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Reproductive Conditioning of the Peruvian Scallop *Argopecten purpuratus* in Different Environments

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Abstract: Obtaining viable *Argopecten purpuratus* seeds faces challenges, especiallyci the unpredictability of the marine environment and high production costs in hatcheries. However, improving the method of "Broodstock Conditioning In Hatcheries" is key to ensure permanent seed supplies by minimizing the dependence on marine conditions and by maximizing economic viability in hatcheries. In an effort to overcome these barriers, broodstock were conditioned into two different environments: (a) Natural Environment: Natural marine conditions located in Bahía Inglesa, Atacama Region, Chile. (b) Hatchery: Laboratory conditions to achieve gonadal maturation, spawning induction, fertilization and larval development. The purpose of this research was to evaluate how the type of reproductive conditioning affects the reproductive potential and nutritional quality of the progeny. Both methods were successful at inducing the necessary maturity for reproduction, obtaining viable gametes and larvae. On the other hand, it was observed that in the natural environment, the oocytes and D larvae reached a greater size and nutritional value, being the most significant differences with ($p < 0.05$): the size of the D larvae reached figures of 95.8 ± 3.1 µm and 91.2 ± 2.7 µm in the environment and hatchery, respectively; the lipid content in dry mass was 25.2 ± 3.1 mg g^{-1} and 13.5 ± 1.9 mg g^{-1} for the natural environment and hatchery, respectively. Although quality indicators in hatcheries were slightly lower compared to the natural environment, the possibility of conditioning *A. purpuratus* broodstock independently of environmental variability highlights the importance of further optimizing broodstock conditioning aspects in hatcheries that would allow more predictable and sustainable production.

Keywords: reproductive conditioning; hatchery; broodstock; *A. purpuratus*

Key Contribution: This study makes a detailed contribution to the conditioning of *A. purpuratus* broodstock to optimize gonadal maturation and facilitate effective spawning, presenting a carefully designed technical strategy that aims to reach continuous improvement in reproduction and in the

nutritional quality of oocytes and D-larvae, which is a key factor for the success and sustainability of seed supply for the aquaculture of this species.

1. Introduction

The cultivation of *A. purpuratus* has been an important economic activity in the regions of Coquimbo and Atacama for more than 30 years [1,2]. Nevertheless, the development of this scallop has been subject to considerable production and commercial fluctuations, which have prevented its consolidation [3,4]. An aspect that has been a constant problem for this culture has been the variability of naturally captured juveniles (seeds), which has always been the main source of seeds used for the farmingo of *A. purpuratus* [5]. An attempt has been made to address this by using larval and juvenile crop systems under laboratory (hatchery) conditions [6,7]. However, for these systems to be viable, it is necessary to have sexually mature broodstock that can provide gametes of adequate quality for a successful seed production [8].

To obtain mature broodstock of this species, two modalities were used: (a) Broodstock were maintained under natural or environmental conditions (natural environment) until favorable environmental conditions, for adequate gametogenesis development, existed. (b) Broodstock were bred under laboratory conditions (hatchery) with temperature controlled at 15 ± 1 °C. Both methods were used to produce seeds of *A. purpuratus* for large-scale cultivation of this species [9]. The broodstock was provided with microalgae as a food source [10]. Microalgae such as *Isochrysis galbana* var (t-iso) and *Nannochloropsis oculata* were used, which are necessary for the sexual maturation of *A. purpuratus* broodstock, They were supplied for the hatchery [9]; these microalgae thrive in a wide range of temperatures, from 15°C to 30 °C [11], and improve the survival of the scallop larvae during in the early stages [12]. This scallop species inhabits semi-enclosed bays and is also cultivated there, close to upwelling areas, where the subsurface water rises and generates sudden changes in nutrients, temperature, and oxygen concentration levels [13–15]; thus, the variability and unpredictability of natural environmental conditions tend to be the greatest disadvantage of conditioning in the sea. In the case of laboratory conditioning, or the hatchery, the main difficulties are related to the high cost of microalgae production, temperature maintenance, and obtaining filtered and sterilized seawater for the process. Temperature and food availability are the most relevant environmental factors for the proper development of gametogenesis (cell proliferation and vitellogenesis) in bivalves [11,16].

Temperature is considered a crucial factor in the regulation of bivalve reproduction, and the seasonal changes it undergoes have often been correlated with gonadal growth [17]. In general, it has been argued that increased temperature, in the marine environment, accelerates the gonadal maturation process in bivalves, since observations of the reproductive cycles of several of these species indicate that the main spawning season occurs during the spring–summer period [15,17] and the reproduction of *A. purpuratus* is only viable at temperatures above 15 °C [18]. It has been found that under laboratory conditions, gonadal maturation is more appropriate under stable conditioning temperatures, where thermal stability would prevent the occurrence of partial spawning that delays the gonadal development process. In general, conditioning with a specific temperature regime must be analyzed in conjunction with other relevant factors of the process, as an individual analysis of isolated factors may lead to erroneous conclusions [17,19,20].

Regarding food availability [21], it was observed that the recovery of gonadal function was accelerated in *Ostrea edulis* broodstock and was also found that the larvae obtained from them were better developed when feeding levels were increased [22–24]. They also postulated that differences in gamete´s quality would depend on the environmental conditions to which the parents were exposed to. In bivalves, ingested food accumulates in the adductor muscle as energy reserve in the form of glycogen, a component that can be used as an energy source for the gametogenesis process [25,26]. In terms of food

quality, it has been observed that the number of eggs and larvae produced is directly related to the nutritional value of the food provided during the parental reproductive conditioning process [12,27]. Even if after parental food reserves have been depleted and the larvae begin to consume exogenous food, growth and survival are positively affected if gonadal maturation conditions are adequate. In this regard, oocyte size is a factor that has been considered by several authors as a valid indicator of progeny quality [25,28,29]. While Working with *O. edulis*, [21], it was found that maximum viability and survival in larval crops are directly related to the lipid content of the broodstock; for this reason, the diet is based on polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), this component is found in concentrations of 31. 31 ± 2.92% in the microalgae *N. oculata* [30] and eicosapentaenoic acid (EPA), which reaches 37.88 ± 0.66% in the microalgae *I. galbana* [31]. These microalgae complement each other and are used in aquaculture as an important source of nutrients to stimulate the gonadal maturation of broodstock.

Interestingly, in [32], a similar result was obtained from *Mercenaria mercenaria* and *Crassostrea virginica* larvae, where they found a high correlation between larval survival and the initial amount of lipids present in the oocytes. Consequently, lipids play a fundamental role both energetical and functionally, as they are not only the energy reserve source but serve as precursors of hormones and are also the main component of cell membranes [33,34].

Based on the information presented above, this study aimed to examine the quality of the offspring by using two methods of reproductive conditioning: natural (environment) and laboratory (hatchery) conditions. It is important to emphasize that the natural condition is characterized by its constant variability and it is unpredictability, although seed production does eventually occur. This contrasts sharply with the second method (laboratory), where factors such as feeding and temperature are more stable, providing a more regular environment for the continuous development and supply of seed. This sustainable approach contributes to the viability of *A. purpuratus* farming.

2. Materials and Methods

2.1. Obtaining Biological Material

A total of 300 *A. purpuratus* oysters were collected at the (CIC-UDA) Coastal Research Center at the University of Atacama marine concession, located in the "El Morro" sector, Bahía Inglesa, in the Atacama Region of Chile, at 27°8′13″ S latitude and 70°54′22″ W longitude, They were transferred to the (CIC-UDA) hatchery, located at 27°8′11″ S latitude and 70°54′18″ W longitude. The oysters were kept in tanks with circulating seawater to reduce the stress caused by transportation.

2.2. Experimental Design

To evaluate the influence of the two different environments on the reproductive capacity of adult *A. purpuratus* and the nutritional quality of the offspring, 180 immature broodstock with an average valve length of 8 ± 1 cm were selected from the biological material previously described. The broodstock were evenly distributed into two treatments: (environment) gonadal maturation in the natural marine environment, in suspended farms such as lantern nets; (hatchery) conditioning in farming tanks in a controlled environment, called as hatchery. Each treatment was replicated three times to estimate outcomes reliably.

2.2.1. Natural Maturation of *A. purpuratus* Broodstock in Marine Conditions

In this treatment (environment), 90 immature broodstock of *A. purpuratus* were selected for gonadal maturation. Coming from the crop in three lantern nets at a rate of 30 individuals per lantern net, each of which represented a replica of the trial. The nets were then submerged under a depth of 5 m below the ocean surface to facilitate optimal exposure of the broodstock to the marine environment. During this stage, the gonadal maturation process was observed every two weeks by SCUBA diving professionals at 5 m in depth (Figure 1).

In the last phase, the sexually mature broodstock of *A. purpuratus* were placed, temporarily, in ponds of 7.2 m³ capacity until their spawning and fertilization were achieved; after this, embryonic development was carried out in tanks until larva D was obtained to estimate reproductive and production indicators.

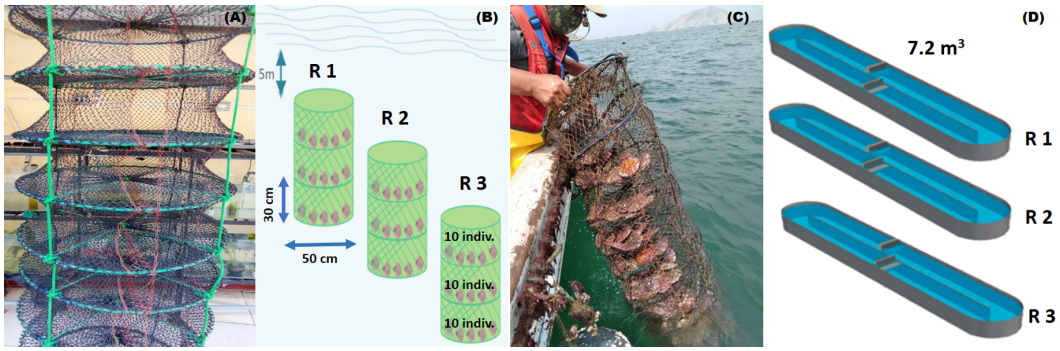

Figure 1. (**A**) Photograph of a lantern-type net where immature broodstock of *A. purpuratus* were placed for gonadal maturation, (**B**) diagram showing three replicates of the treatment (environment) organized in three divisions, in each division, a density of 10 broodstock was placed due to the reduced dimensions of the lantern net of 50 cm in diameter and 30 cm high, (**C**) photograph showing the collection of a lantern net containing mature broodstock, which were transferred to the hatchery of the (CIC-UDA), (**D**) figure showing three 7.2 m³ farming tanks in which the reproduction of scallop was carried out. These tanks were operated by using the recirculatory aquaculture system (RAS).

2.2.2. Conditioning of Broodstock in the Hatchery

In a previous phase, to start the treatment (hatchery) and to feed the broodstock of *A. purpuratus* in the hatchery, the intensive growing of two varieties of marine microalgae was carried out: *I. galbana* (Tiso) and *N. oculata*. Two fiberglass tanks of 12 m in length, 2 m in width, and 0.5 m in useful height, and with a capacity of 9.6 m³ were installed to ensure the proper circulation of seawater, There was also an agitation system installed, consisting of a vane driven by a three-phase motor was installed, generating a rotating flow of water at a speed of 1.57 rad/s. It is also important to note that the operating water level was 0.4 m. Keeping the water temperature constant at 15 ± 1 °C (Figure 2).

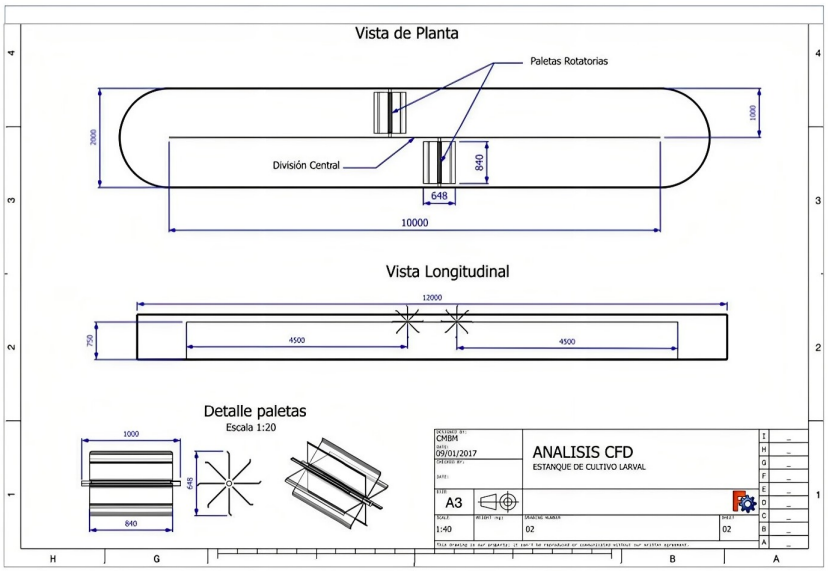

Figure 2. Details of the size of the tank used for the cultivation of microalgae, and the configuration of the propeller paddle used in the cultivation of live feed for the broodstock of *A. purpuratus*.

To carry out the treatment (hatchery), the remaining 90 immature broodstock of *A. purpuratus* from the cultivation lines of the marine concession (CIC-UDA) were conditioned in three fiberglass tanks of 0.3 m³ capacity. These tanks were installed in the hatchery of the (CIC-UDA), whose dimensions were 1.2 m long, 0.6 m wide and 0.6 m high. Each contained thirty scallops and represented a replica. In these tanks, to achieve gonadal maturation, continuous aeration was ensured, and a complete water change was carried out, including cleaning every 48 h, so it was a static water management system, maintaining a constant water temperature at 15 ± 1 °C thanks to a 24,000 BTU air conditioning system (Figure 3).

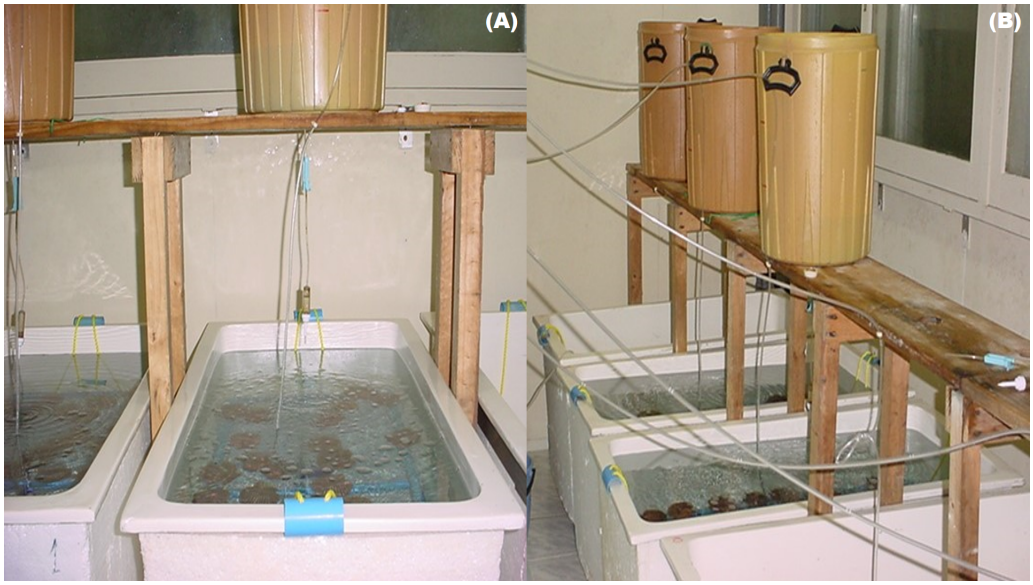

Figure 3. (**A**) Photo of tanks used to condition immature broodstock of *A. purpuratus* for gonadal maturation. They were rectangular to facilitate feed distribution and improve oxygen exchange, (**B**) Photo of the distribution of the culture tanks of the static system.

In a later phase, the gonadally mature broodstock were migrated to three fiberglass crops tanks of 7.2 m³ capacity, 12 m long, 2 m wide, 0.75 m high, each tank representing a replica that served for broodstock spawning, oocyte fertilization, embryogenesis, and obtaining larva D of *A. purpuratus*. The pond was equipped with 2 mechanical water propulsion systems (propeller vanes); the rotation speed of the vanes was set at 1.57 rad/s, and the fluid level in the pond was 0.3 m high. In addition, a heater was installed to regulate the temperature in a range of 18 ± 2 °C; this equipment had a power of 6 kW, voltage of 220 V, current of 16 A, three-phase power, seawater-proof housing, electronic panel for temperature range, temperature sensors for upper and lower limit to prevent overheating or cooling of the water. The culture system model was the Recirculatory Aquaculture System (RAS), maintaining average velocities from 0.02 m/s near the walls to 0.18 m/s near the pallet rotation´s sector. The average flow within the pond was 307 m³/h (Figure 4).

The three phases of hatchery conditioning are summarized in the following diagram, where the first part of the procedure of microalgae farming is represented, the second phase consists of the treatment (hatchery) through conditioning of immature broodstock of *A. purpuratus* for sexual maturation. And the third phase consists of induction to spawn of mature broodstock, fertilization of oocytes, embryonic and larval development, and to measure the reproductive capacity and nutritional quality of the progeny (Figure 5).

2.2.3. Spawning, Fertilization, Early Larval Development and Sampling of Treatments

After 50 days of treatment, 5 mature broodstock (n = 5) of each replicate were sampled to determine gonadal maturity by using the following reproductive parameters:

- The gonadosomatic index (GSI) was calculated by using the following formula: GSI = (Gonad Weight/Total Body Weight) × 100;

- Percentage of spawning assessed using the fertility calculation method: Spawning% = (N° of fertilized eggs/N° of spawning broodstock)
- The number of oocytes per individual was counted in a Sedgewick-Rafter counting chamber: N° of oocytes = (N° of oocyte cells counted/Area of the counting chamber)×gram of gonadal tissue. According to the protocol used by [35]

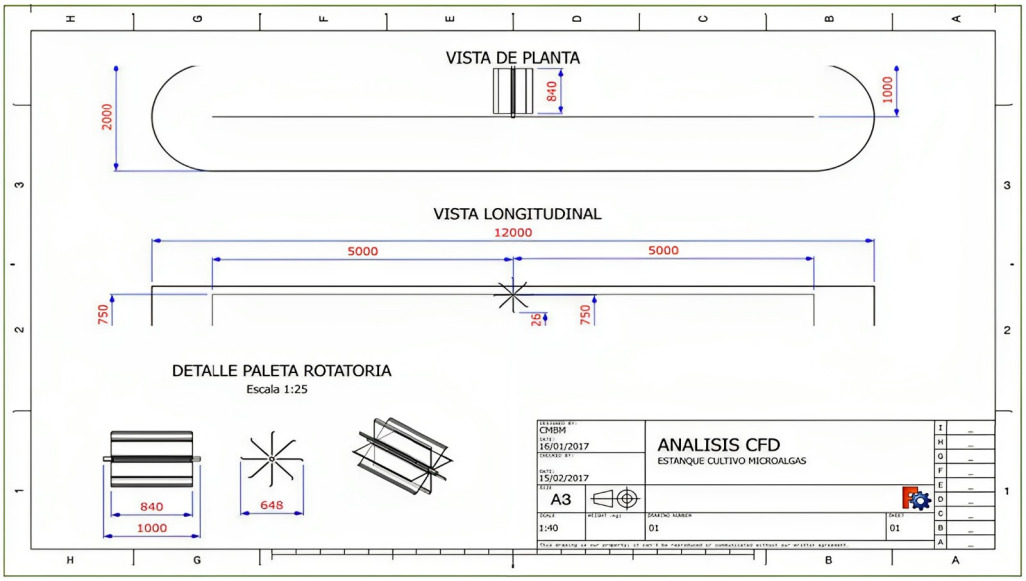

Figure 4. Design of the culture tanks adapted for *A. purpuratus* mature broodstock. In this controlled environment, the process of spawning, fertilization of oocytes, embryonic development, and obtaining D. larvae was successfully accomplished.

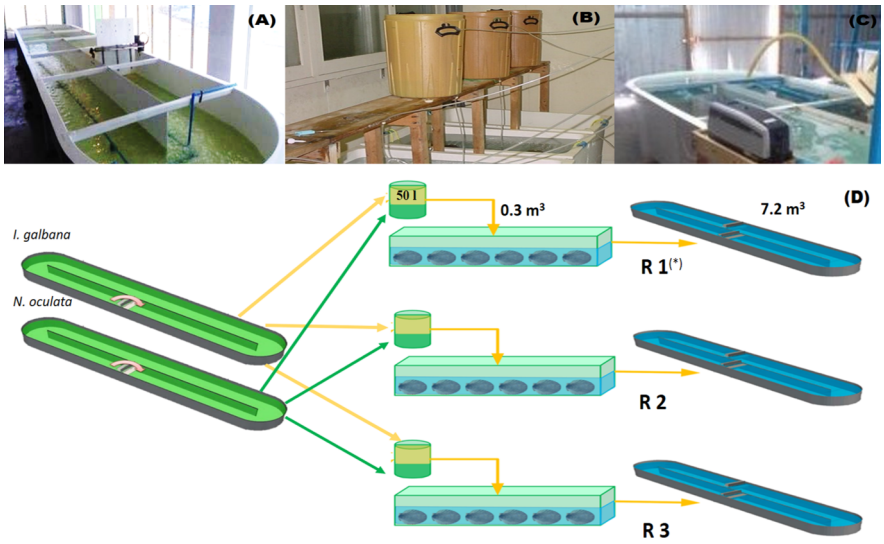

Figure 5. (**A**) Photo of the production of microalgae for broodstock feed, (**B**) photo conditioning of immature broodstock in hatchery, (**C**) spawning photo tank, oocyte/ embryo fertilization and larval development D, (**D**) diagram of the phases of the conditioning treatment of the broodstock in the hatchery (hatchery),where (*) is the number of treatment replicates.

Once the sexual maturation was verified, the broodstock of the two treatments were placed into six cultive tanks with a capacity of 7.2 m³ (Figure 4), where controlled spawning was induced in the hatchery, increasing the water temperature gradually until it reached 18 °C, triggering the spawning and fertilization reflex the eggs. After the reproduction

process was completed, the broodstock of *A. purpuratus* were removed from the tanks. The oocytes were maintained with light aeration and were not fed for 48 h, until they reached the straight hinge stage of larval development D larvae. Subsequently, the water was homogenized, and 20 mL were collected from each of the tanks to determine the following production parameters:

- Percentage of fertilized oocytes, which were counted with the help of a 1 mL (50 × 20 × 1 mm) Sedgewick-Rafter counting camera using a Leica MZ10 stereoscope. The percentage of fertilized eggs was calculated with the following formula:
  Percentage of Fertilized Eggs = (N° of Fertilized Eggs/(N° of Fertilized Eggs + N° of Unfertilized Eggs) × 100
- Larval survival (a). The survival of the D larvae of *A. purpuratus* was determined 48 h after fertilization by taking samples of 20 mL from each tank and using the volumetric method: Larval survival = (N° of surviving larvae/N° of initial larvae) × 100

2.3. Feeding of the Broodstock

The microalgae diet was fed to the broodstock of *A. purpuratus* in the form of living food, consisting of a 50:50 mixed diet by the number of *I. galbana* (T-iso) cells and *N. oculata* cells, using the Neubauer chamber [36,37] through an automatic drip system. The amount of food supplied was equivalent to 6% of the scallop's body weight (shell plus flesh) [17]. The Maintenance of the broodstock at the hatchery ended when more than 80% of the scallops had spawned.

2.4. Size Measurements and Survival

The fertilized eggs and 48 h old D larvae from the two reproductive conditioning treatments were measured ($n = 3$)/They replicate using the Leica ICC50 W microscope with LAS EZ 3.1.0 imaging software. Oocyte diameter and anteroposterior length of D larvae were measured. The D larvae were counted and their survival was determined according to the number of fertilized eggs from each treatment distributed in each 7.2 m^3 incubation tank.

2.5. Proximal Biochemical Composition

The oocytes and straight-hinge larvae ("D" larvae) were analyzed to determine the lipid [38], protein [39], and carbohydrate [40] contents.

2.6. Statistical Analyses

For the statistical analysis, homoscedasticity and normality were established, and later a completely randomized design was applied, establishing two treatments, with three replicates, consisting of the cultivation of *A. purpuratus* broodstock in two conditions: natural (environment) and laboratory-controlled (hatchery). The variables studied were the sexual maturation of *A. purpuratus*; the following were defined as response variables: spawning, number of oocytes/embryo, fertilized eggs, larval size, survival, and progeny quality (biochemical composition of larvae). All evaluated parameters were compared between conditioning treatments by using Students' *t*-test.

3. Results

3.1. Development of Gonadal Maturation

After 50 days of conditioning, both experimental groups indicated an increase in their GSI during the conditioning period. The values obtained in both treatments reflected no significant difference at the end of the process $p < 0.05$, (Table 1).

Table 1. Gonadosomatic index (GSI), percentage of spawning, and fecundity index (expressed as the average number of released oocytes per scallop) of *Argopecten purpuratus* held in two different conditions.

Treatments	GSI	Spawning (%)	Number of Oocytes/ Ind.
Environment	27 ± 2.9	88	1,654,343
Hatchery	25 ± 2.5	82	1,534,576

Each value is a mean ± SD (n = 5).

3.2. Oocyte Evaluation

The size of the oocytes obtained from the spawning of broodstock conditioned in the natural environment (Environment treatment) was significantly greater than those obtained from the broodstock conditioned in the laboratory (Hatchery treatment) ($p < 0.05$, Table 2). Despite this difference in size, the fertilization percentage did not present significant differences ($p > 0.05$, Table 2).

Table 2. Size and fertilization success of oocytes from *Argopecten purpuratus* held in two different conditions.

Treatments	Fertilized Eggs (%)	Oocyte Size (µm)
Environment	93.2 ± 3.3	65.9 ± 1.7 *
Hatchery	91.7 ± 2.8	61.4 ± 1.2

Each value is a mean ± SD (n = 3). * indicates significantly different values (t-test, $p < 0.05$).

Regarding the biochemical composition of the oocytes, the lipid content was significantly higher in those obtained from broodstock conditioned in the natural environment, and there were no significant differences with ($p < 0.05$) in the protein and carbohydrate contents (Table 3).

Table 3. Biochemical components of oocytes from *Argopecten purpuratus* held in two different conditions.

Treatments	Proteins (mg g^{-1} Dry Mass)	Lipids (mg g^{-1} Dry Mass)	Carbohydrates (mg g^{-1} Dry Mass)
Environment	356.7 ± 93.8	98.9 ± 8.3 *	39.4 ± 6.3
Hatchery	342.5 ± 85.7	41.6 ± 6.7	36.7 ± 7.1

Each value is a mean ± SD (n = 3). * indicates significantly different values (t-test, $p < 0.05$).

3.3. Straight-Hinge Larvae ("D" Larvae) Evaluation

The size of D larvae from the spawning of broodstock conditioned in the natural environment (environment treatment) was significantly larger than those obtained from broodstock conditioned in the laboratory (hatchery treatment) ($p < 0.05$, Table 4). Survival showed no significant differences between both experimental groups $p > 0.05$ (Table 4).

Table 4. Size and survival of straight-hinge larvae from *Argopecten purpuratus* held in two different conditions.

Treatments	Larval Size (µm)	Larval Survival (%)
Environment	95.8 ± 3.1 *	52.1 ± 2.5
Hatchery	91.2 ± 2.7	49.5 ± 3.3

Each value is a mean ± SD (n= 3). * indicates significantly different values (t-test, $p < 0.05$)

In relation to the biochemical composition of D larvae, the lipid and protein contents were significantly higher in those from broodstock conditioned in the natural environment

($p < 0.05$, Table 5). There were no significant differences in the carbohydrate content $p > 0.05$, (Table 5).

Table 5. Biochemical components of oocytes from *Argopecten purpuratus* held in two different conditions.

Treatments	Proteins (mg g^{-1} Dry Mass)	Lipids (mg g^{-1} Dry Mass)	Carbohydrates (mg g^{-1} Dry Mass)
Environment	256.5 ± 21.1*	25.2 ± 3.1 *	13.1 ± 3.3
Hatchery	198.8 ± 17.4	13.5 ± 1.9	11.3 ± 3.7

Each value is a mean ± SD ($n = 3$). * indicates significantly different values (t-test, $p < 0.05$).

3.4. Breeding Stock Conditioning in Hatchery

In the hatchery (hatchery), three tanks of 300 L each were installed to create an environment under controlled conditions. Inside these tanks, the broodstock were provided with food using microalgae obtained from 50 L plastic containers. These containers contained cultures of *I. galbana* and *N. oculata*, and constant aeration was maintained (Figure 1).

4. Discussion

Both treatments used for reproductive conditioning of *A. purpuratus* proved to be viable. However, in regions where the temperature drops below 15 °C, it is essential to carry out reproduction under laboratory conditions (hatchery). This helps to reduce the uncertainties associated with natural conditions and ensures a steady supply of seed. It is important to note that the second condition (hatchery) must be carefully calibrated to replicate key aspects of the natural habitat conducive to reproduction. This includes the provision of appropriate microalgal diets rich in essential fatty acids such as polyunsaturated fatty acids (HUFA): C20:5n3 (EPA), C22:6n3 (DHA) and C22:5n6 (DPA). These fatty acids are abundant in different microalgae species, such as *I. galbana*, with concentrations of (EPA) at 37.38 ± 0.66% and 26.47 ± 0.55%, according to [31] and [12], respectively. Similarly, *N. oculata* presents (EPA) levels of 30.77 ± 1.19%, according to [12], and (DHA) levels that reach up to 31.31 ± 2.92%, according to [30]. It is important to highlight that (DHA) is crucial, serving as a precursor for the synthesis of prostaglandins, which plays a fundamental role in processes such as gametogenesis, vitellogenesis, and spawning [41]

In this study, by feeding the broodstock with the microalgae *I. galbana* and *N. oculata*, spawning rates of 88% and 82% were achieved in the natural environment and in hatchery conditions, respectively. Similar results were obtained with [42]: on a high-protein diet, scallops achieved 83.9% reproductive efficiency; on a standard diet, this dropped to 62.6%, and a low-protein diet resulted in suboptimal growth and reproductive performance. In contrast to the lower spawning percentage, [17] achieved 70% under similar laboratory hatchery conditions, maintaining a stable temperature of 15 °C and providing a mixed diet of microalgae, including *I. galbana Chaetoceros gracilis*. Other researchers, such as [43,44], fed broodstock of *A. purpuratus* by using three different treatments. The first diet consisted of a mixture of microalgae species, including *Isochrysis galbana (T-Iso clone), Tetraselmis suecica, Pavlova lutheri and Chaetoceros gracilis*. The second was microalgae supplemented with an emulsion rich in 22:6n-3 (DHA), which resulted in spawning occurring 5 to 7 h earlier than those fed only by microalgae or microalgae supplemented with EPA. The third treatment consisted of microalgae supplemented with 20:5n-3 (EPA), which resulted in a 2% higher lipid content found in the oocytes than in the previous treatments, and *Nannochloropsis* sp. [45]. It appears that gonadal maturation responses are linked to the lipid and protein content of the diet of the purple scallop *A. purpuratus* [46].

Similarly, for oocytes, a larger diameter was observed in the natural condition (environment), indicating a positive correlation between oocyte diameter and subsequent larval viability [12]. Even an increase in oocyte diameter can imply an increase in yolk content [19]. Therefore, the larger oocyte size observed in the natural state is associated with a high accumulation of energy reserves, especially lipids [38,43,46,47], such as polyunsaturated fatty acids (HUFA), which are part of the matrix and cell membranes [10,48]. In

this study, significant differences were found in the lipid content of the oocytes with values of 98.9 ± 8.3 mg g^{-1} dry mass and 41.6 ± 6.7 mg g^{-1} dry mass in the natural (environment) and (hatchery) conditions, respectively. In contrast, proteins and carbohydrates showed no significant differences, suggesting that lipids have a significant effect on fertilization and oocyte survival. This conclusion is supported by the findings of [49], who indicated that lipids contributed the greatest percentage (46.7%) of the energy required for embryogenesis, followed by proteins and carbohydrates, which contributed 43.5% and 9.8%, respectively. A diet rich in a variety of microalgae, particularly diatoms and green algae, is essential to produce high-quality oocytes and to improve the reproductive performance of scallops in hatcheries [50]. Regarding the size of "D" larvae, there were significant differences between the two treatments —natural environment and hatchery conditions— with sizes reaching 95.8 ± 3.1 µm and 91.2 ± 2.7 µm, respectively. In another similar work [51], in which the bivalve *Gari solida* was cultivated in a hatchery, the D-type larvae of the bivalve reached a size of 78 ± 4.7 µm, depending exclusively on microalgae. The ability of phytoplankton to accumulate endogenous reserves in "D" larvae depends on the quality and quantity of proteins, which are essential for larval development [19]. Other authors indicate that lipids, proteins, and carbohydrates contribute up to 47.6%, 44.9%, and 7.5%, respectively, of the energy expended during larval shell formation in the prodissoconcha I phase of the species *Patinopecten yessoensis* [49,52].

As a result, early embryonic and larval development requires a significant amount of lipids and proteins that are rapidly consumed during the formation of new larval structures such as the larval shell and velum [10,29]. In addition to being the primary source of energy during the early stages of development, lipids provide essential polyunsaturated fatty acids that are crucial for the formation of cell membranes [29,45,47]. Therefore, a higher content of lipids stored in the oocytes would increase the developmental rates of the early larvae [10,53], explaining the larger size achieved by the "D" larvae from the naturally conditioned broodstock [45]. Demonstrating that the diet consumed by oysters in the natural environment during reproductive conditioning provides high levels of polyunsaturated fatty acids, suggesting that the resulting larvae have cell membranes with elevated levels of EPA (22:6 n-3), positively influencing their development and survival. However, these contributions would depend on the quality and availability of certain phytoplankton species containing high concentrations of polyunsaturated fatty acids [54], whose presence depends on the prevailing environmental conditions, which are highly variable in upwelling areas [13,14,31].

During the study period, the environmental conditions in the Bahía Inglesa were favorable for the reproductive process of *A. purpuratus*, with recorded phytoplankton blooms that enhanced the conditioning process. However, these conditions are not always present, which limits the availability of high-quality broodstock. Therefore, it is necessary to resort to conditioning of broodstock under laboratory conditions (hatchery) because of the success of the spawning events and the high survival rate of the larvae at 48 h of development, and like that demonstrating the feasibility of conditioning under controlled conditions. Nevertheless, significant advances could be made in this process by identifying native microalgae present in marine phytoplankton with high levels of lipids (polyunsaturated fatty acids) and proteins that can be cultivated in the hatchery to replace or supplement commercial microalgae diets, such as (*I. galbana* var T-iso, *Chaetoceros calcitrans, N. oculata, Pavlova lutheri*) diet. Ultimately, these improvements will increase production and economic benefits for mariculturists [8]. Furthermore, this practice will promote sustainability by reducing dependence on natural resources and mitigating the environmental impacts associated with overexploitation of marine resources [14].

5. Conclusions

This study demonstrates the effectiveness of broodstock conditioning for *A. purpuratus* seed production, both under natural marine (environment) and (hatchery) conditions. Nevertheless, certain indicators of oocyte (size and lipid content) and larval (size, lipid, and

protein content) quality were found to be lower in laboratory-conditioned broodstock in the hatchery than in the natural conditions. However, the successful spawning and robust survival of 'D' larvae at 48 h of development underlines the feasibility of conditioning under controlled conditions. This opens up the possibility of becoming less dependent on the vagaries of the marine conditions (environment) and represents a significant step towards sustainable *A. purpuratus* seed production.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to the following reasons: The main contribution of this research is to develop a controlled environment to facilitate the successful reproduction of the mollusk *A. purpuratus*. This breakthrough boosts the development of sustainable invertebrate aquaculture and, in perspective, promotes future food security. The research also focuses on marine invertebrates belonging to one mollusk species. These organisms, lacking a central nervous system and exhibiting rudimentary behavioral responses, have limited cognitive abilities. Importantly, the methodology employed in our research has been carefully designed to minimize any potential impact on the welfare of the organisms studied. Our approach focuses on practices that ensure maximum respect for animals while maintaining the integrity and health of the individuals involved. Given the context of our study and the nature of the organisms under investigation, we strongly believe that the exemption from ethical approval is relevant and by accepted ethical guidelines in the scientific community.

Data Availability Statement: The data presented in this study are available upon request to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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