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Histological Evaluation of Purple Sea Urchin (*Paracentrotus lividus*) Gonads: Influence of Temperature, Photoperiod Regimes, and Diets

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Abstract: Understanding and controlling reproductive cycles and gonad maturation in cultivated species are crucial in aquaculture. The sea urchin *Paracentrotus lividus*, known for its edible gonads, requires careful maturation control for both reproduction and commercialization. This study explores the impact of temperature, photoperiod, and diet on gonad development to enhance year-round aquaculture practices. Using two independent cultivation systems, we manipulated environmental conditions to mimic different seasons. Sea urchins were exposed to natural or manipulated temperature and photoperiod conditions and fed either natural (*Ulva* spp.) or formulated diets. The gonadosomatic index (GI) and histological analysis were used to assess gonad development. The results revealed a clear correlation between environmental conditions, diet, and gonad maturation. Manipulated conditions accelerated maturation, with sea urchins showing advanced stages compared to natural conditions. Furthermore, sea urchins fed formulated diets exhibited higher GI values, indicating enhanced maturation. Histological analysis confirmed accelerated maturation, particularly in females. This study underscores the feasibility of controlling gonad maturation through environmental manipulation and diet, enabling year-round marketable gonad production. Providing formulated diets rich in polyunsaturated fatty acids, notably docosahexaenoic acid (DHA), enhances the commercial value of sea urchins. These findings optimize aquaculture practices for *P. lividus*, highlighting its adaptability to the maximal production of gonads throughout the year.

Keywords: aquaculture; sea urchins; reproduction; histology; diets; gonadosomatic index

Key Contribution: This study highlights the feasibility of manipulating environmental conditions and diet to control gonad maturation in *Paracentrotus lividus*, enabling year-round marketable gonad production and optimizing aquaculture practices.

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1. Introduction

The sea urchin is an echinoderm of growing interest as a marine resource. In some nations, such as Japan, sea urchin gonads, commonly known as "uni", are an integral component of local culture and cuisine. With almost 126 million residents, Japan is the greatest consumer of sea urchins in the world, consuming 80–90% of the world's sea urchin production [1]. Meanwhile, Chile, with about 6435 km of coastline, is the largest supplier of sea urchins, with more than 55,000 tons per year [2]. The purple sea urchin

Paracentrotus lividus is extensively dispersed in the Mediterranean Sea and throughout the northeastern Atlantic coast, from Scotland and Ireland to southern Morocco [3]. It is a commercially significant species with a strong market demand for its roe, notably in the Mediterranean Basin [4] and in other European non-Mediterranean locations [5,6]. Interest in echinoculture arose because of the growing demand and the unsustainability of capturing wild sea urchins [7–10]. Echinoculture, in addition to responding to the demand increase, has helped to relieve pressure from fishing wild populations [11]. Good control of gonad development in sea urchin farming is extremely important to control the reproductive cycle of captive broodstock, but also to control the quality of the gonads when preparing them for collection and marketing for consumption. In Portugal, *P. lividus* reproduces throughout the year, with gonad maturity and gamete release occurring at the same time for both sexes. The autumn–winter season marks the gonads' maturity, and the spring–summer season is a single, extended spawning season that follows [12,13]. Sea urchins in the Mediterranean Sea have a varied reproductive cycle, with a first vigorous spawning in spring and a second, less intense spawning in October [14]. Variations in environmental conditions, primarily seawater temperature, affect gonad development and nutrient accumulation and also influence spawning duration, gametogenesis, and spawning, which are known to vary between geographical locations along a latitudinal gradient [12,15,16]. Nevertheless, the reproductive cycle might also vary significantly from year to year according to how the environment (primarily temperature and photoperiod, but also hydrodynamics) affects *P. lividus* population dynamics, reproductive biology, and secondary production [12]. The quality of the food also plays a major role in the development of the gonads, and can strongly influence the gonadosomatic indices and biochemical characteristics of the gonads [9,12–17]. The main objectives of this work were to understand the influence of two environmental parameters and feeding on the development and maturation of the gonads. For this reason, two different seasonal periods were produced by adjusting the temperature and photoperiod. Additionally, two diets were tested: an inert feed formulated for sea urchins [18] and a natural food (the macroalga *Ulva* spp.).

2. Materials and Methods

2.1. Spawning and Larval Rearing

The production of purple sea urchins (*P. lividus*) at the Aquaculture Research Station (EPPO/IPMA) was carried out using a set of protocols drawn up by combining bibliographical research and the experience of the research team, as well as the specific characteristics of the EPPO's facilities.

2.1.1. Broodstock and Spawning

The wild broodstock were caught off the south coast of Portugal and acclimatized in fiberglass tanks with open-water circuits, with temperature manipulation whenever necessary, especially to avoid temperatures above 25 °C during the summer period. The broodstock was fed with macroalgae (*Ulva* spp.) produced in earthed ponds at EPPO facilities. Spawning was induced by injecting 1 mL of 0.5 M KCl into the coelom through the peristonial membrane. Fertilization was carried out at a ratio of 500 spermatozoa per 1 egg after determining the number of oocytes. Fertilization took place in 5 L glass beakers filled with filtered, UV-disinfected water. The eggs were then hatched in 220 L cylinder-conical fiber tanks, with no water renewal, soft aeration, and low light. Hatching occurred approximately 48 h after fertilization.

2.1.2. Larval Rearing

The newborn larvae were kept in the same tanks, with an approximate density of 2250 larvae per liter. The tanks remained in a closed-water system, with daily renewals of about 20% of the water in the tank. A 50-micron filter was installed at the water outlet to prevent larvae from escaping. The larvae were fed a mixture of microalgae in different proportions in the number of cells: *Phaeodactylum tricornutum* (50%), *Isochrysis galbana*

Tiso (25%), *Nannochloropsis oculate* (25%). The amount provided varied depending on the stage of larval development, starting with 6000 cells/mL to 30,000 cells/mL per day. The microalgae were produced at EPPO facilities, growing in 60 L plastic bag photobioreactors at 20 ± 0.5 °C with aeration, during the sea urchins' larval cultivation phase.

2.1.3. Post-Larvae and Juveniles' Cultivation

Metamorphosis and settlement began approximately 20 and 30 days after hatching. To increase the surface available for fixation in the tanks, plastic pieces consisting of polyethylene terephthalate were suspended, in which natural biofilm was developed. During the first few weeks after settlement, the tanks were kept in a closed-water system, with small daily renewals. In addition to the food provided by the biofilm, around 2 L of *Phaeodactylum tricornotum* microalgae (roughly 6×10^9 cells) was added to each tank daily. With the progressive growth of the sea urchins, the supply of macroalgae (*Ulva* spp.) "previously washed with fresh water" started. Once they were close to 5 mm in test diameter (a transfer-safe size), the juvenile sea urchins were transferred to 300 L raceway fiber tanks with an open-water system and aeration. The water temperature was kept at room temperature and cooled in the warmer months so as not to exceed 22 °C. From then on, the juveniles were fed exclusively on macroalgae *Ulva* spp. ad libitum. The tanks were cleaned before new food was supplied.

2.2. Experimental Design

In order to analyze the effect of temperature, photoperiod, and food type, four cultivation systems were created: NoM U—no environmental manipulation and natural food (*Ulva* spp.); NoM If—no environmental manipulation and inert feed; M U—manipulated environment and natural food (*Ulva* spp.); M If—manipulated environment and inert feed. Six rectangular fiberglass tanks with a volume of 110 L were used for this test. Three tanks were subjected to manipulated environmental conditions and three were subjected to natural environmental conditions. Plastic boxes measuring 0.57 × 0.38 × 0.08 m were placed in each tank, divided in half by a 3 mm mesh plastic net. Each side of the box was subjected to a different diet (*Ulva* spp. and inert food). Three replicates were created for each treatment. Each tank had an aeration system and open-water system with a renewal rate of approximately 112.5 L/h. The tanks were lit by halogen lamps with an average intensity at the water surface of 100 lux (Figure 1).

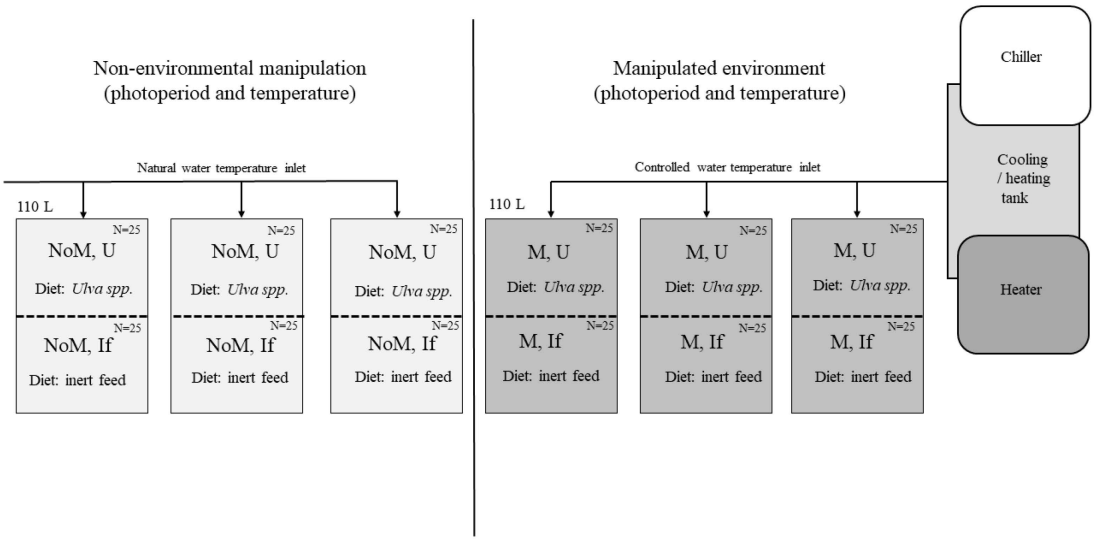

Figure 1. Representative diagram of the system used for the test. On the left are the three tanks (replicates) subjected to non-environmental manipulation (NoM). On the right are the tanks subjected to temperature manipulation and an altered photoperiod (M). Each tank was divided in half and each half was subjected to different diets (U—*Ulva* spp., If—inert feed).

A total of 300 F1generation purple sea urchins (*P. lividus*), approximately 3 years old, were used for this study, with mean weights of 22.73 ± 4.46 g. Fifty individuals were randomly placed in each box; there were twenty-five for each half of the box.

2.2.1. Environment Conditions

Two different environmental conditions were created, with three tanks in each system (Table 1). The "No environmental manipulation" (NoM) system was subjected to normal ambient temperatures for October to February (there was no temperature control). In this system, a photoperiod typical of southern Portugal (37.0265, −7.84045) was regulated for the three months in question (October–February). In the "manipulated environment system", the temperature was controlled using a system of chillers and heaters to recreate the temperature conditions of December to April, which means a two-month anticipation. The photoperiod was regulated for the above-mentioned months (December–April).

Table 1. Photoperiod conditions (number of hours of light per day) and temperature (average monthly temperature) for each of the experimental situations (manipulated and non-manipulated).

	Non-Manipulated (NoM)		**Manipulated (M)**	
	Daylight (hours)	**Mean Temperature (°C)**	**Daylight (hours)**	**Mean Temperature (°C)**
October	10.75	19.2 (±1.4)	10.25	18 (±1.0)
November	10.2	16.0 (±1.3)	10.83	14.2 (±1.3)
December	9.66	14.9 (±1.3)	12	14.6 (±1.7)
January	9.95	13.9 (±0.9)	13.16	16.1 (±1.8)
February	10.6	15.4 (±0.5)	13.95	17.7 (±0.4)

2.2.2. Feeding

Two diets were used in this study. The natural diet (U) consisted of fresh macroalgae (*Ulva* spp.) produced in earthen ponds. The inert diet (If) consisted of pellets formulated and produced by the company SPAROS, R&D (Olhão, Portugal), specifically for sea urchins. The nutritional characteristics are described in Araújo et al. [18]. The sea urchins were fed once every 3–4 days based on a daily consumption for each urchin of 0.40 g of *Ulva* spp. and 0.15 g of inert food at the beginning of the trial. The amount of feed was adjusted every month based on the residual total biomass of each replicate.

2.3. Sampling

Biometric sampling was performed every month (5 sampling moments), measuring the total weight with a KERN PRS/PRJ precision and analytical balance of all sea urchins from the three replicates of each treatment (n = 25 each replicate). Five individuals were randomly selected from each treatment to collect gonads from after recording their total weight. The gonads were weighted and the gonadosomatic index was calculated using the following equation:

$$\text{Gonadosomatic Index (GSI)} = (\text{Gonad wet weight (g)})/(\text{Total wet weight (g)}) \times 100$$

Samples of each gonad were then placed in histological cassettes for later procedures and observation in the laboratory. The cassettes were maintained in 10% formaldehyde for 48 h for fixation. Next, they were transferred to 70% ethanol for storage until paraffin histology.

2.4. Histology

Slide sections (4 µm thick) were prepared using a tissue processor (Model Citadel 2000, Thermo Scientific, Nanjing, China) and a microtome (Model Jung RM 2035, Leica Instruments mb, Etzlar, Germany). Haematoxylin and eosin were used to stain slides using an automatic slide Stainer (Model Shandon Varistain 24-4, Thermo Scientific, Nanjing,

China). Images were visualized and photographed with an NDP View 2 and scanned with a Hamamatsu NanoZoomer (C13140-01). The different stages of maturation were classified according to Byrne's method [5].

2.5. Statistical Analysis

A statistical analysis was carried out to compare the gonadosomatic indices of the sea urchins subjected to the four experimental conditions, for both females and males, for each sampling time. For each point, normality was tested using the Shapiro–Wilk test and the equality of variances was tested using the Brown–Forsythe test. If positive, One Way ANOVA was carried out; if negative, the data were subjected to Kruskal–Wallis One Way Analysis of Variance on Ranks. If there were significant differences ($p > 0.001$), the data were subjected to All Pairwise Multiple Comparison procedures. Following ANOVA on Ranks, Dunn's Method was used. For One Way ANOVA, the Holm–Sidak method was used. Pairs of samples were considered statistically different when $p < 0.05$. The analyses were carried out using the SIGMAPLOT 14.0 software developed by Systat Software, Inc. (Santa Clara, CA, USA).

3. Results

3.1. Gonadosomatic Index

The variation in the GSI was analyzed considering the different treatments and the different sexes. Starting with the male individuals, all treatments showed an increase in this index over the 4 months of work (Figure 2). It also seems clear that animals of both sexes fed inert food always had a higher GSI. At each point, significant differences were seen in pairs of treatments ($p < 0.05$). In all cases of significant differences, it was found that sea urchins fed inert food had higher GSI values than those fed *Ulva* spp. In November, significant differences were found between sea urchins fed different diets under non-manipulated conditions (NoM U and NoM If). In December, these two treatments (NoM U and NoM If) maintained significant differences, and there were also differences between urchins fed different diets under manipulated conditions (M U and M If). In January, there were only differences between sea urchins in non-manipulated conditions fed inert food (NoM If) and those in manipulated conditions fed *Ulva* spp. (M U). In February, there was an increase in the differences between the treatments. In this case, there were significant differences between all the pairs of treatments where feeding was different.

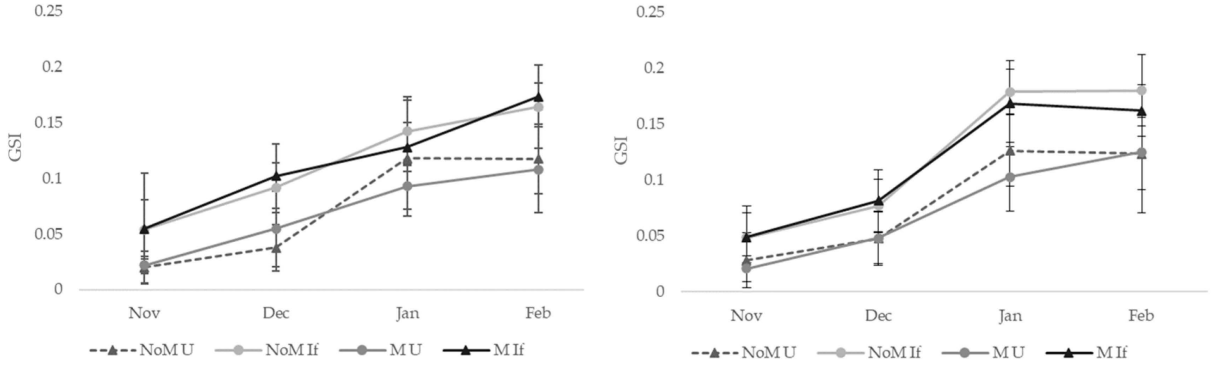

Figure 2. Evolution of the gonadosomatic index of male (**left**) and female (**right**) sea urchins (*Paracentrotus lividus*) under different conditions (NoM U—non-manipulated conditions, fed with *Ulva* spp.; NoM If—non-manipulated conditions, fed with formulated feeds; M U—manipulated conditions, fed with *Ulva* spp.; M If—manipulated conditions, fed with formulated feeds). Equal letters mean pairs of samples, with significant differences ($p < 0.05$) for each month.

In the case of females, the differences were not so obvious. In November and December, the GSI values for the four treatments were not statistically significant ($p = 0.281$ and $p = 0.078$, respectively). In the following months, the differences increased. In January,

there were significant differences in sea urchins under manipulated conditions fed with different diets (M U and M If). In February, the biggest difference was found in sea urchins in manipulated conditions fed with *Ulva* spp. (M U) and individuals in non-manipulated conditions fed with inert food (NoM If). As in the case of males, the differences were due to higher gonadosomatic index values in sea urchins fed with inert food (If).

3.2. Gonad Development

As with the GSI, gonad development was analyzed considering the condition of the test (manipulated and non-manipulated), the diet (*Ulva* spp. and inert food) and both sexes. Figures 3 and 4 describe each stage for males and females, respectively.

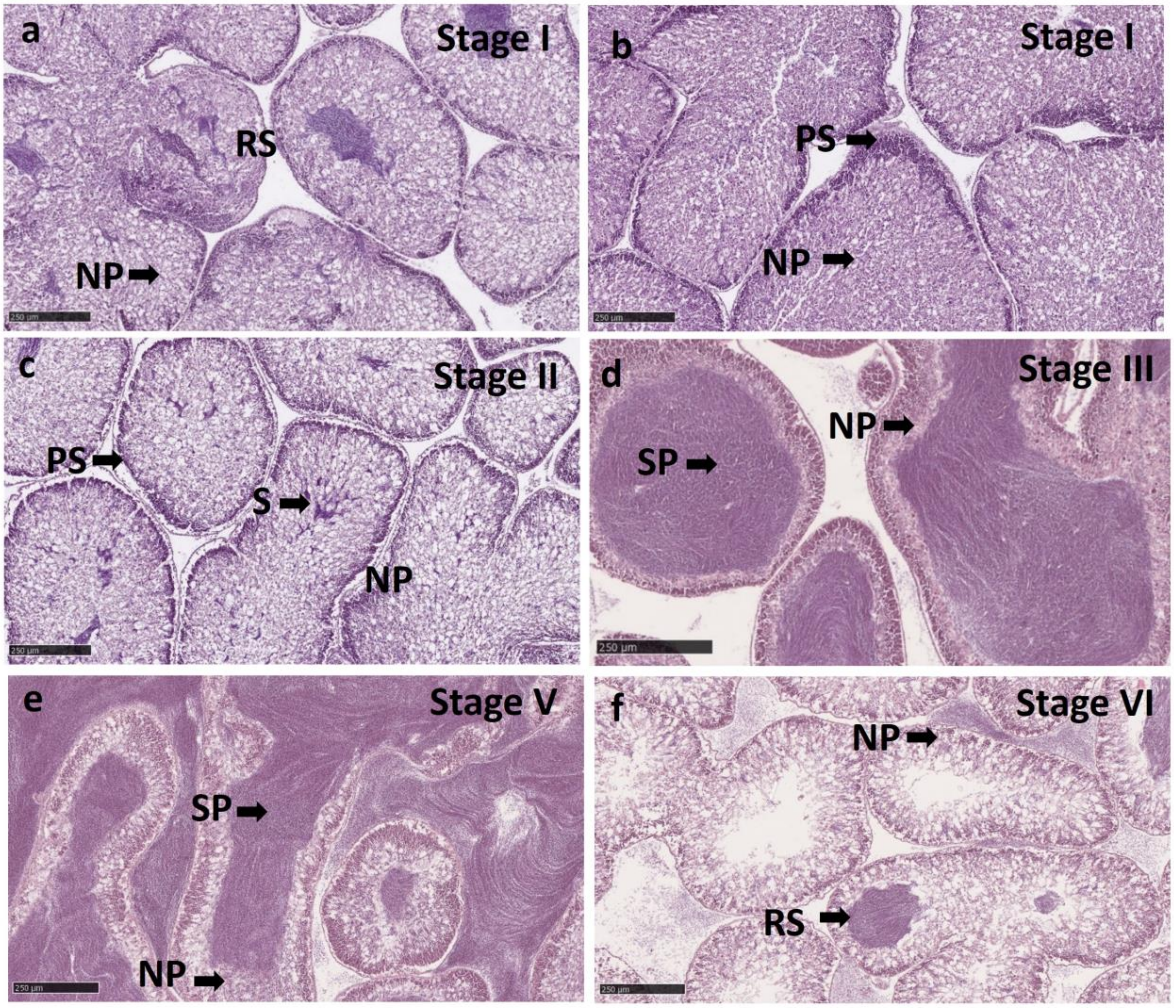

Figure 3. Histology sections of male gonads in *Paracentrotus lividus* at different stages through the trial: (**a**,**b**) Stage I: recovery stage; may have a restored male gonad with relict spermatozoa (RS) and parallel displays of primary spermatocytes along the ascinal wall (PS) and nutritive phagocytes (NPs). (**c**) Stage II: growing stage; displays nutritive phagocytes (NPs), primary spermatocytes (PSs) along the ascinal wall and columns of spermatocytes (Ss) heading toward the center. (**d**) Stage III: the central region contains spermatozoa (SP) and the peripheral region contains nutritive phagocytes (NPs). (**e**) Stage V: the partially spawned stage presents spaces that have been vacated by the spawned spermatozoa. (**f**) Stage VI: This stage presents large spaces devoid of content (arrowheads) with thin ascinal walls and nutritive phagocytes around the periphery. Relict spermatozoa can be found. Scale bars: 250 µm.

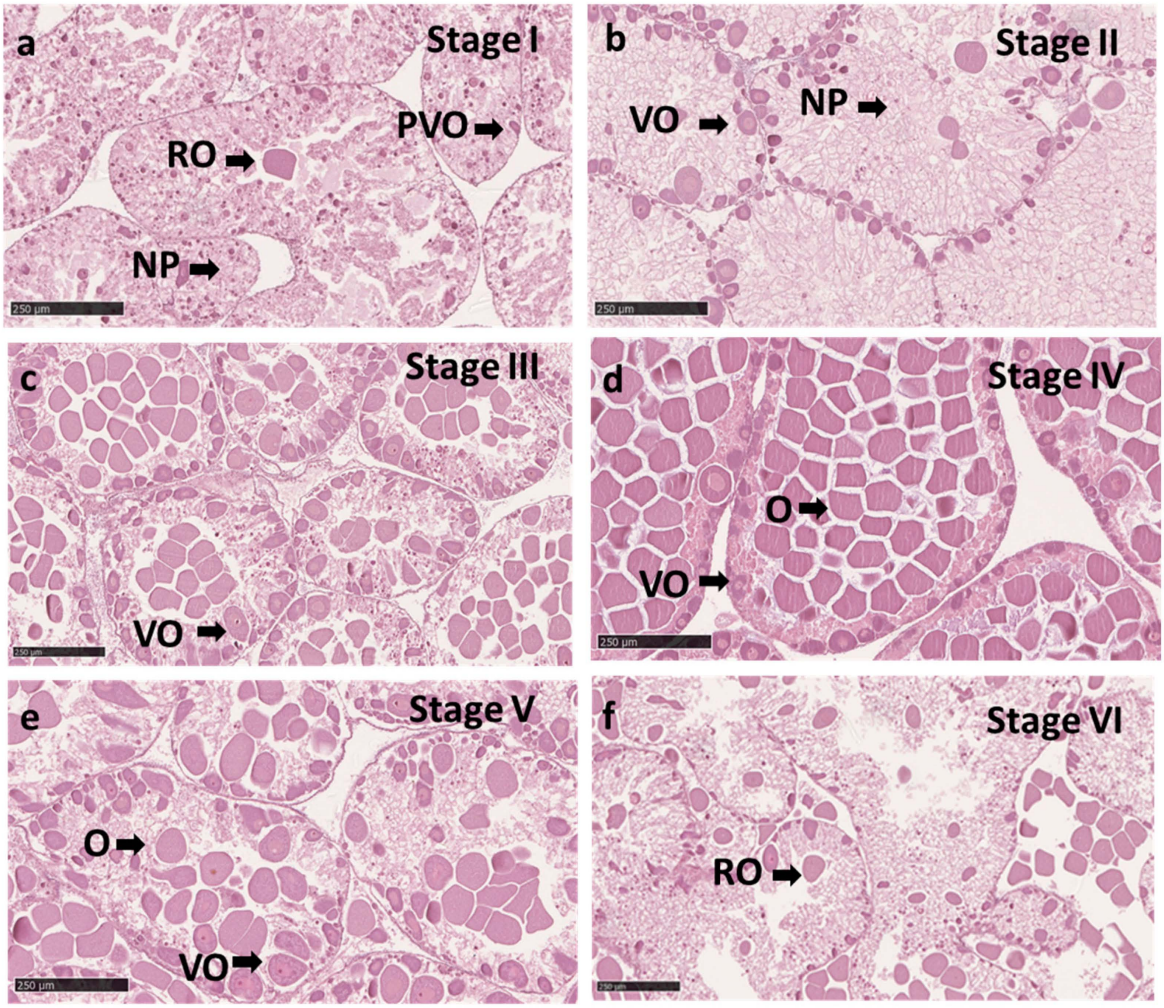

Figure 4. Histology sections of female gonads in *Paracentrotus lividus* onat different stages throughout the trial: (**a**) Stage I: recovery stage; ovary seems vacuole due to the formation of a mesh across the ascinus by nutritive phagocytes (NPs). During the lysis process, the ovary may contain relict ova (RO), which the phagocytes may absorb. Ovary presents previtellogenic oocytes (PVOs) along the acinal wall, which range in diameter from 5 to 30 µm. (**b**) Stage II: growing stage; represents the beginning of vitellogenesis. Vitellogenic oocytes (VOs) increase in size (10 to 50 µm diam). They are found connected on the ascinal wall, surrounded by nutritive phagocytes (NPs). (**c**) Stage III: premature stage; the vitellogeneses continue. The oocytes increase, ranging in size from 10 to 90 µm diameter, with an oval shape, and migrate toward the ascinus's center. (**d**) Stage IV: mature stage; the ovaries contain a dense population of oocytes (Os) that can grow up to 90 µm in size, and on the ascinal wall may exist some primary oocytes (POs) that range to 10 µm in size. (**e**) Stage V: partly spawned stage; the ovaries present space (L) emptied from the release ova. There are cases where oocytes are contained in the ovaries at all stages as mentioned in Stage III and Stage IV. As a result, the females from Stage V may seem to be starting to spawn. (**f**) Stage VI: In this stage, the ascinal wall of spent ovaries is thin and sometimes appears empty (L). Ovaries may be entirely devoid of oocytes or contain relict ova (RO). Reabsorption and phagocytosis are applied to the remaining ova and oocytes. Scale bars: 250 µm.

The characteristics of the gonads and gametophytes were used to classify and quantify each individual in their respective stages of maturity, and this information is shown in Figures 5 and 6

Figure 5 shows the evolution of the maturation stages of the gonads of female sea urchins over the four months of the trial, for the four treatments, keeping in mind that manipulation describes adjustments to temperature and photoperiod in relation to natural

environmental conditions. For all four treatments, there was a notable evolution over the study period, always reaching the last stage of development, Stage VI (spent stage). However, there were some differences in terms of the speed of development. The greatest differences were found between urchins kept in manipulated environmental conditions compared to non-manipulated conditions.

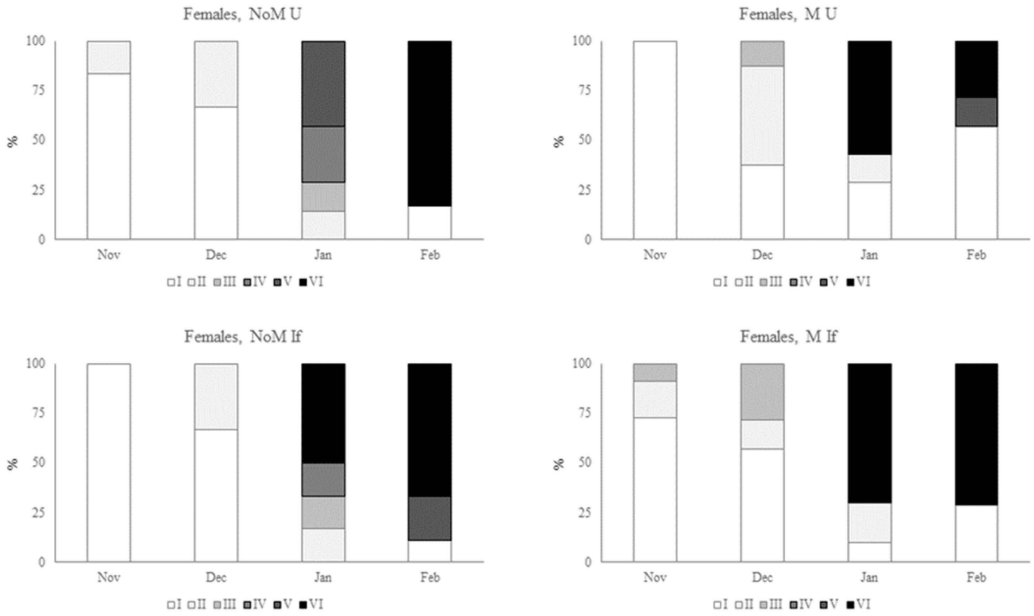

Figure 5. Development of the gonads of female sea urchins (*Paracentrotus lividus*) under different conditions (non-manipulated NoM and manipulated M) and different feedings (*Ulva* spp. U and inert feed If). States of development: recovery stage (I), growing stage (II), premature stage (III), mature stage (IV), partly spawned stage (V), spent stage (VI) (according to Byrne) [5].

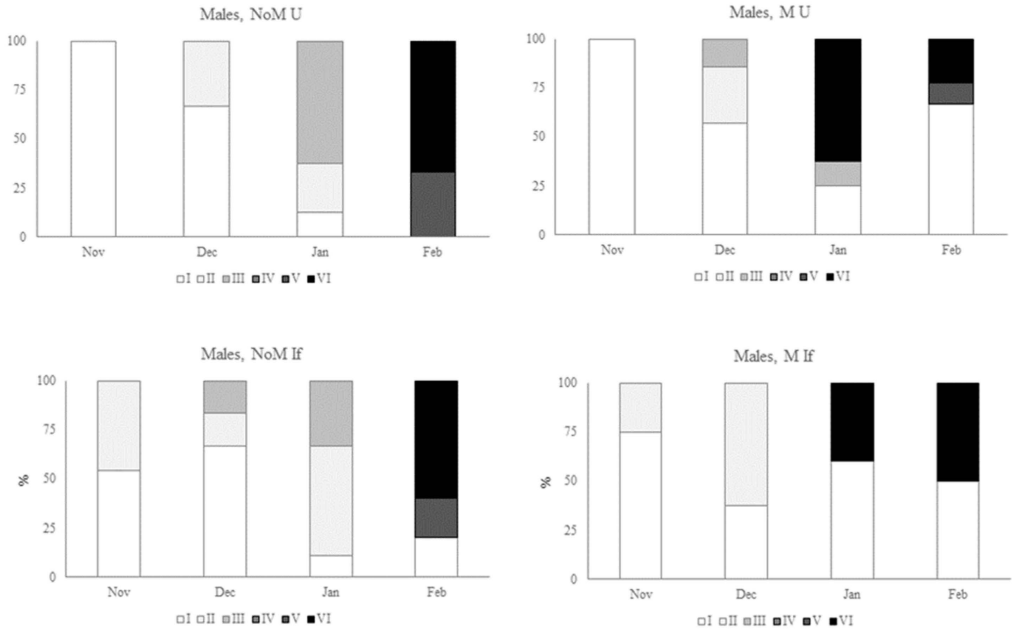

Figure 6. Development of the gonads of male sea urchins (*Paracentrotus lividus*) under different conditions (non-manipulated NoM and manipulated M) and different feedings (*Ulva* spp. U and inert feed If). States of development: recovery stage (I), growing stage (II), premature stage (III), mature stage (IV), partly spawned stage (V), spent stage (VI) (according to Byrne) [5].

When we compare the female sea urchins fed with *Ulva* spp. but under different environmental conditions, we see a more rapid evolution from November onwards. The following month (December), under manipulated conditions, there was a higher percentage of sea urchins in Stage II (growing stage), with some individuals already in Stage III (premature stage). In January, 57.1% of the sea urchins in manipulated conditions were already in Stage VI (spent stage), with no individuals in the same stage in non-manipulated conditions. This trend was also seen in females fed with inert feed. In December, in the manipulated conditions it was already possible to see individuals in Stages II and III, unlike the situation in the non-manipulated conditions, where all observed individuals were in Stage I. In January, the female urchins in non-manipulated conditions were already at an advanced stage of maturation, with a mixture of females in Stages II, III, and IV and some in Stage VI. For the same month, 70% of the individuals in the manipulated situation fed with the same inert feed were already in Stage VI. At the end of the trial, the percentage of individuals in Stage VI was practically the same for both situations (66.6% for NoM If and 71.4% for M If). Although less emphasized, differences were also observed between sea urchins fed with different diets under the same environmental conditions. In non-manipulated conditions, individuals in Stage VI were found in January when fed with inert feed, which was not the case with sea urchins fed *Ulva* spp. In manipulated conditions, there was also an earlier onset of maturation in females fed with inert feed. However, these differences dissolved in January.

As with the females, the greatest differences were seen between the male sea urchins kept in manipulated environmental conditions compared to non-manipulated conditions (Figure 6). In the sea urchins fed with *Ulva* spp., the stages of gonad maturation showed the greatest progression in December, with individuals already at Stage III (premature stage). In January, 63% of the sea urchins fed with algae under manipulated conditions were already at Stage VI (spent stage). In the same month, urchins fed with the same food, but without the manipulation of environmental conditions, still had no males in this stage. The following month, the males in the non-manipulated conditions reached the final stage (VI), with a large percentage (67%) of the manipulated ones already in the recovery stage (I). This situation is similar to that of the sea urchins fed with inert feed, where it can be seen that in January 40% of the males were already in Stage VI, and no individuals were observed in this stage in that month. When we compare the sea urchins under the same environmental conditions but with different feedings, we can also see some differences in terms of gonad maturation. In non-manipulated conditions, males started maturing earlier when fed inert food, but at the end of the trial the percentage of individuals in Stage VI was very similar. Under manipulated conditions, the evolution was apparently superior in the animals fed on algae.

When comparing male and female individuals, females mature earlier than males, and this is particularly relevant in non-manipulated environmental conditions.

4. Discussion

In aquaculture, a good understanding of the maturation of the gonads of cultivated species is necessary. In the case of sea urchins, this control is important not only for reproductive purposes but also for the commercialization process, considering that the gonads are the edible part of sea urchins. One of the strategies in aquaculture in general, and particularly in sea urchins, to control the quality of the gonads is to create formulated feeds that allow more nutrients and energy to be incorporated [19]. In the natural environment, the species *Paracentrotus lividus* has a reproductive cycle that varies with the location of its population. In Portugal, it is considered a species with annual reproduction, with a fairly long spawning period that begins in mid-spring and ends in mid-summer [12,20,21]. On the northern coast of Spain, spawning occurs between March and September, potentially happening at two distinct times during this period, depending on the environmental characteristics of each year [22]. On the coast of Morocco, a more extended spawning period is observed, between March and October [23]. At higher latitudes, such as in Ireland,

spawning begins later, in May and June, and ends in August or September [5]. In the Mediterranean, sea urchins exhibit a varied reproductive cycle, with a primary vigorous spawning in spring and a secondary, less intense spawning in October [14]. Given the variability and reproductive adaptation of this species to geographically distinct conditions, it is considered feasible to control maturation and reproduction. By understanding the factors that influence the reproductive cycle, it may be possible to manipulate farming conditions in aquaculture so that the gonads mature and reproduce at any time of the year. In this way, it may be possible for a commercial aquaculture farm to have several spawning events per year, thus increasing production.

According to the available literature, several factors influence maturation and the sea urchins' reproductive cycle in nature, with the most commonly cited being temperature, photoperiod and food availability [5,12,22–24]. This work was therefore designed taking into account the influence of these three factors. Due to technical impossibility, the environmental parameters temperature and photoperiod were not analyzed in isolation, but were merged together, and their joint action was considered in determining parameters of local seasonality. Therefore, two independent cultivation systems were developed to simulate the environmental conditions of two different seasonal periods. In the non-manipulated condition, the temperature and photoperiod conditions correspond to the natural conditions for the season. In the manipulated condition, the sea urchins were subjected to conditions that correspond to three months ahead of the actual month. Two different diets were used in these two conditions: a natural food (*Ulva* spp.) and a feed formulated for sea urchins. The choice of these two diets comes from previous knowledge of their potential influence on gonad development [18].

4.1. Gonadosomatic Index

The general results of this study reflect the natural evolution of the reproductive cycle of this species, in this case the period after resting until spawning. In all treatments, an increase in the gonadosomatic index was observed between November and January, followed by a downward trend in February. In general, a low GIS indicates gamete release, while a high GIS indicates gonad maturity and the building of energy reserves [21,25]. A higher GIS during the first few months of the year is indicative of growing during the winter and maturing (spring) stages of the gonad development cycle, when the gonads are filled with spermatozoa and eggs. The index declines in late spring or early summer, signaling the start of the spawning period [24,26].

The general results regarding the progression of the stages of gonad development also represent the natural tendency for this species. During the progression of gametogenesis, the number of germ cells increases, and nutrient reserves accumulate in the somatic cells, specifically the nutritive phagocytes, at the onset of this process. This is then visible through histological analysis of the gonads and an increase in the GSI. In this study, we were able to see differences in the development of the gonads between the four treatments. Regarding the GSI, the most significant differences were between sea urchins fed with different diets. Although the pattern of variation is very similar, the gonadosomatic index values are always higher for sea urchins fed with inert feed, with the differences being more significant in the last few months of the trial. Despite showing a similar pattern, the variation in males and females was slightly different, with a more significant increase in the first few months in the case of males, and then a greater increase in females from December onwards. The importance of feed in the GSI has already been verified in other similar studies. It is known that the abundance of food in the wild has a positive correlation with the size of sea urchin gonads [21,27–29]. The nutritional quality of the food will also play a major role in the constitution and development of the gonads. Candeias-Mendes et al. [9] found that sea urchins fed with maize and *Ulva* spp. had GSI values approximately three times higher than animals fed only algae, with a strong relationship between the fatty acid profile of the gonads and the feed provided. Lourenço et al. [30] concluded that a feed with a protein content of 30% DM (dry matter) and 21 mg P kJ^{-1} promotes nutrient utilization and gonad

growth in weight. Raposo et al. [13] tested three different diets and found that animals fed maize and spinach had higher GSI values, suggesting a direct relationship between lipid content and gonad size. Our study used two different diets in terms of presentation and nutritional characteristics: fresh *Ulva* spp. and a dry inert feed presented in the form of disc-shaped pellets. Analyses of its fatty acid profile showed that the inert food had a higher content of the three types of fatty acids: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA). The inert feed had much higher levels of 22:6 ω3 (DHA) than the macroalgae, due to the enrichment of microalgae biomass (*Schizochytrium*), and a high content of this fatty acid was then found in the gonads of the sea urchins fed with this feed, showing the close relationship between the food supplied and the gonads [18].

4.2. Gonad Maturity

The gonadosomatic index is a parameter that allows one to obtain information on the development of the gonads, but it is through histological analysis that is possible to observe the development of gametogenesis and, therefore, the state of maturation of the gonads. This study used the maturation scale proposed by Byrne [5], which has been widely used in studies of the reproductive cycle of the species *P. lividus* [12,22–24,31,32]. The development of the gonads observed in this study reflects the natural pattern of this species' reproductive cycle. In general, in November the vast majority of individuals were found to be in the recovery stage (Stage I), which translates into the presence of developing germ cells, primary oocytes and spermatocytes. In the following month, the differences between the treatments began to become more pronounced, revealing a tendency for the maturation of sea urchins to accelerate when exposed to the manipulation of temperature and photoperiod conditions, especially for the females. In the following month (the non-manipulated situation corresponds to January, while the manipulated conditions correspond to April), a strong evolution of the gonads was observed, which was particularly noticeable again in the females subjected to the manipulation of environmental conditions, where it was possible to observe a large percentage of individuals in Stage VI (spent stage). This stage corresponds to the post-spawning period, when the spent ovaries have thin ascinal walls and appear empty except for relict oocytes [5]. For the same month, most of the males were still in the lower stages of maturation. It is generally assumed that this species shows synchronous gonad maturation. Kahili et al. [31] provided an example where synchronization in the gametogenesis of males and females was verified, although several cases of some anticipation of females or males in terms of gonad maturation have been described. In Ouchene et al. [23], an earlier maturation of the males was observed, and it was also possible to see that in the last month of laying most of them were already in Stage VI. The same authors relate this difference to the environmental conditions that characterized that year's study. In the case of Machado et al. [12], the reproductive cycle of two populations in different locations on the Portuguese coast was characterized; a clear synchronism was observed in one population and an anticipation of females was observed in the other population, which shows that environmental conditions may indeed play a role in the synchronism of gametogenesis.

In the last month of the study, there were individuals in Stage VI in all the treatments, and it was also possible to observe that in the manipulated conditions there was already a significant percentage of animals in Stage I (recovery stage), corresponding to individuals who were already restarting a new gametogenic cycle.

Overall, we can see that the manipulation of environmental conditions (temperature and photoperiod) influenced accelerating gonad maturation. Under the manipulated conditions, the animals were subjected to a cooling of the water and a reduction in the photoperiod in the first month of the trial, in order to reproduce the normal conditions corresponding to the month of January. It is known that lowering the water temperature promotes the storage of nutrients, while increasing it is associated with the stimulation of gametogenesis [5,29]. On the other hand, in relation to photoperiod, it is known that reducing the duration of daily light stimulates nutrient storage, with the shortest days

of the year being associated with the onset of gametogenesis [24]. There are studies that have shown that subjecting sea urchins to darkness promoted a strong stimulus for gonad growth [15]. In this study, manipulated conditions were created in which there was an early decrease in the photoperiod and temperature in the animals under the manipulated conditions, with the minimum temperature and photoperiod values being recreated in the month of October. This manipulation then promoted the start of gametogenesis in these animals earlier than normal. In January, it was possible to observe animals already in Stage VI (spent phase) in the males and females in the manipulated situation (there were also some females in the non-manipulated condition fed inert food, but in smaller quantities). This month in the manipulated condition corresponds to April, a time of year when spawning begins in several Iberian sea urchin populations [12,22,24].

5. Conclusions

Controlling gonad maturation and reproduction is of great importance in the production and commercialization of the gonads. Through this work, it was possible to verify that by feeding with suitable inert formulated feeds and manipulating environmental conditions it is possible to create conditions that allow for spawning all year round and for marketable gonads to be obtained at different times of the year. Controlling the temperature and photoperiod seems to be a viable strategy for controlling gonad maturation. Providing an inert food with a high PUFA content, especially DHA, could optimize the gonadosomatic index, thus enhancing the value of sea urchins at the time of marketing. These results show that this species of sea urchin, in addition to its commercial potential, has plastic characteristics that allow it to adapt to production strategies that optimize its commercial yield.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) and the Portuguese legislation for Laboratory Animal Science (EU Directive 2010/63; Decreto-Lei n° 113/2013) and approved by IPMA's Animal Welfare and Ethics Body (ORBEA), (protocol code 009366 and date of approval 26 June 2023), overseen by the National Authority for the use of live animals, also known as the Directorate-General for Food and Veterinary (DGAV).

Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sun, J.; Chiang, F.S. Use and Exploitation of Sea Urchins. In *Echinoderm Aquaculture*; Brown, N.P., Eddy, S.D., Eds.; Wiley: New York, NY, USA, 2015; pp. 22–45.
2. Carboni, S.; Vignier, J.; Chinatore, M.; Tocher, D.; Migaud, H. Effects of dietary microalgae on growth, survival and fatty acidcomposition of sea urchin *Paracentrotus lividus* throughout larval development. *Aquaculture* **2012**, *324*, 250–258. [CrossRef]
3. Boudouresque, C.F.; Verlaque, M. Ecology of *Paracentrotus lividus*, in: Lawrence, J.M. (Ed.) Edible sea urchins: Biology and ecology. *Dev. Aquac. Fish. Sci.* **2001**, *32*, 177–216.
4. Régis, M.B. Analyses des fluctuations des indices physiologiques chez deux khinoides (*Paracentrotus lividus* (Lmk) et *Arbacia lixula* L.) du Golfe de Marseille. *Thkthys* **1979**, *9*, 167–181.
5. Byrne, M. 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. *Mar. Biol.* **1990**, *104*, 275–289. [CrossRef]

6. Barnes, D.; Crook, A. Quantifying behavioural determinants of the coastal European sea-urchin *Paracentrotus lividus*. *Mar. Biol.* **2001**, *138*, 1205–1212.
7. Cyrus, M.D.; Bolton, J.J.; De Wet, L.; Macey, B.M. The development of a formulated feed containing *Ulva* (Chlorophyta) to promote rapid growth and enhanced production of high quality roe in the sea urchin *Tripneustes gratilla* (Linnaeus). *Aquac. Res.* **2012**, *45*, 159–176. [CrossRef]
8. Mendes, A.; Araújo, J.; Soares, F.; Pousão Ferreira, P. Produção de larvas e juvenis de ouriços-do-mar (*Paracentrotus lividus*) na estação piloto de piscicultura de Olhão (EPPO). *Relat. Cient. Téc. IPMA* **2018**, *2*, 22.
9. Candeias-Mendes, A.; Araújo, J.; Santos, M.; Namora, M.; Soares, F.; Gomes, R.; Cardoso, C.; Afonso, C.; Bandarra, N.M.; Pousão-Ferreira, P. Growth, survival and fatty acids profile of sea urchins, *Paracentrotus lividus* juveniles fed with *Ulva* spp. and maize in aquaculture production. First results using G1 generation in Portugal. *J. Aquac. Mar. Biol.* **2020**, *9*, 208–214.
10. Santos, P.M.; Albano, P.; Raposo, A.; Ferreira, S.M.F.; Costa, J.L.; Pombo, A. The effect of temperature on somatic and gonad development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture* **2020**, *528*, 735487. [CrossRef]
11. Ciriminna, L.; Signa, G.; Vaccaro, A.M.; Messina, C.M.; Mazzola, A.; Vizzini, S. Formulation of a new sustainable feed from food industry discards for rearing the purple sea urchin *Paracentrotus lividus*. *Aquacult. Nutr.* **2020**, *26*, 1046–1057. [CrossRef]
12. Machado, I.; Moura, P.; Pereira, F.; Vasconcelos, P.; Gaspar, M.B. Reproductive cycle of the commercially harvested sea urchin (*Paracentrotus lividus*) along the western coast of Portugal. *Invertebr. Biol.* **2019**, *138*, 40–54. [CrossRef]
13. Raposo, A.; Ferreira, S.; Ramos, R.; Anjos, C.; Baptista, T.; Tecelão, C.; Santos, M.P.; Costa, J.; Pombo, A. Effect of three diets on the gametogenic development and fatty acid profile of *Paracentrotus lividus* (Lamarck, 1816) gonads. *Aquac. Res.* **2019**, *50*, 2023–2038. [CrossRef]
14. Vafidis, D.; Antoniadou, C.; Kyriakouli, K. Reproductive Cycle of the Edible Sea Urchin *Paracentrotus lividus* (Echinodermata: Echinoidae) in the Aegean Sea. *Water* **2019**, *11*, 1029. [CrossRef]
15. McCarron, E.; Burnell, G.; Kerry, J.; Mouzakitis, G. An experimental assessment on the effects of photoperiod treatments on the somatic and gonad growth of the juvenile European purple sea urchin *Paracentrotus lividus*. *Aqua. Res.* **2010**, *41*, 1072–1081.
16. Sellem, F.; Guillou, M. Reproductive biology of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats of northern Tunisia (south-east Mediterranean). *J. Mar. Biol. Ass.* **2007**, *87*, 763–767. [CrossRef]
17. Cyrus, M.; Bolton, J.; Scholtz, R.; Macey, B. The advantages of *Ulva* (Chlorophyta) as an additive in sea urchin formulated feeds: Effects on palatability, consumption and digestibility. *Aquac. Nutr.* **2014**, 21. [CrossRef]
18. Araújo, J.; Loureiro, P.; Candeias-Mendes, A.; Gamboa, A.; Bandarra, N.; Cardoso, C.; Soares, F.; Dias, J.; Pousão-Ferreira, P. The effect of a formulated feed on the body growth and gonads quality of purple sea urchin (*Paracentrotus lividus*) aquaculture produced. *J. Aquac. Mar. Biol.* **2023**, *12*, 11–18. [CrossRef]
19. Schlosser, S.C.; Lupatsch, I.; Lawrence, J.M.; Lawrence, A.L.; Shpigel, M. Protein and energy digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall *Aquac. Res.* **2005**, *36*, 972–982. [CrossRef]
20. Rocha, F.; Baião, L.F.; Moutinho, S.; Reis, B.; Oliveira, A.; Arenas, F.; Maia, M.R.G.; Fonseca, A.J.M.; Pintado, M.; Valente, L.M.P. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal. *Sci. Rep.* **2019**, *28*, 2994. [CrossRef]
21. Nicolau, L.; Vasconcelos, P.; Machado, I.; Pereira, F.; Moura, P.; Carvalho, A.N.; Gaspar, M.B. Morphometric relationships, relative growth and roe yield of the sea urchin (*Paracentrotus lividus*) from the Portuguese coast, *Reg. Stud. Mar. Sci.* **2022**, *52*, 102343.
22. González-Irusta, J.; Cerio, F.; Canteras, J.C. Reproductive cycle of the sea urchin *Paracentrotus lividus* in the Cantabrian Sea (northern Spain): Environmental effects. *J. Mar. Biol. Assoc. UK* **2010**, *90*, 699–709. [CrossRef]
23. Ouchene, H.; Boutgayout, H.; Hermas, J.; Benbani, A.; Oualid, J.A.; Elouizgani, H. Reproductive Cycle of Sea Urchin *Paracentrotus lividus* (Lamarck, 1816) from the South Coast of Morocco: Histology, Gonads Index, and Size at First Sexual Maturity. *Arab. J. Sci. Eng.* **2021**, *46*, 5393–5405. [CrossRef]
24. De la Uz, S.; Carrasco, J.F.; Rodríguez, C. Temporal variability of spawning in the sea urchin *Paracentrotus lividus* from northern Spain. *Reg. Stud. Mar. Sci.* **2018**, *23*, 2–7. [CrossRef]
25. Allain, J.Y. Structure des populations de *Paracentrotus lividus* (Lamarck) (Echinodermata, Echinoidea) soumises à la pêche sur les côtes Nord de Bretagne. *Rev. Trav. Inst. Pêch. Marit.* **1975**, *39*, 171–212.
26. Ouréns, R.; Fernández, L.; Freire, J. Geographic, population, and seasonal patterns in the reproductive parameters of the sea urchin *Paracentrotus lividus*. *Mar. Biol.* **2011**, *158*, 793–804. [CrossRef]
27. Guetaff, M.; San Martin, G.A. Étude de la variabilité de l'indice gonadique de l'oursin comestible *Paracentrotus lividus* (Echinodermata: Echinoidea) en Mediterranée nord-occidental. *Vie Milieu* **1995**, *45*, 129–137.
28. Fernandez, C.; Boudouresque, C. Phenotypic plasticity of *Paracentrotus lividus* (Echinodermata: Echinoidea) in a lagoonal environment. *Mar. Ecol. Prog.* **1997**, *152*, 145–154. [CrossRef]
29. Spirlet, C.; Grosjean, P.; Jangoux, M. Reproductive cycle of the echinoid *Paracentrotus lividus*. *Invertebr. Reprod. Dev.* **1998**, *34*, 69–81. [CrossRef]
30. Lourenço, S.; Cunha, B.; Raposo, A.; Neves, M.; Santos, P.M.; Gomes, A.S.; Tecelão, C.; Ferreira, S.M.F.; Baptista, T.; Gonçalves, S.C.; et al. Somatic growth and gonad development of *Paracentrotus lividus* (Lamarck, 1816) fed with diets of different ingredient sources. *Aquaculture* **2021**, *539*, 736589. [CrossRef]

31. Khaili, A.; Haroufi, O.; Bouzaidi, H.; Maroua, H.; Bouzoubaa, A.; Rharrass, A.; Essalmani, H. Reproductive cycle of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) from the Moroccan western Mediterranean Sea: Histology, gonad index and size at first sexual maturity. *EJABF* **2023**, *27*, 745–771.
32. Cirino, P.; Ciaravolo, M.; Paglialonga, A.; Toscano, A. Long-term maintenance of the sea urchin *Paracentrotus lividus* in culture. *Aquac. Res.* **2017**, *7*, 27–33. [CrossRef]