POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Internship Report at Oceano Fresco

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Internship report for the obtaining of the Master's degree in Aquaculture

Master’s Report realized under the orientation Specialist Teresa Maria Coelho
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Acknowledgment

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Abstract

The increasing global demand for sustainable protein sources has positioned aquaculture as a key solution for food security and environmental conservation. This internship at Oceano Fresco focused on the cultivation of the native European clam species *Venerupis corrugata* and *Ruditapes decussatus* using innovative aquaculture techniques. The internship involved hands-on participation in hatchery operations, including broodstock handling, spawning, larval rearing, and microalgae cultivation, along with microbiological monitoring and biosecurity measures. Additionally, a research project was conducted to evaluate the impact of different conditioning methods on broodstock gonadal maturation and reproductive success. Results showed that broodstock conditioned in a recirculating system (R) exhibited 43.8% higher egg production, a 15.3 percentage point increase in hatching success, and nearly double the number of viable D-larvae compared to those in a flow-through system (FT). Despite no significant differences in broodstock survival or gonadal maturation rates between treatments, the R system facilitated faster reproductive development and improved larval viability. These findings highlight the potential of optimized conditioning protocols to enhance hatchery efficiency and contribute to the sustainable production of bivalves. Implementing such strategies could increase the availability of broodstock year-round, fostering greater independence from natural reproductive cycles and enabling continuous cultivation throughout the year.

Keywords: Aquaculture, Sustainability, Bivalves, Clams, Broodstock conditioning.

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I. Introduction

As of the year 2024 global population has surpassed 8 billion people, an astonishing increase considering an estimated world population of 2.5 billion people in 1950, which roughly translates into a threefold increase since that year, further population projections indicate a likely continuation of this pace of growth meaning that global population would reach close to 10 billion in 2050 (UNDESA, 2022; FAO, 2024a).

The increase in global population since the Industrial Revolution can be tightly linked to a multitude of advances in science and technology in a myriad of areas such as medicine with the surge of vaccines and antibiotics, public infrastructure like access to water and sanitation and food production and distribution, which have ultimately led to an increase in the rate of births comparatively to the rate of deaths (Kinder, 1998). Although taking the increase in global population up for face value may make it seem insignificant, when considering the challenges that may arise with such an increase, we begin to paint the picture for actions needed to pursue global sustainable development, such as the ones described in the Sustainable Development Goals (SDGs) envisioned by the United Nations 2030 Sustainable Development Agenda.

The consequences of population growth are wide-ranging and multifaceted, impacting various aspects of human existence such as resource utilization, environmental sustainability, and socio-economic development. Rapid population expansion has been identified early on to place a strain on finite resources and exacerbating environmental degradation (Pimentel, 1991). Moreover, it has posed significant challenges for global food security, necessitating the production of ever-increasing quantities of food to meet the nutritional needs of a growing population. This matter is further highlighted under many reviews and research articles such as Gupta (2019), Jamaludin (2022) and Misselhorn et al., (2012). The UN 2030 agenda approach this issue under goal number two “Zero Hunger” which aims to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture by providing a blueprint for collaborative action to be adopted by all United Nations Member states and by making an urgent call for action by all countries, developed and developing, in a global partnership (UN, 2023).

Against this backdrop, the quest for sustainable sources of protein has emerged as a pressing matter worldwide, albeit food industries are facing a vast array of challenges as competition with other industries for land, water and energy intensifies (Harvey & Pilgrim, 2011). As such, there is an ever increasing need to explore and embrace alternative sources of animal protein that are environmentally sustainable, socially equitable, and nutritionally sound. By reducing reliance on resource-intensive livestock farming and promoting more diverse, efficient and ecologically friendly protein production systems, these innovations hold the potential to transform the global food landscape and contribute to a more sustainable future.

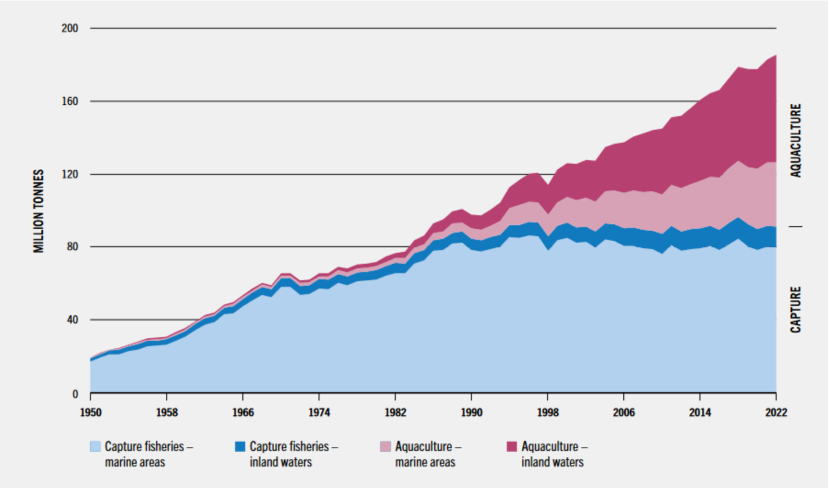
In the quest for sustainable solutions to meet the burgeoning global demand for protein, aquaculture has emerged as a promising solution, offering a diverse array of aquatic species cultivated in controlled environments to provide nutritious food while minimizing environmental impact (Henriksson et al., 2021). As the human population continues to expand, exerting pressure on traditional protein sources and exacerbating concerns over food security, aquaculture stands poised to play a pivotal role in addressing these challenges. The following introductory chapters seek to explore the multifaceted role of aquaculture in meeting the need for sustainable sources of protein, examining its current development and future projections in production growth, understand the panorama of the industry in Europe and Portugal, and approach bivalve aquaculture and Oceano Fresco's role in producing sustainable bivalve protein.

1. Aquaculture's current projections

In 2022, global production reached a record high of 185 million tonnes (live weight equivalent) (Figure 1). Farming of aquatic animals contributed approximately 94 million tonnes, accounting for 51% of total production, surpassing capture fisheries for the first time, which produced 91 million tonnes (49%) (FAO, 2024a, p.1). This milestone underscores the rising demand for aquatic animal protein and highlights aquaculture's crucial role in meeting that demand.

Since 2000, global aquaculture production has grown at an average annual rate of 5.2%, whereas capture fisheries have stagnated since 1990, despite increased fishing efforts (FAO, 2024a; Watson & Tidd, 2018; Pauly & Zeller, 2016). This trend reinforces aquaculture's potential to bridge the supply gap that capture fisheries can no longer fulfil.

Even in the face of economic disruptions caused by the COVID-19 pandemic, when consumer spending declined significantly (Coibion et al., 2020), global aquaculture production continued to rise throughout 2020, 2021, and 2022. This resilience further emphasizes the sector's growing stability and importance in global food security.



NOTES: Aquatic animals excluding aquatic mammals, crocodiles, alligators, caimans, aquatic products (corals, pearls, shells and sponges) and algae. Data expressed in live weight equivalent

Figure 1- World fisheries and aquaculture production of aquatic animals (Source: FAO, 2024a).

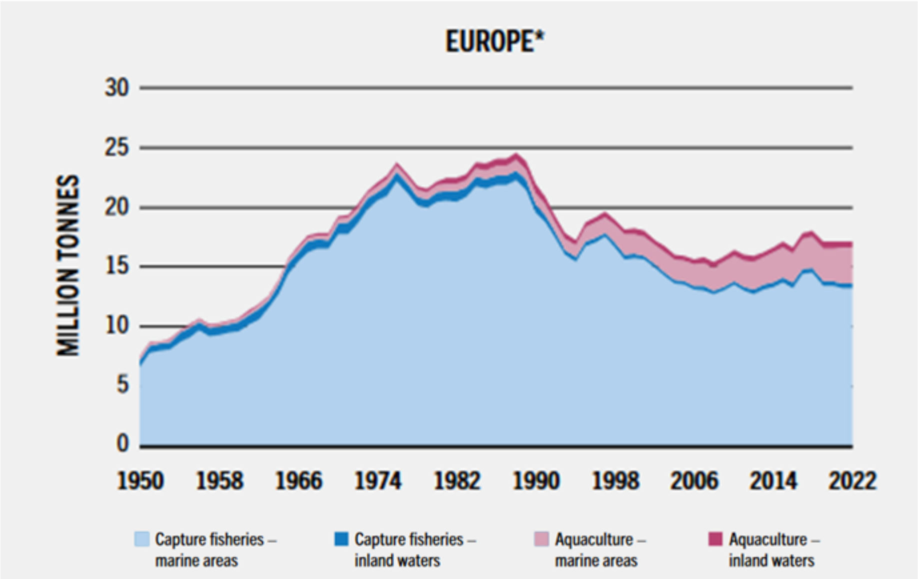
2. Future Projections

FAO's latest projections suggest that global fisheries and aquaculture production will continue to expand, reaching 205 million tonnes (live weight equivalent) by 2032. This represents an increase of 19 million tonnes (10%) compared to 2022.

Aquaculture is expected to drive most of this growth, with production projected to reach 111 million tonnes, an overall increase of 16 million tonnes (17%) relative to 2022. These projections highlight the industry's critical role in meeting future seafood demand and suggest continued advancements in aquaculture practices, technologies, and sustainability efforts to support this expansion.

3. Europe and Portugal’s role in the global aquaculture market

Europe's role in global total fisheries and aquaculture production in 2022 was 9 percent, or around 16 million tonnes, of which only 3.5 million tonnes (21.9 percent) resulted from aquaculture production of farmed animal species (Figure 2), which seems pale in comparison to the previously mentioned global output of 51 percent of the total aquatic animal production originating from aquaculture (FAO, 2024a).



NOTES: Aquatic animals excluding aquatic mammals, crocodiles, alligators, caimans, aquatic products (corals, pearls, shells and sponges) and algae. Data expressed in live weight equivalent.

Figure 2 - Europe's fisheries and aquaculture production of farmed animals (Source: FAO, 2024a).

The European aquaculture industry faces significant challenges, including disease management, environmental concerns, and social impacts (Kaiser & Stead, 2002; Lieke et al., 2020; Read & Fernandes, 2003). However, restrictive and cumbersome regulatory structures are also a major contributor to the industry's struggles (Guillen et al., 2019). High labour costs, prioritization of commercial fishing and tourism, and high coastal property values further hinder growth. Despite these challenges, countries like Norway have shown that a more streamlined regulatory framework can facilitate aquaculture development, even in regions with strict standards and high labour costs. This raises the question of whether similar approaches can be successfully implemented in other European countries with densely populated coastlines and concentrated tourism industries (Garlock et al., 2019).

European aquaculture is primarily focused on the cultivation of fish and shellfish, with a select group of species dominating the industry. In 2018, eight key species accounted for 93 percent of total production. These species include five types of finfish: Atlantic salmon, rainbow trout, European seabass, gilthead seabream, and common carp. The remaining three species are shellfish, consisting of mussels, oysters, and clams (FAO, 2024b).

Geographically, Northern Europe plays a significant role in global aquaculture (Figure 3), driven mainly by its salmon and trout production (FAO, 2024b). Southern Europe, on the other hand, is notable for its shellfish, marine fish, and trout supplies. In contrast, many Eastern and Western European countries, particularly those without direct access to the sea, rely heavily on freshwater aquaculture. However, France stands out as an exception, with a significant focus on oyster culture (Hough, 2022).

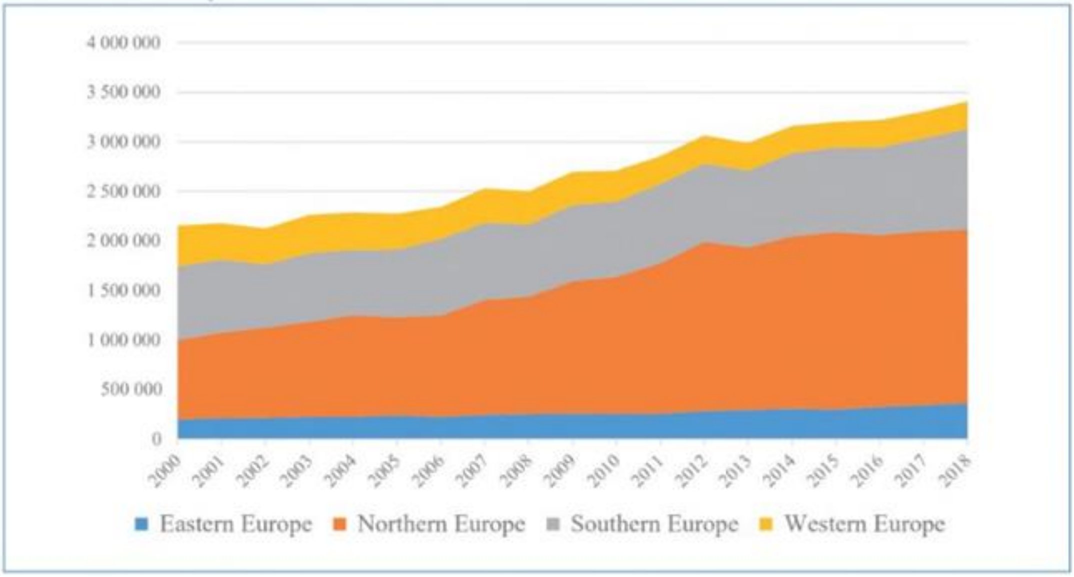


Figure 3 - Development of aquaculture production in the European region and sub-regions 2000-2018 (tonnes/year) (Source: FAO, 2024b).

From Europe’s 3,5 million tonnes, Portugal’s contribution to total aquaculture production of farmed animal species was 18 822 tonnes, a 4.8 percent increase in total production volume from the previous year, 2021 (INE, 2023) and, as well as Europe, has been on an increasing trend since 2000 (FAO, 2024a; INE, 2023). In Portugal, aquaculture is a strategically important sector, serving as an alternative to traditional forms of fish supply. The country's high *per capita* fish consumption and favourable geographical and climatic conditions make it an attractive market for aquaculture development (INE 2023).

Production in brackish and marine waters remained the most significant for the country, accounting for about 98 percent of total production in 2022 (INE, 2023). Fish production in these waters represented 43.9 percent of production, with close to 80 percent of this production coming from gilt-head sea bream, European sea bass and turbot (INE, 2023). Mollusc and crustacean production reached 53.9 percent of total production in 2022. Clams were the most produced species (4,346 tonnes), followed by mussels (3,189 tonnes), which recorded production increases of 21.2 percent and 3.8 percent, respectively (INE, 2023).

The low production of aquaculture in Europe does not reflect an equally low consumption of aquatic animal foods by Europeans, this imbalance between consumption and production has made Europe heavily dependent on the importation of fisheries, with the ratio between own production (fishing plus aquaculture) and consumption being only 38 percent in 2021 (Altmayer, 2024). With this contextualization, it’s easy to understand Europe’s push to increase its total aquaculture production, decreasing its reliance on fisheries imports, and furthering its food security. Currently the EU holds a wide range of funding programmes potentially available for investments in the field of aquaculture, accounting for a total of EUR 2.018 trillion in 2023 prices (ECIEEA, 2023).

4. Bivalve aquaculture

Bivalves, which include clams, oysters, and mussels, are filter feeders that play a crucial role in maintaining the health of marine ecosystems, by filtering water, they remove excess nutrients and particulate matter, thereby improving water quality and promoting biodiversity. This natural filtration process not only benefits the aquatic environment but also enhances the overall health of coastal ecosystems, making bivalves an essential component of marine sustainability efforts (Vaughn & Hoellein, 2018).

The production of bivalves through aquaculture presents a unique opportunity to meet the growing demand for seafood while minimizing the ecological footprint associated with traditional fishing practices. Unlike many forms of animal agriculture, bivalve farming requires no artificial feed, as these organisms thrive on naturally occurring phytoplankton and microalgae. This characteristic significantly reduces the environmental impact of their production, as it eliminates the need for resource-intensive feed inputs and the associated land and water use (Cranford et al., 2003).

In Europe, the interest in bivalve aquaculture is particularly pronounced due to the continent's rich marine resources and a growing consumer preference for sustainable seafood. European coastal regions, provide ideal conditions for bivalve farming (Laing & Spencer, 2006; Zander & Feucht, 2017). Countries such as France, Spain, and Italy have long-standing traditions of bivalve cultivation, and there is a renewed focus on expanding these practices to meet both local and international demand.

Moreover, bivalve aquaculture aligns with the European Union's commitment to sustainable development and the Green Deal, which aims to make Europe the first climate-neutral continent by 2050. By investing in sustainable aquaculture practices, Europe can enhance food security, create jobs in coastal communities, and promote environmental stewardship. The cultivation of bivalves not only supports local economies but also contributes to the restoration of marine habitats, as well-managed farms can serve as sanctuaries for various marine species.

Production of bivalves represents around 54 percent of Portugal’s total aquatic production, with the main three cultivated species being clams, mussels, and oysters. Despite clams being the most produced bivalve species in volume, 4, 346 tonnes in 2022 (INE, 2023), their production derives exclusively from extensive practices (DGRM, 2023). Among many bivalves Portugal’s sandbanks accommodate two native clam species *Ruditapes decussatus* and *Venerupis corrugata* (IPMA, 2023).

V. corrugata, commonly known as the pullet carpet shell, and *R. decussatus*, known as the grooved carpet shell, are two species of marine bivalve molluscs that belong to the family Veneridae. These species are widely distributed in the coastal waters of the

Atlantic Ocean, the Mediterranean Sea, and the Black Sea, and are of significant economic and ecological importance. *V. corrugata* is a medium-sized bivalve, typically growing up to 4-6 cm in length, with a rounded, oval-shaped shell that is often yellowish-brown in colour with darker brown stripes (Rayment, 2007). The shell is characterized by a distinctive corrugated pattern, with numerous concentric ridges and grooves that provide a unique texture. *R. decussatus*, on the other hand, is slightly larger, reaching lengths of up to 7-8 cm, with a more elongated shell shape and a distinctive groove that runs along the length of the shell (FAO, 2025).

Both species are filter feeders, using their siphons to draw in water and filter out plankton, algae, and small invertebrates. They are typically found in shallow, coastal waters, often in areas with sandy or muddy substrates, and are capable of burrowing into the sediment to escape predators or extreme environmental conditions (Rayment, 2007, FAO, 2025).

V. corrugata and *R. decussatus* are both dioecies, meaning that they possess either male or female reproductive organs, and reproduce sexually (Cerviño, 2011; FAO, 2025). Spawning occurs in the spring and summer months, with larvae drifting in the water column before settling on the seafloor and metamorphosing into juvenile bivalves (Cerviño, 2011; FAO, 2025).

Both species are of significant commercial importance, being widely harvested for their meat (Fahy et al., 2010; Macho et al., 2016). However, both species are also vulnerable to a range of threats, including overfishing, habitat degradation, and climate change, which can impact their populations and ecosystems (Joaquim et al., 2010; Domínguez et al., 2023).

In recent years, there has been growing interest in the potential of *V. corrugata* and *R. decussatus* for aquaculture, since demand for them is high, and captures have been decreasing due to overfishing, many farms and hatcheries are cultivating these species for food and other products (Matias et al., 2009; Joaquim et al., 2016). However, more research is needed to fully understand the biology and ecology of these species, and to develop sustainable and responsible aquaculture practices that minimize the impacts on wild populations and ecosystems.

5. Oceano Fresco

Oceano Fresco is a sustainable seafood enterprise that employs innovative aquaculture methods to cultivate high-quality bivalve species. The company is committed to regenerating nature by transforming our eating habits and farming practices. They exclusively farm native European bivalves, beginning with the pullet carpet shell (*V. corrugata*) and the grooved carpet shell (*R. decussatus*), Figure 4, which are currently declining and threatened by invasive species, yet are highly sought after (Habtemariam et al., 2015; Joaquim et al., 2016). Oceano Fresco believes their clams represent some of the most sustainable, naturally nutritious, and delicious food options available globally.

With vertically integrated operations, they ensure sustainable and traceable farming practices. The clams are initially bred in the hatchery at the cutting-edge BioMarine Center in Nazaré, Portugal, before being transferred to the world's first open-sea clam farm off the coast of Lagos, Portugal, where they are nourished with naturally occurring microalgae until they reach adulthood. This process is supported by a dedicated team of scientists who lead research and development as well as selective breeding programs, which have earned a 'Seal of Excellence' from the European Commission.

The innovation stems from the biology of low trophic animals. Clams have no known negative inputs (such as artificial feed or antibiotics) and provide several positive benefits, including CO_2 capture in their shells, water filtration, and the creation of a "shelter" effect in the open-sea farm. As a largely untapped source of food and protein, clams present a promising opportunity. The company aims to establish a reliable, year-round supply and foster business partnerships to promote the consumption of premium clams, both in restaurants and at home. They plan to serve customers in Spain and Portugal, with aspirations to expand into other European markets. Oceano Fresco has been financed by its founders, public grants, and venture capital investors who share their vision of a transformative 'Blue Economy' for Europe and beyond.

Figure 4 – *Venerupis corrugata* (left) and *Ruditapes decussatus* (right) (Source: Oceano Fresco).

The company is divided between two locations, one being the Biomarine Center, located in the Port of Nazaré (Figure 5), in which broodstock is kept and spawned, and the resulting larvae are brought until they are of sufficient size to head to the grow-out facility, which is around 2.4 mm. The other location, the company's grow out facility, is located offshore, 3.5 nautical off the coast of Lagos, it spans 100 hectares. Once in the open sea farm, the clams remain there until they're the commercial size of 32 mm or higher.

Figure 5 - Biomarine Center, Nazaré Port, Nazaré, Portugal (Source: Oceano Fresco, 2024).

II. Internship

The internship in question took place solely on the Biomarine Center, which encompasses the hatchery, in which I was able to learn and participate in all operational areas that comprise it. The duration of the internship was of 10 months. During these 10 months the objective for the internship was to learn and work collaboratively among the other hatchery technicians, assuring the well-functioning of the hatchery and acquiring knowledge of the techniques used inhouse, as well as implement an improvement project, with the aim of assisting the hatchery and get a chance to apply knowledge acquired during the master program's formative year.

1. Production Operations

For the purposes of this report, the following chapter will provide a brief overview of the hatchery's facilities, followed by a more detailed explanation of all production operations carried out during the internship. It is also important to note that the productive processes described refer solely to the farming of *V. corrugata*, since *R. decussatus* was not under production during my time in the hatchery.

The hatchery is composed of a microalgae chamber, for preservation of inoculums, initial scale-up and microbiology analysis, a microalgae bag-room, for the last scale-up phase. This room holds capacity for 84 bags of 400 L capacity in which the selected strains of microalgae will grow until they are collected continuously to feed the clams. A broodstock room, to keep broodstock to be used for spawns, two larvae rooms, in which the larvae grow until they are at the settling phase, a post larvae room, where the settling larvae are put in non-recirculating setting systems, two seed rooms, where the seed are kept until they are big enough to go to Lagos's open sea farm, two labs, one for production and one for R&D, an exterior water filtration area, a maintenance building, as well as a locker area and offices.

1.1. Water inlet and treatment

Seawater utilized within the hatchery is initially sourced directly from the ocean and subsequently pumped into two 30 cubic meter storage tanks located in the external water filtration area (Figure 6). These tanks serve as reservoirs to supply the hatchery with saltwater on demand. To prevent the buildup of biofilm and other unwanted deposits, these tanks require disinfection with chlorine every two weeks.

Figure 6- Exterior reservoirs (30 m³ each) that supply the hatchery with saltwater

Following this, the seawater undergoes sand filtration, effectively removing most particulate matter larger than 20–40 µm. This process is further enhanced by a series of three cartridge filters with progressively smaller pore sizes, followed by UV lights that destroy any remaining DNA (Figure 7). A well-maintained filtration system is essential for eliminating a significant portion of detritus and organisms that could potentially disrupt the development of bivalve larvae. Additionally, this filtration process aids in the removal of various fouling organisms that may settle and proliferate within the hatchery's piping system. Such organisms can obstruct water flow and, upon decomposition, create anaerobic conditions that may be detrimental to bivalve larvae. Furthermore, they can act as reservoirs for bacteria that pose risks to larval health.

Figure 7- Exterior filtration system for the hatchery's seawater supply composed of sand filters, cartridge filters and UV lights

After the filtration process, the water can be directed along three different pathways: one that maintains the water at ambient temperature, another that routes it to a heater, and the last that directs it to a cooler. This configuration allows the hatchery to achieve a wide range of water temperatures, as these lines can be mixed to reach specific desired temperatures.

Additionally, these three water lines must be periodically cleaned with oxalic acid to prevent the proliferation of *Vibrio spp.* and other unwanted microorganisms. Regular water quality tests are also conducted to ensure that the water sourced meets established standards.

1.2. Microalgae culture and scale-up

To produce the microalgae necessary for feeding both species of clams, *V. corrugata* and *R. decussatus*, throughout all growth phases in the hatchery (broodstock, larvae, post-larvae, and seedlings), monospecific intensive culture systems are employed. The culture process begins in 40 ml T-flasks and culminates in a 350 L vertical polyethylene bags (Figure 8).

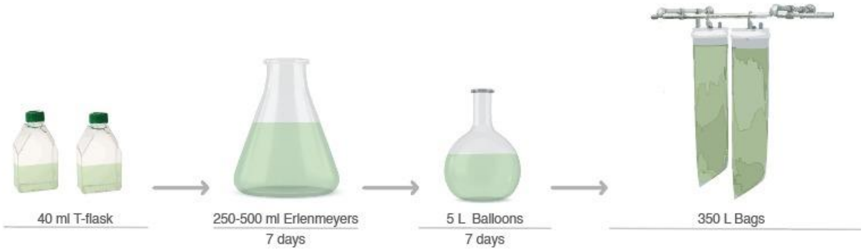


Figure 8 – Schematic representation of the hatchery's microalgae scale-up process, starting at 40 ml T-flasks, to 250-500 ml Erlenmeyer flasks, 5L Balloon flasks, and ending in 350 L vertical polyethylene bags

The microalgae in these T-flasks are sourced from other laboratories and maintained as stock cultures (Figure 9a). These flasks serve as inoculum for 250 ml and 500 ml Erlenmeyer flasks (Figure 9b), which are then used to inoculate 5 L balloon flasks (Figure 9c). The Erlenmeyer flasks were divided once per week, with inoculation of new flasks occurring every 15 days. Each Erlenmeyer contained 250 ml of nutrient medium, to which 50 ml of culture was added. Two Erlenmeyer flasks were prepared for each species and origin.

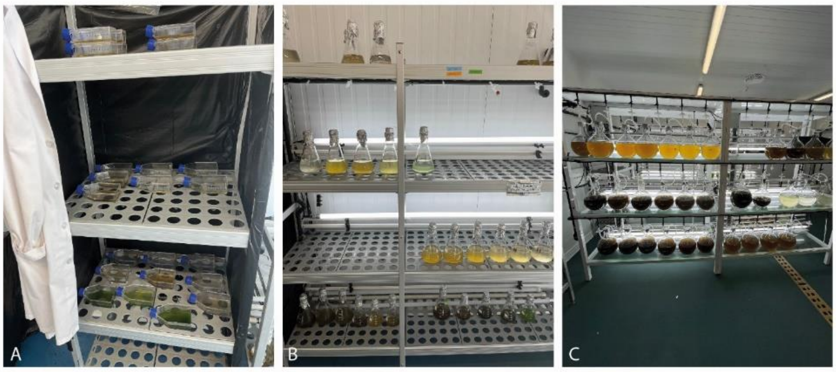


Figure 9 – Inoculum chamber scale-up, composed of 40 ml T-flasks (a), 250-500 ml Erlenmeyer flasks (b) and 5L Balloon flasks (c)

The seawater used for cultivation is maintained at approximately 35 ppm salinity. All containers and water for phytoplankton culture are sterilized using an autoclave. The water is enriched with a commercial nutrient solution composed of potassium nitrate, monosodium phosphate, trace elements (Fe, Zn, Mn, Mo, Co, Cu, and EDTA), and vitamins. In diatom cultures, sodium silicate is also added.

The initial stages of microalgae cultivation occur in an isothermal chamber, featuring constant artificial illumination and a temperature of 19±1ºC. The flasks are aerated with CO_2-enriched air. This phytoplankton not only serves as inoculum for larger volumes but is also used for feeding larvae and post-larvae due to its higher purity. The species cultured in these flasks include *Chaetoceros gracilis*, *Chaetoceros calcitrans*, *Tisochrysis lutea*, *Diacronema lutheri*, *Isochrysis galbana* and *Tetraselmis suecica*.

The division of *C. calcitrans* for larval feeding occurs daily to ensure the availability of 3 to 4 fresh flasks per day. This process involves using two Erlenmeyer flasks to generate a new Erlenmeyer flask, while the remaining volume is used to inoculate a balloon flask. After seven days, this balloon flask serves as the source for inoculating 3 to 4 new balloon flasks for larval feeding. This cycle is repeated daily.

The division of balloon flasks for the other microalgae species currently follows a biweekly schedule, with half of the species being processed on Mondays and the other half on Wednesdays, aligning with the designated inoculation days for the polyethylene bags. Previously, daily inoculations were performed based on the condition of the cultures and the growth stage of the balloon flasks.

Each species has multiple culture origins maintained in T-flasks. From these, selected cultures were scaled up to larger volumes. Typically, one selected origin was used for inoculation on one day, while another was inoculated 2–3 days later to maintain a seven-day culture cycle. One of the balloon flasks was always utilized to generate the next set of 3–5 balloon flasks.

The next stage of cultivation involves 350 L polyethylene bags supported with steel mesh, which are maintained with CO_2-enriched aeration and artificial illumination. The culture in the bags begins with a volume of 50 L, which is gradually increased through a drip inlet supplying a mix of seawater and nutrients at an initial rate of 1 ml/s. The culture medium used in the bags is like that in the inoculum chamber, providing the necessary nutrients, including phosphates and trace metals (Fe, Mn, Zn, Cu, Co, and Mo). Additionally, diatom cultures are also supplemented with sodium metasilicate. The water used for bag culture is run by a pasteurizer beforehand to eliminate any existent microorganisms. Once the culture reaches approximately 300 L, it will continuously drip into an algae collection tank. The microalgae production consists of 84 bags arranged in rows, allowing for a maximum daily production of 4200 L of microalgae mix. This microalgae mix is then distributed to various production areas through a series of pvc piping. Additionally, for the purposes of more thoroughly providing a consist amount of feed to all clams in the hatchery, this microalgae mix is sampled every morning for counting and estimating the number of microalgae cells per litre of mix, this way the amount of microalgae mix being used for any hatchery operation may vary, but the number of microalgae cells being consumed by each one should be similar. The microalgae cell count is performed using a modified version of the Neubauer chamber method. For microalgae culture samples, a standard four-square counting method is applied. The mean cell count from these squares is used in the following formula:

$$Sample = \frac{Mean\ cell\ count}{0.1} \times Dilution\ factor$$

For example, if a *C. gracilis* sample has a dilution factor of 1:8 (100 µL of sample mixed with 700 µL of seawater) and yields cell counts of 78, 69, 75, and 76, the mean count is 74.5. Applying the formula:

$$\frac{74.5}{0.1} \times 8 \ = 5960\ cells/\mu L$$

This system ensures accurate monitoring of microalgae concentrations, allowing precise adjustments in feeding regimens.

The cultivation system used for the scale-up enables a continuous supply of microalgae, a vital characteristic for the hatchery's needs, at a relatively low installation cost compared to other photobioreactors. However, it offers lower cultivation density and less environmental control than more advanced systems, like a vertical column or horizontal tubular PBR, whilst, on the other hand, providing a more controlled environment and higher cultivation density than a raceway system (Helm et al., 2004).

1.3. *Venerupis corrugata*

a. Anatomy

V. corrugata has an oval, elongated shell composed of two symmetrical valves connected by a hinge with three cardinal teeth, closure is controlled by two adductor muscles, which counteract the hinge ligament (Kahler et al., 1976). Externally, the shell has concentric ridges, while the interior features a pallial line, a sinus near the siphons, and adductor muscle impressions (Cerviño, 2011).

The body comprises the visceral mass, gills, foot, and mantle. The visceral mass contains the digestive, reproductive, circulatory, and excretory systems (da Costa, 2012). The foot, located posteriorly, aids in movement and burrowing (Ansell & Trueman, 1967), while in juveniles, a gland at its base secretes filaments for attachment. The mantle, consisting of two lobes, secretes the shell and encloses the pallial cavity. Posteriorly, it forms two siphons essential for feeding and respiration (Figure 10) (Ansell, 1961).

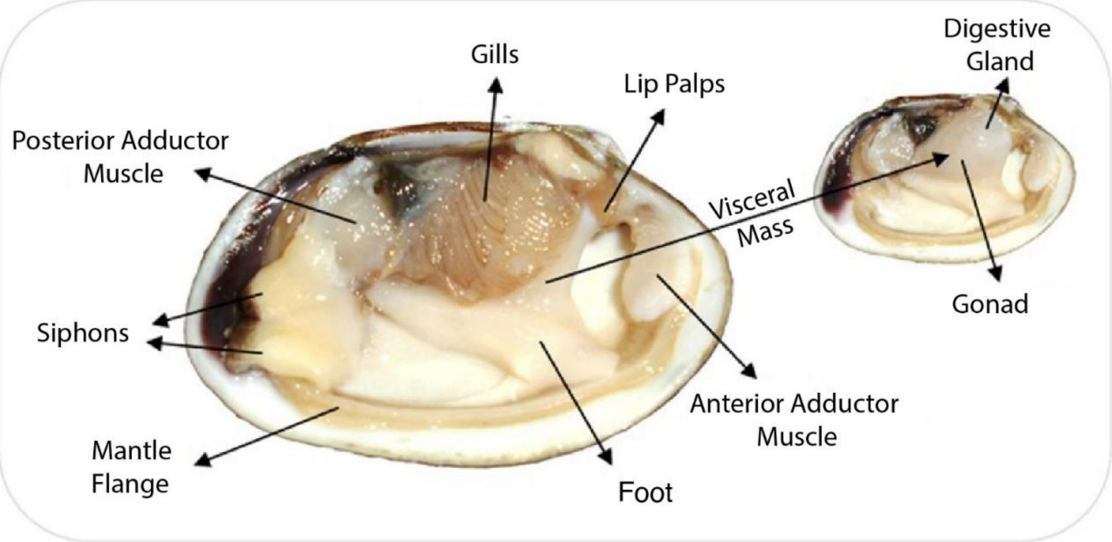


Figure 10- Internal anatomy of *V. corrugata*. Adapted from Cerviño (2011).

b. Physiology

V. corrugata breathes and feeds through paired gills, which filter food particles carried by ciliary movement to the labial palps and mouth, undigested material is expelled as pseudo-feces (Yonge et al., 1923). The digestive system includes a crystalline style in the stomach, which grinds food and releases digestive enzymes (Yonge et al., 1923). Nutrients are absorbed in the digestive gland, while waste exits near the exhalant siphon (da Costa, 2012).

The open circulatory system has a dorsally located heart with two auricles and a ventricle that pumps hemolymph through arterial networks ending in sinuses (Wirkner & Richter, 2013). Excretion is managed by kidneys that filter hemolymph before oxygenation in the gills (da Costa, 2012). The nervous system consists of cerebral, pedal, and visceral ganglia, regulating sensory, motor, and visceral functions (Ruiz-Velásquez et al., 2018).

c. Reproductive Cycle and Development

V. corrugata is dioecious with no sexual dimorphism. Gonads, composed of gonoducts and follicles, expand during reproductive maturity. Gametes are transported by ciliary movement, and fertilization occurs externally (Quayle, 1952).

Oogenesis and spermatogenesis follow standard gametogenic pathways, with spermatozoa and oocytes developing in follicles. Fertilized eggs undergo spiral cleavage, forming a trochophore larva that transitions into a D-larva, secreting a larval shell (prodisoconch I). As growth progresses, it forms prodisoconch II with visible growth lines, then develops a foot in the umbonated larval stage (Cerviño, 2011).

During the pediveliger stage, the larva settles, transitioning from a planktonic to a benthic lifestyle. Metamorphosis involves velum resorption, gill development, and shell formation, leading to the juvenile clam’s final morphology (da Costa, 2012).

1.4. Broodstock handling and spawning

The broodstock in the hatchery are kept in rectangular polyethylene tanks of 120 L capacity (Figure 11). The clams are placed in perforated trays so that pseudo faeces and any other debris flows out the tanks, accommodating stocking densities of anywhere from 3 kg to 5 kg, depending on how big a specific batch is, and how full the hatchery's broodstock room is. These tanks are supplied with a continuous supply of seawater at a flow rate of 60 L/h and constant aeration. The microalgae are put into the boxes diluted with a flow that allows for a specific percentage of feed, meaning number of microalgae cells, per total fresh weight in the box, this percentage can vary between 3 and 6 percent depending on when and for what purposes the broodstock is needed, the microalgae is kept in a 1000 L tank and is then pumped to the 120 L tanks, this pump is set on a timer, leaving it to operate 15 minutes on and 15 minutes off, so that broodstock can better filter the microalgae in the 120 L tank, avoiding excessive feed waste.

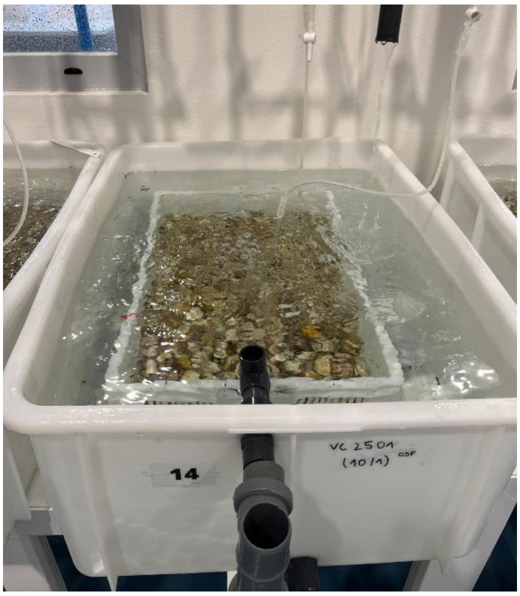


Figure 11 – Broodstock placed in perforated trays kept in rectangular 120 L polyethene tanks

Before a spawn various batches will go under a ripeness assessment test, in which broodstock are open and the gonad is assessed and later scarified, so to evaluate the quality of eggs and sperm under the microscope. According to these parameters the broodstock is qualified with a ripeness score to help choose which batches could be used for spawn (Table 1).

In cases where there are spontaneous spawns, or where a batch is very ripe, a set of sieves is placed at the exit of the broodstock tanks so to collect the spawn, one of 90 µm and another of 25 µm, the first one to capture any debris or pseudo faeces and the latter to collect the eggs, these eggs will then be counted and assessed, so to decide if it is kept or not, depending on the size and quality of the spawn.

Once the batches for spawn are chosen, the broodstock is placed dry in an isothermal box with ice packs up to one whole day (Figure 12).

Figure 12 – Broodstock placed dry in an isothermal box with ice packs 24 hours before spawn

Then, on the day of the spawn the broodstock are submitted to temperature shocks with cold water (10-15°C) and hot water (24-28°C), in periods of between 30 minutes and an hour and a half. This procedure is done in a 450 L cylindroconical tank supplied with constant aeration, with the broodstock placed on trays near the surface so to be visible (Figure 13).

Figure 13- Broodstock placed in 450 L cylindroconical tanks for spawning

Once the broodstock start to spawn the temperature shock cycles stop and the clams are left in the tank until they stop spawning. After this the broodstocks are removed from the tank and placed back in the broodstock tanks with no food for 2 days.

1.5 Embryonic, larval and post larval development

Following spawning, external fertilization initiates cell division. Within 13 to 14 hours, the larvae reach the trochophore stage, becoming more active. On the first day, they are separated from residual material using sieves (90 µm for residues and 25 µm for larvae) and transferred to disinfected cylindroconical tanks, where they remain until reaching the pediveliger stage (Figure 14).

Figure 14- Separation of larvae from residual materials from spawn using sieves (90 µm for residues and 25 µm for larvae)

a. Feeding and Drop Schedule

Larvae are fed twice daily, in the morning and afternoon, except on drop days. Drops occur every two days, starting on day 2 (Figure 15). On these days, morning feeding is omitted to allow for tank exchanges, but fresh food is added to the new tanks before the afternoon feeding. Prior to the drop, the replacement tanks are prepared by filling them with water.

Figure 15 – Larvae in 2000 L cylindroconical tanks with fresh algae mix

b. Drop Procedure and Larval Counting

Drops are performed one tank at a time using sieves of different sizes (50–175 µm) to separate larvae by size. Each drop includes a cleaning sieve and a safety sieve. The larvae are then collected in a beaker, and the volume is adjusted with seawater until the density appears uniform (~4L initially).

To estimate the total larval population, samples are taken using a micropipette (20–100 µl, depending on density). Three samples are analysed under a microscope. If the counts vary significantly, outliers are excluded, and the average is calculated. The total number of larvae in the tank is estimated using the formula:

Average larvae count × Beaker volume × Sample volume = Total larvae per tank

Only the two largest size classes are typically counted, unless growth is uneven, in which case three sizes may be assessed. If the target for the day is 8 million larvae and the largest size accounts for 7 million, only 1 million of the next size down is added, with a proportion used to determine the exact volume needed. Any excess larvae are discarded.

Tanks are never mixed, except on the first drop when all larvae are pooled and redistributed into 3 to 4 new 2000L tanks.

c. Larval Diet and Monitoring

Larvae are fed a microalgae mixture following a progressively adjusted diet. The composition shifts over time, increasing diversity and adjusting the proportion of each species to support development. The diet consists of a changing percentage of the following algae through the different larval stages: *Chaetoceros calcitrans* (Chc), *Isochrysis galbana* (Iso), *Dunaliella sp*. (Dl), and *Chaetoceros gracilis* (Chg).

Microalgae cell counts are performed using a modified Neubauer chamber method. Residual counts are calculated by summing the cells from all nine squares of the grid and applying the formula:

$$Residuals = \frac{Total\ cell\ count}{9} \times \frac{1}{0.1} \times 1000$$

Throughout the larval period, daily assessments monitor gut content, lipid reserves, velum/swimming activity, deformities, and growth (Figure 16). Tank deposits are checked during each water exchange, as their presence may indicate mortality. Survival rates are also regularly calculated.

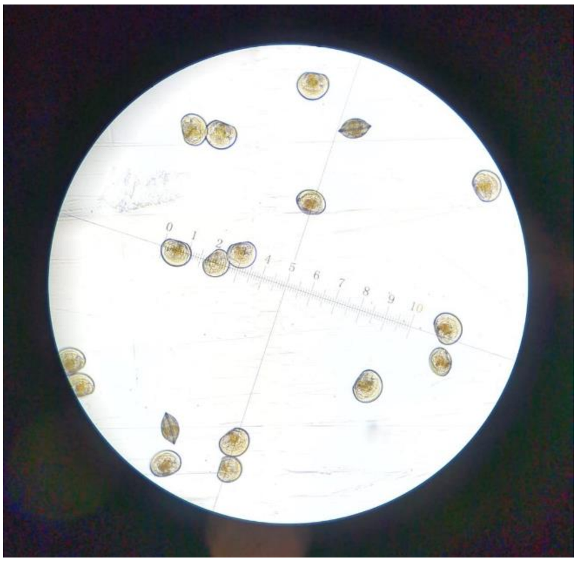


Figure 16- Observation of larvae under microscope to assess gut content, lipid reserves, velum/swimming activity, deformities, and growth

d. Transition to Post-Larval and Spat Stages

When larvae reach approximately 150–175 µm, typically between days 12–14, they are transferred to the post-larval system to prevent premature settlement. This system consists of 50 cm diameter fiberglass cylinders with 125 µm mesh and controlled airlift flow (Figure 17). At this stage, they transition from a planktonic to a benthic lifestyle, reabsorbing the velum and developing gills.

Figure 17 – Post-larval settling systems consisting of 50 cm diameter fiberglass cylinders with 125 µm mesh and controlled airlift flow

By day 22, the post-larva, now equipped with a fully developed foot and no velum, reaches approximately 250–270 µm and begins forming its definitive shell (disoconch). The transition to spat tanks occurs at 300–400 µm, typically between days 22–24, though variations depend on growth rates. At this stage, there are often three different size classes present.

1.6 Spat rearing

Spat are initially reared in 50 cm diameter fiberglass cylinders, beginning at a 125 µm mesh with a gentle downward airlift. These cylinders are placed within 3000 L spat rearing tanks, which receive a constant flow of seawater at a rate of 60 L/h, along with a continuous supply of microalgae (Figure 18).

Grading is performed periodically to separate individuals by size and assess biomass, enabling appropriate food supply adjustments (Figure 18). Currently, each batch undergoes grading approximately every three to four weeks, depending on workload and the number of batches being processed.

Figure 18- Spat reared in 50 cm diameter fiberglass cylinders with downward airlift inside 3000 L spat rearing tanks and spat grading (Source: Jornal de Leiria).

The tanks are cleaned three times per week using a commercial disinfectant to maintain optimal water quality and hygiene.

Additionally, the spat's food line undergoes monthly cleaning and disinfection with acid, bleach, and sodium thiosulfate to prevent biofouling and ensure a healthy rearing environment.

Once spat reach a size of 2.4 mm, and there is enough available, they are prepared for transport to Lagos.

1.7 Microbiology tracking

As the hatchery constantly houses larvae, spat, and broodstock, tracking potential pathogens and preventing outbreaks is essential. Known pathogens affecting *R. decussatus* and *V. corrugata* are monitored closely. To identify possible sources of contamination, weekly microbiological sampling is conducted at various locations within the hatchery, with at least one sampling point in each room.

Microbiological analysis is carried out using Marine Agar and TCBS Agar. Routine sampling is scheduled as follows:

- Mondays: Marine Agar and TCBS samples are taken post-UV in the water treatment area, from the bag culture room (post-pasteurization), and from the six production lines (one sample per line). Additional samples are collected from running water in larvae, post-larvae, broodstock, and seed tanks.
- Wednesdays and Fridays: Rapid TCBS testing is performed on the collection tanks in the bag culture room. This method involves adding 1 mL of sample directly onto the plate, mixing, pouring, and incubating.
- Daily (on drop days): Rapid TCBS testing is conducted on larval tanks using the same method as for collection tanks.

This structured microbiological monitoring system helps ensure early detection of potential threats, maintaining optimal health conditions within the hatchery.

1.8 Biosecurity

Maintaining strict biosecurity protocols is crucial in hatchery operations to prevent disease outbreaks and ensure the health of all cultured organisms. In this hatchery, technicians are prohibited from entering other rooms after handling broodstock to minimize the risk of cross-contamination. Extra precautions are taken when working with larvae, and foot baths are installed at the entrance of every room to reduce the risk of pathogen introduction. These measures collectively help maintain a controlled and disease-free environment within the facility.

1.9 Open Sea grow-out

After the spat reaches 2.4 mm it is dispatched to the company's open sea grow-out facility, to prepare for shipping spat are placed in bags, dry, in an isothermal box kept with ice for maintaining a cool temperature and a wet cloth over them to maintain some humidity. Once at the facility, they are lotted and placed in lanterns, which are displaced along longlines (Figure 19). In these lanterns they have all the conditions necessary for the development until the desired commercial size, which is typically around 35 mm for *V. corrugata*, and larger. Once in the lanterns the clams feed off naturally available phytoplankton. Periodically the lanterns need to be rotated for cleaning, mainly due to biofouling. The grow-out consists of an area of 100 ha.

Figure 19 – Open sea grow-out consisting of lanterns hanging in longlines (Source: Barlavento (left), and Oceano Fresco (right)).

III. R&D project

The following project was proposed by the teacher as a strategic initiative to drive improvement within the company, with the intention of documenting and analysing the outcomes in this report. Following a collaborative discussion with the hatchery manager, we identified broodstock conditioning as a key area of focus for experimentation. This decision was based on its potential to yield valuable insights and tangible benefits for the hatchery.

The Effect of Broodstock Conditioning systems on Reproductive Performance in *Venerupis corrugata*

Abstract

The success of bivalve hatcheries relies on effective broodstock conditioning strategies that enhance reproductive performance and ensure a steady supply of larvae. This study evaluates the impact of two conditioning methods—Recirculating (R) feeding and Flow-Through (FT) conditioning—on the reproductive performance of *Venerupis corrugata*. A total of 1308 broodstock individuals were allocated equally into the two treatment groups, with gonadal maturation, gonadosomatic index (GSI), spawning success, and larval viability assessed over a 31-day conditioning period. Results indicated that while both conditioning methods supported similar survival rates and gonadal maturation trajectories, R conditioning led to a significant increase in egg production (43.8% higher), hatching success (15.3 percentage points higher), and larval output (approximately twice the number of viable D-larvae per broodstock individual compared to FT). No statistically significant differences were observed in GSI, suggesting that while R conditioning accelerated early gonadal development, overall reproductive readiness was ultimately similar between the two methods. These findings underscore the potential of R conditioning to enhance reproductive efficiency in hatchery settings, reducing reliance on wild broodstock and optimizing larval production. However, logistical and economic considerations associated with increased feed input warrant further investigation to refine conditioning protocols for sustainable aquaculture.

Keywords: Broodstock conditioning, reproductive performance, *Venerupis corrugata*, aquaculture, gonadal maturation, larval viability, hatchery management.

1. Introduction

The success of commercial bivalve hatcheries is largely dependent on the quality and availability of broodstock, ensuring a reliable supply of reproductively mature individuals is essential for maintaining consistent larvae production (Joaquim et al., 2016). However, many hatcheries rely on wild-caught broodstock (Ikhwanuddin & Abol-Munafi, 2016), sourced from diverse sandbanks across different geographical regions. While this approach supports genetic diversity, it also introduces significant challenges, including unpredictable reproductive readiness and increased pressure on natural populations. These constraints underscore the need for improved broodstock conditioning strategies that enhance reproductive efficiency while reducing dependence on wild stocks.

Broodstock conditioning programs play a critical role in optimizing reproductive success by manipulating environmental and nutritional factors to promote gonadal maturation (Nascimento-Schulze, 2021). Conditioning methods such as flow-through (FT) systems, provide improved water quality but may not fully optimize feed utilization, potentially limiting reproductive output. An alternative approach, Recirculating system (R) conditioning, seeks to improve gonadal development by ensuring greater feed availability by preventing feed waste.

This study was initiated as a strategic research initiative within the hatchery, aiming to evaluate the impact of R and FT conditioning on broodstock maturation and subsequent larvae production. By systematically comparing these two approaches, we seek to determine whether increased feed availability leads to improved reproductive metrics, including gonadosomatic index (GSI), spawning success, and larval viability. Our hypothesis is that broodstock subjected to R conditioning will exhibit faster gonadal maturation, higher fecundity, and improved offspring quality compared to those reared under standard FT protocols.

The findings from this research will contribute to the optimization of hatchery broodstock management practices, supporting more sustainable and efficient production systems. Additionally, by refining conditioning protocols, particularly using recirculating systems, this study aims to enhance the contact time between algae and broodstock, leading to improved conditioning results with reduced algae usage compared to flow-through systems. This approach has the potential to reduce reliance on wild broodstock, addressing both ecological and operational challenges faced by the industry.

2. Materials and Methods

2.1 Experimental Design

This study aimed to evaluate the effects of two broodstock conditioning methods, Recirculating (R) conditioning and Flow-Through (FT) conditioning, on gonadal maturation, reproductive output, and larvae production in *Venerupis corrugata* broodstock. The experiment was conducted at the company's hatchery facility, following a controlled design with randomized allocation of broodstock into experimental groups.

A total of 1308 broodstock individuals were collected and randomly divided into two treatment groups (n = 654 per group):

R Group: Conditioned using a recirculation system. (Figure 1)

FT Group: Conditioned using the hatchery's standard flow-through system, serving as the control.

Each group was further subdivided into three replicate tanks (n = 218 per tank), ensuring statistical reliability of the results. The conditioning period lasted 31 days, with periodic sampling at Day 0, Day 16, and Day 31 to assess gonadal development and reproductive performance.

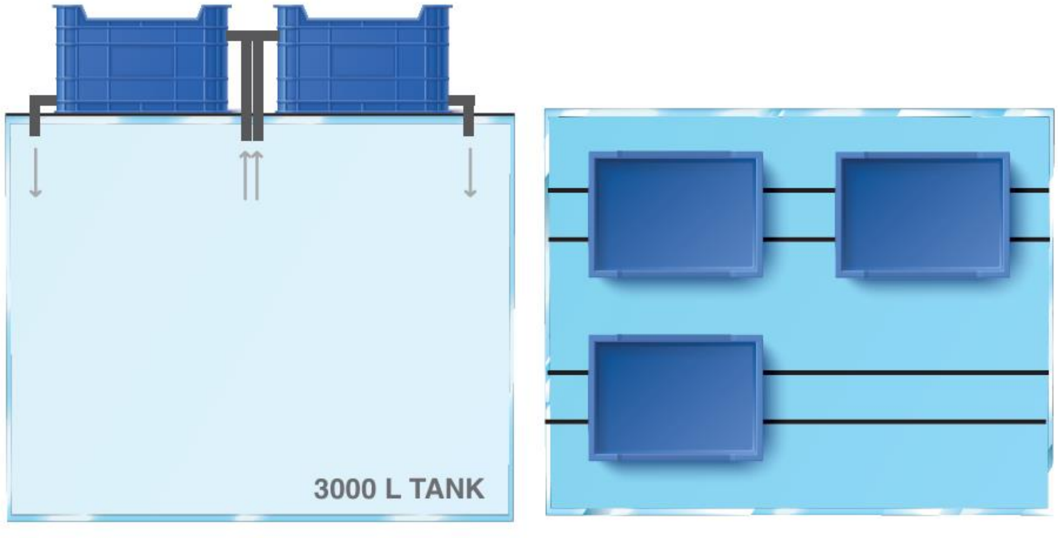


Figure 1 – Broodstock recirculation tank design

2.2 Experimental System Setup

a. Broodstock Selection and Acclimation

Broodstock individuals were collected from natural sandbanks from Ria de Aveiro, Portugal, and transported to the hatchery under controlled conditions. Upon arrival, they underwent a seven-day acclimation period in the hatchery's flow-through system to ensure stabilization of physiological conditions before the experiment began.

b. Conditioning Systems

The two conditioning systems were designed to compare the effects of feed availability and water flow dynamics on broodstock maturation:

Recirculation (R) Conditioning: Broodstock were placed in a recirculating system where a microalgae mix was given daily (Figure 2).

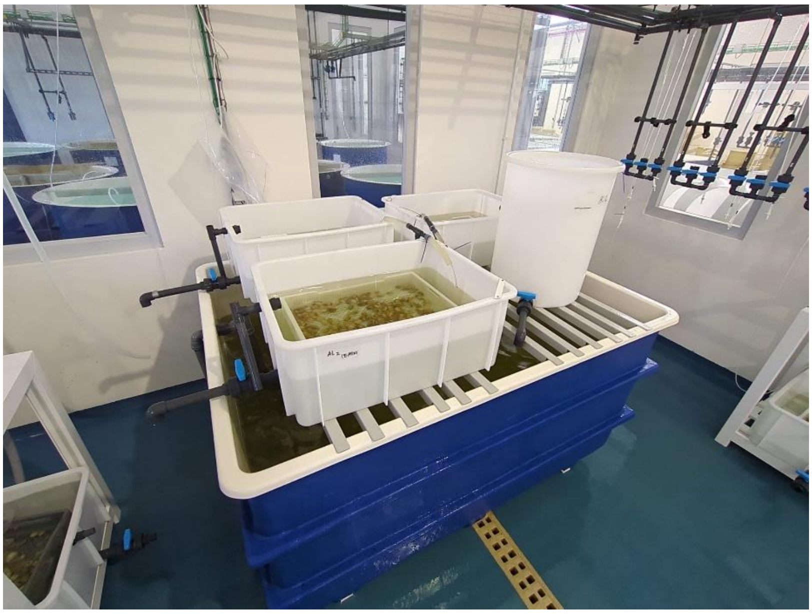


Figure 2- Broodstock recirculation tank used for the trial

Flow-Through (FT) Conditioning: Broodstock were placed in standard flow-through tanks, where a continuous supply of fresh seawater and a microalgae mix was provided, simulating conventional hatchery conditions (Figure 3).

Figure 3- Broodstock flow-through tanks used for the trial

Both systems operated under controlled temperature (18–20°C) and salinity (32–35 ppt), with dissolved oxygen levels kept above 6.0 mg/L through aeration systems.

c. Feeding Protocol

Broodstock in both treatments were fed a controlled mixture of live microalgae species (*Chaetoceros gracilis*, *Chaetoceros calcitrans*, *Tisochrysis lutea*, *Diacronema lutheri*, *Isochrysis galbana* and *Tetraselmis suecica*), adjusted to match estimated metabolic demands. Both conditions were fed 80L of this phytoplankton mix per day.

R Group: Microalgae were provided once daily, following the hatchery's standard feeding regime.

FT Group: Microalgae availability was kept constant through dosing in the flow-through system.

2.3 Data Collection and Measurements

a. Broodstock Survival

All replicates were monitored daily to record any occurrences of mortality. Dead individuals were promptly removed, and mortality events were documented throughout the experiment. The recorded data were used to calculate the cumulative mortality rate over the duration of the trial, providing insights into the survival dynamics of broodstock under different conditioning treatments.

b. Gonadal Maturation and Conditioning

To evaluate gonadal development and maturation, broodstock were sampled at three key time points: Day 0, Day 16, and Day 31. These assessments aimed to track reproductive progression throughout the experimental period and determine the effects of different conditioning treatments on gametogenesis. At each sampling point, 15 individuals per condition were randomly selected, five from each replicate, to undergo the following measurements:

Total wet weight: Each individual was removed from the tank, gently dried with a paper cloth, and weighed using a precision scale.

Gonad wet weight: Individuals were dissected, and their gonads were carefully separated from the visceral mass using a scalpel before being weighed on a precision scale.

Gonadosomatic Index (GSI): The GSI was calculated using the following formula:

$$\text{GSI} = \frac{Gonad\ wet\ weight}{Total\ wet\ weight} \times 100$$

Gonadal smears: After weighing, small portions of the gonads were excised and spread onto clean microscopic slides. Cover slips were gently placed to prevent tissue distortion, and the smears were examined under a microscope. The reproductive stage of each individual was classified according to Table 1, providing a detailed characterization of gonadal maturation throughout the experiment.

Table 1 -Description of each maturation level Source: Oceano Fresco

Maturation level	*Description*		
Stage	Denomination	Macroscopic appearance	Fresh smear
0	Resting	The visceral mass is flat, whitish and transparent (intestines can be observed)	Absence of gametes
1	Early developing	The visceral mass continues to be small	Gametes are beginning to appear
2	Late developing	Gonad size begins to increase	Gametes are becoming more abundant
3	Ripe and Spawning	The gonad reaches its maximum development occupying most of the visceral mass	Abundance of gametes
4	Spent	The gonad is in regression and flaccid	Residual gametes

c. Algae Consumption

Daily algal consumption was quantified for the R condition using a Neubauer chamber, recording the difference between initial and residual algal using a modified version of the Neubauer chamber method.

d. Larvae Production and Reproductive Output

Upon spawning, reproductive success evaluated by assessing total egg count per broodstock individual and hatching rate, as a percentage of fertilized eggs developing into D-larvae. Additionally, total D-larvae survival at 48 hours post-hatch was assessed.

e. Statistical Analysis

All data were analysed using SPSS v29.0, with a significance level set at $p < 0.05$. Chi-square tests were used to compare gonadal maturation stages and mortality rates between R and FT. Two-way ANOVA was conducted to assess differences in GSI over time between treatments. Post-hoc Tukey’s tests were performed where significant differences were detected. Graphical representations were generated using SPSS and GraphPad Prism, ensuring clear visualization of trends.

3. Results

3.1. Broodstock Survival

Mortality rates were similar between the two conditioning methods, with no significant difference observed between FT and AL ($\chi^2 = 0.232$, $p = 0.630$) (Figure 4). The FT group exhibited a mortality rate of 11% for the duration of the trial, whereas the R group had a mortality rate of 9%, indicating that the conditioning method did not significantly influence broodstock survival over the study period.

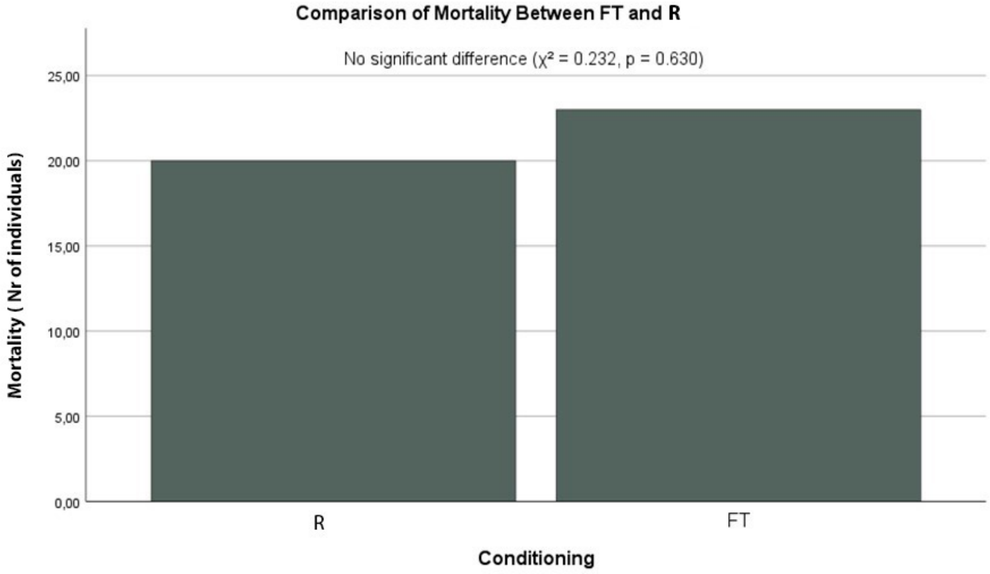


Figure 4- Comparison of mortality between two conditioning systems, recirculating and flow-through

3.2. Gonadal Maturation and Conditioning

a. Gonadal Maturation

The progression of gonadal maturation was assessed at three time points: Day 0, 16, and 31 as represented in Figure 5. At the beginning of the trial (Day 0), all individuals were classified as Stage 4 (Spent Gonads). By Day 16, the R-conditioned individuals had predominantly reached Stage 3 (Ripe and Spawning), while those in the FT group were primarily at Stage 2 (Late Developing), with some reaching Stage 3. By Day 31, individuals in both groups were predominantly at Stage 3, indicating a comparable state of reproductive maturity.

Despite the observed differences in maturation rates at Day 16, statistical analysis using a chi-square test revealed no significant differences in the distribution of gonadal stages between FT and R at any time point. These findings suggest that, although R conditioning facilitated a more rapid transition to ripe gonads in the early stages, both conditioning methods ultimately led to similar reproductive development by the end of the study.

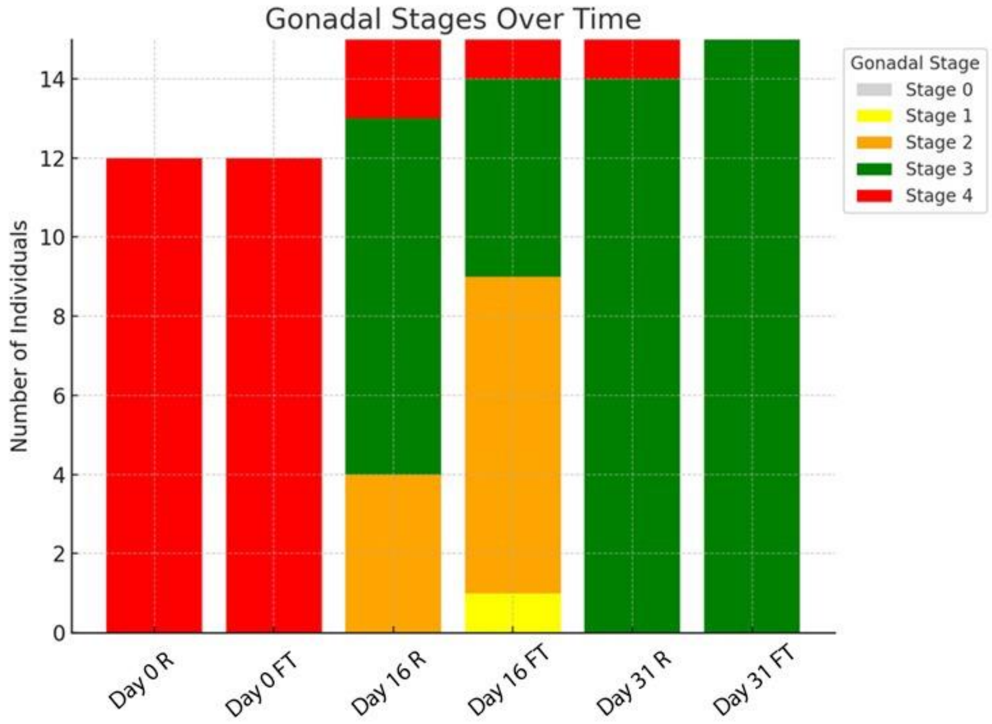


Figure 5- Maturation level of assessed gonads for two different conditioning systems, recirculating and flow-through, taking place at day 0, 16 and 31.

b. Gonadosomatic Index

The Gonadosomatic Index (GSI) was used to evaluate reproductive development over time and are represented in Figure 6 and Table 2. The results indicated a general increase in GSI from Day 0 to Day 16 in both conditions, but no further increase from Day 16 to Day 31. The R group exhibited slightly higher GSI values at Days 16 and 31 compared to the FT group; however, no significant differences were detected between treatments at any time point ($p > 0.05$). These results suggest that both conditioning methods resulted in similar gonadal development, with no apparent advantage of one method over the other in terms of GSI trends.

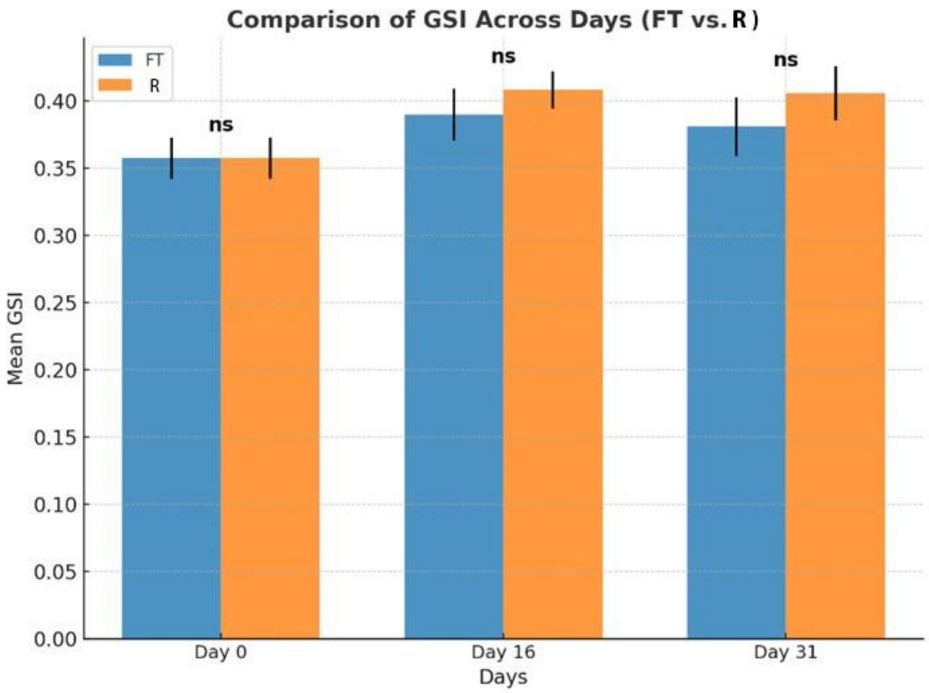


Figure 6 – Comparison of GSI at three different time points (Day 0,16 and 31) between both conditioning systems, recirculating and flow-through (ns stands for not significant).

Table 2 - Average gonadosomatic index of both conditions (FT and R) at day 0, 16 and 31.

Day	Average GSI (FT)	Average GSI (R)
0	35.7	35.7
16	39.0	40.8
31	38.0	40.6

3.3 Algae Consumption

The number of algal cells consumed per hour per kilogram of clams was calculated based on the difference between the initial and residual algal concentrations on the recirculating condition. The average algal consumption was 1.11×10^{6} cells per kilogram of broodstock. This data provide insight into the feeding dynamics of the broodstock but cannot be directly linked to reproductive performance parameters since only one condition could be accounted for.

3.4 Larvae Production and Reproductive Output

a. Egg Production

The total number of eggs produced differed between conditioning methods. Broodstock conditioned under Recirculating (R) system produced 1.021×10^8 eggs, whereas those under the Flow-Through (FT) system produced 7.1×10^7 eggs, representing a 43.8% increase in total egg production for the R treatment. When standardized per individual, R broodstock produced 1.75×10^5 eggs per clam, while FT broodstock produced 1.26×10^5 eggs per clam, a difference of approximately 39% (Table 3).

Table 3 – Spawn data for both conditioning systems, recirculating and flow-through.

Condition	**Number of individuals to spawn**	**Number of Eggs collected (10^6)**	**Number of Eggs per Individual (10^3)**	**Number of Day 2 D larvae produced (10^6)**	**Hatching rate (%)**	**Number of D larvae per individual**
R	585	102.1	175	52.1	51.0%	89114
FT	565	71.0	126	25.3	35.7%	44836

b. Hatching Rate and Larval Production

Hatching success and subsequent larval output were also influenced by the conditioning method (Table 3). The R group achieved a hatching rate of 51.0%, whereas the FT group had a lower success rate of 35.7%, reflecting a 15.3 percentage point increase in the R group. Consequently, the total number of viable D-larvae produced was significantly higher in R (5.21×10^7 larvae) compared to FT (2.53×10^7 larvae). When normalized per broodstock individual, R broodstock produced 8.91×10^4 larvae per clam, while FT broodstock produced 4.4836×10^4 larvae per clam, nearly half as much (Table 3).

4. Discussion

This study evaluated the effects of two broodstock conditioning methods, Recirculating (R) system and a Flow-Through (FT) system, on reproductive performance in *Venerupis corrugata*. The results demonstrated that while both conditioning methods supported similar broodstock survival and gonadal development, R conditioning led to significantly higher egg production, hatching success, and larval output. These findings highlight the role of nutritional availability in optimizing reproductive output and provide insights into hatchery management strategies.

4.1 Effects of Conditioning on Broodstock Survival and Gonadal Development

Broodstock survival rates were comparable between the two conditioning treatments, suggesting that feed availability did not influence overall survival under hatchery conditions. The observed mortality rates (11% in FT and 9% in R) were relatively low and within expected ranges for bivalve broodstock maintenance (Cerviño, 2011).

Gonadal maturation followed a similar trajectory in both conditioning groups, with individuals progressing from a spent (Stage 4) to a ripe (Stage 3) condition over the course of the trial. However, R-conditioned broodstock exhibited a faster rate of maturation, with a higher proportion reaching Stage 3 by Day 16. Despite this apparent acceleration in gonadal development, no statistically significant differences were detected in maturation stages between treatments. This suggests that while R conditioning may facilitate earlier maturation, both methods ultimately lead to similar reproductive readiness by the end of the conditioning period.

The Gonadosomatic Index (GSI) results further reinforce these findings, showing a steady increase in gonadal mass from Day 0 to Day 16, followed by a plateau between Day 16 and Day 31. The absence of significant differences in GSI between the R and FT conditions suggests that nutritional availability alone may not be the primary driver of gonadal mass accumulation. Instead, factors such as temperature and photoperiod likely play a crucial role in regulating reproductive cycles in *V. corrugata*, like as in other bivalves, demonstrated by studies such as Villalejo-Fuerte & Ochoa-Báez (1993) and Joyce *et al.* (2013). Similarly, a study on *Ruditapes decussatus* by Delgado & Camacho (2005) found that under food scarcity, energy depletion was more closely associated with the loss of reserve tissue rather than variations in the amount of reproductive tissue. This could also explain the lack of significant differences in gonadal maturation and GSI in the present study, as broodstock may prioritize reproductive investment even under varying nutritional conditions.

4.2 Influence of Conditioning on Spawning Performance and Larval Output

A key finding of this study was the significant impact of conditioning method on reproductive output. Broodstock maintained under R conditions produced 43.8% more eggs than those in the FT system, with 1.75×10^5 eggs per individual compared to 1.26×10^5 in FT. This increase in fecundity is consistent with previous studies on bivalve species, where enhanced feeding regimes resulted in greater spawning success (Pronker et al., 2008).

In addition to higher egg production, R conditioning significantly improved hatching success and larval viability. Hatching rates were 15.3 percentage points higher in the R group, leading to a more than twofold increase in viable D-larvae production compared to FT broodstock. These results suggest that adequate nutritional intake during conditioning not only enhances fecundity but also improves egg quality and embryonic development. The mechanisms underlying this effect may be linked to the increased availability of essential fatty acids and total lipids in R-fed broodstock, which are known to influence oocyte quality and larval survival (Joaquim et al., 2016).

4.3 Implications for Hatchery Practices

The results of this study provide important implications for broodstock management in bivalve hatcheries. While both R and FT conditioning methods supported reproductive development, R conditioning resulted in superior reproductive performance in terms of egg quantity, hatching success, and larval output. This suggests that hatcheries aiming to optimize larvae production may benefit from adopting R conditioning strategies or modifying current FT systems to allow for increased feed availability.

However, it is important to consider the economic and logistical challenges associated with R conditioning. Increased feed supply can increase operational costs, and recirculating systems may lead to higher maintenance requirements due to potential water quality deterioration. Future studies should explore cost-benefit analyses to determine whether the increased larvae output justifies the additional resource investment associated with such conditioning.

4.4. Study Limitations and Future Research Directions

While this study provides valuable insights, some limitations should be acknowledged. First, gonadal lipid and protein composition were not analysed, which could provide a more detailed understanding of how nutritional intake influences reproductive quality. Future research should assess the biochemical composition of gametes to determine whether R-conditioned broodstock exhibit higher lipid reserves or improved energy transfer to offspring.

The physiological status of the broodstock upon arrival plays a crucial role in their reproductive potential, as it is influenced by seasonal variations. It is possible that the broodstock used in the experiment had sufficient glycogen reserves to support gametogenesis, regardless of the conditioning treatment applied in the hatchery. This could suggest that external environmental factors prior to capture may have had a significant impact on their reproductive development.

Additionally, the study did not evaluate post-hatch larval survival beyond 48 hours. Although R conditioning resulted in higher initial larval production, it remains unknown whether this advantage persists throughout the larval development stages. A study by Beekey & Karlson (2003), observed that in *Sphaerium striatinum*, a freshwater clam species, individuals provided with higher food availability reproduced more frequently, produced larger broods, and had more offspring per reproductive attempt. However, despite these advantages, there was no significant difference in the number of offspring surviving to later developmental stages among treatments. In fact, clams with greater food availability experienced proportionally higher offspring loss, suggesting that physical constraints, rather than solely nutritional input, may be more influential in determining reproductive success.

Long-term monitoring of growth rates, settlement success, and juvenile survival would provide a more comprehensive evaluation of conditioning effects on hatchery productivity.

4.5 Conclusion

This study demonstrates that broodstock conditioning significantly influences reproductive success in *V. corrugata*, with R conditioning leading to enhanced fecundity, higher hatching success, and increased larval viability. These findings highlight the importance of optimized nutritional strategies in hatchery settings and suggest that increased feed availability can significantly improve reproductive outcomes. Further research into the biochemical and economic aspects of R conditioning will be essential in refining hatchery protocols for sustainable bivalve aquaculture.

IV. References

Anne, A., 2024. *EU aquaculture: State of play*, EPRS: European Parliamentary Research Service. Belgium. Retrieved from https://coilink.org/20.500.12592/k0p2vn0 on 17 Mar 2025. COI: 20.500.12592/k0p2vn0.

Ansell, A. D. (1961). The functional morphology of the British species of *Veneracea* (*Eulamellibranchia*). Journal of the Marine Biological Association of the United Kingdom, 41(2), 489-517.

Ansell, A. D., & Trueman, E. R. (1967). Burrowing in *Mercenaria mercenaria* (L.) (Bivalvia, Veneridae). Journal of Experimental Biology, 46(1), 105-115.

Beekey, M.A., & Karlson, R.H. (2003). Effect of food availability on reproduction and brood size in a freshwater brooding bivalve. Canadian Journal of Zoology, 81, 1168-1173.

Cerviño Otero, A. (2011). Ciclo reproductivo, cultivo en criadero y en el medio natural de la almeja babosa *Venerupis pullastra* (Montagu, 1803).

Coibion, O., Gorodnichenko, Y., & Weber, M. (2020). The cost of the covid-19 crisis: Lockdowns, macroeconomic expectations, and consumer spending (No. w27141). National Bureau of Economic Research. 10.3386/w27141

Cranford, P., Dowd, M., Grant, J., Hargrave, B., & McGladdery, S. (2003). Ecosystem level effects of marine bivalve aquaculture. A scientific review of the potential environmental effects of aquaculture in aquatic ecosystems, 1, 51-95.

da Costa, F. (2012). Introduction to the biology of clams. Clam Fisheries and Aquaculture, 1.

Delgado, M.N., & Camacho, A.P. (2005). Histological study of the gonadal development of *Ruditapes decussatus* (L.) (Mollusca: Bivalvia) and its relationship with available food. Scientia Marina, 69, 87-97.

Direção-geral de Recursos Naturais, Segurança e Serviços Marítimos. (2023). Recursos da Pesca. Série estatística, Volume 36 A-B.

Domínguez, R., Olabarria, C., & Vázquez, E. (2023). Assessment of risks associated with extreme climate events in small-scale bivalve fisheries: conceptual maps for decision-making based on a review of recent studies. *Journal of Marine Science and Engineering*, *11*(6), 1216.

European Climate, Infrastructure and Environment Executive Agency (ECIEEA). (2023). EU funding opportunities available for aquaculture, Background paper. ISBN 978-92-95231-45-0, PDF HZ-04-23-181-EN-N, doi:10.2926/207560

Fahy, E., Carroll, J., Rafferty, J., Roantree, V., Reid, C., Norman, M., & Clarke, S. (2010). Observations on the local distribution, biology and ecology of palourde T*apes decussatus* (L.) of relevance to its exploitation in the Republic of Ireland. In *Biology and Environment: Proceedings of the Royal Irish Academy* (Vol. 110, No. 2, pp. 95-108). Royal Irish Academy.

FAO. (2024a). The state of world fisheries and aquaculture 2024: Blue transformation in action. Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/cd0683en

FAO Fisheries and Aquaculture Division. (2024b). Fishery and Aquaculture Statistics: Global production by production source 1950-2018 (FishstatJ). Retrieved from https://www.fao.org/fishery/statistics-query/en/global_production

FAO (2025), *Ruditapes decussatus*. Cultured Aquatic Species Information Programme. Text by Figueras, A.. In: *Fisheries and Aquaculture*. Updated 2006-10-23 [Cited Thursday, March 6th 2025].

https://www.fao.org/fishery/en/culturedspecies/ruditapes_decussatus/en

Federation of European Aquaculture Producers (FEAP). (2023). European aquaculture producers report 2016-2022. https://feap.info/wp-content/uploads/2022/03/production-report-v1.1.pdf

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., … Tveterås, R. (2019). A Global Blue Revolution: Aquaculture Growth Across Regions, Species, and Countries. Reviews in Fisheries Science & Aquaculture, 28(1), 107–116. https://doi.org/10.1080/23308249.2019.1678111

Guillen, J., Asche, F., Carvalho, N., Polanco, J. M. F., Llorente, I., Nielsen, R., ... & Villasante, S. (2019). Aquaculture subsidies in the European Union: Evolution, impact and future potential for growth. Marine Policy, 104, 19-28. https://doi.org/10.1016/j.marpol.2019.02.045

Gupta, G. S. (2019). Land degradation and challenges of food security. Rev. Eur. Stud., 11, 63. . https://doi.org/10.5539/res.v11n1p63

Habtemariam, B. T., Arias, A., García-Vázquez, E., & Borrell, Y. J. (2015). Impacts of supplementation aquaculture on the genetic diversity of wild *Ruditapes decussatus* from northern Spain. Aquaculture Environment Interactions, 6(3), 241-254. https://doi.org/10.3354/aei00128

Harvey, M., & Pilgrim, S. (2011). The new competition for land: Food, energy, and climate change. Food policy, 36, S40-S51. https://doi.org/10.1016/j.foodpol.2010.11.009

Helm, M. M., Bourne, N., & Lovatelli, A. (2004). *The hatchery culture of bivalves: a practical manual*. FAO. https://doi.org/10.3354/aei00128

Henriksson, P. J. G., Troell, M., Banks, L. K., Belton, B., Beveridge, M. C. M., Klinger, D. H., ... & Tran, N. (2021). Interventions for improving the productivity and environmental performance of global aquaculture for future food security. One Earth, 4(9), 1220-1232. https://doi.org/10.1016/j.oneear.2021.08.009

Hough, C. (2022). Regional review on status and trends in aquaculture development in Europe–2020. FAO Fisheries and Aquaculture Circular N0.1232/1. Rome, FAO. https://doi.rg/10.4060/cb7809en

Instituto Nacional de Estatística. (2023). Estatísticas da Pesca. Lisboa. https://www.ine.pt/xurl/pub/439542305

IPMA. (2023). Lista de Espécies. https://www.ipma.pt/pt/bivalves/docs/files/Lista_de_espxcies_em_26_06_2023.pdf

Ikhwanuddin, M., & Abol-Munafi, A. B. (2016). Fish and shellfish domestication and stock enhancement: current status and future directions. 10.3923/ajsr.2016.167.170

Jamaludin, M. (2022). Indonesia's Food Security Challenges: How Food SOE Optimizes its Role?. Research Horizon, 2(3), 394-401. https://doi.org10.54518/rh.2.3.2022.394-401

Joaquim, S., Pereira, J., Leitao, A., Matias, D., Chaves, R., Guedes-Pinto, H., ... & Gaspar, M. (2010). Genetic diversity of two Portuguese populations of the pullet carpet shell *Venerupis senegalensis*, based on RAPD markers: contribution to a sustainable restocking program. *Helgoland Marine Research*, *64*, 289-295.

Joaquim, S., Matias, D., Matias, A. M., Gonçalves, R., Vera, C., Chícharo, L., & Gaspar, M. B. (2016). Relationships between broodstock condition, oocyte quality, and 24 h D-larval survival during the spawning season of the pullet carpet shell *Venerupis corrugata* (Gmelin, 1791). *Invertebrate reproductIon & development*, *60*(4), 271-280.

Joyce, A., Holthuis, T. D., Charrier, G., & Lindegarth, S. (2013). Experimental effects of temperature and photoperiod on synchrony of gametogenesis and sex ratio in the European oyster *Ostrea edulis* (*Linnaeus*). *Journal of Shellfish Research*, *32*(2), 447-458. 10.2983/035.032.0225

Kahler, G. A., Fisher Jr, F. M., & Sass, R. L. (1976). The chemical composition and mechanical properties of the hinge ligament in bivalve molluscs. The Biological Bulletin, 151(1), 161-181.

Kaiser, M., Stead, S. (2002). Uncertainties and values in European aquaculture: communication, management and policy issues in times of “changing public perceptions”. Aquaculture International, 10, 469–490. https://doi.org/10.1023/A:1023963326201

Kinder, C. (1998). The population explosion: causes and consequences. Yale-New Haven institute, The population explosion. https://teachersinstitute.yale.edu/curriculum/units/1998/7/98.07.02.x.html

Laing, I., & Spencer, B. E. (2006). Bivalve cultivation: criteria for selecting a site. SCIENCE SERIES TECHNICAL REPORT-CENTRE FOR ENVIRONMENT FISHERIES AND AQUACULTURE SCIENCE, 136.

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. Reviews in Aquaculture, 12(2), 943-965. https://doi.org/10.1111/raq.12365

Macho, G., Woodin, S. A., Wethey, D. S., & Vázquez, E. (2016). Impacts of sublethal and lethal high temperatures on clams exploited in European fisheries. *Journal of Shellfish Research*, *35*(2), 405-419.

Matias, D., Joaquim, S., Leitao, A., & Massapina, C. (2009). Effect of geographic origin, temperature and timing of broodstock collection on conditioning, spawning success and larval viability of *R. decussatus* (Linné, 1758). Aquaculture International, 17(3), 257-271. https://doi.org/10.1007/s10499-008-9197-3

Misselhorn, A., Aggarwal, P., Ericksen, P., Gregory, P., Horn-Phathanothai, L., Ingram, J., & Wiebe, K. (2012). A vision for attaining food security. Current opinion in environmental sustainability, 4(1), 7-17. https://doi.org/10.1016/j.cosust.2012.01.008

Nascimento-Schulze, J. C., Bean, T. P., Houston, R. D., Santos, E. M., Sanders, M. B., Lewis, C., & Ellis, R. P. (2021). Optimizing hatchery practices for genetic improvement of marine bivalves. *Reviews in Aquaculture*, *13*(4), 2289-2304.

Pauly, D., & Zeller, D. (2016). Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. Nature communications, 7(1), 10244.

Pimentel, D. (1991). Global warming, population growth, and natural resources for food production. Society & Natural Resources, 4(4), 347–363. doi:10.1080/08941929109380766

Pronker, A. E., Nevejan, N. M., Peene, F., Geijsen, P., & Sorgeloos, P. (2008). Hatchery broodstock conditioning of the blue mussel Mytilus edulis (Linnaeus 1758). Part I. Impact of different micro-algae mixtures on broodstock performance. *Aquaculture International*, *16*, 297-307. 10.1007/s10499-007-9143-9

Quayle, D. B. (1952). VIII.—Structure and Biology of the Larva and Spat of *Venerupis pullastra* (Montagu). Earth and Environmental Science Transactions of The Royal Society of Edinburgh, 62(1), 255-297.

Rayment, W.J. (2007). *V. corrugata* Pullet carpet shell. In Tyler-Walters H. and Hiscock K. (eds) Marine Life Information Network: Biology and Sensitivity Key Information Reviews, [on-line]. Plymouth: Marine Biological Association of the United Kingdom. DOI https://dx.doi.org/10.17031/marlinsp.1558.1

Read, P., & Fernandes, T. (2003). Management of environmental impacts of marine aquaculture in Europe. Aquaculture, 226(1-4), 139-163. https://doi.org/10.1016/S0044-8486(03)00474-5

Ruiz-Velasquez, M., Montecinos, H., & Tarifeño, E. (2018). Características Anatómicas e Histológicas del Sistema Nervioso del Mejillón Gigante, *Choromytilus chorus* (Molina 1782) (Bivalvia, Mytilidae). International Journal of Morphology, 36(4), 1262-1267.

United Nations (2023), The Sustainable Development Goals Report 2023: Special Edition. The Sustainable Development Goals Report. https://doi.org/10.18356/9789210024914

United Nations Department of Economic and Social Affairs (UNDESA), Population Division (2022). World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3.

Vaughn, C. C., & Hoellein, T. J. (2018). Bivalve impacts in freshwater and marine ecosystems. Annual review of ecology, evolution, and systematics, 49(1), 183-208. https://doi.org/10.1146/annurev-ecolsys-110617-062703

Villalejo-Fuerte, M., & Ochoa-Báez, R. I. (1993). The reproductive cycle of the scallop *Argopecten circularis* (Sowerby, 1835) in relation to temperature and photoperiod, in Bahia Concepcion, BCS, Mexico. *Ciencias Marinas*, *19*(2), 181-202. 10.7773/CM.V19I2.929

Watson, R. A., & Tidd, A. (2018). Mapping nearly a century and a half of global marine fishing: 1869–2015. Marine Policy, 93, 171-177.

Wirkner, C. S., Richter, S., Watling, L., & Thiel, L. (2013). Circulatory system and respiration. Natural History of Crustacea, 1, 376-412.

Yonge, C. M. (1923). Studies on the comparative physiology of digestion. Journal of Experimental Biology, 1(1), 15-64.

Zander, K., & Feucht, Y. (2017). Consumers' Willingness to Pay for Sustainable Seafood Made in Europe. Journal of International Food & Agribusiness Marketing, 30(3), 251–275. https://doi.org/10.1080/08974438.2017.1413611