.

Suckling, C.C., Symonds, R.C., Kelly, M.S., and Young, A.J., (2011). The effect of artificial diet on gonad color and biomass in the edible sea urchin *Psammechinus miliaris. Aquaculture, 318*,335-342.

Sun, J., and Chiang, F. S. (2015). Use and exploitation of sea urchins. Echinoderm *aquaculture*, 25-45.

Thiviya, P., Gamage, A., Gama-Arachchige, N. S., Merah, O., and Madhujith, T. (2022). Seaweeds as a Source of Functional Proteins. *Phycology*, *2*(2), 216-243.

Tomas, F., Turon, X., and Romero, J., (2005a). Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Marine Ecology Progress Series, 301*, 95–107.

Taylor, E., and Heyland, A. (2018). Thyroid hormones accelerate initiation of skeletogenesis via MAPK (ERK1/2) in larval sea urchins (*Strongylocentrotus purpuratus*). *Frontiers in Endocrinology*, *9*, 439.

Tortonese, E., (1965). *Fauna d'Italia, Echinodermata*. Edizioni Calderini, Bologna

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

Unuma, T., Sakai, Y., Agatsuma, Y. and Kayaba, T., (2015). Sea urchin aquaculture in Japan. In: Brown N. P. and Eddy S. D. (eds), *Echinoderm Aquaculture*, John Wiley and Sons, Inc, New Jersey, 77-126.

Verling, E., Crook, A., and Barnes, D. (2002). Covering behaviour in *Paracentrotus lividus*: is light important? *Marine Biology*, *140*(2), 391-396.

Vizzini S., Miccichè L., Vaccaro A., and Mazzola A. (2015). Use of fresh vegetable discards as sea urchin diet: effect on gonad index and quality. *Aquaculture International, 23*, 127-139.

Walker C.W., McGinn N.A., Harrington L.M., and Lesser M.P. (1998). New perspective on sea urchin gametogenesis and their relevance to aquaculture. *Journal of Shellfish Research, 17*: 1507-1514.

Walker C.W., Unuma T., McGinn N.A., Harrington L.M., and Leser M.P. (2001). Reproduction of sea urchin. In: *Lawrence J.M. Edible Sea urchins: biology and ecology.* Elsevier Science B.V., Amsterdam, 5-26.

Walker, C.W., Unuma, T., and Lesser, M.P., (2007). Gametogenesis and Reproduction of Sea Urchins. In: *Lawrence, J.M. (ed.), Edible Sea Urchins: Biology and Ecology*. Amsterdam – London - New York – Oxford- Paris- Shannon-Tokyo, Elsevier Science, p.11-33.

Wangensteen, O.S., Dupont, S., Casties, I., Turon, X., and Palacín, C., (2013). Some like it hot: temperature and pH modulate larval development and settlement of the sea urchin *Arbacia lixula*. *Journal of Experimental Marine Biology and Ecology. 449*, 304–311.

Williams, H. (2002) Draft Background Paper - Sea Urchin Fisheries of the World: A Review of Their Status, Management Strategies and Biology of the Principal Species. *Marine Resources, Department of Primary Industries, Water and Environment*. Tasmania.

Wray, G. A. (1992). The evolution of larval morphology during the post-Paleozoic radiation of echinoids. *Paleobiology*, 258-287.

Wright, J.T., and Dworjanyn, S.A., (2005). Density-dependent Sea urchin grazing: differential removal of species, changes in community composition and alternative community states. *Marine Ecology Progressive Series, 298*, 143–156.

Zupo, V., Glaviano, F., Caramiello, D., and Mutalipassi, M., (2018). Effect of five benthic diatoms on the survival and development of *Paracentrotus lividus* post-larvae in the laboratory. *Aquaculture, 495*,13–20.

Zupo, V., Glaviano, F., Paolucci, M., Ruocco, N., Polese, G., Di Cosmo, A., ... and Mutalipassi, M. (2019). Roe enhancement of *Paracentrotus lividus*: Nutritional effects of fresh and formulated diets. *Aquaculture nutrition*, *25*(1), 26-38.