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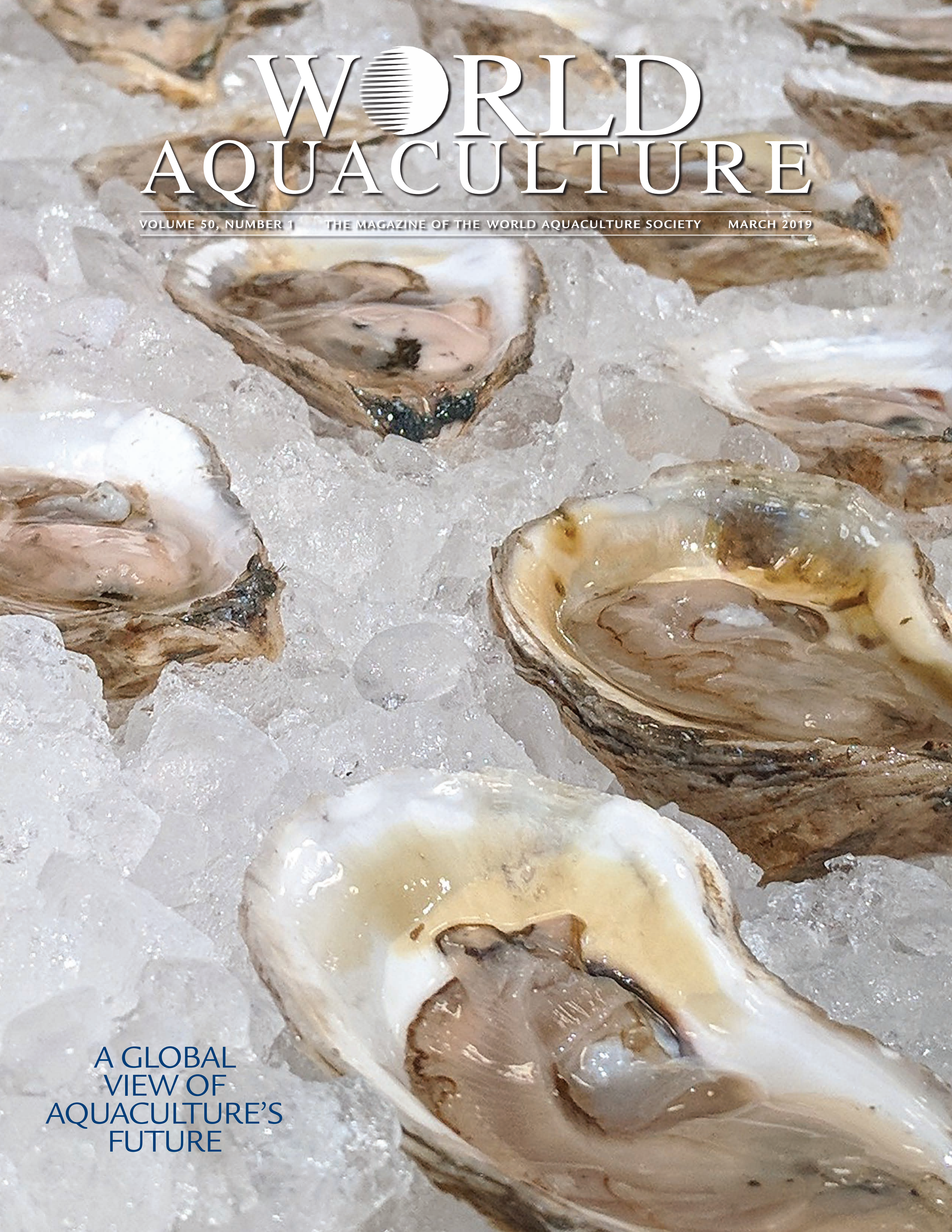

A GLOBAL VIEW OF AQUACULTURE'S FUTURE

Production of Purple Sea Urchin *Paracentrotus lividus* in Portugal

Ana Mendes, João Araújo, Florbela Soares,
Narcisa Bandarra and Pedro Pousão-Ferreira

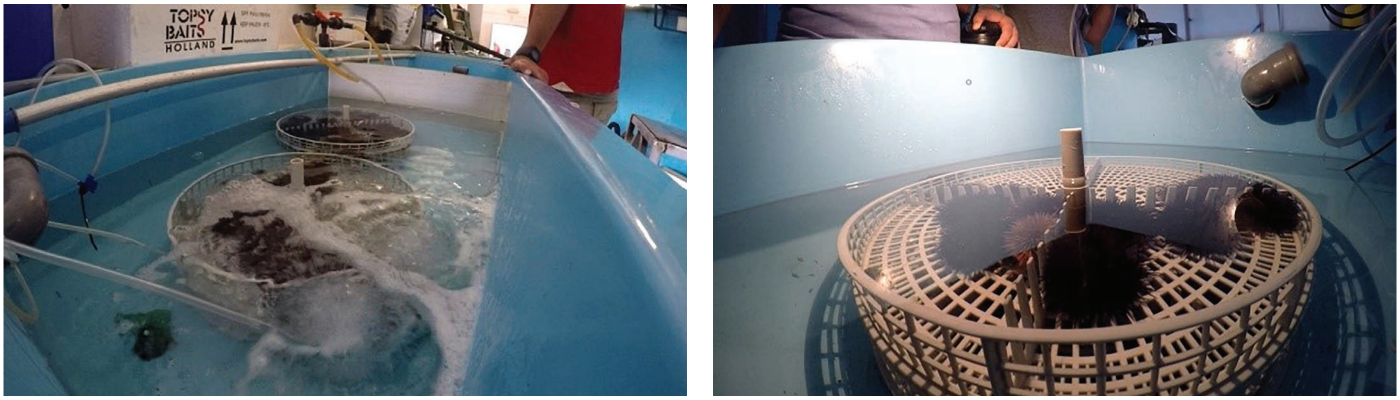

FIGURE 1. *Raceway tanks (left) and lanterns (right) used in acclimation and conditioning of purple sea urchin broodstock (Photo: João Araújo).*

The roe (gonads) of sea urchin is considered a delicacy in Asian countries, especially Japan and South Korea, but it also is consumed worldwide in diverse locations that include Chile, Polynesia, New Zealand, France and Spain. In the last five decades, demand for this product has gradually increased, causing a consequent expansion of industrial fishing activity, reaching a historical peak of 108,969 t in 1995 (FAO 2012). Catches have been decreasing since then (Cirino *et al*. 2017), mainly from collapse of fisheries and policy measures of population control in some fishing areas.

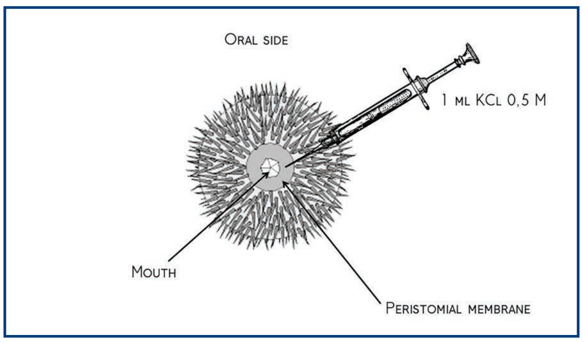

FIGURE 2. *Schematic of induced spawning technique of purple sea urchins by injection with 0.5 M KCl.*

The purple sea urchin *Paracentrotus lividus* is an echinoderm with a wide distribution that covers the Northeast Atlantic from Scotland to southern Morocco, and the Mediterranean (Boudouresque and Verlaque 2013). At present, overall catches of purple sea urchin from the Eastern Atlantic are relatively low but there are no data specific to this species (FAO 2012).

In Europe, this species was consumed mostly in the Mediterranean countries, especially France (Carboni *et al*. 2014). In Spain, the consumption of sea urchin features notable regional differences, being more significant in the Asturias region where is part of traditional cuisine, and in some of the coastal areas of Galicia, Andalusia, Catalonia and Valencia. In Portugal, there is no tradition of consumption and therefore low demand in the domestic market.

However, there was a major capture activity, mainly from the rocky beaches of the north coast of Portugal, for export (Bertocci *et al*. 2014, Domínguez *et al*. 2015). Traditional artisanal fisheries consumption is permitted, with a licensed limit of 5 kg/d, but production was not mentioned in the official bulletin of fisheries statistics (Ouréns 2013). According to FAO (2012), the greatest landings of sea urchin in the Atlantic region occur in Spain.

The increase in global demand and consequently an increase in fishing effort, associated with biological and ecological factors of the species, led to instability of the stocks and annual recruitment indicated by a decrease in the abundance and biomass (Domínguez *et al*. 2015). The sea urchin fishery was, therefore, not sustainable and its continuous exploitation could jeopardize natural populations. In this context, aquaculture production in the short term may contribute to its sustainability (Ouréns 2013).

The production of echinoderms constitutes a challenge to the scientific community and aquaculture producers, mainly related to the existence of different phases over their lifecycle and the complex biotic relationships that occur during phase metamorphosis. There were several studies describing the development of the sea urchin, especially production in the Bay of Biscay (Galicia and Cantabria) and in the Mediterranean Sea (Boudouresque and Verlaque 2000, Ouréns 2013). In Portugal, the most recent research studies are related to ecology and population dynamics (Domínguez *et al*. 2015), sea urchins as a fisheries resource (Bertocci *et al*. 2014) and the fattening of individuals caught in the wild (Silva 2012). Gago (2009) studied the potential of eggs and endotrophic larvae for marine fish larval feeding.

The Aquaculture Research Center (EPPO) of the Portuguese

FIGURE 3. *Emission of purple sea urchin gametes after induction with 0.5 M KCl (Photo: João Araújo).*

Institute for the Sea and Atmosphere (IPMA) in Olhão recently started scientific work to develop techniques for breeding, larval development and post-larvae, growth and survival of the purple sea urchin, with special emphasis on the development of cultivation protocols to promote larval survival.

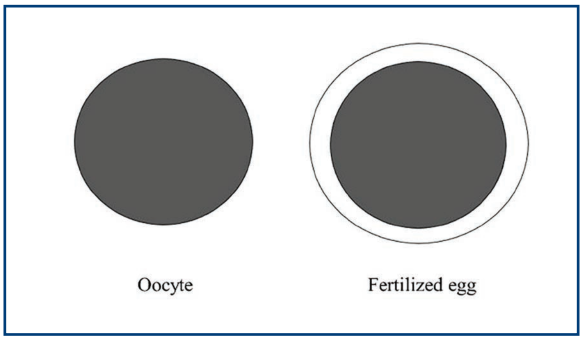

FIGURE 4. *Schematic illustration of the difference between non-fertilized and fertilized eggs in sea urchin.*

Broodstock Collection and Conditioning

Wild broodstock were collected manually in rocky areas during low tide. Urchins were transported in boxes at low density to avoid excessive contact between animals. Boxes were covered to protect urchins from direct sunlight and to keep them moist, and then quickly transported to EPPO.

At EPPO, animals were placed in fiberglass tanks in an open system with filtered water (Fig. 1). The cultivation water temperature followed natural seasonal variation; however, cooling during the warmer months was needed so that temperature would not exceed 22-23 C. Aeration was relatively strong because these animals occupy turbulent habitats, heavily influenced by wave action.

The provided diet was primarily green seaweed (*Ulva* spp.) and this was readily accepted by urchins the day after individuals were collected. Other species of macroalgae were tested, such as *Saccorhiza polyschides* (Laminaria), *Cystoseira usneoides*, *Codium sp*. and *Asparagopsis armata*) that were collected in the intertidal zone on the southwest rocky coast of Portugal. However, purple sea urchins did not have any interest in consuming any of these macroalgae. Another advantage of using *Ulva* spp. as the main food for sea urchin production was the availability of vast quantities of this seaweed in earthen ponds at EPPO.

Spawning Induction

Echinoidea class members are dioecious (individuals are one sex) animals. However, sea urchins do not exhibit sexual dimorphism and so it was possible to differentiate sexes only during the emission of gametes. There are several approaches to induce spawning referenced in the literature but the most efficient method was osmotic shock (Gago *et al*. 2009). To provoke this osmotic shock, 1 mL of 0.5 M potassium chloride was injected into the celomic space through the peristomial membrane (Fig. 2).

After injection, sea urchins were stirred manually for 1-2 min to facilitate diffusion of the solution injected into the celomic cavity and then placed inverted on top of a glass to carry out gamete collection (Fig. 3). If the sea urchin was sexually mature, gamete emission would occur in a few minutes. The oocytes were orange, while the sperm was whitish. Sperm was collected using a micropipette.

Oocytes were diluted in sterile sea water for counting. When the total number of oocytes was determined, sperm could be added. Dilution was important because an imbalance between gametes could compromise fertilization success by the occurrence of polyspermy. There is still work needed in this area, but in a very simplistic way, the use of a ratio of 500 male gametes for every oocyte can be used with good results. Fertilization rate was assessed through microscopic observation of samples two hours after mixing gametes. The presence of eggs with a fertilization membrane (Fig. 4) meant successful fertilization (McBride 2005).

Hatching and Larval Culture

Fertilized eggs were then transferred to a tank for hatching. Sufficient water renewal to maintain good water quality for the entire column is necessary, a flow equivalent to 10 percent water exchange per hour is advised. The water inlet should be preferably located on the tank bottom and the outlet at the surface should be provided with a 55-μm plankton net to prevent egg loss. Regular monitoring is required to avoid an overflow. Aeration was carried out by a small air diffuser and crystal tubes of small diameter without a diffuser to promote a greater turbulence. Embryonic development (Fig. 5) was followed by regular observation with a stereoscopic microscope.

After hatching, and to decrease handling, the larvae (planktonic phase) could be kept in the same tank but all residual organic material, such as capsules and non-fertilized eggs, must be removed. Larval cultivation at EPPO was carried out at a controlled temperature of 21 to 23 C. Dissolved oxygen was monitored by

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probes and maintained near saturation. The photoperiod was natural daylight but luminous intensity was reduced partially by covering the tanks.

The diet in the larval phase was composed of a mixture of microalgae produced in 80-L plastic sleeves. In this phase, several microalgae species were supplied to cover the nutritional requirements of the sea urchin larvae: *Isochrysis aff. galbana, Nannochloropsis occulata* and *Tetraselmis* sp. imp3 and diatoms *Chaetoceros calcitrans, Phaeodactylum tricornutum*, *Skeletonoma costatum*. Algae was provided 2-3 times throughout the day to provide an algae concentration between 75,000 and 300,000 cells/mL for each tank.

Water renewal of rearing tanks was low (5 percent/hr) because of cultivation constraints. The small size and morphology of urchin larvae required the use of a very small mesh for larval retention, with periods of no water exchange when adding the microalgae, resulting in less waste. Microalgae consumption was 5-20 L/ day during the larval period and, with flow-through operation, the culturist may need a larger supply of microalgae. Tanks were cleaned by siphoning and mortality evaluated.

Metamorphosis and Early Growth

Larval stages (Fig. 6) presented major difficulties. On the cultivation of the sea urchin, which was still in a preliminary phase and with only incipient knowledge of requirements, survival was the biggest challenge to the development of aquaculture of this species. At EPPO, samples from tanks were collected daily for counting and estimating survival. A 70 percent survival rate at 7 DAH decreased to about 30 percent at 14 DAH. After this period, the estimation of survival by counting was no longer practical because of the onset of settlement and consequent passage to a benthic life stage. During metamorphosis, there is high mortality associated with the failure of settling and transition to the benthic stage. This is one of the aspects that lacked good research work to improve production performance.

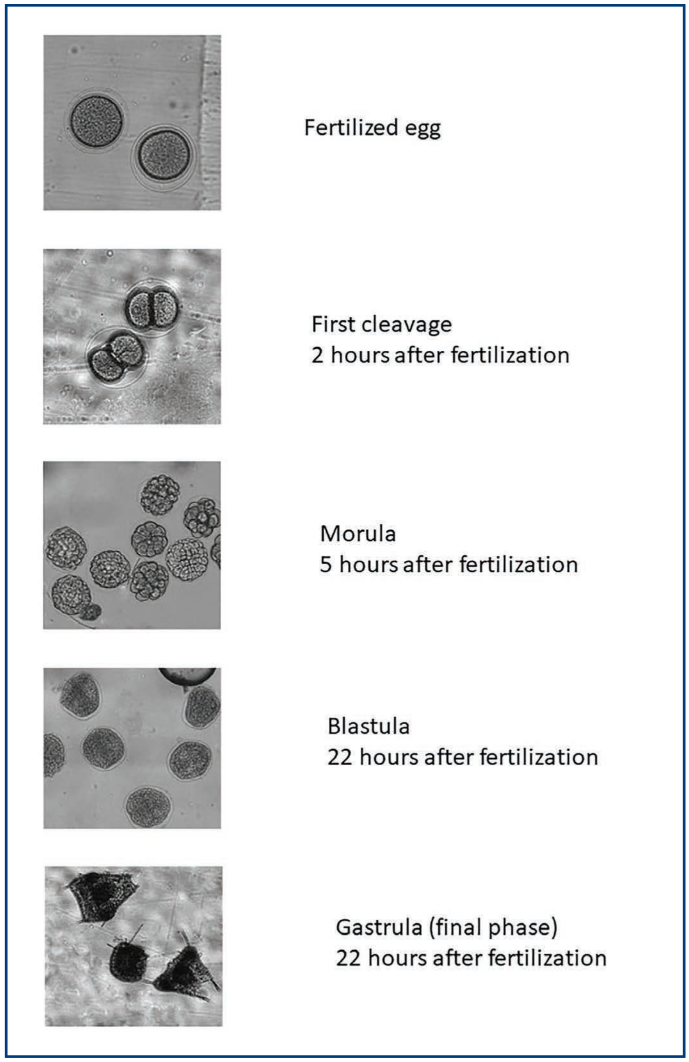

FIGURE 5. *Embryonic development of purple sea urchin at 22 C. The fertilized egg diameter varies between 70-75.5 µm.*

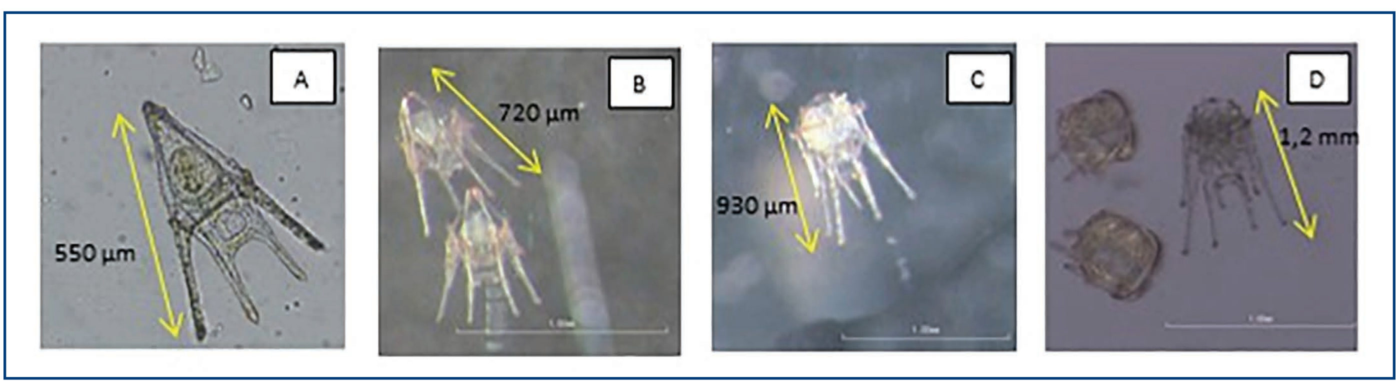

FIGURE 6. *Larval development of purple sea urchin. (A and B) The 4-arm stage occurs from 0-12 days after hatch (DAH), (C) the 6-arm stage begins 12 DAH, and (D) the 8-arm phase begins 16 DAH.*

Relating to sea urchin larval morphology, two measurements were chosen as standard throughout the first phase: total length and the diameter measured by the maximum amplitude of the arms (Castilla-Gavilán *et al*. 2018). Larval growth was not continuous over time (Fig. 7), presenting growth that reflected morphological alterations of the body, including the emergence of new arms (front side) and enlargement of the upper part (pre-adult).

Growing Benthic Juveniles

Between 14 and 17 days old, depending on water temperature, larvae started settlement. At 1 month old, all sea urchins reached the juvenile and benthic stage (Fig. 8). At that time, it was essential to increase the surface area available in tanks for post-larvae by adding structures such as plates and PVC pipes. After settling it was necessary to clean tanks daily through siphoning because there was a greater accumulation of debris (feces, uneaten food, dead individuals) and animals had greater contact with the sediment.

After establishment of post-larvae, the diet consisted of seaweed (*Ulva* spp.) produced and collected from earthen ponds at EPPO and a mixture of microalgae (*Isochrysis galbana*, *Nannochloropsis occulata*, *Tetraselmis* spp., *Chaetoceros calcitrans imp3* and the diatom *Skeletonoma costatum*). In this way, a nutritionally varied diet was guaranteed by favoring of different ways of eating food: scraping with teeth (seaweed) or by passive intake (microalgae).

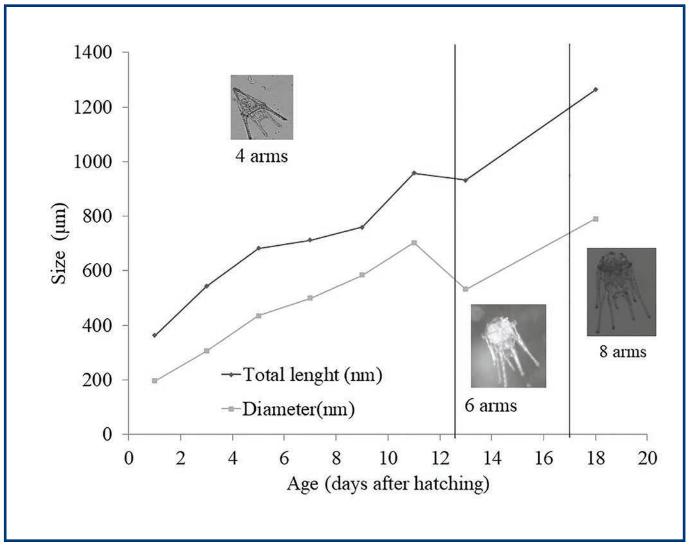

FIGURE 7. *Variation in total length and diameter of purple sea urchin during the planktonic phase. Main changes in larval metamorphosis indicated.*

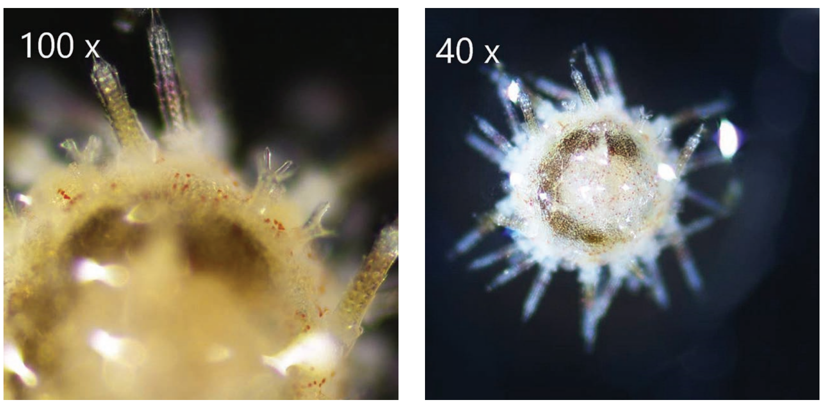

FIGURE 8. *Purple sea urchin at 30 DAH (Photos: João Araújo).*

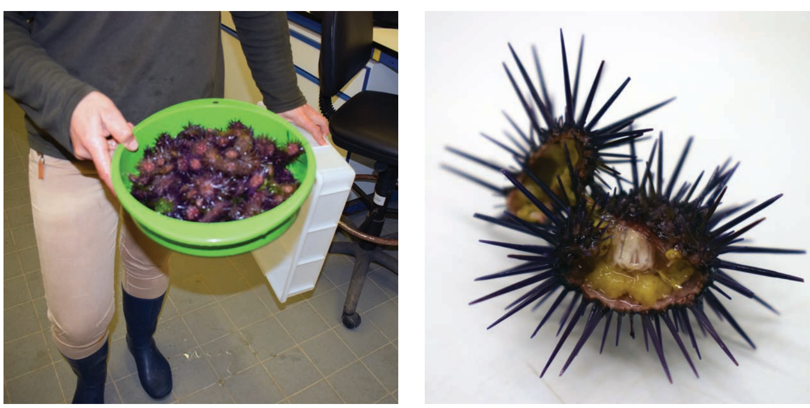

FIGURE 9. *Juvenile purple sea urchins at approximately 220 DAH, reproduced in captivity (left). Mature gonad of purple sea urchin at 220 DAH (right) (Photo: João Araújo).*

The growth in diameter of the shell was low (0.03 mm/day) until 58 DAH, when a significant increase occurred between 58 and 77 DAH (0.22 mm/day). This may have been cause by the ingestion of macroalgae (*Ulva* spp.), which was provided daily after 58 DAH.

Fattening

Fattening tanks for juveniles are managed in flow-through mode with filtered seawater, with a water exchange of about 100 percent/h with seasonally variable temperature. Dissolved oxygen concentration was maintained above 7 mg/L.

After four weeks of grow-out (sea urchins of 150 DAH), the biomass reached 1.4 g/L. At this stage, the cultivation was done at a density of 294 individuals/m^2 and a biomass of 2.8 g/L. After 10 weeks of cultivation, densities reached 10 g/L (approximately 4 individuals/L) with an average weight of 2.3 g. The feeding supply was between 5 and 10 percent/d of total biomass and consisted of macroalgae (*Ulva* spp.) and corn grain.

According to Boudouresque and Verlaque (2013), the sea urchin *P. lividus* reaches an average diameter of 2 cm in two years. However, in our trials, 35 percent of individuals were greater than 2 cm in diameter in only seven months. The greater growth rate observed may be the result of not only the food provided, but also the higher cultivation temperature.

In a trial with four species of seaweed, Cyrus *et al*. (2015) obtained the best results with *Ulva*.

The size of first maturity for purple sea urchins is reached at a diameter of 20-28 mm (Ouréns *et al*. 2013), well above the verified size in our work. Sea urchins at 230 days (Fig. 9) had a 30 percent sexually mature individuals (with diameter up to 20.4 mm) and a gonadosomatic index at this age of around 2-4 percent. When fed with good quality nutritious diets, sea urchins improved their reproductive capacity and may direct energy to gonadal development (Fernandez and Boudouresque 2000, Schlosser *et al*. 2005, Watts *et al*. 2013). The type of feed should be formulated with consideration to development stage and cultivation objective. An early stage of fattening will be important to the greater development of shell size, allowing the development of gonads in a stage closer to marketing or reproduction.

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FIGURE 10. *Boxes of green seaweed (*Ulva *spp.) to support offshore production of purple sea urchin (Photo: João Araújo).*

Offshore Cultivation Trial

In July 2018, IPMA started a trial of offshore sea urchin cultivation off the south coast of Portugal. Nearly 4,000 approximately one-year old sea urchins were placed in boxes suspended on longlines that are usually used to produce mussels. The purpose of this test was to evaluate the potential of offshore production and compare the survival, growth and quality at different depths and densities. The animals were fed regularly with macroalgae *Ulva* spp. (Fig. 10) and corn grain.

Preliminary results were promising, with low mortality and fine growth so far. In less than four months, the average diameter of sea urchins increased from 2.3 to 3.0 cm, and weight nearly doubled. Through the results obtained in these pilot tests, it will be possible to develop technologies and methodologies for a future production of sea urchins on a commercial scale in Portugal.

Notes

Ana Mendes, João Araújo, Florbela Soares, Narcisa Bandarra and Pedro Pousão-Ferreira, IPMA – Portuguese Institute for the Sea and Atmosphere; EPPO – Aquaculture Research Center, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal

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